EXPLORING THE LATEST

LABORATORY SCIENCE INNOVATIONS AT PITTCON

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CONFERENCE & EXPO

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Introduction

This e-book highlights the latest advances in genomic analysis, bioengineering and analytical technologies presented at Pittcon 2017.

It includes interviews with world-renowned experts, including Dr Deisseroth, Dr Mirkin, and Professor Sweedler.

WHAT IS PITTCON?

Pittcon is a world-leading annual event that allows laboratory researchers from all disciplines to learn about the latest trends in the field and share details of developments with their own research. It is also an arena for companies to demonstrate their latest technologies and instrumentation.

Pittcon attracts a wide range of experts from across the globe who characterize, quantify, or analyze the chemical or biological properties of compounds or molecules. The congress has attendees from industry, academia and government.

PITTCON 2017

Pittcon 2017 included a range of symposia, oral presentations, short courses, poster sessions and industry-sponsored demonstrations of cutting-edge technologies. The topics discussed covered **ground-breaking technological advances across a wide range of disciplines, highlighting the current strength of scientific research**. Researchers are constantly adapting and enhancing existing technologies and devising innovative new solutions to meet the challenges set by the ever-changing research, medical, monitoring and security demands that arise.

ADVANCES IN GENOMIC ANALYSIS

Sequencing of the human genome in 2003 was heralded as a ground-breaking achievement in scientific research. A once-unthinkable task had been successfully accomplished and many thought this was the pinnacle of genomic research. However, it proved to be the opening of a door to a multitude of advances that have totally changed, indeed revolutionized, genetic research.

The expensive, labor-intensive Sanger gene sequencing methodology used in the Human Genome Project has been eclipsed with the introduction of next-generation sequencing. **This technique allows millions or even billions of DNA fragments to be sequenced in parallel in a fraction of the time and at a fraction of the cost**. Furthermore, it made gene sequencing technology accessible for every genetics research laboratory whereby accelerating their research programs.

It is now also **possible to edit genes**. Gene sequences can be inserted, deleted, or replaced in a specific and targeted manner, opening the way for studies of gene function and expression and providing the means for curing disorders that arise as a consequence of genetic mutations —gene therapy.

MASS SPECTROMETRY DEVELOPMENTS

Mass spectrometry has been considered a standard tool for analyzing the components of complex mixtures.

It is a sophisticated tool used across a broad range of fields and applications and has been pivotal in gaining knowledge of protein structure, function, modification and global protein dynamics.

However, the technique is now available in various guises with modifications and enhancements that have tailored its capabilities to specific applications.

In addition to its utility in research, mass spectrometry is a valuable tool for ensuring our safety, be it in quality assurance of food products, monitoring of air pollution or scanning for explosives. It is also fundamental to numerous medical diagnostic tests.

This technology, like gene sequencing has been made accessible to each and every laboratory with the development of bench-top models that can be accurately operated without a specialist operator. Scientists have now achieved further compaction of the component parts of mass spectrometers without compromising efficacy, making the dream of portable devices a reality.

1 GENOMIC ANALYSIS TECHNOLOGIES & BIOENGINEERING

Each year at Pittcon, we learn of the latest advances in genomic analysis and bioengineering technologies.

INTRODUCTION

Over 25 years ago, **The Human Genome Project**, the world's largest collaborative biological project, was launched. The international team aimed to determine the sequence of the nucleotide base pairs that make up human DNA.

In April 2003, they reported that they had achieved their goal. They also identified and mapped all the genes within "euchromatic" regions of the human genome. These represent **92%** of the entire genome, and subsequent audits reported that the sequencing accuracy exceeding **99.99%.**

This mammoth undertaking, which took 13 years to complete, besides being a technological breakthrough, accelerated advancements in numerous scientific fields from human evolution to the etiology of a host of disorders and the development of new treatments and the tailoring of treatments to maximize positive outcomes, especially in oncology.

Since the human genome was sequenced, genomic technologies have advanced at an unprecedented rate. It is now possible to obtain DNA sequences in a fraction of the time at a fraction of the cost. The Human Genome Project will always be a landmark scientific achievement and now has the additional accolade of providing the catalyst for the explosion in new genetic methodologies.

A key contributor to the genomic revolution was **next-generation sequencing (NGS)**, which allows DNA sequencing to be conducted quickly and affordably in laboratories across the world. Using NGS, millions of DNA fragments to be sequenced concurrently using much smaller sample sizes. Consequently, DNA sequencing has become an everyday process so mutations can be identified more readily and DNA screening for mutations has become commonplace for assessing the risk of inheriting debilitating or life-limiting conditions, for diagnosing cancer and tailoring treatment according to which mutations a patient has.

An added benefit of NGS over the Sanger technique (the sequencing technique used in the Human Genome Project) is that sequencing can be completed without prior knowledge of the target gene.

Similarly, the development of the gene-editing technology gene known as **CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)** has made genome editing a widely accessible technique. CRISPR uses the innate immune system of bacteria to specifically cleave at a unique location of the genome, **allowing DNA sequences to be inserted, deleted, or replaced**. This has facilitated research into the function and modulation of specific genes and the proteins they encode and opened up the possibility of gene therapy and target gene sequences can be removed, replaced or modulated with relative ease.

This chapter discusses these techniques in more detail and explores the capabilities of some of the newer technologies currently available.

It includes an interview with the world-renowned **Dr Deisseroth** who gave the Wallace H. Coulter Lecture at Pittcon 2017 about the development and applications of optogenetics, a technique that renders individual neurons photosensitive. It allows investigations at a previously unthinkable level of precision and control into how the brain processes information and drives behavior.

Another contributor is the pioneer of nanoparticles in biological sensing, **Dr Chad Mirkin**, who spoke at Pittcon 2017 on the use of spherical nucleic acids in the development of high sensitivity and selectivity detection systems.

The speed and quantity of innovations and the myriad of potential applications, which may become reality in the near future, makes genomic analysis and bioengineering a particularly exciting field of research today.

ADVANCES IN OPTOGENETICS

Optogenetics and CLARITY are novel techniques that enable rapid analysis of the wiring and fine structure of the brain whilst it is functioning under usual conditions.

Their development and application were led by **Dr Deisseroth, DH Chen** Professor in the Bioengineering and Psychiatry Departments at Stanford University and a practicing psychiatrist. Dr Deisseroth has gained many awards for this work and is internationally renowned in his field.

During his presentation at Pittcon 2017, Dr Deisseroth described how optogenetics works and how it complements the existing range of neuroscience tools. In the following interview, he gives an overview of the development and uses of optogenetics and **CLARITY** and his thoughts about future research.

An interview with Dr. Karl Deisseroth, M.D., Ph.D., conducted by April Cashin-Garbutt, MA (Cantab)

Q **Please can you give an introduction to optogenetics? How was this technology inspired by psychiatry?**

A Optogenetics is a way of introducing information into the brain using light, it targets specific cells or connections across the brain. It is fast, like brain signaling, and helps you communicate with neural circuitry in a language more similar to what is normally used within the brain.

It can be carried out in behaving mammals carrying out complex tasks, even social behavior and memory, and that then opens the door to understanding at a deeper level, not only how things work, but how they can fail to work in psychology and neurology.

It is commonly thought amongst psychiatrists and neurologists that there is not a deep enough understanding of the brain to build the kinds of new treatments with the specificity and precision needed to be successful. It is felt in the whole community, whether you're doing talk therapy for patients, giving medications in the form of pills, or giving brain stimulation treatments.

Fortunately, the goal of building precise ways of interfacing with the brain is not just important for psychiatry, but it's also important for basic neuroscience too, and that's been one of the most exciting applications of optogenetics.

My lab is primarily a basic science lab, investigating what we can do in terms of advancing our fundamental understanding of the brain.

Q **How does optogenetics fit into the broader context of experiments in neuroscience?**

A Optogenetics has become a fairly standard technique, there are thousands and thousands of labs and papers around the world that have emerged using it. It's part of the causal arm of the toolkit used to test whether something is necessary or sufficient to a process, for example whether an activity pattern in a particular kind of cell is necessary or sufficient for physiology or behavior.

There's a lot of other wonderful technology in neuroscience, there's very powerful genetic and anatomy tools for example. Where optogenetics fits in is this is how you can test whether activity is causally important.

Q What challenges does optogenetics allow us to overcome?

A For many years, neuroscientists have effectively been studying correlations. Optogenetics takes you away from observation and correlations and takes you to causality.

This limitation is something that optogenetics addressed for the field, and is very synergistic with all the other beautiful and foundational work in neuroscience. It also provides cell type specificity and speed that prior approaches couldn't quite provide.

Q What aspect of optogenetics did you cover in your Wallace H. Coulter Lecture at Pittcon 2017?

A I provided an overview of the emerging insight into how optogenetic proteins themselves work. I'm actually a chemist and a biochemist before that, and I have devoted a lot of time over the past eight years or so to understanding these light activated ion channels.

> "... deeper understanding of those proteins has allowed us to build new optogenetic approaches. We've been able to make mutants and new versions of these proteins..."

These are the proteins that we put into cells in order to make them respond to light, they are beautiful proteins even separated from their optogenetic applications. They come from algae and other microorganisms allowing the animals to respond to light, so at Pittcon, I talked about how these proteins work, which is of particular interest to molecular type investigators.

I also talked about how our deeper understanding of those proteins has allowed us to build new optogenetic approaches. We've been able to make mutants and new versions of these proteins because we have a deep structural and mechanistic understanding of the protein itself. That has led us to totally new kinds of optogenetics experiments. I explained how this basic chemistry has led to new advances in neuroscience.

Q **Which other topics did you discuss in your talk? Did it include the CLARITY method?**

A Recently, there's been a lot of work on CLARITY and all the methods that have come from it and so I did include it in my talk. What a lot of people are doing, including my own lab, is merging the techniques into a common workflow.

Once you've established a particular cell type is important in the behavior of an animal using optogenetics, you can then obtain the networks of those same cells using CLARITY. That enables you to see the connectivity, the circuit diagram, of those same cells that you know are important in the activity causing behavior.

That's a big step forward and a lot of labs are using this technique, it has helped us start to move toward with an integrative understanding of how neural circuitry gives rise to complex behaviors.

Q **Which techniques have been most important to your work?**

A These days, we use a lot of optics, imaging, electrophysiology and computation analysis and they have become particularly important creating beautiful but complex data streams that we couldn't even imagine before.

From a single mouse we get terabytes of structural and activity data and then when we start to do numerous experiments and test a lot of population dynamics studied across many animals, this gets to be quite challenging, and so we need very advanced computation methods, which have been important too.

Q **How have advances in technology impacted your research?**

A We've helped push a lot of things forward, but a lot of it has built on pioneering work in other disciplines, for example, lasers. The advances in laser and LED technology have been extremely important for optogenetics.

Higher speed computing, GPU devices, cloud computing, and then advances in genetics as well. Basic virology has also been very valuable. A lot of what we've been able to do, even if we'd wanted to, we couldn't have done it 20 years ago, because these other supporting fields hadn't reached their current state.

Q **What further developments would you like to see?**

A We're getting better at matching or precisely altering naturally occurring patterns of activity. We can play in any pattern of activity we want, but the question is how closely does that resemble a natural pattern?

To answer that question, you've first got to detect natural patterns to see what's naturally happening. We've gotten much better at that, and so these days you can now match naturally occurring activity patterns using optogenetics and that is something I'd like to see more of.

That requires yet another union of fields combining imaging, collection of light, with playing in or providing light, and doing that all through the same device, and the same system at a high speed, and even in a closed loop fashion which is very powerful.

Q **What do you look forward to at Pittcon?**

A I like the broad, interdisciplinary, quantitative meetings where I can learn different things, maybe not something that I'm looking for specifically. I look forward to that more than anything, just getting exposed to something that would not be in my normal path.

Q **What are your future research plans?**

A We want to keep advancing optogenetics and CLARITY, and using them together with the goal of understanding how adaptive and maladaptive behaviors arise from circuit activity patterns. There's a lot more to do.

As a psychiatrist, I'm hoping that we can continue understanding these deep questions about anxiety, depression, drug addiction, and get to a level where we can get to the nature of a patient's problem very precisely. Then to follow this with individualized treatments, but this may be much later on.

> "We want to be able to move past the ambiguity and be able to pinpoint exactly the biology behind what patients are intensely suffering from. This will be a big step forward for the patients, families, and society."

Q **Where can readers find more information?**

A **http://web.stanford.edu/group/dlab/about_pi.html**
http://web.stanford.edu/group/dlab/

1.2 ADVANCES IN GENOMIC ANALYSIS TECHNOLOGIES

Sequencing the human genome was heralded as a breakthrough in genetics, allowing at-risk patients to be screened for chronic disabling disease. Since then genetic sequencing technologies have advanced so greatly that the work carried out over 10 years in the human genome project can now be completed in a single day.

Consequently, genetic information is now a key factor in determining precise diagnoses and deciding treatment strategies for individual patients. These tools have also facilitated safe and effective prenatal and cancer screening tests. In addition, technologies for precise and rapid genetic-editing have been developed, allowing the DNA of patients to be manipulated to free them from a range of genetic disorders. This section provides an overview of these new technologies and the companies that have made them possible.

INTRODUCTION

The structure of DNA was discovered over 60 years ago, yet many mysteries remain on how our genome functions. In order to understand what the genome means we need to know, not just its sequence, but how this relates to phenotype. Our genome is over three billion bases long, meaning that genome sequencing produces vast quantities of data that must be deciphered and, historically, this has been a slow process. But, advances in genetic sequencing technology mean that we stand on the edge of a revolution that will see genetic sequencing data be quicker to obtain, easier to decipher and increasingly informing medical diagnosis and treatment.

Not only have recent years seen major advances in genetic sequencing but they have also witnessed the emergence of the genetic-editing technology **CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)**. The arrival of CRISPR has given scientists their most versatile genome-editing tool yet, allowing us to edit the genome at specific locations quickly, cheaply and accurately. As a result, the realms of genetic engineering that have been hypothesized and anticipated for the future have suddenly become today's reality.

Genomics – the study of genomes – is a branch of science that is finding its own in the 21st century. Although our understanding of genetics has informed a multitude of medical discoveries and treatment, in the coming years this role is going to explode to become a central pillar of healthcare. The catalyst that has fueled this is next-generation-sequencing and the company that has been at the forefront of this revolution is **Illumina.**

This year's Pittcon, which took place in Chicago from 5-9 March, 2017, hosted The Twenty-Eighth James L Waters Symposium on Genomic Analysis Technologies. This prestigious annual symposium was founded to explore the origin, development, implementation, and commercialization of scientific instrumentation of established and major significance. And in 2017, it highlighted Illumina's technologies.

The Pittcon exposition was supported by a number of leading companies delivering genetic sequencing solutions, including **Thermo Fisher Scientific, Eppendorf, Beckman Coulter, Vitl Life Science Solutions, Panasonic Healthcare, and Malvern Instruments**.

Personnel from Illumina itself, including co-founder **David Walt** and long-time CEO and now executive chairman, **Jay Flatley**, gave presentations at Pittcon2017 alongside leading genetic analysis companies that are using Illumina's instruments to facilitate consumer and healthcare genetic testing.

NEXT GENERATION SEQUENCING

On 26th June 2000, **Bill Clinton**, then President of the United States, announced to a press conference at the White House that the first "rough draft" of the complete human genome had been sequenced as part of the **Human Genome Project.**

But while this was a landmark achievement in genomics, in the intervening years such remarkable progress has been made in the field of genetic sequencing, that it essentially belongs to another era.

The entire Human Genome Project took over 10 years to complete at a cost of **$3 billion.** Now the technology we have can process **20,000x** as much data, and a person can have their entire genome sequenced for just a thousand dollars.

Genetic information is now poised to play a central role in our healthcare, with decisions made in doctors' offices day-to-day expected to increasingly be informed by sequencing results. Direct-to-consumer genetic testing is also on the rise, and soon we will see prenatal and cancer diagnoses routinely made via blood tests rather than invasive procedures.

Furthermore, the vast wealth of data that can now be rapidly acquired is going to inform our genetic understanding of health and disease at an unprecedented level.

Since the first publication of the human genome sequence, whole genetic technologies have come and gone. But one has emerged to dominate over the rest: **Illumina sequencing**. The company is now thought to hold around **90%** of the genetic sequencing market share.

Pittcon 2017 hosted The Twenty-Eighth James L Waters Symposium on Genomic Analysis Technologies. The symposium focused on **Illumina**'s history and technologies, while looking to the future applications of this science.

The symposium was fortunate to have as one of their speakers **Dr David Walt**, one of Illumina's co-founders, who described the journey from academic discovery, to startup, to market-leading company. Also present at the symposium was **Jay Flatley**, executive chairman of the board of directors, who served as CEO for 17 years and oversaw the company's acquisition of its flagship next generation sequencing technology **Solexa**, as well as its rise to dominance in the market.

Although the principles of Illumina's sequencing by synthesis, or SBS, technology have remained the same, since acquiring Solexa 10 years ago, the company has continued to invest in its innovation to keep pushing the limits of what it can do.

The most recent generation of Illumina SBS instruments can generate **1.8 terabases** of data per run compared with **one gigabyte** just a decade ago.

Representatives from many other genetic sequencing technology companies were also at the **Pittcon** exposition, including **Thermo Fisher Scientific, Beckman Coulter and Eppendorf.**

Thermo Fisher Scientific provides next-generation sequencing technology through its Ion Torrent semiconductor sequencing products. In addition to hardware, **Thermo** also offers a complete next-generation sequencing data analysis package in the form of the Ion Reporter software and server. Applications for the technology include targeted DNA, transcriptome, targeted RNA, and exome sequencing.

The Solid Phase Reversible Immobilization technology **Beckman Coulter** developed the SPRIworks HT kit that provides high-throughput sample preparation for Illumina next-generation sequencing platforms. This technology helps to facilitate the increased throughput of next-generation sequencing by reducing the labor intensity and hands-on time required for library preparation. This is achieved through an automated workflow using **Solid Phase Reversible Immobilization (SPRI)** paramagnetic bead based technology. It is able to process up to **96 samples in as little as three hours**.

Eppendorf presented their automated workstations, such as the epMotion **5075t / 5075m**, which can be incorporated into an Illumina sequencing workflow. The automated pipetting system comes with built-in ThermoMixer and a range of pre-programmed and validated next-generation sequencing

SPRI

96 samples

≤ 3 hours

Eppendorf

96 samples

a day

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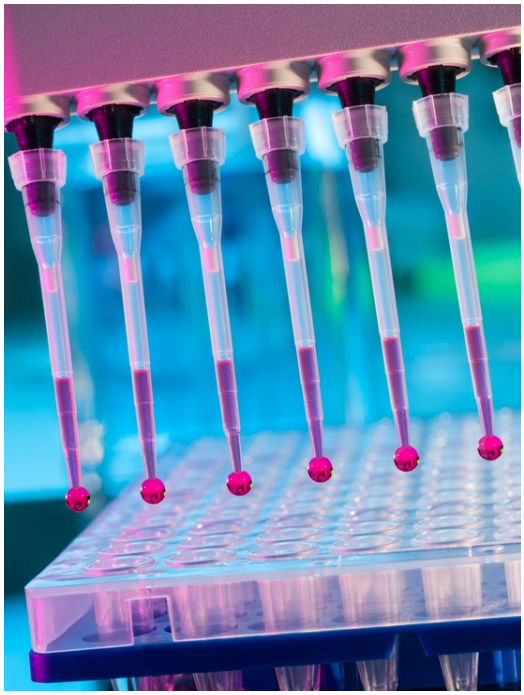
Advances in Genomic Analysis Technologies

DNA SCREENING



The advent of low-cost next-generation-sequencing is opening new doors in DNA screening, creating the possibility of non-invasive cancer detection, prenatal diagnosis and genetic carrier testing via blood samples.

The James L Waters Symposium on Genomic Analysis Technologies, included presentations by some of the leading players in this burgeoning industry.

For example, **GRAIL**, an Illumina spinout, presented their blood screening tests for cancer in asymptomatic people, sometimes referred to as 'liquid biopsies'. In addition, **LabCorp** discussed their development of **non-invasive prenatal testing, or NIPT**.

These tests work by detecting DNA shed from the placenta and could help lower the use of amniocentesis for prenatal diagnosis – an invasive procedure that carries a substantial risk of miscarriage.

Genetic screening is also increasingly being chosen by consumers to make decisions about their health.

One of the frontrunners in this field is **Counsyl**, whose Chief Medical Officer **Dr Jim Goldberg**, presented at the James L Waters Symposium.

Counsyl are trying to make genetic screening cheaper and more accessible to all. The company offer DNA screening and genetic counseling for inherited conditions and cancer risk genes, and prenatal screening, with some of their services available online.

Counsyl have three main products:

- a genetic carrier screen that covers more than 100 inherited disorders
- a non-invasive pregnancy screening test
- a hereditary cancer screen that looks at 36 cancer-related genes, including for breast, ovarian and colon cancer.

At the James L Waters Symposium, **Alex Aravanis** who heads up research and development at GRAIL spoke about the challenges of developing a safe and effective cancer screening test. He also explained how advances in next-generation-sequencing are enabling the detection of circulating nucleic acids shed from tumor cells in blood samples.

> The company's aim is to produce ways of detecting cancers much earlier, to increase the likelihood that they can be effectively treated and enhance survival.

Daniel Grosu from **LabCorp** presented a similar approach being used in the development of non-invasive prenatal testing, or NIPT. The company launched their own InformaSeq test in 2014 and in 2016 completed acquisition of Sequenom, who were the first to offer prenatal non-invasive commercial sequencing for chromosomal aneuploidy.

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CRISPR

During the last five years, one major development in gene editing technology has seized the world of biology with excitement. **CRISPR/Cas9** has made genome editing more specific and easier than ever before, giving us **an unprecedented ability to manipulate DNA**.

The arrival of **CRISPR** has opened up myriad possibilities, including the potential to conduct experiments that were previously impossible. It can give us new insight into disease by allowing scientists to knock out or modulate specific genes and study their function, it can be used in drug development, such as the creation of new antimicrobials, and work has already begun to apply the technique clinically to cells of the human body.

Outside of biomedical applications, CRISPR will also find uses in agriculture and livestock breeding, and in environmental settings such as control of disease-vector insects using gene drives.

The companies who are delivering the tools and technology to put CRISPR to use in your own lab were present at Pittcon 2017. They included **ThermoFisher Scientific, Eppendorf, Beckman Coulter, Vitl Life Science Solutions, Panasonic Healthcare, and Malvern Instruments.**

WHAT IS CRISPR?

CRISPR stands for **Clustered Regularly Interspaced Short Palindromic Repeat.** These are clusters of DNA repeats that were first identified in bacterial genomes in the 1980s. Research revealed that they are part of the bacterial adaptive immune system.

When a bacterium is infected by a virus, it can incorporate short sequences of DNA from the genome of the invading virus into its own genome. As such, they serve as a memory of past viral invaders. These sequences are stored as “spacers” in between CRISPR sequences.

Then, if the same virus attacks the bacterium at a later date, it can recognize and cut any section of the invaders genome matching the spacer sequence. To do this, the transcribed CRISPR sequences, composed of single-stranded RNA, act as guides for the enzyme Cas9, which has the ability to cut through DNA. As a result, **it is able to disrupt and disable the viral genome at very specific locations.**

The CRISPR/Cas9 gene-editing system hijacks this ancient process by using guide RNAs, which are short synthetic stretches of RNA, to guide the Cas9 enzyme to a target location in the genome. This has created a powerful tool that allows specific genomic sequences to be inserted, deleted or replaced.

FACILITATING CRISPR EXPERIMENTS

Pittcon 2017 was attended by some of the world's leading biotechnology companies that provide solutions for each stage of the CRISPR protocol.

These included:

Eppendorf	Thermo Fisher Scientific	Illumina
offering a range of equipment for multiple stages of CRISPR preparation including thermo-mixing devices, centrifuges, thermocyclers and CO2 incubators	offering ready-to-use CRISPR/Cas9 products including vectors, Cas9 mRNA and protein and CRISPR libraries	offering next-generation sequencing products that are ideal for library verification and validating genome edit success.

THE CRISPR PROCESS

Vector Generation

Pittcon exhibitor **Thermo Fisher Scientific** offer the GeneArt CRISPR Nuclease Vector Kit, which is a ready-to-use, all-in-one system that makes it easy to encode a guide RNA using a vector that also expresses the Cas9 enzyme.

Transformation and Cultivation

Centrifugation is an ideal method to concentrate lentiviral vectors for use in CRISPR experiments. Pittcon exhibitor **Beckman Coulter** offer a range of centrifuge devices for this purpose including their Optima X series ultracentrifuges which range from comprehensive floor models to the tabletop Optima MAX-XP.

Vitl Life Science Solutions, demonstrated the Co-Mix laboratory mixer at this year's Pittcon exhibition. The device allows you to mix plates and vortex them at the same time, thanks to its unique independent vortex pad, and can be used in a variety of genetics experiments and PCR.

Preparation and Purification

The Vitl Life Sciences Ther-Mix is a fully programmable heater laboratory mixer with numerous applications including in DNA isolation, plasmid isolation, and sample prep for PCR. It allows you to **combine multiple heating and mixing steps in one device** to reduce variability and contamination risk.

Transfection and Cultivation

One key application of CRISPR is for the generation of genetically modified animals, which can provide insights into gene function, disease mechanisms and assist drug development. The **Eppendorf TransferMan 4r** is a multifunctional manipulator that can be used for the injection of CRISPR/Cas9 components into the cytoplasm or pronucleus of oocytes or embryos.

In an ideal workstation setup, it can be used alongside the **Eppendorf PiezoXpert**, which uses piezo impulses to ensure gentle and effective penetration of the cell membrane. Piezo-assisted micromanipulation improves results of a variety of applications, including transfer of embryonic or induced pluripotent stem cells into blastocysts, mouse ICSI (intracytoplasmic sperm injection), and enucleation/nuclear transfer.

Pittcon 2017 was also attended by **Panasonic**, that offers a range of CO2 incubators that can provide accurate and tightly controlled conditions for cell incubation.

Harvesting and Preparation

When the time comes to harvest cells during CRISPR experiments ready for analysis, a complete set of tools and technologies are on offer from the leading manufacturers.

> For example, **Eppendorf** provide centrifuges, tubes and plates, and a range of purity grade consumables to safeguard against contamination by any biological substance.

The Eppendorf ThermoMixer C can also heat, cool, and mix almost any lab vessel.

Analysis and Detection

A photometer is an ideal tool for verifying RNA and DNA concentration during CRISPR experiments. Eppendorf, an exhibitor at Pittcon 2017, offers a well-established line of photometers, including the BioPhotometer D30 and the Biospectrometer Basic, for rapid and accurate nucleic acid photometry.

And when using PCR to amplify mutated regions and target sites, the Eppendorf Dual Block Thermocycler Mastercycler nexus X2 can help reduce variability and ensure consistent results. The device is perfect for busy labs by providing two separate thermoblocks, allowing you to run t**wo separate PCR experiments simultaneously, but completely independently.**

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CRISPR APPLICATIONS

Via the application of **CRISPR**, life sciences research can generate a better understanding of how genes and regulatory elements function and reveal how the genome is organized within the cell.

The ability to easily and efficiently modify genetic sequences within cells holds enormous potential across basic science, biotechnology and medicine. In the field of biotechnology, genetic engineering could be used to create infection-resistant crops. And within medicine, genome engineering could lead to a whole new generation of therapies, and improved models of disease. There is also the possibility to apply the technology directly as gene therapy to correct harmful mutations.

Previously studying the physical organization of the genome required denaturation, making it virtually impossible to study dynamic processes in living cells, and fluorescent tagging of DNA in live cells has mostly been restricted to specific chromosomal regions, such as the centromere or telomere.

CRISPR has created an opportunity to study DNA within living cells through the adaptation of genome-editing tools. Due to the relationship between genome structure and function, such methods could therefore offer invaluable insight into the genome's workings.

Some of the leading manufacturers who are providing the tools needed to put CRISPR into action in the lab, discussed their work at Pittcon 2017. For example, **Eppendorf** presented the micromanipulation system, with PiezoXpert, which can help successful pronuclear injection.

Piezo–assisted injection methods can significantly increase productivity and enhance yields of cell micromanipulation experiments. The system works with piezo impulses which are directly transferred to the capillary to generate perforation of the cell membrane.

It can be applied to a variety of applications including enucleation/ nuclear transfer, as well as transfer of embryonic or induced pluripotent stem cells into blastocysts and morulae, and injection into mammalian oocytes and zygotes.

Pittcon 2017 exhibitor **Thermo Fisher Scientific** provides a complete toolset for every step of the genome editing workflow, whether for generating animal disease models, stem cell engineering or creating transgenic plants.

Thermo Fisher produce an all-in-one CRISPR genome editing solution that includes an online CRISPR search and design tool, four formats of CRISPR/Cas9 (Cas9 protein, Cas9 mRNA, all-in-one expression vector, and CRISPR Libraries) as well as cell culture reagents, delivery method, and analysis tools.

They provide a demonstrated protocol for the use of their CRISPR/ Cas9 products in human induced pluripotent stem cells, one of the major applications of the genome-editing technology in research.

The company have shown that their GeneArt CRISPR-Cas9 System is able to perform edits in both mouse embryonic stem cells and human induced pluripotent cells with over **50%** efficiency.

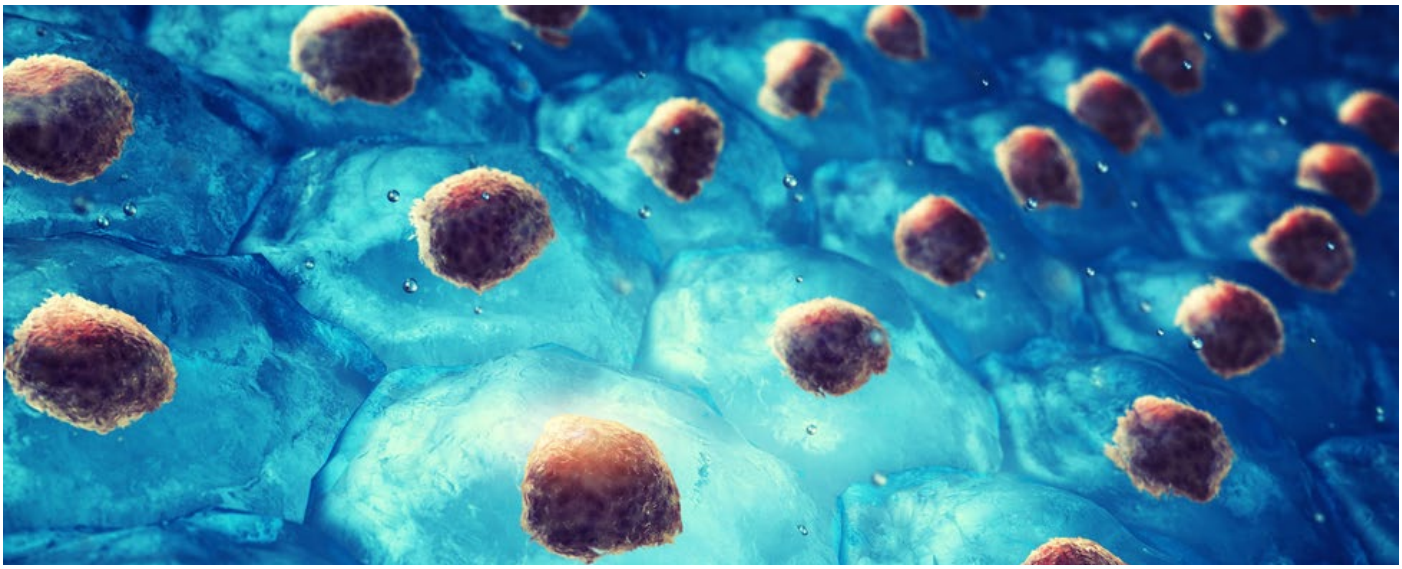
Thermo Fisher Scientific have also demonstrated the use of genome-editing tools in the creation of animal models.

The team say the approach is suitable for multiplexing and they have had success in testing between six and eight separate CRISPR/guide RNAs in a single experiment.

A team tested the efficiency of both Invitrogen GeneArt Cas9 recombinant protein, or Cas9 mRNA and in vitro-transcribed short guide RNA (sgRNA) in the generation of genetically engineered mouse models by carrying out pronuclear injections and culture of zygotes at the blastocyst stage.

They have also used the **GeneArt Cas9 Nuclease** in fertilized zebrafish eggs to create mutant alleles via microinjection of CRISPR/guide RNA stock volume. The team generated a guide RNA to target the tyrosinase gene involved in melanin synthesis. Thus, they were able to visualize the impact of the editing, which produced a range of phenotypes with varying mosaic-like levels of melanin pigmentation in the body and eyes.

One particular medical application for CRISPR/Cas9 that has generated significant excitement is in the use of stem cells as a source for cell replacement therapies.



In a paper published in Cell Stem Cell, a team led by **Vijay Sankaran** showed that editing hematopoietic stem and pregenitor cells using CRISPR could increase their expansion and differentiation into red blood cells.

The researchers employed a clever approach, by using population genetic data to identify mutations associated with increased hemoglobin levels. They identified several rare variants in a gene, SH2B3, that suppressed its function. People with these variants had higher hemoglobin levels compared with controls.

The researchers showed that inactivation of SH2B3 in human embryonic stem cell lines using CRISPR/Cas9 increased red blood cell production more than three-fold. The researchers say the findings demonstrate proof-of-concept that targeting the gene could improve red blood cell differentiation and expansion.

With further optimization and cost reductions, it is hoped that in future, engineered stem cells could provide a sustainable source of red blood cells, as an alternative or substitute for donated blood.

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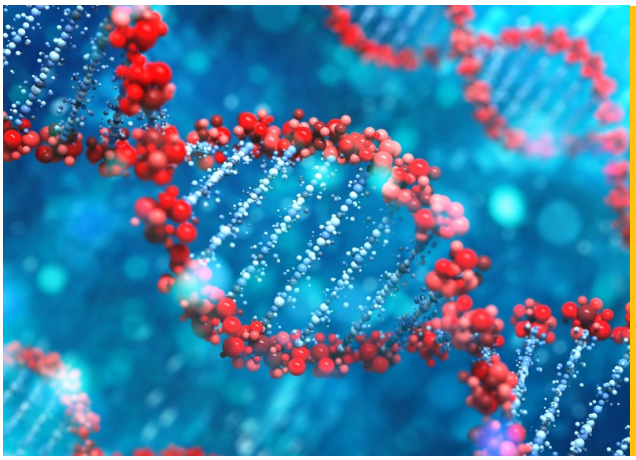
CONCLUSION



Since the turn of the millennium, we have seen major developments in genome analysis technologies that have, and will continue to, make transformations from basic science through to clinical care.

The arrival of next-generation sequencing has increased the speed of genome sequencing **1000s** of times over, multiplying the amount of genetic information we are able to obtain and process. This has already taken center stage in genetic research and will underpin the coming personalized medicine revolution.

In the coming years, more and more people will have their whole genomes sequenced and DNA screening, such as for cancer or prenatally, will take on a greater role in the diagnosis of disease.

CRISPR gives us a new level of specificity and simplicity in genome editing

Meanwhile, **CRISPR** gives us a new level of specificity and simplicity in genome editing that one day we will likely see applied to gene therapy. But this is only just one use of this powerful tool, which is already informing discoveries in basic science and drug development, and will also find uses in fields outside of biomedicine, such as environment and agriculture.

1.3 GENOMIC ANALYSIS TECHNOLOGIES: PAST, PRESENT AND FUTURE

Illumina provided the technology that has enabled researchers to understand genetics in ways that were previously thought to be impossible.

Illumina is the market leader in genetic sequencing technology, holding around **90%** of the market share. The company is the largest maker of the DNA sequencing machines that have transformed biological research and the diagnosis and treatment of numerous diseases.

Jay Flatley was the CEO of Illumina for 17 years, during which time he oversaw the company's massive expansion. At Pittcon 2017, Executive Chairman, **Jay Flatley,** described the path to Illumina's success, the applications of their technology and challenges for the future.

An interview with Jay Flatley, Executive Chairman of Illumina, conducted by April Cashin-Garbutt, MA (Cantab)

Q **Can you please give a brief history of Illumina?**

A **Illumina** was founded in 1998. It was based on technology invented by **Dr. David Walt** that was licensed from Tufts University and formed the basis of our microarray business. The fundamental invention was a unique way to put beads in wells that were created on fiber optic bundles, with the beads being imaged through the fibers.

I joined the company in the October of 1999 and we went public in July of 2000, raising a **$100 million**, giving us the capital to develop our initial products.

We launched our first product in 2002, called the **Illumina BeadLab**. **The BeadLab** was designed to perform large-scale analysis of genetic variation. We then continued to build a whole series of microarray products through the course of 2009 up to ones that included five million individual markers on a single BeadChip.

We entered the DNA sequencing business through an acquisition of a company called Solexa in 2007. We spent a year improving this technology, which was not yet quite ready for market. We grew that business from zero to **$100 million** in one year.

Over the course of the next five or six years we continued to improve that technology through the launch of a platform technology called **HiSeq**, which has taken the sequencing output from **1 gigabase (Gb)** pairs per run on the initial Solexa product up to a system we just announced in January called **NovaSeq**, which does **6 terabase (Tb)**

1	**HiSeq** **gigabase (Gb)** pairs per run
6	**NovaSeq** **terabase (Tb)** pairs per run

Our sequencers now operate at the rate of about one whole human genome per hour, in comparison to 2001 it required all the sequencers in the world to complete, taking a year to sequence a single human genome!

From all of that, we've now created a whole series of clinical applications in markets such as reproductive health, oncology screening and infectious disease. We've in turn spun off a number of companies, including **GRAIL** and **Helix**. In July of last year, I stepped down as the CEO of Illumina and I am now Executive Chairman.

Q **What impact have next generation genomics tools had on our understanding of biology?**

A The microarray technology through the middle part of the last decade was responsible for the first large scale associations of generic variations with human disease. It was called the **GWAS era, genome-wide association studies.**

As a result, there have been many thousands of publications and genetic associations of locations where we vary in our genome and associated likelihood of getting diseases, or in the case of Mendelian diseases, the definitive relationships between genetic variation and development of a particular Mendelian disease.

Q **What are the main healthcare applications of the technologies developed by Illumina?**

A We now have many applications that are purely clinical, the flagship application being **non-invasive prenatal testing (NIPT).**

> “We’ve also made tremendous strides in non-human applications, such as agriculture and microbiology, and in understanding the microbiome. So, this technology has been applied very broadly outside of human genetics as well.”

In NIPT, a blood sample from a pregnant mother can be used to determine whether her baby has any number of genetic diseases, with Downs syndrome being the most common. We do this by sequencing the entire genome of the DNA that we extract from the mother’s blood, and looking for extra copies of chromosomes that are contributed to that sequence. This can be achieved because the baby’s DNA is present in the mother’s blood.

NIPT replaces the invasive amniocentesis procedure, and over the course of the last four or five years has become the frontline screen. We think it's the fastest ever adopted diagnostic test.

There are also many applications of our technology in sequencing cancer tumors and some of our customers use our sequencing technology for germline analysis.

For example, Myriad Genetics does this for identifying variants in the BRCA gene to predict a women's likelihood of getting breast or ovarian cancer.

We also have a range of products that are used in sequencing solid tumors for determining the best therapeutic choice. And as we mentioned in our symposium, our spin off company **GRAIL** is using sequencing to create a cancer screen for asymptomatic patients.

> "Our view in the long run is that everyone is going to get sequenced. It's going to become part of the standard health record, and used to manage healthcare throughout an individual's lifetime."

Q **What do you think the future holds for genomic information?**

A The size of genomic data that's being created now is quite astonishing. GRAIL alone over the next five to eight years will create more data than any other commercial company in the cloud. That's a critically important challenge in the storage of information.

Deep learning is increasingly important in our field because of the amount of genomic data that we're accumulating with phenotypic information from medical records, but also the genotypic information from the genome is now at a scale that no human can figure out exactly what questions to ask.

We're relying more and more on deep learning technologies in order to analyze those databases and create the correlations and extractions that are going to become medically relevant in the future.

Q **Over what time frame are these changes likely to occur?**

A They've been happening over the last ten years and sequencing has now become amazingly economical. We can sequence a human genome for **$1,000**, which makes sequencing practical on a large scale.

Over the next five years, it's likely we're going to see very important developments in the technology and the direct insertion of genomic information in healthcare records, deeply embedding sequencing in the healthcare system.

In the ten-year timeframe, we'll begin to see babies sequenced at birth on a reasonably routine basis. And a complete human genome sequence will be at a price point under **$100**.

> "In the next five years, we'll probably have a true cancer screen on the market that many people will be able to afford as part of their normal health physical."

Q **What challenges will need to be overcome?**

A There certainly are many challenges, probably the biggest are reimbursement-related because these technologies are new, the payer system tends to respond reasonably slowly to change in healthcare, and it takes a compilation of lots of clinical evidence to convince payers to pay for these tests.

> For example, now we have the ability to diagnose rare or undiagnosed disease in children incredibly effectively, but almost nobody reimburses that test today. Overcoming that hurdle is vital.

There are also regulatory barriers to overcome, and those barriers are different in each country. The FDA prevents us from creating certain types of information and returning it to consumers. That regulatory burden is quite high, and prevents many companies from actually developing and marketing their test at the rate that they could otherwise.

I think there will always ethical questions as well. In the long run, as the power of this technology becomes more understood, we'll have to ask ourselves ethical questions such as is it appropriate to run a complete genetic sequence of a baby that's unborn? And what is the appropriate use of that information and what isn't?

How do we prevent discrimination from the use of somebody's genetic information, whether that has to do with employment, or whether it has to do with medical or life insurance? There are many issues that will need to be carefully thought through.

> "There certainly are many challenges, probably the biggest are reimbursement-related...There are also regulatory barriers to overcome... there will always ethical questions as well...There are many issues that will need to be carefully thought through."

Q **What is Illumina's vision?**

A The mission of our company is to **improve human health by unlocking the power of the genome** and what that means is that we're continuing to push ever more aggressively on the technology front to make these technologies economically affordable and easier to use, so that they can become deeply embedded in the healthcare system. Because it's only then that we'll be able to use the power of understanding our genomes in order to improve human health. That's what Illumina is all about.

Q **What was the focus of your talk at the Twenty-Eighth James L Waters Symposium on Genomic Analysis Technologies at Pittcon 2017?**

A I was charged with giving a high-speed history of Illumina. **David Walt** began by talking about the founding technology that he invented and how it got into Illumina. I then took it from there all the way up until today, and painted a quick history of what has happened to our company, what the major milestones have been, and where we're headed in the future.

We also had three of our customers talk about how they're using our technology. One of them was a company called **Counsyl**, who uses our technology for doing carrier screening. They described the evolution of what's happened in carrier screening, some of the historical limitations in doing point analysis or small panel analysis of carrier screens. And why sequencing is a much more powerful tool.

Then we heard from a consultant who's involved in implementations of non-invasive prenatal testing and who has previously been with Sequenom and was the CMO of Illumina. He also talked about the history of that test and its effectiveness, and showed some data on the sensitivity and specificity of that test and why sequencing is such an important tool.

Then we wrapped up with the Chief Technology Officer for GRAIL, who used to be the head of research at Illumina. He talked about the mission of GRAIL, the challenges of developing a cancer screen, and showed a reasonable amount of data about the different approaches and some of the challenges involved in developing that product.

Q **Where can readers find more information?**

A Our website, **https://www.illumina.com/**, is a fantastic resource for getting more information. As well as hundreds of articles about our company that can be found using a Google search for Illumina.

1.4 CARRIER SCREENING: PAST, PRESENT AND FUTURE

One of the company's that uses Illumina's genome sequencing technology is Counsyl, a technology company that offers DNA screening for a range of diseases. At Pittcon 2017 Counsyl's Chief Medical Officer **Dr. James Goldberg**, explained the benefits of screening for carriers of recessive genetic disorders. Such screening gives potential parents the opportunity to avoid passing two copies of the gene for a devastating condition to their offspring. He also detailed new guidelines that identify the diseases for which screening is currently recommended and summarizes ongoing research into expanding carrier screening.

A transcript of Dr. Jim Goldberg's talk at the twenty-eighth James L Waters Symposium on Genomic Analysis Technologies at Pittcon 2017. Disclosure: Dr. Goldberg is the Chief Medical Officer for Counsyl who use Illumina equipment for screening.

The history of carrier screening

Tay-Sachs disease has been the model for carrier screening for recessive genetic disease and is a disease we've known about for a long time. It's a severe disease, especially devastating because an infant appears normal for the first six months and then develops a progressive, degenerative, neurologic condition, which results in their death, typically within four to five years.

There is, what is referred to as, the cherry-red spot of Tay-Sachs disease, but it is not actually the abnormality, it's the accumulation of lipid in the macula that forms that cherry-red spot.

The incidents of Tay-Sachs disease has gone down because of screening, frequently children are suspected of having Tay-Sachs disease because of a cherry-red spot seen by their ophthalmologist. Tay-Sachs disease is not often thought about because it has become increasingly rarer.

Another reason to which Tay-Sachs is a model for genetic disease screening, is that it has a Jewish predilection, where the carrier frequency in the Jewish population is about **1 in 27 to 1 in 30.** In comparison, the carrier frequency in the general population is about **1 in 250 to 1 in 300.** There is a defined group that has a higher risk of being carriers for the disease.

Jewish Population
1 in 27 to 1 in 30

General Population
1 in 250 to 1 in 300

Tay-Sachs disease was described a long time ago, the enzyme, and disease description was found in the 1880's by Tay and Sachs. However, it wasn't until the late 1960's, that **Okada and O'Brien** described the enzymatic defect, and the deficiency of hexosaminidase A in this disorder. **O'Brien** described shortly after discovery of the genetic defect was that carriers of the disorder had intermediate levels of the enzyme. This made the disease particularly amenable to screening because it could easily and cheaply be measured, making it an easy approach for diagnosing these carriers. In the early 70's, **Mike Kaback** started a number of screening programs in the Washington Baltimore area, which gradually spread around the country.

They involved the religious leaders and the at-risk population and became an extremely successful model for the identification of carriers and it is the paradigm that people often compare other types of screening to.

Although it has been a model for many years, it does have some issues. The instance, following the screening program that started in the mid-70's, Tay-Sachs disease in the non-Jewish population has dropped significantly, however, most cases now are found in the non-Jewish population. This is due to a couple of things, first of all, we've effectively screened in many places the Jewish population, the USA is pan-ethnic and it's getting more difficult to identify who has an Ashkenazi Jewish background.

Current guidelines for screening

New guidelines came out last week, I think they're important, something we've been looking for a long time. Two committee opinions came out, and guidelines for us in this area of medicine come from two major organizations: **the American Congress of Obstetricians and Gynecologists** and **the American College of Medical Genetics**. Those are the two primary organizations that I'll be talking about.

This first opinion, that came out last week, covers **carrier screening for genetic conditions** and here is a summary of what the guidelines look like. These are the new guidelines and it combines both the American College of Medical Genetics and the American Congress of Obstetrics and Gynecology. There are now two diseases that both organizations recommend pan-ethnic screening for, **Cystic Fibrosis** and **Spinal Muscular Atrophy**.

Ashkenazi Jewish screening, which has been around since the early 70's, both organizations recommend screening for that but the **American College of Medical Genetics** has much larger panels of disorders. Hemoglobinopathies aren't mentioned at all by the American College of Medical Genetics but **ACOG** goes recommend screening for them.

Not much has changed from the old guidelines. The name difference between the new guidelines and the old guidelines was the inclusion of Spinal Muscular Atrophy in the ACOG guidelines. Just remember that old and new the only difference was the addition of Spinal Muscular Atrophy and that Spinal Muscular Atrophy was already part of the American College of Medical Genetics guidelines.

Looking at data that looks at screening and application of expanded carrier screening is important, because we combine it to the old guidelines. Cystic Fibrosis was the first disease that was recommended for screening in the first guidelines back in 2001. At that point, the recommendation was that testing should be offered to Caucasians and Ashkenazi Jews and made available to all other ethnic groups. At that point, they recommended a **25-mutation** panel.

In 2011, both **ACOG** and **ACMG** recommended that it is becoming increasingly difficult to assign a single ethnicity to individuals. Unfortunately, this hasn't been recognized for Ashkenazi Jewish screening. It is reasonable therefore to offer CF screening to all patients.

One of the problems with this type of screening is that. The ACOG and the ACMG guidelines on screening is based on using a **23-mutation** screening panel. The detection rate works well for Ashkenazi Jews and non-Hispanic whites but drops off for other ethnicities, especially Asian-Americans who only a **49%** detection rate.

This **23-mutation** panel is still the official recommendation, even in the new guidelines. The implication of this is a problem we call residual risk.

Say you have run the test and you're identified as not being a carrier, if the detection rate is only **49%**, you still have a significant residual risk of being a carrier. This is a serious problem, but there are some new technologies NGS approaches that can address some of these issues in terms of lowering the detection rate, I will discuss in detail later on.

Increasing the number on the panel of abnormalities, genetic abnormalities for Cystic Fibrosis, is problematic because, right now, there are over **1800** variants that have been reported. The guidelines call that just a **23-mutation** panel. Another thing looking at screening in the Ashkenazi Jewish population, the other major category that both of the organizations recommend screening.

When looking at our Jewish disease panel results from our own screening information, we found that **45%** of people who were reported were carriers of a Jewish disease did not report Jewish ancestry. This is a real problem in terms of identifying these people. Much of it is based on our now multi-ethnic culture population.

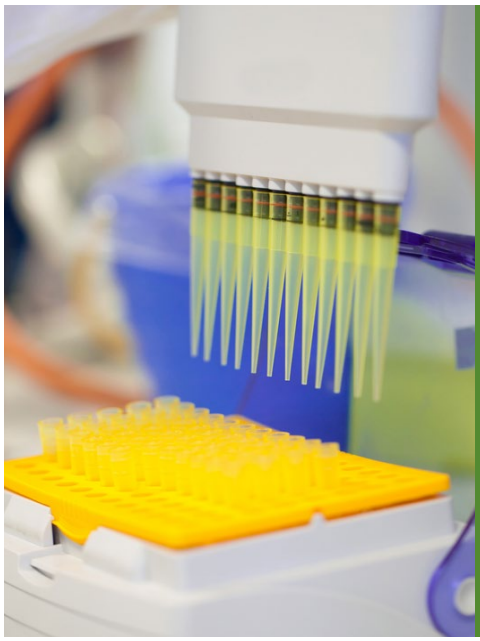
ACOG and **ACMG** give very little guidance for ancestry, **ACOG** simply refers to ancestry, it's hard to know what that means in terms of trying to assess whether they should screen for a couple. **ACMG** on the other hand says one Jewish grandparent is sufficient to offer testing, however, if someone is unsure as to their precise lineage, it is recommended to offer testing.

One in seven marriages are between spouses of different ethnicities, multiracial children have increased **50%** over the last decade and there are many publications now that support the pitfalls of relying only on self-reported ethnicity.

How many people are precisely sure of their lineage? From my own experiences, when I was in practice, there were not many who knew their lineage and this supports pan-ethnic screening.

Another example of why pan-ethnic screening is important is the increase in incidents of Tay-Sachs disease in French-Canadian's Cajun, and there is also data now emerging that the carrier frequency in the Irish population is about **1 in 40**.

Expanding carrier screening



Expanding carrier screening was introduced by **Counsyl** in 2010 by adopting a pan-ethnic approach, which avoids the problem of determining ethnicities. At the time, there were more than 100 diseases, and it was initially carried out by targeting mutation analysis. This occurred some of the **Illumina**'s early products, but now all our testing is done by **next generation sequencing (NGS)**.

At the time of the first papers published that looked at the approach for expanded carrier screening, the reason that people started doing expanded carrier screening was because the cost of an expanded carrier test was about the same as testing for just one disorder. This meant that you could test for **100** disorders for the same cost as just one.

> It wasn't until we published data in 2013, which looked at an outcome study of **23,000** individuals, that it became clear that, at the time, **77%** of carriers would have been missed by the **ACOG** guidelines, and **69%** would have been missed by **ACMG** guidelines.

We were detecting a lot more carriers, which translates into carrier couples who are risk of having an affected offspring. A few years after that paper, the Perinatal Quality Foundation recommended that a lot of expanded carrier screening was to be done. A small group of us brought together a number of organizations; ACOG, ACMG, the National Society of Genetic Counselors, and the Society of Maternal Fetal Medicine, to talk about and hopefully to come to a consensus about expanded carrier screening. However, trying to work among five different organizations to get consensus is not easy but we managed to.

While this was not a recommendation, we came out with a document called **"Points to Consider if you're going to do Expanded Carrier Screening."** The premise of this was that all individuals, regardless of race or ethnicity, are offered screening for the same set of conditions.

We also address the clinical utility of expanded carrier screening, the increased detection rates we talked about, the importance of pre-test education and post-test education and the types of conditions to be included.

The types of conditions was a difficult topic to come to an agreement on, and a complete consensus was not made with specific conditions, but the type of conditions and we felt that serious conditions with significant disability should be included. We felt that late onset conditions were non-appropriate to be included.

More data was needed to facilitate a decision or a move towards expanded carrier screening, so we went and looked at more data. We published our findings in **JAMA** in August of this year, we looked at the outcome of utilizing expanded carrier screening in almost **350,000** patients. This is a mix, again, of both targeted mutation screening and **NGS** screening.

We were relying on self-reported ethnicities, with 15 different ethnic categories for participants to choose from. We excluded known disease carriers, or those with a family history of a condition or infertility and we included only severe or profound diseases.

Technology and the analysis we looked at was same ethnicity couples, but we do have in an addendum, in the **JAMA** article, of 150 pages, that includes all the tables with all the various ethnic mating groups, so all that information is available.

The outcome measure we looked at was not carrier frequency, because at least for some disorders, like Fragile X, it's not a carrier couple that determines it, but the size of the number of genetic repeats that you have in the Fragile X gene.

The population was almost **350,000** and you can see that even in the less common ethnic groups there were over **5,000** individuals in those groups, which was much more than had ever been published with this type of screening, looking at a large panel of conditions.

What we found was that ACMG and ACOG guideline based carrier screening, missed significant percentages of pregnancies.

We decided to make it a level the playing field, and used a measurement that we could use for all the disorders. We looked at the modeled risk of having an affected offspring and used real data to calculate it, and that's the outcome number that we looked at.

This varied by ethnic group, for example, in East Asians, **94%** of significant disorders were missed by using traditional approaches to screening; in the Hispanic population, **79%**; of Northern Europeans, where the screening was designed, **65%**; and Ashkenazi Jewish, **55%**. This goes to show that this is extended through a number of other ethnic groups.

We saw that it was slightly better in the Southeast Asian and the African American groups, because much of the burden of disease in those groups was hemoglobinopathies and those were picked up in those groups.

Overall, the chance of having an affected offspring after expanded carrier screening is about **1 in 550**. It's not high, but that is higher than the chance, for example, of having Down Syndrome in the general population, which is recommended that everyone be screened for. The chance of Down Syndrome in the general population is about 1 in 700, and goes up with maternal age. The chances of neural tube defects and what everyone screens for, either by ultrasound or Alpha-Fetoprotein, is about **1 in 1000**.

Another example, **Cystic Fibrosis** is about **1 in 3500** and across ethnicities, this number is in general higher than things we're already routinely screening for.

Another way of looking at it is the increase detection of affected pregnancies, we were 17 times more likely to find an affected pregnancy for less than **8 in 100,000** to **130 in 100,000** in East Asians, and in the Ashkenazi Jewish population we were twice as likely to find an affected pregnancy.

Another opinion that came out last week was companion opinion to the one that looked at carrier screening. It was a general statement on carrier screening in the age of genomic medicine.

I have to commend ACOG for looking at our data and analyzing it and then incorporating it into this statement. What they say is that ethnic specific, which is traditional screening pan-ethnic, and what they recommend now just for two disorders and expanded carrier screening, are acceptable strategies. This shows support for doing expanded carrier screening.

Unfortunately, they specifically say that complete analysis of the CFTR gene, Cystic Fibrosis by DNA sequencing, is not appropriate for routine carrier screening.

After looking at data and considering the detection rates and the residual risk I mentioned earlier from just using the **23-mutation** panel. **Faux exome sequencing** is what we're doing now versus targeted genotyping.

When looking at our data from **Cystic Fibrosis** using the **23-mutation** panel among different ethnic groups versus the 1.0 detection by sequencing. In Southeast Asians, for example, you're detecting at less than **20%** of affected individuals that are picked up by using targeted mutation screening.

> To me, it seems illogical, especially in our pan-ethnic population to say that the **23-mutation** panel should still be used. I think one of the arguments is the cost, which is rapidly dropping and so I this is not much of argument anymore.

Another argument they have is about variant curation. When carrying out sequencing, variance of uncertain significance will get picked up, as compared to target genotyping. Again, we have a solution for that and its variant curation, which is made up of a variety of different approaches and steps that go through to decide whether a variant is pathogenic or not.

Our philosophy is for carrier screening, we do not report the US's, but that doesn't mean we don't curate every single one of them. We do real time curation as we're running our assay, to make a decision whether it's deleterious or not and then decide to report it.

> I think that problem is solvable, for instance, **Collins**, a number of years ago, in his book, **"The Language of Life"**, said it's likely within a few decades people will look back at our current circumstances with a sense of disbelief that we screen for so few conditions. I think that's really true.

Parents who go for a carrier screening during pregnancy and are discovered to be carriers of **Wilson's disease.**

> **Wilson's disease** is a disease that has a variety of manifestations and is very difficult to diagnose after birth because the manifestations aren't necessarily characteristic.

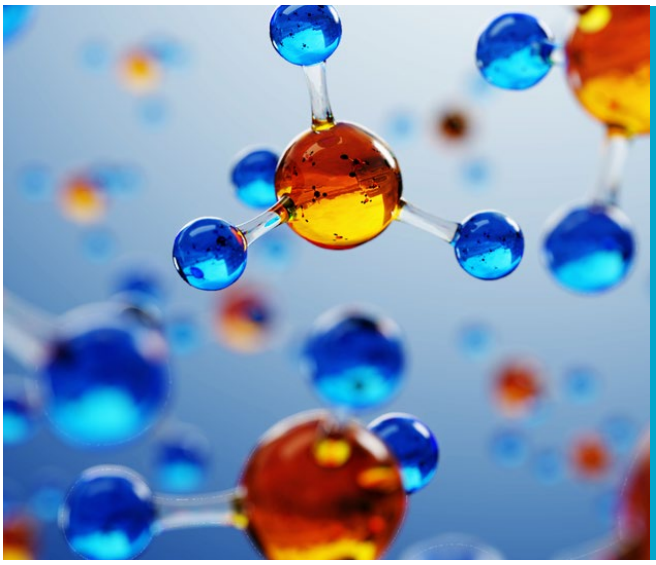
It's a disease we call as part of the diagnostic odyssey that could go on for years before the actual diagnosis is made. During this time, copper is accumulating in the patient and it is absorbed causing organ damage and multiple issues.

However, a couple who had the screening and knew that their child had Wilson's disease, allows them to be put on low copper, and added zinc to help excrete copper. They could even remove all copper plumbing and to hopefully never have manifestations of Wilson's disease.

This illustrated that there are incredible benefits at risk of not expanding carrier screening and with the new technologies we be enhancing the detection of more at risk carrier couples.

1.5 SINGLE MOLECULE DETECTION OF PROTEINS IN SINGLE CELLS

David Walt is Robinson Professor of Chemistry and has made many groundbreaking inventions in the biological applications of microwell arrays. He applies micro- and nanotechnology to solve urgent biological problems and is one of the most successful life science entrepreneurs of recent times. It was his miniature lab platform that allowed researchers to conduct genetic screening quickly and cheaply.



At Pittcon 2017 he described the single molecule array, a technique that allows researchers to count the number of molecules present in a solution. It detects nucleic acids and proteins with high sensitivity and could also be used to analyze biopsies for different types of cancer cells.

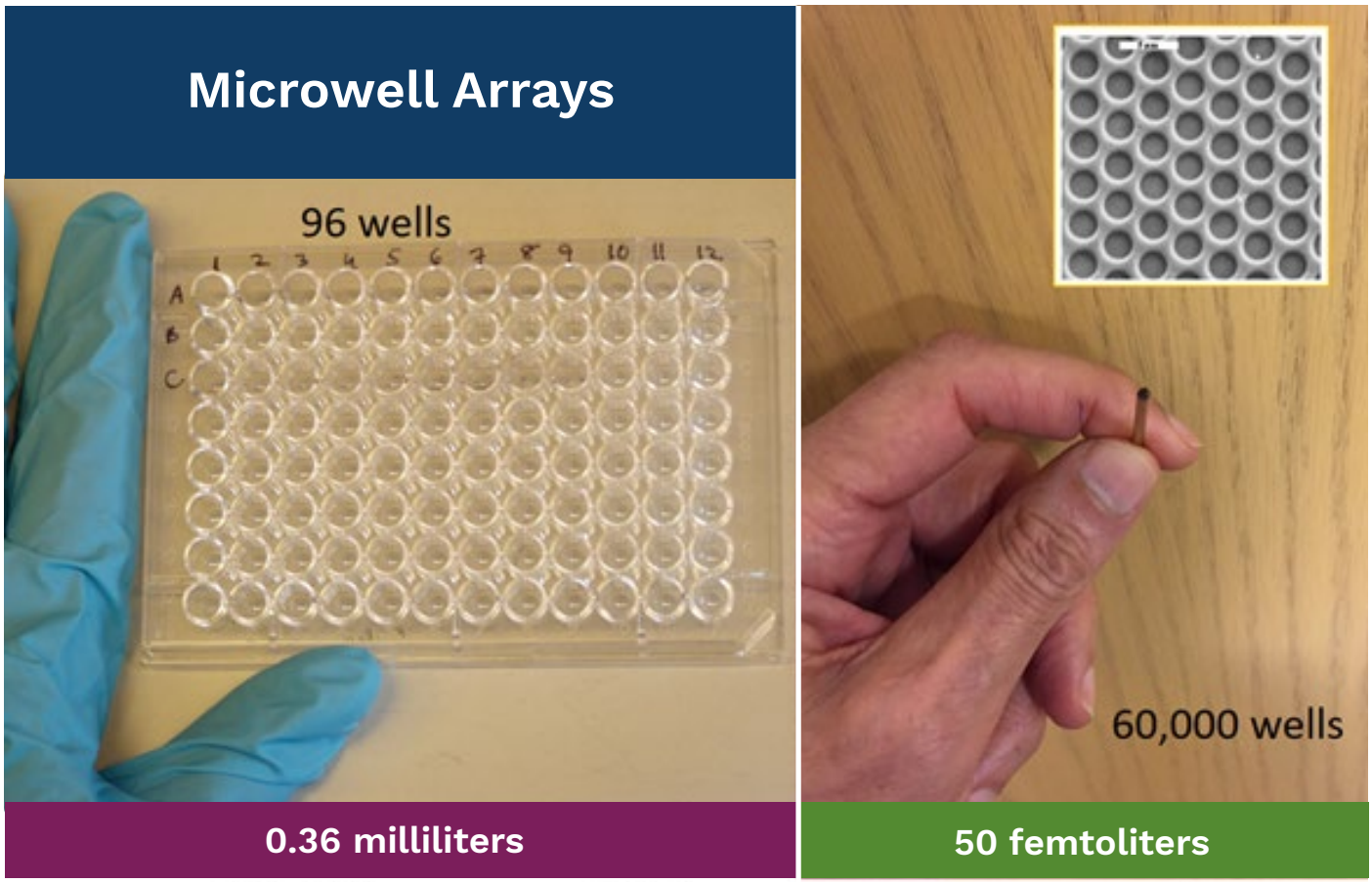
An interview with Professor David Walt, Tufts University, conducted by April Cashin-Garbutt, MA (Cantab)

Q **Can you please give an introduction to the single molecule array (Simoa) that you developed and discussed in your talk at Pittcon 2017?**

A We developed this technology almost 10 years ago. It was developed after a key experiment was carried out by **David Rissin**, who was a PhD candidate in my laboratory at the time.

At the time, we were working with microwell arrays for a different project. At a lab meeting I posed the question: “How many molecules of a fluorescent molecule would it take to bring the concentration in one of these very tiny microwells to a level that would be easily detectable?” We performed the calculation and it turned out to be about 100 molecules of a fluorescent dye.

David carried out an experiment using a fiber optic microwell array, which had approximately **60,000** microwells on the end of an optical fiber. We trapped a very dilute solution of an enzyme in these microwells–each microwell had a volume of the order of **40 femtoliters (10-15 liters)**—a very tiny volume.



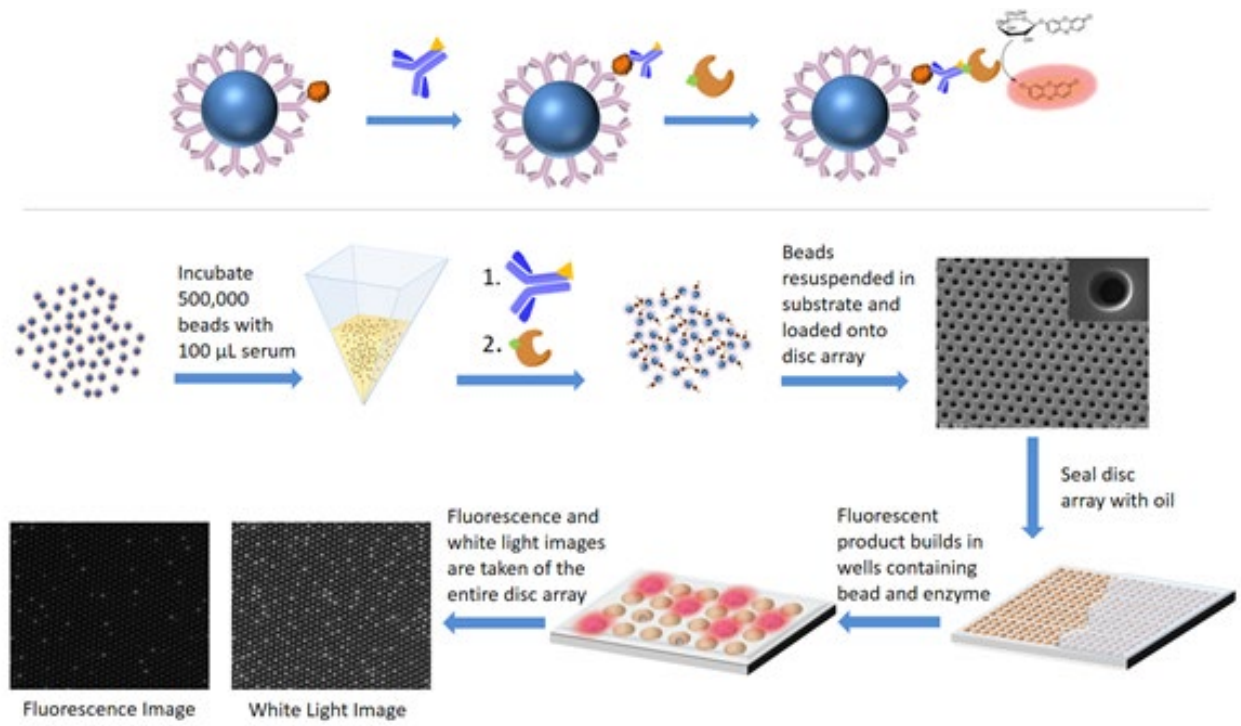
1000 times as many
5,000,000,000 times smaller

The experiment was designed to trap a very dilute solution of an enzyme containing a high concentration of a substrate. Substrates are the starting materials for enzymes, such that if there were an enzyme present, the enzyme would catalyze the conversion of that substrate into many molecules of a fluorescent product. This process would occur quite rapidly with β-galactosidase, which is a fairly active enzyme.

> The idea was that we would run this reaction at such a dilute concentration of enzyme that only a fraction of the wells would contain an enzyme molecule and only those wells that did would fluoresce.

After **David Rissin** worked out the technical details required to carry out the experiment, he demonstrated that one could clearly see which wells contained an enzyme molecule because they turned very bright red in contrast to the wells that contained no enzyme molecule, which remained black.

Single Molecule Arrays (SiMoA)



After this demonstration, we observed that as we decreased the enzyme concentration, we observed fewer fluorescent wells because there were fewer enzyme molecules in the array. We published a paper in Nano Letters and claimed that this was the first time that anyone had measured concentration by counting molecules.

> So this experiment was the first demonstration that you could take a solution, confine it into very tiny volumes and literally count the number of molecules present in that solution. In this case, the β-galactosidase concentration could be measured by simply counting the number of bright wells relative to the number of dark wells.

Simoa was developed because we wondered if we could use this approach to actually measure things that people cared about. For example, biologists are particularly interested in measuring molecules such as nucleic acids and proteins.

Over time, the single molecule array technology developed where binding reagents are now attached to microspheres or 'beads' in order to capture the target molecules of interest from solution. The beads containing the bound target molecules are then labeled with an enzyme molecule and loaded into the wells and the wells are sealed.

If there is an enzyme molecule attached to a bead, it acts as a reporter for the nucleic acid molecule or the protein molecule of interest. The attached enzyme molecule catalyzes the conversion of many molecules of substrate into product such that wells containing enzyme will build up a locally high concentration of product and fluoresce. The wells containing beads but no bound molecules will stay dark. That's the entire evolution of the technology.

Q **How sensitive is Simoa at detecting proteins and nucleic acids?**

A With respect to nucleic acids, it's close to other sensitive techniques such as the **polymerase chain reaction (PCR).**

For example, there have been demonstrations where a particular nucleic acid sequence can be detected at femtomolar (10-15) concentration so the sensitivity may even be higher than that.

The same goes for proteins where the Simoa limit of detection is typically about a factor of **100 to 1000** times more sensitive than the traditional method of performing a protein assay—the enzyme-linked immunosorbent assay (ELISA) technique.

Q **Can you please explain how you have applied the method to the analysis of cultured cancer cells?**

A We've simply changed the sample handling and preparation so that it can be applied to a single cell assay. It's still a conventional Simoa assay that uses capture antibodies attached to beads in order to bind proteins of interest in individual cells.

For sample preparation, we first isolate individual cells, either manually or with a variety of single cell isolation procedures such as flow cytometry or microfluidics. The key is to isolate individual cells in very small volumes. We then take the isolated cells and lyse them to disrupt their membranes and release their contents into a small volume of buffer. We then add beads and capture the molecules that we're interested in detecting. Everything then follows the conventional workflow for Simoa.

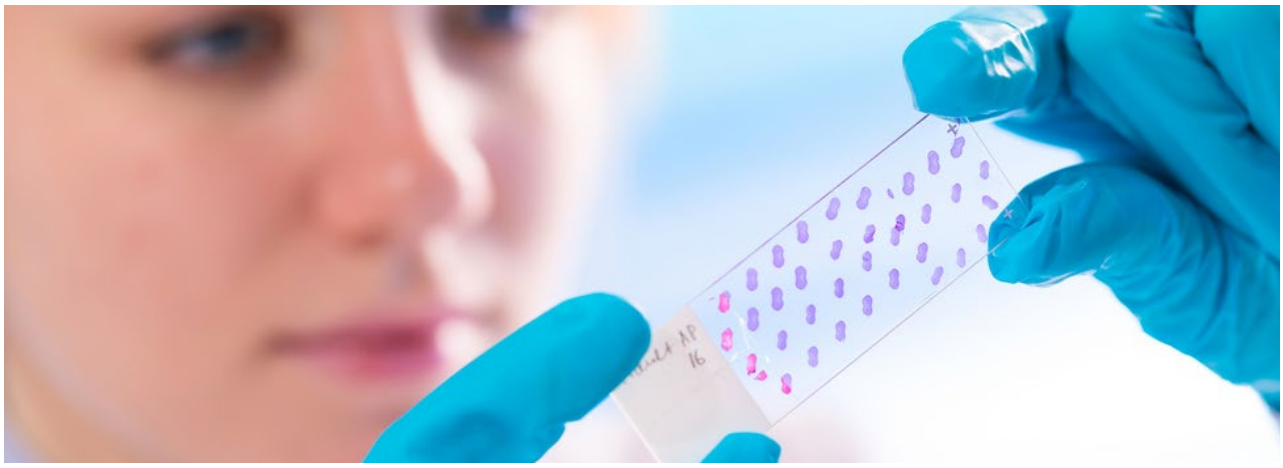
The goal is to be able to study individual cells within a population. It is now recognized that homogenizing a population of cells, typically thousands if not millions of cells, when making a measurement gives an average concentration of the protein or the nucleic acid that's in the cells. Such measurements mask the heterogeneity of the cell population.

Using **Simoa** we can measure the protein concentrations in individual cancer cells. Observing hundreds of cells at a time at the single cell level provides insight into the heterogeneity of the cell population. By developing a technology that can measure the protein concentrations in individual cells, we are able to look at the distribution of protein concentrations within the population.

One possible use such a technology would be in a clinical setting to study tissue biopsies. For example, if one took a needle biopsy from a woman who had an abnormal mammogram, the individual cells could then be dissociated from that biopsy tissue and analyzed, as opposed to looking at the population average.

This approach is important because if there were any rare cells in the sample that happened to have a particularly aggressive phenotype, even **one out of 1,000** or **one out of 10,000** cells in that tissue sample, it would be difficult to detect that cell if you were to homogenize the tissue first and were looking only at the average of the cell population. This particularly rare aggressive cell is going to divide rapidly and could end up leading to metastatic disease and ultimately a poor outcome for the patient.

When pathologists evaluate biopsy samples, they look at the overall cell population and they don't get the level of granularity that's necessary to determine if there is a particularly aggressive rare cell present that will give the patient a bad prognosis. By looking at the average, you might conclude, "Well, **9,999** cells are normal" because that rare **10,000th** cell is averaged into the results and the results are therefore biased in favor of the population average.



Q **What other applications of Simoa are there?**

A There are a wide variety of clinical indications being investigated. This technology has now been commercialized by **Quanterix**—a company based in Massachusetts of which I am the scientific founder. They licensed the patents that came out of my Tufts laboratory and are commercializing the technology.

My lab has been focused primarily on breast cancer and infectious disease detection. We're now beginning to look at **Parkinson's disease** using the technology.

Other researchers are looking at various inflammatory diseases. There's also a fairly substantial community that's using the technology to look at neurological disorders; in particular, things like traumatic brain injury.

The National Football League in the US has funded some of the research that's looking at markers released from the brain when the brain experiences a traumatic injury. These proteins are released and appear in the bloodstream, and appear to be highly predictive of the extent of the brain trauma.

> "My lab has been focused primarily on breast cancer and infectious disease detection. We're now beginning to look at **Parkinson's disease** ... Other researchers are looking at various inflammatory diseases...to look at neurological disorders;... traumatic brain injury...for Alzheimer's disease...to detect early signs of heart attacks"

Other researchers have used the Simoa technology to begin to look at blood markers for **Alzheimer's disease**. Still others are using Simoa to detect early signs of heart attacks, by measuring certain proteins that are released in the early stages when the heart is beginning to have issues.

Q **What did audience members learn from your talk at Pittcon 2017?**

A At Pittcon I spoke about some of the technical details of sample preparation for getting single cells isolated to make meaningful biological measurements.

I also presented some of our latest work with various cultured cancer cells where we've been able to multiplex the assays and I will show some meaningful measurements that indicate that we have a good chance of detecting rare cells when we apply this technology to clinical samples.

Q **What impact do you think Simoa will have in the future with regards to detecting rare cells?**

A I have already mentioned the detection of aggressive rare cells in tissue biopsies in a clinical setting but there's also a research component in addition to the clinical component.

In a research setting, one of the interesting questions from our perspective is what do these rare cells look like? How do they differ from the population average? There's been a lot of effort in single cell genomics as a result of the wide variety of genetic technologies, including sequencing, that are now available. As a result of these technologies, there's the capability to isolate individual cells, tag them and then look at how the genetics differs from cell to cell.

> Of course, that is giving us some interesting insight into cell-to-cell variability but one of the things that people are extremely interested in is how do these genetic changes manifest in terms of how the cell expresses different proteins and how the cell actually differs from its surrounding cells?

Again, getting from the genotype to the phenotype is a much less explored area, and is what my lab is focused on—trying to understand how large are these differences between cells.

If we can now combine both technologies and correlate the genotype—the genetic differences—with the phenotype—the way proteins are expressed and the physical appearance of the cell—then we're going to have a much better understanding of how rare cells have the ability to do things that most of the other cells in the population cannot do.

> "Then of course, the next step is to figure out a way to scale up this technology such that it can become a diagnostic technique, so that it can be used in the clinic as well as a research tool."

Q **What do you think the future holds for single molecule detection and single cell analysis?**

A I think both of these areas are incredibly promising. The thing that unites both single molecule detection and single cell analysis is that the molecule is the fundamental unit of chemistry and the cell is the fundamental unit of biology.

> We're in the very early days of being able to analyze single molecules, look at populations of molecules, and with some of the more biophysical technologies, look at single molecules and observe them over time. My lab has demonstrated this capability with relatively large single molecules such as enzymes.

Single cells are the fundamental units of biology. Just as single cells have differences when you begin to look at the levels of proteins in them or the sequence of DNA in ostensibly identical cells, when you look at single molecules, you also see a distribution of behaviors.

We're now at the stage where we have the technologies and the resolution to begin to observe these kinds of individual differences between the behaviors of individual molecules and individual cells. I think it's going to certainly rewrite some of the textbooks in fields such as biochemistry and biology because there's no such thing as an average molecule or an average cell. **Every cell has its own identity. Every molecule has its own identity.**

These behaviors have previously been treated as average properties of molecules and cells. The stochastic behavior of individual molecules can have significant implications that affect biochemical processes, pathways and ultimately the properties of individual cells. And you can begin to look at these pathways and processes and surmise how the properties of individual molecules manifest

Q **What advances in technology would you like to see and why?**

A One thing that I'd like to continue in my lab is to push the boundary of sensitivity to detect even lower concentrations than we've been able to achieve so far.

> "What we've been able to accomplish so far is to measure proteins at two to three orders of magnitude lower concentration. It would be great to seek out another ten to hundred-fold more sensitivity."

There are a lot more things that we will be able to look at with a higher level of sensitivity. Even today, when we are measuring blood samples, we don't see certain proteins because the Simoa assay technology, even with its thousand-fold higher sensitivity, does not give us the sensitivity that we need to measure some of the molecules present in the blood at lower expression levels. I would like to see continued movement of that lower limit of detection to even more sensitive levels.

I also think that it would be great to be able to increase the level of multiplexing so that more molecules of different types can be measured simultaneously.

Then finally, and this is something that I don't have a technology solution for but certainly something that I'd like to see, I would like to be able to begin to apply this kind of molecular counting technology to small molecules. We use immunoassays and nucleic acid assays that are capturing large molecules, but the technology that we've developed is really not amenable to being able to measure small molecules such as metabolites. Obviously, it would be great to be able to do that.

> "I would like to see continued movement of that lower limit of detection to even more sensitive levels. I also think that it would be great to be able to increase the level of multiplexing... to be able to begin to apply this kind of molecular counting technology to small molecules."

Q **Where can readers find more information?**

A The field is changing so rapidly that the best thing to do is simply search for "**Simoa**", and there will be a hundred articles where the technology's been used for various applications.

1.6 SINGLE CELL ANALYSIS FOR PRECISION MEDICINE

David Walt is Robinson Professor of Chemistry and has made many groundbreaking inventions in the biological applications of microwell arrays. In the previous section, he described the technique of single molecule arrays. This section builds on this, highlighting the importance of the technique in identifying rare cell types or heterogeneity in a sample.

For example, it could help identify particularly aggressive or metastasizing cells in the diagnosis and management of cancer. An automated digital assay system is available so analyses can be conducted quickly. Furthermore, the sensitivity is higher than that achieved with a standard immunoassays and much smaller sample sizes are needed.

A transcript of Professor David Walt's talk at the twenty-eighth James L Waters Symposium on Genomic Analysis Technologies at Pittcon 2017.

INTRODUCTION

There are multiple reasons why single cell measurements are important. When making average measurements of many cells, the full heterogeneity of the population is not captured. A good example of this is when handling the bimodal distribution of gene expression. Calculating the average in this scenario, it can't be known fully what is being measuring, simply the average expression level is being measured.

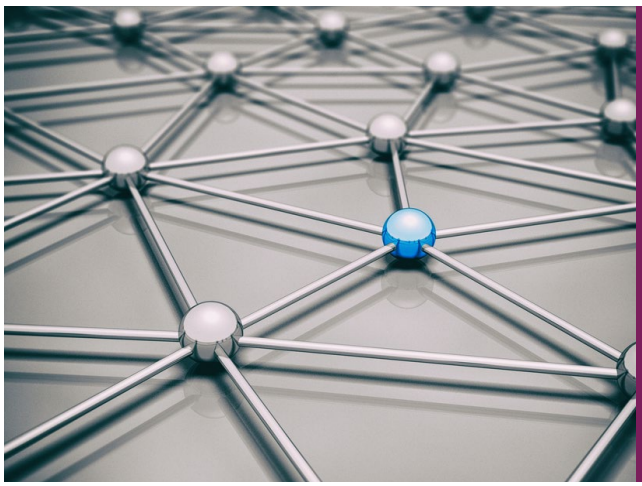
This can be especially important when working with populations that contain rare cells, such as a tumor, whether this is gene or protein expression. Taking a bulk measurement of this population might make you conclude that all cells are virtually identical, and the true identity of the population is overlooked. The rare cells that are of most interest and might have expression levels that are very different, get swamped out by the other cells in the population.

> What we're interested in is looking at protein expression at the single cell level, because of the masking of subpopulation that occurs from taking bulk measurements. We think that the single cells are key to giving us an insight at the rare cell level.

The challenge for single cell analysis as applied to proteins. There have been a number of studies that have investigated correlations between gene expression in single cells, using messenger RNA and protein expression. In some cases, correlation is observed, but in other cases it is not. This is a consequence of two processes, one is the **Poissonian process** of transcription and the other is **burst kinetics of protein production in a cell.** In burst kinetics messenger RNA binds to ribosome and then in a burst type fashion, generates dozens to hundreds of molecules at a time.

Going from mammalian cells to bacteria, the total protein content decreases, as well as the average number of molecules of a given protein in the cell. In a typical mammalian cell, there are roughly ten thousand proteins being expressed, with approximately a hundred thousand copies of each of those molecules being expressed, and ten to the power of nine, billion total protein molecules. There's a very wide distribution of how many molecules of a given protein are exhibited in each cell, this is gone into more detail later in the document.

WHAT IS SIMOA?



Single molecule arrays, or **SiMoA**, technology was developed in my laboratory about ten years ago, and is like a digital **ELISA**. It has tremendous value, being able to carry out digital measurements as opposed to analog.

As the concentration of protein increases, in what is similar to individual wells in a 96-well microtiter plate, more protein is captured onto a detection antibody, which is either labeled with a flora form or enzyme. As you add substrate or as more fluorescent labeled antibodies become attached to the sandwich, the intensity increases. There must be some kind of calibration, because the bindings are going to be different.

In digital immunoassay, measurements produced are intensity independent i.e. either a molecule is present, or not. This means that molecules can simply be counted and calibration is not necessary, however we still calibrate in our lab, because we're still developing the technology. In this field, for the molecules of interest the question is not 'What is the intensity?' it's more about finding an absolute measurement.

The technology works using a bead based immunoassay, there are three micron beads, to which we attach specific capture antibodies. There's roughly two-hundred-fifty thousand copies of each capture antibody attached to each bead, and this is put into a solution. The target protein binds to the beads using a biotinylated detection antibody. The detection antibody then creates the sandwich, which is labelled with a strep avenue labeled enzyme, for example beta galactosidase.

Where a protein molecule has been captured, a single molecule sandwich is created, which can be detected by looking at the catalysis, that was described earlier, in very small volumes. The beads are put into wells and will generate, locally, a very high concentration (nanomolar to micromolar) in this flora form.

This is made into a digital immunoassay by drowning out the number of protein target molecules that are being measured. This is done by adding many more capture beads than there are molecules in the solution. A typical one femtomolar concentration of a sample that has a hundred microliters, will contain sixty thousand molecules.

For example, if one cell has ten thousand or fifty thousand molecules of a given protein in a hundred microliters, you would add five-hundred thousand beads. The Poisson distribution will dictate that roughly one out of ten beads will capture a molecule and therefore as you do the labeling process, as long as the dissociation constant is sufficient to prevent the labeling region or the target protein from dissociating, you now have a population of beads that contains either one or zero molecules on it.

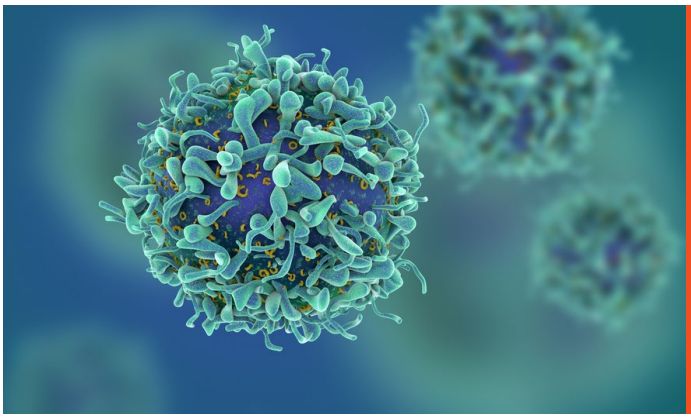
The beads are then distributed into the wells, which are loaded with substrate and sealed from each other, using for example with a fluorinated oil. The white light image is used to find where the beads are and the fluorescent image shows us where the labeled molecules are. A ratio of these, tells us the percentage that correlates to the original concentration in the solution, which can be used as an absolute measure of the concentration or number of molecules in the individual cells.

The **SiMoA** assays, is an automated instrument, sold by **Quanterix**. I'm a scientific founder of Quanterix and on the board of directors. We have an open platform that allows us to do different things than what the company recommends.

APPLICATIONS OF SIMOA

Most applications of this technology, that I will discuss in this document, are to individual cells and cultures. First of all, in this process we must isolate the individual cells, this is usually done by a relatively manual process, which has a low throughput. We then lyse the cells in a relatively small volume of buffer, typically ten microliters. Next, we add the beads, along with the lyse buffer; capture the proteins of interest; add detection antibody enzyme congregate; and run through the immunoassay process.

Prostate cancer cells



In my first example, we applied this process to a culture of LNCaP cells, which are a **prostate cancer cell** line that produces **prostate specific antigen (PSA)**.

We were able to produce a great PSA calibration curve and a **LOD (limits of detection)** 3 thousand times lower than the standard of PSA enzyme immunoassay. It's available commercially and so it can be used as a proof of concept.

> We originally had a cell line obtained from a collaborator, we call this our LNCaP bead cells. Initially we got measurements that did not correlate with previous reports in the literature from bulk measurements of how many molecules would be in an average cell.

This cell line was highly sub cultured and high passage, so we went back to ATCC and got a new low passage line and began to grow it. We then sequenced the two and found that there was a twelve percent genetic drift between the ATCC line and the one that had a high passage. Then we began to measure the PSA in these individual cells.

The PSA was carried out by students who did a dilution, so that when they took a microliter and put it into a PCR cap, they observed either a cell or no cell under the microscope. This is then done with hundreds of cells from each of the different cell lines.

When measuring the PSA of these individual cells, we found more than two log difference in the concentration of PSA from ostensibly identical cell lines. This is clearly a consequence of the stochastic nature of transcription and the bursting of individual proteins.

The ensemble measurement, that is the bulk measurement, hides both the distribution information and underestimates the expression level of the PSA.

We also found that a high passage cell line, compared to a low passage cell line, had a significantly lower (thirty-fold) expression level. This allows us to begin to quantify how the genetic drift impacts protein expression.

Next, we made comparisons with the bulk analysis. Following serial dilution, going from a hundred cells per microliter down to single cells per microliter, the variation increased, and these cell lines expressed different concentrations.

When looking at single cell measurements and quantifying them, numbers produced were higher in the case of the single cell measurements than in the bulk measurements. We think that this is a consequence of inaccurate cell counting when using hemocytometer counting. This illustrates the power of getting higher resolution measurements with single cells.

Some of the advantages of using SiMoA technology is having a wide dynamic range. SiMoA has a dynamic range that spans both the digital and the analog range. We have between 5 and 6 logs of dynamic range. I would say this is not efficient, but not high throughput, it is straightforward to sample things and you don't have to do lots of extraneous measurements.

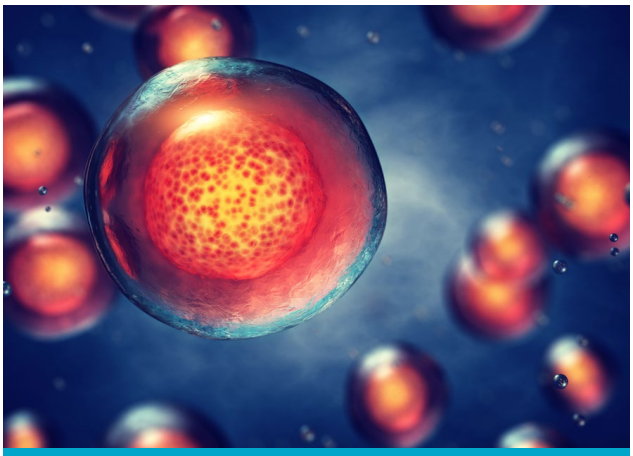
Breast cancer cells

We're also interested in looking at the heterogeneity within tissues. In the clinic, for example, when taking a tissue biopsy there is only a limited amount of sample, it would be useful to be able to dissociate those cells into individual ones without perturbing the biology or chemistry. Being able to preserve the integrity of those individual cells through the isolation process is important, but requires freezing them in time.

If those cells could be dissociated and then analyzed individually, it might allow the identification of a rare cell that may lead to highly invasive metastatic disease and that would be missed in a traditional immunohistochemistry type of assay.

The differentiation of the single cell in this field of view is a tough challenge in an image based system because of the heterogeneity of the tissue. Currently, the chemistry or biochemistry that's inherent in those cells are not being looked at in enough detail.

Measuring one protein in a cell has limits, ideally we need to measure many of them, and this can be easily done with similar technology. This would be done by using fluorescent labeled encoded beads that allow you to barcode the different kinds of proteins in those cells when they're lysed.



Some of the super resolution methods should address this in a different way. Going beyond model studies, we'd like to begin to look at real cells and multiplex the measurements.

The idea is to take the cell, lyse it in the presence of multiple beads, each of which have a different fluorescent bar code on them, distribute those after labeling them appropriately and then look at the expression levels of multiple cell types.

Using these as models, we have six different cell lines representing many different kinds of cancer e.g. breast cancer, inflammatory and invasive breast cancers. These different breast cancers have different kinds of receptor status, usually a steroid, estrogen, progestogen, or HER2 receptor.

In these different cell lines there are several representative of triple negative breast cancer, which is the most aggressive and invasive form of breast cancer. The question we asked is: as we begin to look with finer resolution at protein expression in these individual cells, can we identify and discriminate different cells from one another using a biopsy sample?

We wanted to know whether we would be able to find that single cell that represents a very aggressive phenotype in a population that has a lower risk potential.

Initially, we had a hundred cells and looked at the expression level of the different biomarkers by carrying out serial dilutions, these were our bulk measurements. We looked at inflammatory biomarkers, this included markers that are responsible for immune cell recognition as well as steroid receptor subtypes and we found three different cell lines.

The particular cell line, NB231 is one of the triple negative cell lines and even with a hundred cells and ultra-sensitive measurements, the presence of the three receptor subtypes is not detectable below the detection limit of the SiMoA assay, despite having very good limits of detection.

We start seeing what the concentration in these individual cell lines are, what the assay LOB is, and now what the assay LOD is in terms of absolute numbers of molecules.

The conclusion we made from this is that these should be relatively easy for us to measure using single cells.

We then carried out single cell measurements and began to measure the individual proteins in the individual cell lines. We found that MCF7 was almost undetectable. The percentages of cells that can be measured above the limit of detection, for the MCF cells, only one out of a hundred and twenty cells gave a measurable level of curve 2, whereas the other cell lines gave a more common distribution.

CYR61 single cell analysis, gave much higher measurements of all those in all the different cells. Once plotted, the data showed a gamma distribution, which is a two-parameter distribution function, and a good measure of protein expression due to the stochastic nature of the gene expression, the transcription and the bursting of proteins. Next, we applied the same kinds of measurements to a messenger RNA, to avoid amplification.

Digital measurements have the benefit of not requiring amplification, so we can first capture the messenger RNA on a bead. Conversion of the RNA into a cDNA is required, but it can be done in a single step.

To label the cDNA with biotin, we carried out the reverse transcription in the presence of biotinylated nucleotides. We then captured the biotinylated messenger RNA on beads that have a particular sequence for the sequence of interest and labeled that again with the streptavidin beta galactosidase.

This allowed us to use the same format assay for single molecule transcript measurements as we did with the protein, therefore making the work flow the same.

These messenger RNAs can be measured in a single cell using SiMoA. Standard RNA from the human brain is used as a reference sample. The amount taken corresponds to ten cells worth of RNA and we dilute that by ten. The measurements from 6 individual cells, looking at the same four markers, including the gap DH housekeeping gene.

COLLABORATIONS

We've been engaging some of the leaders in generating higher throughput systems. We have been developing a commercial system that is a cell selector and is primarily an automated picker. It does exactly the same thing that we were doing with our manual pipette of putting them in PCR tubes.

We're also working with another laboratory on their microrafts using this system, that's an array of twelve thousand rafts. Being able to pick the cells that we want of these rafts and deposit them. We can also sort cells using facts e.g. on the basis of size and fluorescence, putting one cell in each well of a 96-well microtiter plate. This is the most efficient way to do it at the **Tuffs** facility.

We've also being doing some work in collaboration with **Daniel Chiu**'s group. He's been very interested in selecting **circulating tumor cells (CTCs)** and has a high-sensitivity technique for isolating and analyzing the cells.

The technology is called **ensemble decision aliquot ranking (eDAR)** and is used to isolate the CTCs. They use fluorescent antibodies to bind to biomarkers, select the cancer cells and put them into a 96-well plate. The droplets produced by induction, and then deposited into the wells.

At our laboratory, we use **Daniel Chiu**'s isolated CTCs and even though we receive them dry, they remain intact enough to recognize their binding epitopes. We then run assays on these cells and we measured PSA in one of the cell lines.

The idea is to move the CTCs to a multiplex, similar to our breast cancer cell work. We were able to recover a similar distribution from the PSA levels, from the cells that we obtained from Chiu's lab, however this was not of the same throughput that the Waits' lab has for sequencing. I think improvements can be made to be able to isolate the cells better.

From the perspective of establishing variations between cell lines and beginning to look at those variations within real cells and real human samples, a more predictive measure is needed, and one where you can measure five cells at a time to look at the outliers, because the baseline levels are going to be in a much lower level of expression.

This is not work that I carried out, most of the work was done by three people, and primarily through breast cancer research program funded through the **Department of Defense (DoD)**.

1.7 STUDYING ALZHEIMER'S AT SINGLE CELL RESOLUTION

Single cell analysis has also proved invaluable in the assessment of neurological disease. Since a cell's behavior is controlled by the effects of its immediate environment on gene expression, individual cells within the same population may be functioning differently and releasing different analytes.

Neurological diseases, such as Alzheimer's disease, are particularly complex and involve a many different types of neurons. The ability to characterize individual neurons and the interactions between them may therefore improve understanding of these neurons and how they differ in healthy and diseased states. This section discusses the techniques that have made such investigations possible.

Cells vary considerably within cell populations, including within a particular type of tissue or cell. No two cells have the same response to their surroundings, since each cell's behavior is dictated by the particular genes it expresses and at what level. This unique gene expression is what controls how the cell performs in the body.

Traditional gene expression analysis involved profiling whole cell populations and averaging measurements across those populations. Today, however, it is possible to study cells at single-cell resolution, which has opened up new possibilities in terms of understanding cells on an individual level.

SINGLE-CELL ANALYSIS IN NEUROLOGICAL DISEASE

Even within a single brain region, there is significant variation between the morphology, connectivity and electrophysical properties of individual neurons. A key step towards understanding the basic components of the nervous system is systematic classification of individual neurons. For cells to be classified on a molecular basis, gene expression must be assessed at single-cell resolution.

Neurological diseases such as Alzheimer's are often too complex for researchers to be able to develop effective treatments, due to the heterogeneity of the neurons underlying the disease.

Using single-cell tool kits, researchers can study heterogeneity within cell populations, single out rare cells, study interactions between diverse cell types and improve their understanding of how such interactions are relevant to health and disease states.

CELL ISOLATION AND CAPTURE

Several techniques are available for isolating and capturing cells for single-cell analysis, including manual or automated micropipetting, laser capture microdissection, **fluorescence-activated cell sorting (FACS)** and microdroplet devices. Another example is microfluidic platforms, which not only enable single-cell capture, but also automation of certain downstream biochemical reactions.

Future development of microfluidics technology will lead to ever greater increases in the throughput of microfluidic cell capture and the isolation of single cells.

At Pittcon, high quality analytical equipment is showcased by leading suppliers from more than 30 countries across the globe.

Among the companies that have exhibited are **LabSmith. Inc,** a world leader in microfluidic platforms for over 15 years. LabSmith gave live demonstrations of their products, including a new line of agile and thermal control products, specifically designed for applications in microfluidics. Also showcasing their products was IDEX Health & Science, which boasts a comprehensive portfolio of fluidics, microfluidics and optics devices and ExtraGene Inc, a professional manufacturer of micropipettes, minicentrifuges and PCR thermocyclers.

MICROENGRAVING

One type of microfluidics technology, namely **microengraving**, enables multiple secreted analytes to be quantified, by culturing cells in a dense array of nanowells.

In a study by **Tracy Young-Pearse** and colleagues (2016), the team adapted microengraving to create a new technology that can, for the first time, detect secreted analytes that are relevant to Alzheimer's disease.

Secreted factors are known to play an important role in both healthy and pathological processes, across all types of body tissue. Using their new technique, the researchers were able to uncover the dynamic range of secretion profiles of the analytes from single, living human neurons and astrocytes. They identified subpopulations of these cells that secrete the analytes in high concentrations and then molecularly characterized them using immunostaining and **RNA sequencing (RNA-seq).**

RNA-SEQ DEVELOPMENT

RNA-seq is a recently developed high-throughput technique for gene expression analysis. Although RNA-seq has clear advantages over previous techniques, capturing rare dynamic processes, such as adult neurogenesis, can be challenging, since isolating rare neurons is difficult and there are limited markers for each phase.

In a paper by **Naomi Habib and Yinqing Li** (2016), the researchers report on their new method for studying rare cell types in the brain. The technique combines the sequencing of RNA from isolated nuclei (sNuc-Seq) with tagging of regenerating cells (Div-Seq). **Habib and Li** stress that targeted and effective therapies can only be developed once it is possible to achieve a full atlas of every type of neuron at single-cell resolution and establish exactly which cells are causing the disease.

Pittcon 2016 featured more than **2,000** presentations on the latest research findings and technical advancements in laboratory science, including a session on the impacts of single cell analysis on biology and medicine. Among the presenters was **Young-Pearse**, who explained more about their exciting new technology for detecting and characterizing secreted analytes relevant to Alzheimer's disease.

Further presentations explained how the technology can be easily adapted to detect other analytes secreted by neural cells, which could open up new avenues for exploring human CNS development and dysfunction, and how nanomedicine can be used for functional imaging and therapy of the brain.

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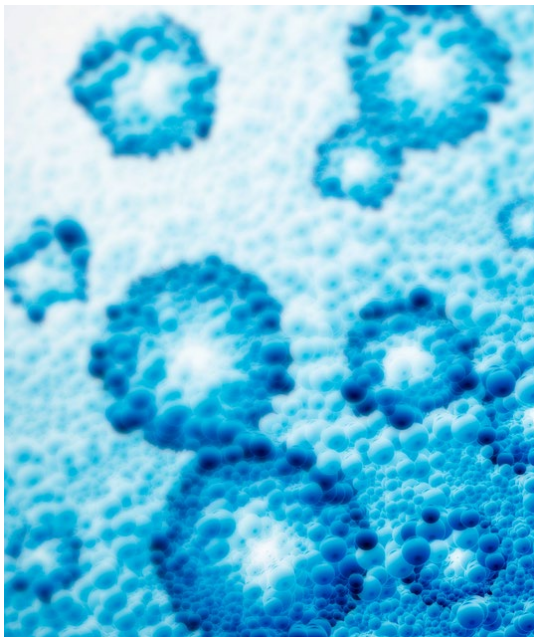
1.8 USING SPHERICAL NUCLEIC ACIDS TO TRACK AND TREAT DISEASE



Adding fragments of DNA or RNA to spherical nanoparticles creates spherical nucleic acids, which have much greater binding affinities than linear nucleic acids of the same sequence.

The development of spherical nucleic acids has thus increased the sensitivity of nucleic acid assays. This has proved particularly valuable in identifying infection before symptoms have developed, whereby massively improving the chance of successful treatment.

In addition to their use in diagnostic assays, spherical nucleic acids are being investigated as therapeutic agents and as potential cancer vaccines. This section details available spherical nucleic acid assays and both current and developmental therapeutic applications of spherical nucleic acids.

An interview with Dr. Chad Mirkin, Northwestern University, conducted by April Cashin-Garbutt, MA (Cantab)

Q **What are spherical nucleic acids (SNAs)? What do they consist of and how do they differ from linear nucleic acids?**

A **Spherical nucleic acids** are structures that are made by taking a nanoparticle template and using chemistry to arrange short strands of DNA or RNA on the surface of those particles. The spherical core of the nanoparticle creates a spherical arrangement of DNA or RNA, similar to tiny little balls of nucleic acids.

Even though the sequences can be identical, the properties of spherical nucleic acids are very different from linear nucleic acids. For example, SNAs bind complementary DNA or RNA much more tightly than linear nucleic acids.

This means that in the context of detection and the use of SNAs as diagnostic probes, you can use a lower concentration of a nucleic acid target, for example, associated with a given disease. And so, these have become the basis for high sensitivity and also very high selectivity probes, in molecular diagnostic tools.

Q **How can SNAs be used for the detection of infections?**

A There's a technology called the **Verigene system** that was commercialized by **Nanosphere**, a company I had started, which was then sold to Luminex. The Verigene system is used to sort signatures associated with disease, infectious disease in particular, and at very low concentrations, meaning at very early time points, to measure the presence of a particular infection. For example, in blood.

> This is important because it can then be used, for example, to diagnose patients with sepsis, where being able to diagnose very early is really important because for every hour that a patient goes undiagnosed and untreated, the chance of mortality increases substantially.

Technology like this is changing the way molecular diagnostics is carried out. It is a very simple and rapid point-of-care medical diagnostic tool that allows for the detection of bacterial infections way before conventional tests. It is not necessary to go through the process of culturing the sample, which takes a long time and, therefore, increases patient risk.

So ultimately, you have a tool that is better for the patient because you get an accurate diagnosis earlier and better for the doctor, because the doctor is not needlessly prescribing a lot of unnecessary antibiotics, wasting money, and contributing to antibiotic resistance. Instead the tool can be used to figure out who has a bacterial infection and who doesn't and the appropriate treatment with effective measures then can be taken.

Q **What does SNA synthesis involve?**

A In the case of developing a biological label, a gold nanoparticle is used for the template, and the SNA is made by bringing the template in contact with short strands of DNA that can be chemically anchored to it. In the case of gold, the anchoring groups are thiols.

We have developed a process that allows you to load the DNA or RNA on the surface of a particle to very high extents. The reason that's important is that it forces the orientation and gives the architecture both its spherical shape and also the properties that I've been mentioning.

Q **Can you please outline your talk at Pittcon 2017 on 'Nano-Enabled In Vitro and In Vivo Diagnostic Tools for Tracking and Treating Disease'? Which bioassays did you focus on?**

A At Pittcon I focused on two different types of bioassays:

- One based upon the Verigene system
- A new technology that allows one to measure intra-cellular nucleic acid targets -- mRNA

Both technologies are based on SNAs, which are structures that can enter a live cell, bind to a particular target, in this case an mRNA target, and elicit or liberate a fluorophore signaling entity that lights up the cell.

This allows you to then measure for the first time, the genetic content of live cells. In addition to measuring the genetic content, cells can be differentiated based on mRNA expression levels. The location of the RNA within the cell can also be measured, which is especially exciting because nobody has ever been able to do that in live cells before now.

This is especially interesting because when coupled with a technology like flow cytometry, you are able to sort cells based upon genetic differences. **Millipore** is a company that has commercialized this technology and produced many variations of these types of architectures, so that researchers can begin to look, for example, for rare cell populations and pick out circulating tumor cells, in the presence of healthy cells.

This becomes a way of studying the cells and the number of them. It also allows you to isolate them so that you can study them after the fact.

You can pull them away from majority cell populations, culture them, and use them to understand the origins of genetic differences. For example, looking at how a cancer patient's cells respond to different types of therapeutics.

This is a major step towards personalized medicine and increasing our capabilities with respect to probing cellular systems. It's also potentially useful for high throughput drug screening, where you can look at how different types of drug molecule activate or suppress different types of genes. You can get a visual readout in this case, based upon the use of this technology, we refer to as nano-flare technology. Millipore has commercialized a form of nanoflares they refer to as smart-flares.

Q **What was the focus of your second talk at Pittcon 2017, 'Spherical Nucleic Acids as Potent Immunomodulation Agents for Cancer Therapy'?**

A SNA structures also represent the basis for an entire new class of nucleic acid therapeutics. There are three central arteries of drug development:

SMALL MOLECULES

Benefits are well known, aspirin being a great example.

NUCLEIC ACID MEDICINES

Here short snippets of DNA or RNA are used to treat disease and attack it at its genetic roots.

BIOLOGICS

Seven of the top ten drugs are based upon biologics; these are antibodies, protein-based architectures. They have a lot of advantages and capabilities that go beyond what small molecules offer.

Antisense drugs are based upon DNA and are used to soak up mRNA in cells and stop translation of that RNA and production of proteins that we associate with disease. The idea behind antisense is that you can regulate a person's cells and convert an unhealthy cell into a healthy cell by knocking down the production of a specific type of protein.

Then came along siRNA technology – a similar concept in the sense that you're knocking down the production of specific types of proteins, but via different pathways.

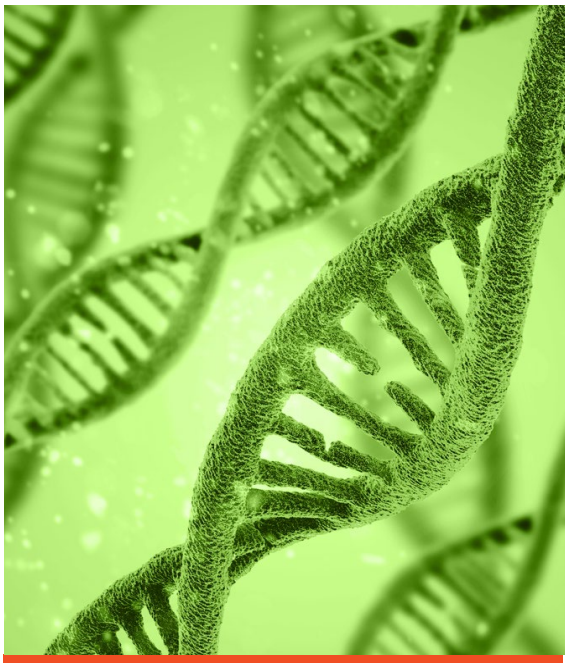
The idea of developing genetic medicine is really the concept of a type of digital medicine, where instead of every time you need a new drug you don't look for a new small molecule, you change the sequence of DNA or RNA being used based upon an understanding of biological pathways.

From a conceptual standpoint, these were really powerful technologies. They led to the development of many commercial approaches but have had limited success.

The reason being, to truly realize digital medicine you need multiple things in play. One is you have to be able to synthesize DNA and RNA, and two, you have to be able to understand pathways.

These two issues have now been overcome; we know how to synthesize DNA and RNA, and thanks to the human genome project, we also know a lot about the pathways of disease and how to attack different types of pathways to treat disease.

But the third, and perhaps most important requirement, is the ability to get the DNA or RNA to the site that matters. And that's where most attempts have fallen short.



> "...to truly realize digital medicine you need multiple things in play. One is you have to be able to synthesize DNA and RNA, and two, you have to be able to understand pathways... the third, is the ability to get the DNA or RNA to the site that matters."

This is where spherical nucleic acids are very important. SNA structures, which have no natural equivalent, can interact with natural systems completely differently from the native DNA and RNA from which they're derived.

Almost every cell type in your body, other than mature red blood cells, recognize **SNAs** and rapidly internalize them without the need for transfection agents.

This is particularly interesting because, for example, putting normal DNA or RNA in creams and putting them on your skin won't make them go into your skin cells; but with spherical nucleic acids they'll rapidly take them up. This discovery therefore opens up the ability to create topical medicines, local medicines, that allow you to treat a lot of diseases.

And so we've been looking at this capability in terms of developing new types of treatments for skin disease. There are over 200 diseases with a known genetic basis. One can begin to think about creating therapeutics for the eye, ear, lung, bladder, and colon via similar approaches.

The fundamental properties of SNAs make nucleic acids relevant for treating a wide range of medical conditions not addressable with conventional nucleic acids. The first SNA constructs are in human trials for treating psoriasis.

Q **How could SNAs be used in cancer vaccines?**

A Another application we've been researching is the use of structures as potent regulators of the immune system. SNAs will enter immune cells, dendritic cells, and if the sequence is correct, they will activate toll-like receptors, so that you can take an animal, or a patient in principle, and selectively activate their immune system.

This allows for the creation of new forms of vaccines, for example, where you can train a person's body to fight a specific type of cancer. This is what is happening right now, we have a whole series of drug candidates based upon this approach, and I talked primarily about prostate cancer at Pittcon.

In principle vaccines like this could be developed to treat many different types of cancers, including cancer of the brain, bladder, colon, and melanoma.

Q **What stage of development are SNA cancer vaccines currently at and what hurdles still need to be overcome?**

A The cancer vaccine work is just about to go into human clinical trials this year. The technology has been extensively vetted in animals and proven to be safe, for example, in primates.

The human trials are extremely important. With a cancer vaccine, you are modulating a person's immune system and there is a risk of creating autoimmune responses.

Q **What are the next steps in your research?**

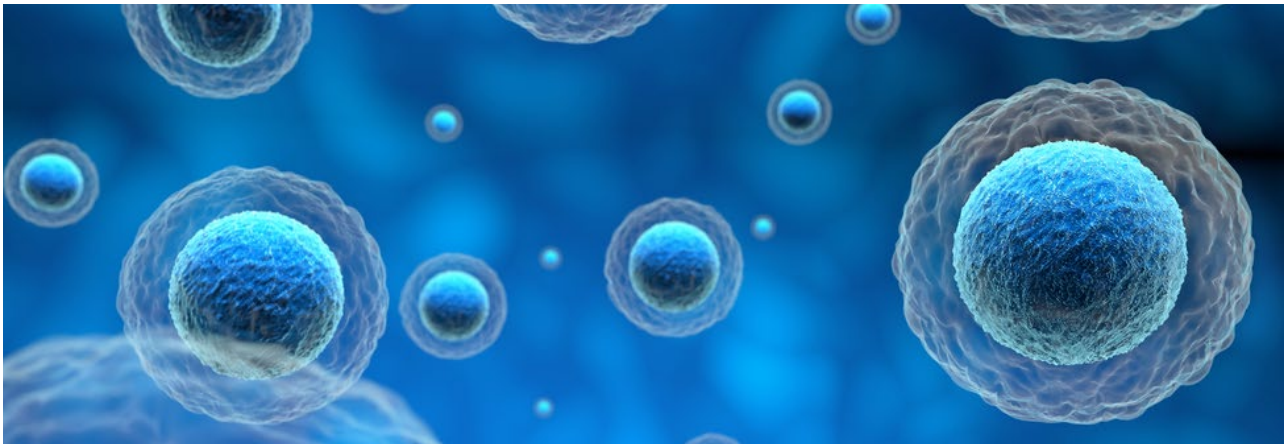
A For me, it's all about understanding what makes these structures so special and continuing to understand how we can build different forms of spherical nucleic acids, and use the unique properties of them to solve major problems in medicine and other areas of research.

Q **Do we currently know why the spherical nucleic acids are internalized or is further research needed to fully understand this?**

A At the moment, we believe that they are recognized by what are called scavenger receptors; these are structures common to many cell types, and they're used to move cargo in and out of cells.

They have also been shown to recognize and bind to spherical nucleic acids much more tightly than linear nucleic acids, and so effectively we have, in part by accident, discovered and designed an architecture that is recognized by natural biological machinery, scavenger receptors that lead to their internalization into a cell.

There are several papers that explore this for different cells types, and all our research thus far is consistent with that conclusion.



Q **What did you enjoy most at Pittcon 2017?**

A It's honestly a really exciting venue for anybody interested in analytical chemistry, new instrumentation, or new techniques associated with that instrumentation, and so, I particularly enjoyed the frontier talks. But of course, I also enjoyed the expo hall and seeing all the new technology on display.

Q Where can readers find more information?

A

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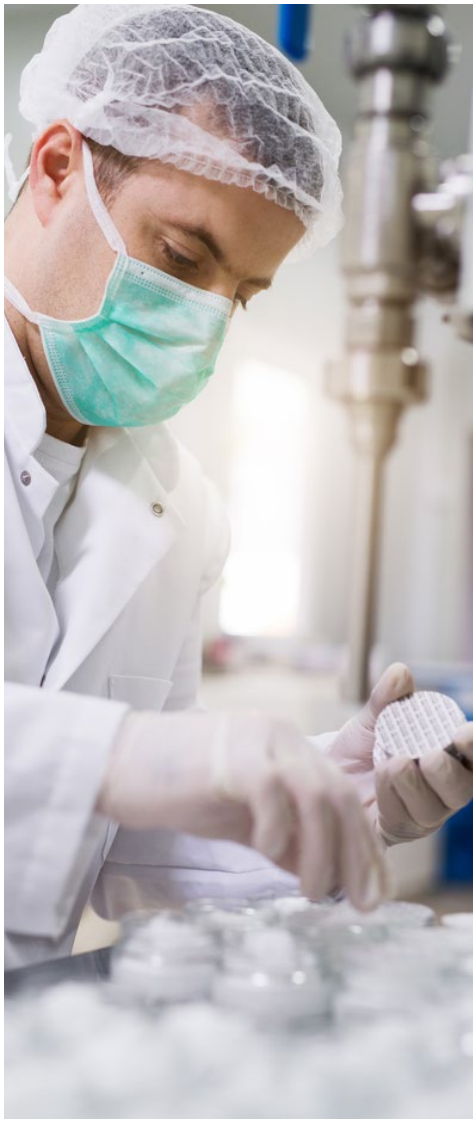

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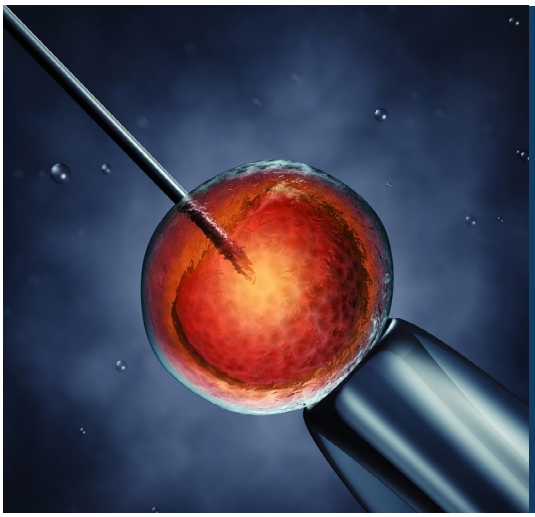
1.9 NANO-ENABLED IN VITRO AND IN VIVO DIAGNOSTIC TOOLS FOR TRACKING AND TREATING DISEASE



Dr Chad Mirkin is George B. Rathmann Professor of Chemistry, Materials Science and Engineering at Northwestern University. He pioneered the use of biomolecules in materials science and is renowned for the use of nanoparticles in the development of analytical tools for biological sensing.

Here he explains how nano techniques form the basis for a new class of medical modalities that have the potential to changing the way we study, track, and ultimately treat disease. In particular, he describes the value of spherical nucleic acids in diagnostic and detection methodologies. Tracking particular, or even multiple, species within a cell using nano spherical nucleic acids could be especially valuable in developmental or neurobiology investigations.

A transcript of one of Dr. Chad Mirkin's talks at Pittcon 2017.

In my talk, I'm going to discuss how we're developing a whole series of new constructs that make a lot of, currently very difficult, intracellular measurements routine.

WHY NANO?

We spent a lot of time at Northwestern University developing nanotechnologies for lots of different purposes, such as making and manipulating structures, and making new measurements.

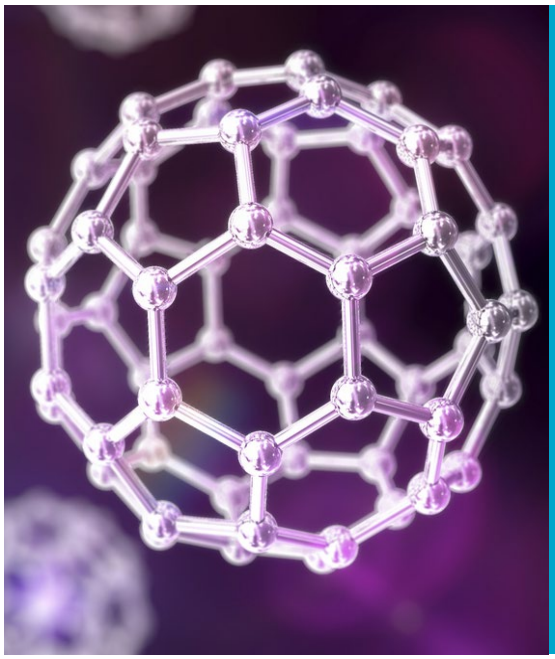
To me, the reasons for using nano rather than molecular techniques is the ability to take materials and miniaturize them to get new properties. In addition, you can take existing molecular structures and rearrange them into new forms on the nano scale and get new properties. This means that you have the ability to discover interesting things and also exploit those new properties in the development of new technologies.

At Northwestern, we've been doing this in the area of cancer research, under the Cancer Center for Nanotechnology Excellence, which is funded by the NCI.

The goal was to learn how to take these materials and begin to develop new ways of carrying out detection, creating high sensitivity and selectivity detection systems. A couple of examples of doing in-vivo imaging where you can get better contrast in principle and theranostic capabilities in certain cases, the ability to both diagnose and treat.

We can use these types of constructs in the development of new drugs and types of therapies, and are examples of technologies that are now deep into the clinic. They're being used for human health applications, especially in diagnostic therapeutic areas. They form the basis for a new class of medical modalities that have a very good chance of changing the way we study, track, and ultimately treat disease.

SPHERICAL NUCLEIC ACIDS



One of the constructs that we're particularly enthusiastic about is an area that we discovered back in the 90s and it is making well-defined nanoparticle bio-conjugates. This can be done by taking nullifying nanoparticles and interfacing them with oligonucleotides. At the time, we were thinking about making materials, not developing diagnostic or therapeutic tools.

You can synthesize DNA with a, for example, alkaliphile end group, and under the right conditions, you can attach it to a gold nanoparticle. This can then load up the oligonucleotides to the point that they are very densely packed standing upright and highly oriented.

Using an electron microscopy image of the resulting particle, stained with uranyl acetate, you can very clearly see the gold nanoparticle core and the shell of oligonucleotide surrounding the structure. This architecture can be tailored with subnanometer precision. Every base that is added, adds about two point angstroms of radius distance to the particle, and we know that through a lot of very sophisticated, x-ray measurements in other areas that also exploit the use of these types of particles.

> These constructs were interesting, not only because we were the first people to interface an inorganic nanomaterial with a biological structure, but because they exhibit unusual properties.

It wasn't until about 4 or 5 years ago, that we began to think of them in a very different way, when we had a chance to watch and look at their properties as we discovered and characterized them and began to think about them, not as a nanoparticle oligonucleotide conjugate, but as a new form of nucleic acid, one that has no natural equivalent but one that interacts with natural systems in a completely different way.

> We don't think of **spherical nucleic acids** as a biologically important construct from a natural standpoint, yet if you look at how these types of structures interact with biological systems, complementary oligonucleotides, living cells, and we compare them to linear nucleic acids of identical sequences, they have different properties.

They bind complementary oligonucleotides about 100 times more tightly. This is understood as an entropic effect, that involves pre-orientation of the oligonucleotide, dialing down the entropic penalty of binding that you pay from bringing two oligonucleotides together to form a double helix, and that's reflected in a larger binding constant. They exhibit very narrow melting transitions, cooperative melting, sometimes down to a single degree, whereas the same oligonucleotide free in solution, linear oligonucleotide, would melt over a transition that spans 20 degrees.

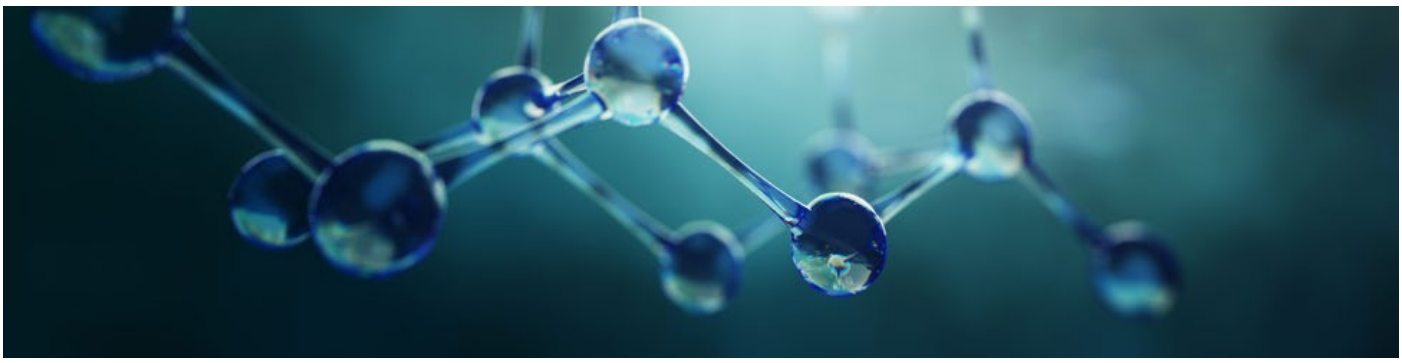
This is important from a diagnostic standpoint, there are the particle probes that soak up lower concentrations, taking advantage of the higher affinity constant. The sharp melting transition means that you can get higher selectivity, and there are conditions where you can get near perfect selectivity. Some of the early uses of these types of particles were in the development of probes that are now used in medical diagnostic tools used all over the world.

> The other thing that's important is the concept of a **spherical nucleic acid**, which is extendable to lots of different materials. You don't have to have inorganic cores, you can have organic cores, and those are the basis for therapeutic agents that rely on antisense siRNA, and amino therapy strategies with respect to treatment.

On the inorganic side, you can change the properties and impart plasmonic, catalytic, magnetic, or luminescent properties dependent upon the compositions that you choose.

Different structures also have different capabilities for entering a cell, for example some won't enter living cells, they need transfection agents, but others will naturally enter cells, and they engage the cellular machinery on the surface of the cell, scavenger cells in particular, and they're rapidly internalized.

Once inside the cell, these structures produce almost no immune response, entering in a very stealth-like manner, whereas others exhibit a very significant immune response as measured by interfering with beta levels. This is important to consider for their use as probes and when it is important not to perturb the cell, for example.



Another benefit is because of the electrostatics and sterics of this type of architecture, they're packed in there very tightly, and resist nucleus degradation. They're more stable and they have a longer half-life in the cell than a native oligonucleotide. That's important in terms of life time and their use to make intracellular measurements.

We've been trying to use these types of structures to create new capabilities in terms of diagnostics. We want to be able to make intracellular measurements with them and take advantage of their rapid internalization to measure the analytes within a cell and quantify them.

On the in-vitro diagnostic side of things, we can use these as high affinity labels to sort oligonucleotide signatures that are in whole blood, either that's been processed or not processed very much, identify those signatures and begin to use them to track disease in unique ways. That's the basis for something we call the **VERIGENE system**, which was commercialized by Nanosphere and then sold to Luminex recently, and is now used in half the nation's top hospitals.

We're also moving towards being able to do this in living systems, such as animals, where we can make diagnostic measurements using the unique characteristics and fundamental properties that define spherical nucleic acids and differentiate them from their linear cousins.

MEASURING MRNA IN LIVING CELLS

Taking an example of the issue of detecting an analyte in the cell, for example, mRNA. This is an important marker, that's involved both in transcription and translation, and knowing mRNA levels in cells is critical for understanding how cells function and in certain cases for ultimately diagnosing and treating a disease.

Currently, there is no technology that allows you to measure mRNA levels in live cells and to quantify it routinely. Some techniques available are fluorescence in-situ hybridization, fish-type techniques etc., but you must work with fixed cells, meaning you have to kill the cells.

Molecular beacon technology in principle may come to mind for using and you could carry it in with a transfection agent, then make a measurement as it binds to the mRNA and target nucleic acid sequence to restore fluorescence, but there are a couple of problems.

> One is that if you use a transfection agent, you have to live with the toxicity associated to those agents, and they're often highly toxic. The second is these are not spherically protected and they are rapidly degraded, therefore every time a probe gets degraded it gives you background signal, and increases the fluorescence associated to background measurement, making it a challenging technique, which has not yet been successful to date.

Real time PCR could also be considered, and this is very quantitative. It's extremely powerful, but again, it requires that you lyse the cells, and so killing the cells and its signal averaging, you have to take many cells and then average them and figure out on average what you have at the signal cell level. There is no technology that allows you to routinely make such measurements on live cells.

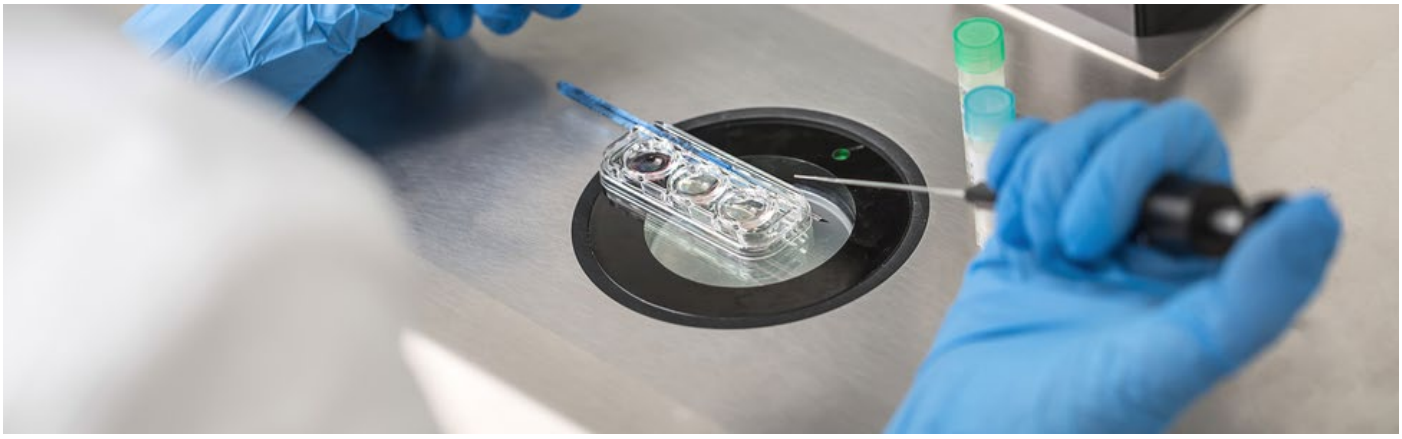
> As these types of structures will naturally enter cells, a spherical nucleic acid with a fluorophore probe permeated on it, will rapidly fill the cytoplasm. They cannot go into the nucleus because they're too large to enter the nuclear pores, but they fill the cytoplasm.

Over 17 hours, you get **100,000**s of particles into the cell and this works across many cell lines. We used a flow cytometry experiment where we measured uptake for different cell lines, and we've looked at over **60** different cell types, primary cells included. Tissues and neurons will take them up, even though they're notoriously difficult to transfect. No transfection agent is being used, it is the architecture itself being recognized by receptors on the surface of the cell and facilitating endocytosis, releasing the particles in small amounts into the cytoplasm. Those are your active probes in terms of mRNA detection.

We did this using a turn type of mechanism when you wanted fluorescence to be in the off state and we didn't have a large concentration of the analyte present. When the analyte was present, target mRNA for example wanted to be turned on.

We initially developed construct of a spherical nucleic acid with a gold core. We had a short oligonucleotide with a fluorophore hybridized tube with the spherical nucleic acid. That's important because the gold quenches fluorescence, that's off, spectroscopically.

This, like a normal spherical nucleic acid, will rapidly enter cells and it can be designed to have an overhang that can recognize a target mRNA of interest. If that mRNA is present in high enough levels, it will bind and we have a longer overlap region so thermodynamically this is favored and it peels off the flare, moves it away from the gold and turns on fluorescence. What you now have are a whole series of different types of nanoflare structures that go after different types of mRNA target based on sequence.



We carried out an old example with survivin, looking at cancer cells, we used SKBR3 cells that over express survivin, a gene associative cancer. We have a sequence present that is designed to recognize it, creating a very large fluorescence that can be seen with a naked eye, and you can very easily differentiate it from the control, where we have the non-complementary flare.

There will always be some florescence due to degradation but it's significantly reduced compared to a molecular beacon or single stranded oligonucleotide probe type of architecture.

This experiment shows the importance of that, so you may think that you could do the same experiment by using a molecular beacon and a transfection agent.

Ignoring toxicity, you can't do this, because of that lack of stability, so the architecture here is important. It showed the ability to measure the target with a nanoflare compared to the molecular beacon. Although there was a lot of background due to degradation of the probe and I can't differentiate the complementary from the non-complementary state.

> Unless you come up with a strategy to dramatically stabilize, and can replace the use of a transfection agent to get into the cell, which can lead to a lot of perturbation of the cell that could compound the interpretation of results.

What we have is a technology that for the first time allows you to make measurements in live cells, sort them, collect the cells and keep them to be used for other purposes, which can't be done, for example, with techniques like real time PCR. If you compare the time required to do this, but then also think about the advantages, single cell resolution and analysis of live cells.

> This makes for a very compelling argument to begin to think about developing a technology that could provide some important information about how cellular systems work and ultimately lead to a series of new types of diagnostic tools. That's what we've been exploring here.

CHALLENGES FACED DEVELOPING THIS TECHNOLOGY

We hope to be able to not measure one particular target in a cell but multiple targets and there are a couple of reasons to do that, one being more information. One of the drawbacks about nanoflares is that cells, even the same cell population, will all pick up different levels of the flares, even when fed the same concentration. They pick them up at slightly different rates and there's a heterogeneity associated with that.

This leads to a difficulty in terms of quantification and a heterogeneity in terms of the types of results that are produced. It makes it more qualitative than quantitative, which is a bit of an issue. We have tried to come up with solutions to this problem, such as structures that could go into cells and measure two different targets.

One that would be consistent from cell to cell in terms of amount, and one that might vary in terms of concentration depending upon its state. If we could do this, then you could ratio the two and take into account differences in terms of cellular uptake and even get more quantitative information in the process.

We began by looking at actin, which is common to cells and at comparable levels from cell to cell, and survivin, which is elevated in cancer cells versus non-cancer type cells. Compared to the type of profile when looking at only survivin, taking a ratio of the two gives a much tighter profile and we get more quantitative information. That becomes a good strategy for improving the understanding of how this works and also improving the quality of data that comes out of it.

An example of how we tested our theory, is we went after two different targets in one cell type, we had elevated levels of survivin and actin. We used **siRNA** to knock down survivin in these types of cells so that **mRNA** levels go down. We used the flares to see that they had gone down, but the signal from the actin remained relatively constant.

Following this, we can quantify how much we have and correlate this with real time PCR, and we can support our theory that the quantitative and semi quantitative data is valid. This allows us to believe in using these as probes in a variety of applications where we're trying to both understand new things in cells but also use them for medical diagnostic purposes.

An example of where these types of structures can be used is to look for rare cells and create new types of assays, for example, to look at circulating tumor cells. Current assays being used rely on looking at external protein markers on cells, **EPCAM** for example as one of the types of markers that people go after. The problem with that, is in the process you pick out the few cells that you're trying to link or correlate with, for example, breast cancer, you also kill the cells. The process requires that the cells are effectively fixed.

Our method however is looking for from an external protein marker to an internal oligonucleotide marker. We could get in principle a lot more information, we have a lot more targets, and we can customize based upon the different types of rare cells that we're looking for. In addition, for flow cytometry, we can take a sample of blood where the vast majority of the cells are healthy cells, look for these circulating tumor cells and collect them in living form.

In principle, you can then take those cells and on a very personalized basis begin to look at how they respond to different types of therapeutics. It radically changes the way you think about developing a protocol for not only diagnosing disease but also figuring out what the cocktail of drugs that are going to be used.

Breast cancer's a good example of that because you might have a situation where **70%** of the population responds to a given drug combination, but **30%** doesn't. This, we think, is going to allow us, if we can get to the requisite levels, to create a diagnostic tool that could address that problem. We figured out that we can get down to a 100 cells circulating in a milliliter of blood using nanoflares, and in this case, going after markers that are unique to different forms of breast cancer.

TWIST is one of the markers that we used. We hope to get down to 10 cells and eventually one. We are not there yet, but we are hopeful that with some refinements in terms of how we design these structures we can increase signaling noise and achieve this.

The nanoflare technology has been commercialized, and it's one of the early examples of translation in nano in diagnostics. Millipore produced over **1600** different versions of these and they sell them for biological studies and they're all different, primarily based upon the sequences of interest. What's powerful about this is that this is a scalable technology and one that can be utilized across many different types of cellular systems, paving the way for thinking about how we can mass produce other types of nanotechnologies based upon similar concepts.

LOCATING MRNA

It would also be good if you could not only measure **mRNA** in live cells but also determine the location of it, which currently nobody can do. Knowing the location would make a huge difference. Proteins are being produced at different levels as well as **mRNA** expression in different compartments of a cell.

In a nanoflare type of experiment and certainly all the other types of experiments I shared with you, real time PCR, you get none of that information. You get the average amount in certain cases and in one case you get amount per individual cell, but you do not get location. In respect to understanding how a cell works and the role of mRNA within the cell. We think this issue of trying to identify not amount but location could be very meaningful as we move forward.

In nanoflare design, **mRNA** binds and it releases a flare sequence, so the target and the flare are far away from one another. The flare is also far away from the gold and that's why it turns on. That was the original idea behind this.

If we could change the architecture and make a longer flare sequence that is the overhang and that will be the sequence that recognizes the target. When these encounter one another, they bind but this time, the flare sequence stays intimately connected through hybridization with the mRNA target sequence, so now fluorescence turns on and the locations, they're co-localized in this case.

In principle, we can get amount and also location within cells and then we have to go through and make a series of measurements and make sure they work like we think they're going to.

We looked at differences in concentration, starting with extracellular to convince ourselves that we can get a quantitative response and we did. We then began to do it in cells with the sticky flares, and the point here is we can look at normal cells that have not been treated with a knock down agent. In this case, we can knock down a gene, we used beta-actin to knock down using siRNA and we showed that there's a significant signal change when we knock down siRNA as measured by the sticky flare type of structure.

One of the things that we don't understand fully is we how they are picked up by scavenger receptors, and internalized via a process called caveolae mediated endocytosis, they go into endosomes and then we know the endosomes break to some extent. We know this from sequencing specificity in terms of nanoflare activity.

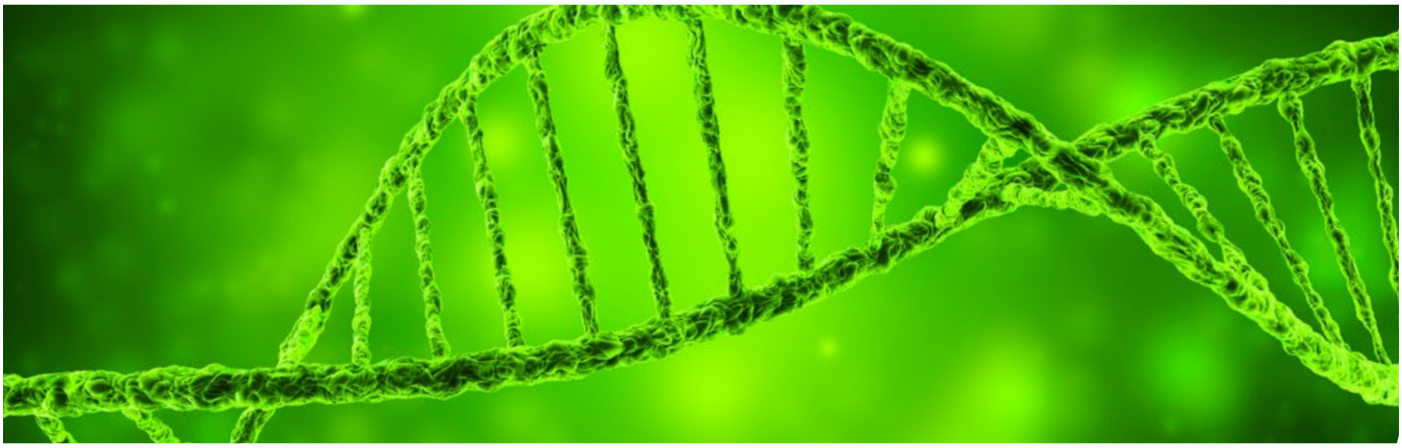
You don't get into the cytoplasm, you cannot be getting that type of response, but it's a small percentage that break. We also know that in the case of the nanoflares, they do not go to the nucleus, as the spherical nucleic acid, is too big to enter, but in this case, when target binding takes place, the flare sequence stays with the target and if the target is processed in the nucleus, it is now small enough to be taken into the nucleus, in principle.

We looked at this and we developed ways of monitoring beta-actin, so looking at the actin mRNA on what generates the fibrils of the cells that give it structure, we also went after a small interfering nuclear RNA.

A population of it is in the cytoplasm but it is ultimately processed in the nucleus. If sticky flares are working and we can see flare activity in the nucleus, then we know that the sticky flares as working the way we think they're working. We clearly saw the actin outside the nucleus, but we also that we end up getting activity with the U1 nuclear RNA also in the nucleus which could only happen with a sticky flare and not a nanoflare type of case. That tells us that we're getting processing in the nucleus.

You could see this where we'd labelled chromatin with a blue dye and we were using a sticky flare to label the U1 short nuclear RNA. We found co-localization in the nucleus, but in different parts of the nucleus, which can't occur with a nanoflare and it can't happen unless you have the sticky flare type of architecture. This is the first piece of evidence that we can measure not only amount of RNA but also the location of RNA in a highly-controlled manner.

We can then begin to look at, for example, where beta-actin mRNA is and correlate it with the generation of actin fibrils, and show it in real time in a live cell, showing it moving down the fibrils. It's a co-localization that you would expect if you're measuring RNA.



We don't know why this is, but it turns out that if we use MitoTracker, we find out that there's a co-localization of mitochondria with the beta-actin RNA and you can rationalize it and say that's where the energy's being generated and this is needed for generating these fibrils. Providing evidence of co-localization and the sticky flare type of architecture which is binding to the RNA and staying connected with it at least over a reasonable period of time that allows us to image it.

To sum up, sticky flares are these new SNA based nano-conjugates capable of detecting and tracking intracellular localization of RNA in living cells. This is going to be an important analytical development that will allow us to understand RNA localization in embryonic development, especially in neurobiology.

MEDICAL DIAGNOSTIC APPLICATIONS

We've developed this technology that allows you to sort oligonucleotides associated with disease targets and then develop probes based upon spherical nucleic acid and nanoparticle conjugates, to produce colorimetric readouts of the presence or lack thereof of a given target. That is the basic technology minus one thing that underlies the **Nanosphere Luminex** technology.

In addition to that, they then use the catalytic properties, a third property that facilitates the reduction of either silver or gold onto the particle probes that signal the presence of a particular target on the chip. This allows you to amplify the signal associated with this recognition event by a factor of a **100,000** in less than 5 minutes, this process is all automated now. You get a sample result based upon this type of chemistry and the ability to very selectively capture target but also amplify using this trick of gold catalyzed reduction of silver or gold one, both of which lead to significant increase in signaling.

Recently we've shown that you can take commercial gene chips and, for example, use these to look for different types of nucleic acid markers in mass. Using a spot or signal that you would get from a Nanosphere type of detection system, we're effectively using the ship as a wave guide, it's a glass slide, and we're measuring the scattered light from each of these spots post development of either silver or gold.

It's false color scale shows a high and low signal. This shows us that massive multiplexing, which is a task in itself, can be done. It also illustrates the power of this technology.

PCR is a more sensitive technology than this, but this is a lot simpler and it enough sensitivity that allows you to consider eliminating PCR in many cases. What it really does well that PCR can't do is it allows you sort short oligonucleotides at relatively low concentration. When you think of that strength, that screams for the development of a technology for looking for micro RNAs.

Micros RNAs are short oligonucleotides, free flowing in the blood in many cases. If you process blood, you can release many more of them, and they can be good biomarkers of the disease. Detecting them even though they're short, and at a relevant concentration where you can use them to make a diagnosis, is a challenge.

If I use this type of chip and try to use fluorescent spaced assays, without PCR, I would miss **85%** of the targets that are identified using this type of amplified approach. To me, that's the difference between being able to have a true diagnostic and not missing a large amount of the information or capturing, this lead us to think about how we can utilize this.

Dr. Colby Shad Thaxton was a medical doctor in my group, he's now a professor at Northwestern, he's a urologist, and we thought about this in the context of prostate cancer. We wanted to come up with a new type of test for prostate cancer based upon looking for these micro RNA signatures.

The current tests for prostate cancer are not very good, PSA testing doesn't give much information, and can even be wrong. In addition, it cannot differentiate between the different forms of prostate cancer, aggressive versus indolent forms etc. A patient with an indolent form, does not want to go through a lot of the procedures that are recommended for aggressive forms. We want to create a diagnostic tool that allows you to differentiate all of these different states.

In our hypothesis, we began to look at these types of biomarkers, micro RNAs, and create a profile of healthy people and compare them to profiles of those that have prostate cancer. We also looked at those who had aggressive forms and indolent forms of prostate cancer, and tried to look for markers that would allow us to uniquely differentiate the three different states.

The assay that we've used is the one I've described here and has been published in a paper. It shows how you can rapidly use a gene chip type of capability, to sort through the micro RNAs and use the gold amplification trick to detect them at very low levels in patient serum.

> We can look at patients and stratify populations based upon whether they have aggressive or indolent forms of the disease and compare those to healthy patients. We then made profiles where we can identify markers that are uniquely associated with aggressive forms of prostate cancer using different cohorts.

The levels were relatively small, but in certain cases, micro RNAs were up regulated and in other cases they're down regulated. What we've found from this, is that there are whole series of micro RNAs, 5 of them, that we think allow you to, definitively diagnose the disease and also allows you to pick and differentiate the aggressive from the non-aggressive forms.

There's no single marker that gives you that predictive capability but a combined analysis of 5 different markers, some of them; mere 135a, mere 106a, mere 605 and mere 433, along with mere 200, are the key to this.

> What we're hoping is going to come out of this is not a test of one particular biomarker but a panel of tests that allows you to create a score that gives the doctor the ability to tell a patient whether or not they have an aggressive or non-aggressive state of the disease. This is going to be tested over the next couple years as we do a very large retrospective study based upon a much bigger population of patients than here.

CONCLUSION

Since the sequence of the human genome was determined, developments in genomic technologies have increased exponentially. Genetic information plays an increasingly important role in decisions relating to the prevention, diagnosis and management of disease.

Much of the information used by physicians in their selection of the best disease management strategy is based on data obtained through gene sequencing. As this chapter has shown, many biotechnology companies are striving to optimize techniques in order to improve the lives of patients.

This can be achieved through less invasive testing procedures, more accurate or earlier diagnoses, or more effective tailored treatment strategies. In addition, the ease with which genetic analyses and manipulations can now be conducted is rapidly increasing our genetic understanding of health and disease, which in turn will lead to better treatments.

A great example of this is the development of low-cost next-generation-sequencing. The sequencing of the human genome that took 13 years to complete can now be done in a day and at a fraction of the cost. This has made it possible to screen for carriers of disease and avoid the risk of genetic disorders being passed on to future generations.

The incidence of **Tay Sachs disease** has been slashed through genetic screening programs and it is hoped that similar results can soon be achieved for cystic fibrosis. It has also made it possible for cancer diagnosis and prognosis predictions to be made from blood tests rather than invasive surgical investigations.

Simple gene editing in the form of **CRISPR** has provided new insight into many disease processes by allowing scientists to knock out or modulate specific genes and study their function. It has also allowed the development of disease models to further understanding and inform drug development. There is also the possibility to apply the technology directly as gene therapy to correct harmful mutations.

These techniques are only the start; myriad others have been invented as our understanding and available technologies grow. It is now possible to measure the function of individual cells rather than using the average activity of the whole population.

This is effective in the early diagnosis of infection and is particularly valuable for identifying rare aggressive cancer cells in biopsy samples. In both cases, such early detection before they spread can improve the chances of successful treatment. Similarly, applications of nanotechnology have increased the sensitivity of nucleic acid assays.

Novel techniques are also rapidly advancing our fundamental understanding of the brain. **Optogenetics** and **CLARITY** provide rapid analysis of the wiring and fine structure of the brain and can be carried out in behaving mammals carrying out complex tasks. It is hoped that work conducted using these methodologies will help in the development of treatments for conditions such as **Parkinson's disease** and **Alzheimer's disease**.

The capabilities of biotechnologies are increasing so rapidly that society cannot keep up. There are many issues that need to be resolved before we can reap the full benefit of these advances. What are the acceptable limits of human genetic intervention and selection? How can we ensure that genetic information is not used to discriminate against an individual, e.g., in obtaining medical or life insurance?

Furthermore, for these technologies to become routinely available, somebody has to pay for them and persuading payers can be a lengthy process. For example, we have the ability to diagnose rare or undiagnosed disease in children incredibly effectively, but almost nobody reimburses that test today.

In addition to providing a space for leading experts to speak on such topics and companies to showcase the latest advances in their technology, Pittcon provides a space for scientists to discuss the key issues and the future of bioengineering and genomic analysis technologies.

2 ADVANCES IN MASS SPECTROMETRY

INTRODUCTION

Mass spectrometry is an invaluable analytical tool across a broad range of fields and applications. It allows identification and quantification of the component molecules in a complex mixture, detection of impurities in a sample, and the detailed study of proteins and cell function.

It has proved especially useful in proteomics studies. During the last twenty years, high-throughput and quantitative **mass spectrometry** has exponentially increased our knowledge of protein structure, function, modification and global protein dynamics.

Until recently, mass spectrometers were bulky pieces of equipment that required specialist operators to perform analyses. Consequently, samples for evaluation by mass spectroscopy had to be sent to centralized laboratories for analysis, which meant researchers had to wait patiently for their results.

Advances in technology enabled smaller mass spectrometers to be produced, in turn allowing every research laboratory to have its own bench-top spectrometer. Results could then be obtained more easily and research flourished.

Today, further reductions in the size of mass spectrometry technology have been made possible by the latest technological advances. Miniaturization of mass spectrometers has now been achieved, making this powerful analytical tool even more portable and even more accessible. Miniature mass spectrometers are readily available from numerous specialist suppliers and are becoming increasingly mainstream.

Initially, the reduction in size of mass spectrometers was accompanied with a reduction in performance. Now, the miniaturization of the various elements comprising a mass spectrometer has been fine-tuned and there is no need for compromise in the quality of data when choosing a smaller mass spectroscopy unit. Scientists have managed to develop smaller individual components that provide the same high level of specificity as conventional mass spectrometers.

Pittcon is renowned for its coverage of the latest advances in genomic analysis and bioengineering technologies and the program for Pittcon 2017 included presentations by researchers from every aspect of mass spectrometry analysis.

Furthermore, the technologies have been tailored to meet the specific requirements of particular application.

Leaders in their field shared the latest developments in mass spectrometry, including demonstrations of the newest tools, details of advances in mass spectrometry miniaturization, and data from the current areas being researched for new applications of mass spectrometry analysis.

2.1 MINIATURE MASS SPECTROMETRY INSTRUMENTS FOR BIOMEDICAL APPLICATIONS

This chapter outlines the advances in **mass spectrometry** instrumentation, discusses methodologies and details new and potential future biomedical uses of these technologies.

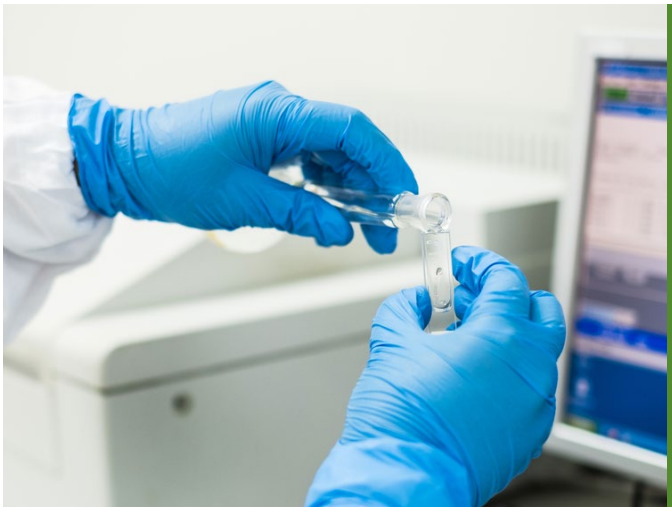
It includes an interview with **Prof. Sweedler**, the James R. Eiszner Family Endowed Chair in Chemistry, in which he discusses his talk at Pittcon 2017 about advances in single-cell profiling using mass spectrometry techniques that allow the identification of rare cells within large cellular populations.

He also explains how the technique has enabled him to characterize neurotransmitters and enhance the understanding of brain chemistry. Such increases in knowledge about how the brain works normally and the changes that occur when things are not working optimally will facilitate the development of restorative treatments. 2.1 Miniature Mass Spectrometry Instruments for Biomedical Applications.

Mass spectrometry is a valuable tool for characterizing the components of complex solutions and for identifying substances of interest in samples. Due to the huge size of mass spectrometry equipment, such analyses have required the use of specialist laboratories. Recent advances however have facilitated the miniaturization of mass spectrometer components, whereby broadening access to this highly sensitive technology.

Initially, miniaturized mass spectrometers were associated with poorer performance, but reliable results can now be obtained using portable, and even handheld mass spectrometers. This section describes the technologies showcased at Pittcon 2017 that have enabled reduced-size mass spectrometers to be produced without compromising performance.

INTRODUCTION



A multitude of disciplines within the biomedical, chemical, and pharmaceutical fields often rely on **mass spectrometry (MS)** as a means for identifying compound structure, quantifying metabolites, and measuring molecules in mixtures of varying complexities.

This highly sensitive approach for the study of biological systems is also used in drug discovery and is crucial in the development of potentially life-saving therapeutics. Large system size is perhaps the most common limiting factor preventing widespread application of MS in the clinical environment. Additionally, complicated analytical methods can make the system impractical for some healthcare practitioners and nonmedical professionals.

Miniature MS has recently been introduced to help overcome size and weight limitations inherent in conventional MS tools. **Benchtop MS** instruments have become condensed and modified for portability and accessibility, and some miniature MS systems have been adapted for handheld use.

Miniature MS technology has significant utility for chemical process control and environmental monitoring, among other applications.

Being able to use miniature MS for in situ analysis, for example, has been one significant reason for developing miniature systems. Also, having a MS system that is approachable and easy-to-use by nonmedical professionals, like firefighters and inspectors of food safety, is also a driving force behind the expansion of miniature MS.

This technology was highlighted in numerous talks at Pittcon in Chicago, IL, March 5-9, 2017. Sessions were led by leading researchers in the field of MS and miniature MS, including **R. Graham Cooks** of Purdue University and **Daniel Austin** of Brigham Young University. Talks were given on the subjects of ion traps and the miniaturization of MS, and numerous companies demonstrated their mass spectrometer products and how they can be used in a variety of scientific applications.

> A miniature MS is revolutionary in that it provides quick, easy clinical diagnostics and can sit in a physician's office without sacrificing space.

Mini 12, a miniature MS with an ambient ionization source and developed by Purdue University researchers, is an example of a successful miniature MS system. This miniature MS instrument has been designed for physicians or nurses who require a simple MS analysis in the clinical setting. A finger prick blood sample can be loaded into a cartridge and into the Mini 12 MS, automatically producing analysis of data.

> The cartridge contains a barcode that is read by a camera in the system, initiating the required analysis. Following a solvent spray onto the cartridge and a number of other processes, the MS scans are performed. This occurs without any operator intervention.

Minimizing MS size, while beneficial in some aspects, also has its own set of limitations. Size reduction of MS can lead to compromised performance of a MS; however, miniature MS has been specifically constructed to maintain a high level of accuracy and sufficient resolutions while offering automatic operation. All mass spectrometers work in a vacuum to avoid intermolecular collision events and remove background signal. Since vacuum systems have considerable weight and are fairly large in size, the vacuum represents one of the biggest challenges for shrinking a mass spectrometer.

An exciting application of miniature MS in surgery has been in the field of oncology, specifically brain cancer. A study from Purdue University and Brigham and Women's Hospital led by **Robert Graham Cooks** found that a tool that relied on desorption electrospray ionization, an ambient mass spectrometry analysis technique, was able to test brain tissue to identify cancer grade and type as well as tumor margins in brain surgery patients. Potentially, a miniature MS system that is being developed by this research team may be used for the same study of cancerous tissue, particularly in regard to their molecular structure.

Robert Graham Cooks, one of the authors of the paper, gave two talks at Pittcon 2017 in Chicago, Illinois, introduced a session about miniature mass spectrometers and presented his study, '**Searching for Biomarkers Using Ambient Ionization Mass Spectrometry**'.

Pittcon 2017 also presented new, innovative studies in the field of miniature MS, including specific ion traps and novel ionization procedures. Bruker, Photonis, Waters, Hamamatsu, and Thermo Fisher Scientific were just a few of the key exhibitors this year, each provided information and demonstrations of their MS systems and new measurement technologies.

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MINIATURIZING MASS SPECTROMETRY

The use of miniaturized **mass spectrometers** is quickly becoming mainstream, particularly by physicians at the bed side. Their small size allows portability and helps provide answers to real-world scientific- and medical-related questions in a simple and efficient manner.

Miniaturized, portable **mass spectrometry** has also become widely utilized in forensics and security to gather and identify substances and chemical threats quickly. Instruments such as these provide a reliable and relatively quick way to obtain answers without compromising the quality of results.

Although effective, the actual construction of miniaturized mass spectrometers can be a challenge to designers of these instruments. Often, the vacuum in these devices is poor due to the instrument's size and power constraints.

Large sample sizes, which are typical of larger instruments, produce more certain results, compared to smaller sample sizes that are often analyzed by miniaturized machines. Fortunately, developers and companies have become more adept at developing technology to circumvent these possible caveats.

Miniaturized mass spectrometry was frequently discussed at Pittcon 2017, including during the following sessions:

- Novel Scan Methods Using Miniature Ion Trap Mass Spectrometers
- Integrated Miniature Mass Spectrometry Systems
- Cartridge-Based Sampling Ionization Methods for Miniature POC Mass Spectrometry Analysis Systems
- Embedded Analytics and Automation Challenges and Opportunities with Miniature Field Analyzers

University researchers are also on the forefront of mass spectrometry miniaturization, using these efficient and effective tools for research across all science specialties. Since the construction of the first miniature ion trap in 1991, researchers at Purdue University have developed two basic, effective approaches for miniaturizing mass spectrometry. These two methods include the "**bottom-up approach**" and the "**top-down approach**."

Bottom-Up Approach

In the bottom-up approach, researchers shrink the micro-scale mass analyzer first before constructing the actual instrument. The instrument is essentially built around the miniaturized micro-scale mass analyzer.

Due to the relatively small trap size, developers of the spectrometer must arrange the traps in a parallel to each other in an effort to trap and increase the number of stored ions.

Top-Down Approach

Contrary to the bottom-up approach, the top-down approach starts with an existing lab-scale instrument, and developers work on first miniaturizing the instrument followed by shrinking the individual components of that instrument. This approach, utilizing the advancements in current technology, can reduce instrumentation and doesn't sacrifice performance in the process. At Purdue University, the top-down approach helped to develop the mini10 and mini11 miniature spectrometers, with no hindrance in overall performance when compared to standard mass spectrometers.

R. Graham Cooks, a speaker at Pittcon 2017, and **Raymond E. Kaiser, Jr.**, have previously reported on their success with building the first miniature ion trap at the University of Purdue. Their paper, published in a 1991 edition of the International Journal of Mass Spectrometry and Ion Processes, discusses methods for overcoming the inherent constraint limitations associated with the quadrupole ion trap mass spectrometer.

The authors demonstrated that the benefits of extending the charge and mass range of the quadrupole ion trap include:

- Using smaller electrodes
- Operating the device at lower radio frequencies
- Resonance ion ejection with the use of a selected voltage at an appropriate frequency

In conclusion, the authors believe frequency reduction with axial modulation and a modest size of the device yields a more effective high-mass biological mass spectrometer.

Photonis, a significantly large supplier of the world's ion electron detectors and amplifiers used in mass spectrometers and an exhibitor at Pittcon 2017, works to miniaturize mass spectrometers successfully, with the ability to:

- Reduce machine size overall
- Provide reliable results in poor elevated pressure and vacuum environments

The detectors offered by Photonis are aimed to support many different types of mass spectrometry, including quadrupole, Time of Flight, and ion trap. Photonis' Spiraltron™ technology is comprised of compact detectors that achieve high gain without the excessive noise. Additionally, Photonis' MegaSpiraltron™ technology, another compact detector, have been designed for poor vacuum environments for portable mass spectrometers.

Both Spiraltron™ and MegaSpiraltron™ allow detectors to feature up to 6 input channels that are contained within one detector, substantially increasing the sample size. This is essential for high-quality experiments seeking reliable results, as a larger sample size tends to produce results that are more accurate when compared with sample sizes on a smaller scale. The "spiraling" of various channels together in the technology significantly reduces the chance of ion loss or feedback.

Mini-spectrometers offered by **Hamamatsu**, another company who exhibited at Pittcon 2017, feature condensed optical systems, circuits, and image sensors that are fitted into a small case. The company also provides ultra-compact spectrometer types that can be connected to mobile devices, offering a greater ease-of-use benefit for physicians and other scientific professionals who wish to analyze data remotely.

More than 20 types of mini-spectrometers are offered by Hamamatsu. The company's technology, micro-opto-electro-mechanical-systems (MOEMS), combines both circuits and software with optical technology to provide in-depth analysis and quick measurement without having to bring in large samples or equipment.

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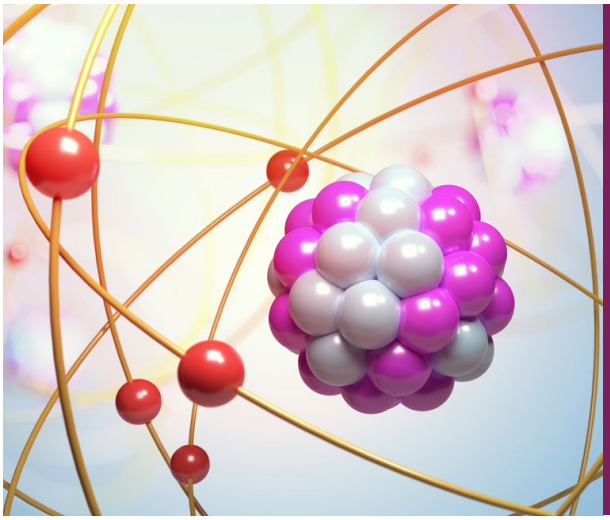
2.1b BIOMEDICAL APPLICATIONS OF MINIATURE MASS SPECTROMETRY



Desorption electrospray ionization (DESI) is a method commonly used for MS and works by creating molecular maps to tissue sections, allowing researchers to identify the state of disease within a selected tissue without requiring a long sample preparation or labelling time.

Typically, **DESI** is used to determine disease state of tissue, which can be helpful in the surgical room. As a technique that can be performed directly on biological tissue, DESI helps to provide imaging of fatty acids, hormones, lipids, and various other compounds. Promising for detecting and identifying certain types of cancer in situ, DESI can also be used for mass spectrometry when using a miniaturized mass spectrometer.

Paper spray (PS) ionization, however, is a different ionization method that uses electrospray to produce ions to a paper substrate. This technique's speed and simplicity makes it attractive for small samples in mass spectrometry and offers a quick, effective method for drug monitoring in biofluids (among other applications).

Thermo Fisher Scientific, an American biotechnology company and an exhibitor at Pittcon 2017, has developed a PS ionization system called the Prosolia Velox 360™ PaperSpray™ System. This technology is highly sensitive and uses a triple stage quadrupole mass spectrometer for targeted quantitation. High-Resolution Accurate-Mass (HRAM™) Q Exactive™ and PaperSpray is combined together in this system for screening and identification of compounds.

This technology can also be used to identify drug abuse by analyzing urine and blood samples. One study was able to identify up to 6 drugs by testing dried blood sports with the PaperSpray system.

Bruker Scientific, one of the leading MS companies who presented at Pittcon 2017, also uses DESI ionization. Handheld, portable mass spectrometers from Bruker, useful for metal and gas detection, may have the potential for DESI application. These products can analyze samples in a nondestructive manner and with sample preparation in the laboratory.

J. Michael Ramsey of the University of North Carolina (UNC) at Chapel Hill discussed miniaturized handheld analyzers during his talk at Pittcon 2017. His session titled "**High Pressure Mass Spectrometry: A Path to Handheld Analyzers with Specificity and Sensitivity**" explored the emergence of portable MS tools for the identification of low-concentrated compounds in a variety of conditions, including conditions of low pressures.

The work presented also focused on the miniaturization of Paul trap mass analyzers, the use of small ion traps to perform MS at unknown pressures, and the most recent advancements in ion source options as it relates to miniature MS.

R. Graham Cooks, a featured speaker at Pittcon 2017, and **Zheng Ouyang** described in their paper "**Miniature Mass Spectrometers**" the mass analyzer as well as the total analytical system and its many applications.

In general, the authors described how ion traps have been the primary focus when it comes to mass analyzer miniaturization. The vacuum system and the radio frequency electronics are greatly reduced when decreasing the size of the mass analyzer, and this can greatly diminish performance. Introducing optimization systems can be helpful for improving performance following miniaturization.

Cooks and **Ouyang** explained that the judgement of a miniature mass spectrometer for optimal performance is based on these key criteria:

a. adequate operation in detection limits, specificity, and resolution;
b. reliability;
c. ruggedness;
d. autonomous performance.

SURGERY APPLICATIONS

Miniature MS can also be a useful tool in the decision-making process during surgery. **Cooks et al** described the often-common issue clinicians are faced with when deciding to remove tissue from a patient, particularly if the observable health of the tissue is ambiguous. Tissue characterization in a fast, comprehensive manner relies on MS, particular MS methods such as DESI.

In a study by Cooks et al, 12 samples resected from a patient in surgery were evaluated and diagnosed as a tumor or necrotic tissue via histopathology. MS were correlated to histopathology.

Using DESI, researchers were able to classify tumor types and aid in the diagnostics of tissue state. A similar study using DESI was also able to differentiate between normal and tumor breast tissue. These findings indicate that miniature MS can be used safely in the surgical environment to identify cancerous cells quickly and effectively.

Lixian Li gave a talk at Pittcon 2017 on the identification of serum biomarkers in triple negative breast cancer, which provided insight on how physicians can use quadrupole time-of-flight mass spectrometry for the detection of these biomarkers.

While **magnetic resonance imaging (MRI)** can be helpful for identifying and diagnosing brain tumors, miniature MS may provide faster diagnostics, especially during surgery.

A study from Purdue University and Brigham and Women's Hospital study and led by Cooks successfully utilized DESI to identify cancer grade and type in 5 brain surgery patients. DESI helped to identify the distribution and quantity of lipids within brain tissue, and software was employed to examine the results in an effort to identify brain tumors.

Lipid patterns were also detected and analyzed to determine tumor grade. While the researchers analyzed specimens removed from the brain, there's hope that miniature MS will evolve to a point where it can evaluate specimens in-tact during surgery.

Pancreatic cancer, which often relies on surgical resection as a potential cure for some patients, can also obtain help from miniaturized MS. **DESI** as used in miniature MS can be helpful for real-time diagnosis of pancreatic cancer, according to one study.

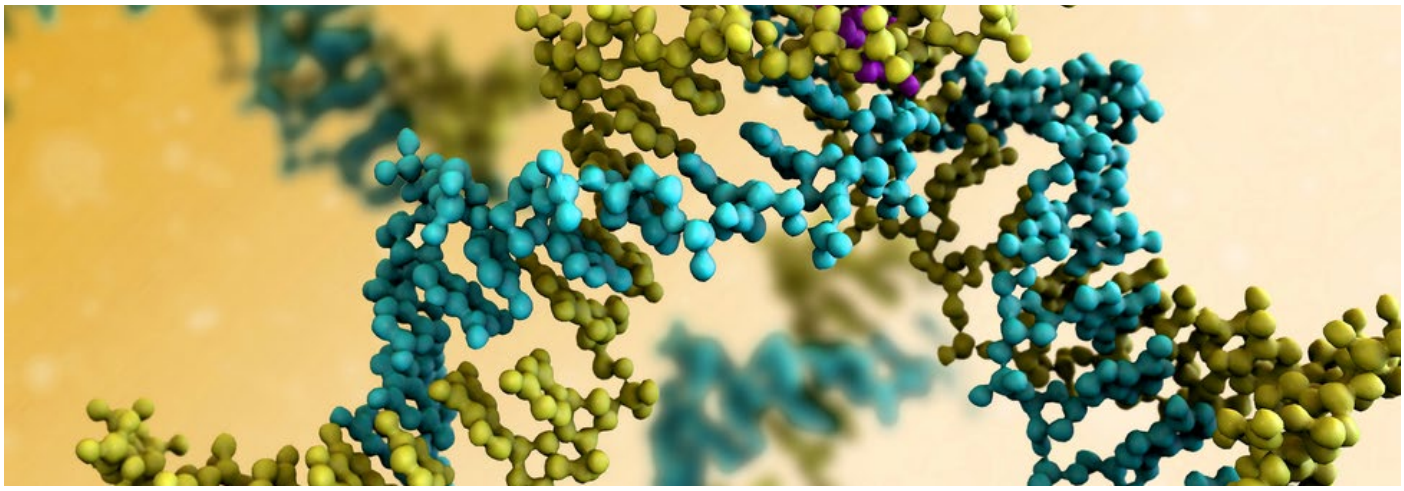
Researchers combined DESI with absolute shrinkage and selection operator (Lasso) statistical method for the diagnosis of pancreatic tissue. They then examined margins of surgical resections from pancreatic surgery.

The findings of this study provided some evidence that both DESI and Lasso techniques applied to pancreatic tissue samples may transform the examination and diagnosis surgical specimens. Essentially, these techniques may be used in the surgery room to assess surgical margins of pancreatic cancer.

PROTEIN ANALYSIS

The study of proteins in biological systems, often termed “proteomics,” can be assisted by miniature MS. Many proteins undergo some type of post-translational modification, which can increase the complexity of the proteins.

Miniature MS has revolutionized the way researchers see biological systems, particularly since techniques for miniature MS have been developed to identify and study proteins. Using high-throughput, quantitative MS proteomics workflows, researchers have virtually broadened the knowledge base on protein structure and function.



THE ENVIRONMENT

Miniature MS can also be used to evaluate the microbes underwater, as a Harvard University study previously reported. This study used a miniature mass spectrometer to examine the effect of microbes on hydrogen and methane content of the ocean.

Stanford Research Systems worked with the researchers to develop a commercial quadrupole mass analyzer and custom gas extractor to complete the mission.

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2.1c IONIZATION METHODS FOR MINIATURE MASS SPECTROMETRY

Adjustments to the way MS is performed by miniaturized mass spectrometers has naturally followed suit after conformation to a portable size. Improvements in simplicity of design and size reductions of these spectrometers have allowed for improvements in medical diagnoses, contraband discovery, and high-consequence fieldable applications, just to name a few examples of miniaturized mass spectrometer applicability.

An advancement in reconceptualizing mass spectrometers include microfabricated arrays of mass analyzers on a chip, enabling higher sensitivity and inherit selectivity of mass spectrometry for data analysis.

According to **Blain MG et al**, the miniaturization of the mass analyzer itself, including its design, characterization, and fabrication, is the first step towards building a complete and efficient micro-MS system. The authors' paper briefly describes considerations in design of miniaturized mass spectrometers and include results from ion trapping simulations for a small-scale cylindrical ion trap mass analyzer. Their results have been incorporated into the overall miniaturization of small-scale mass spectrometers.

At Pittcon 2017, **R. Graham Cooks** discussed a number of miniature MS-related topics associated with ionization methods for miniature mass spectrometry. "**Novel Scan Methods Using Miniature Ion Trap Mass Spectrometers**," one of his featured sessions, provided information regarding the newer scan methods for miniature mass spectrometers, methods that emphasize AC over RF scans for MS experiments in a signal ion trap mass analyzer.

The session also featured discussions about extended mass ranges and linear mass scans, and the illustration of data of biological and forensic applications were also demonstrated. Cooks also explored **paper spray (PS)** and **desorption electrospray (DESI)** ionization methods that are used to assist in diagnosing and monitoring treatments with miniature mass spectrometry.

Other topics covered by speakers at Pittcon 2017 included:

- Integrated Microfabricated Systems for Performing Capillary Electrophoresis – Mass Spectrometry
- Enabling Large-Scale Discovery, Characterization and Quantitation of Neuropeptides via Multiple Tandem Mass Spectrometry Fragmentation Techniques

IONIZATION TECHNIQUES FOR MS

Desorption electrospray ionization (DESI), low temperature plasma (LTP), and paper spray (PS) ionization represent three prime examples of the complementary ionization techniques used in miniature MS sampling and data analyzation. DESI is a versatile analytical method for a variety of compounds and helpful for tissue imaging; LTP is important for in-field applications; PS is highly compatible for sample cartridge design and is attractive for quantitative analysis in regulatory and medical situations.

DESI

DESI has been used for direct analysis of explosives, pharmaceutical ingredients, drugs found within body fluids, and agrochemicals. For this ionization method, charge droplets are used for ionizing the analyte molecule in a small sample. To generate a high-velocity-charged droplet that will affect the sample, sheath gas and a high DC voltage electrospray are used.

Analytes are then extracted into a liquid, are ionized (usually through proton transfer), and are then moved away from secondary droplets' surfaces. Analytes' dry ions form in the air and are moved into a mass spectrometer for analysis.

LTP

Explosives found on surfaces, ingredients in seed and fruit oils, and agrochemical identification are all examples of the applicability of LTP. This ambient ionization method uses active species produced in low-power plasma to both desorb and ionize analytes in samples that are untreated. Low-temperature plasma is produced by dielectric barrier discharge; helium, nitrogen, argon, or air is transferred through an alternating electric field.

A device is used to enable extraction of the plasma species out of the discharge region to allow for sampling chemicals on a surface. Low gas flow rate, the ability to use air as discharge gas, ability to sample large areas, and minimal to now sampling angle requirement make LTP an advantageous ionization method.

PS IONIZATION

For a quick, low-cost ionization and sampling method, PS is often the first choice for quantitative and qualitative MS study of mixtures featuring highly complex structures. This method produces ions from samples directly and is applied on a paper substrate.

Paper has been widely accepted as a good material for storage of samples and is often used in methods of chromatographic separation. The application of a spray solvent featuring high voltage and small volume onto a porous substrate generates analyte ions. Then, the sample is either mixed into the spray solution or is preloaded onto the paper. The observation of a spray plume occurs. Signal intensity depends on the geometry of the paper; change the cut angle on the paper tip can affect the efficiency of the ionization.

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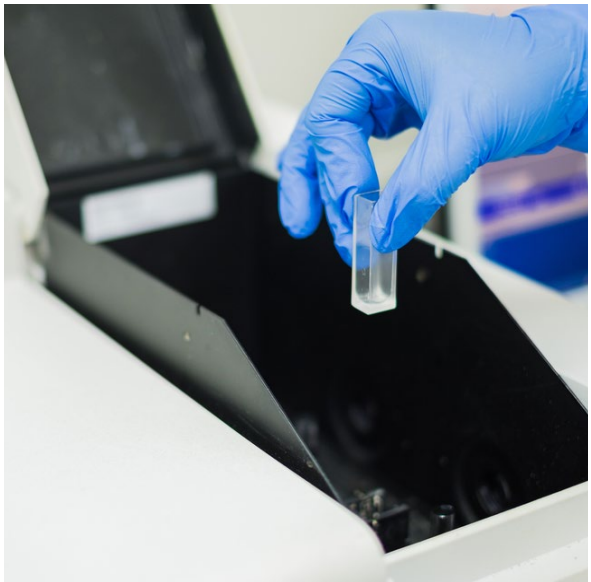
2.1d NEW COMPONENTS FOR MINIATURE MASS SPECTROMETRY

Just as miniature mass spectrometers continue to experience greater miniaturization than ever before, the applications for miniature mass spectrometers continues to evolve. Beyond the science of miniature MS, the structures of the spectrometers continue to get smaller and more reliable in terms of analytical results.



Continual improvements in the simplicity of miniaturizing **mass spectrometers** have been essential for a wide range of fieldable applications.

Daniel Austin, researcher at Brigham Young University and speaker at Pittcon 2017 and recipient of the Spectroscopy Society of Pittsburgh (SSP) Starter Grant in 2008, has been one of the leading pioneers in the development of new miniature MS technology. Austin and his research team have developed approaches using lithographically patterned plates as a unique method for producing mass analyzers.

Additionally, this approach has been used to shrink linear ion traps, radiofrequency quadrupole, electrostatic ion beam traps, and toroidal. Currently, their team is working on making charge detector arrays using the patterned-plates method, bypassing using machined electrodes as a means for producing cost-efficient products.

Austin's 2017 Pittcon talk, "**Wire Ion Trap**," discussed the miniaturized linear ion trap that utilizes sets of wires between two support plates. His research team discussed further the production of the wire ion trap, describing how replacing four of the hyperbolic electrodes of the conventional linear ion trap with wires of 80-120 microns in diameter is a step toward trap production.

Their development approach of the trap involves using 2-dimensional positioning without interfering with trap accuracy or trapping capacity. Austin also described the trap's immunity to things associated with mechanical misalignment, which is common in miniaturized ion traps.

Miniature MS tools can also be applied in electrophoresis applications. **Ramsey** of UNC at Chapel Hill led a talk at **Pittcon 2017** that provided an overview of miniature MS analyzers for the performance of capillary electrophoresis.

His talk discussed his group's recent developments in miniaturizing ion trap MS systems that include microfabricated sections. The analysis of blood for glycated hemoglobin and the examination of bioreactor broth for the detection and identification of amino acids are two such examples of applications that were explored.

One of the most popular mass analyzer choices for producing miniature MS systems are quadrupole mass analyzers. Toroidal ion traps can help reduce the size of mass analyzers, yet they can't be described by mathematical equations of the quadrupole device.

Stephen Lammert of PerkinElmer highlighted recent research in trapping fields and miniature mass spectrometers in his Pittcon 2017 talk, "**Describing and Optimizing Toroidal Trapping Fields for the Development of Miniature Mass Spectrometers**." In his talk, he described a recent effort in developing analytical tools necessary for studying and optimizing trapping fields in a toroidal coordinate system.

Research and product development teams at PerkinElmer, in turn, have produced a portable device containing a mass analyzing trapping field. The Torion® T-9 GC/MS is a small, portable MS, offering quick and accurate information. The product features a low thermal mass capillary gas chromatograph and high-speed temperature programming. Additionally, The Torion® T-9 GC/MS features a miniaturized toroidal ion trap mass spectrometer, as described by **Lammert** during his talk at Pittcon 2017.

Customized miniature mass spectrometers are also being offered by some companies, including Hamamatsu, a manufacturer of products used in spectroscopy. Hamamatsu's micro-spectrometers have been produced using MEMS technology and have been miniaturized to the size of a fingertip. The head of these fingertip-sized spectrometers support a long wavelength region (**850 nm**) and are highly sensitive.

This unique and minute size has allowed easy incorporation into a variety of instruments in the medical and scientific fields. Hamamatsu's ultra-compact spectrometer heads have adopted a newly developed optical system, which is innovative for the market.

The miniature spectrometers from Hamamatsu are also compatible with portable mobile devices. Also, Hamamatsu offers a series of spectrometer heads that can be integrated into equipment, providing a reflective grating and CMOS image sensor that is designed for use in visible measurements.

Miniature spectrometers with light guidance into optical fibers allow for measurement of the light spectrum by a detector, with output from the spectrometer's USB port transferred to a computer for data collection and evaluation. The size of these mini spectrometers, like the ones by Hamamatsu, measure **20.1 x 12.5 x 10.1 mm** and weigh up to 5 grams. The spectral response range is between **340 and 850 nm** (**340 to 780 nm** for the C12666MA) and are trigger-compatible.

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FUTURE OF MINIATURE MASS SPECTROMETRY

Mass spectrometry's future is unknown; however, its rate of utilization growth has shown that its evolution and advancement is certain. In chemical analysis, pharmaceutical applications, and forensics, MS serves a powerful study tool that will certainly continue toward improving research on all fronts.

Miniature mass spectrometers are quickly emerging as a powerful adjunct in research and are easy to use and highly sensitive. Their ability to be used outside of the laboratory by untrained professionals across many different subsets of science broadens its use.

In addition, the development and swift adoption of Purdue University's Mini 10, Mini 11, and Mini 12 systems may be a useful tool in biomedicine, pharmaceutical, and agrochemical industries, both now and in the future. The development and implantation of these systems will help these industries' nurses, biologists, and doctors access information rapidly without needing to send samples to a laboratory.

Smaller systems than those that are currently available must be made to perform more complex, intricate procedures. For example, identification of a smoker using blood tests, while feasible with a miniature mass spectrometer, can't always be performed easily with the current technology available. With smaller devices, physicians may also be able to monitor therapeutic drug administration during surgery in at-risk patients.

Similar to what Purdue's Mini 12 miniature mass spectrometer has demonstrated, future direction for miniature MS systems include the inclusion of ambient ionization methods with the devices.

In something like the Mini 12, a miniature ion trap mass spectrometer is combined with paper spray ionization. Inside a sample cartridge, a blood sample is placed on the paper substrate. Researchers can push this sample into the system, which then adds an organic solvent to the cartridge. Then, a 4 kV voltage is applied. The elution of organic compounds and spray ionization then occurs on the paper substrate at its tip. Exactly two MS-MS scans are performed on the analyte and internal standard.

Future miniature analytical systems may also be used more frequently by non-professionals, or non-instrumentalists. Analytical chemists can apply their knowledge regarding chromatography to these systems in time, enabling for further development of miniature mass spectrometers. Helping these chemists move beyond liquid chromatography columns to sample cartridges that can provide real-time extraction and ionization may also be important in the next few years.

While the future of miniature MS systems appears promising, selling these products on a large scale may be challenging. Many instrument companies have difficulty transitioning into the production of small systems. Also, data regarding these systems aren't impressive or "groundbreaking," which can reduce the likelihood of these companies moving into production.

One study previously described how combining DESI with a miniature mass spectrometer could potentially develop a brand new instrument in the near future. This instrument may enable direct evaluation of samples of practically any type in an ambient environment.

The integration of ambient ionization into miniature mass spectrometers has advanced in the past few years and continues to be used to develop systems that can be operated by non-experts.

Applications, according to authors of the paper, may be diverse. For example, such a device could be used to indicate disease type, detect the content and toxicity of dangerous compounds on surfaces and in water, and examine tissues for tumor margins via lipid distribution examination in tissue sections. The device could also analyze skin lesions and needle biopsies right in the physician's office.

MINIATURE MS IN SPACE

There are some initiatives in place for using miniature mass spectrometers to advance the United States space program. Studying planetary atmospheres and their composition as well as monitoring the quality of air on space missions are two primary application fields for miniature MS.

Since systems used in the space program need to be portable, lightweight, and have a high level of sensitivity, miniature mass spectrometers make for the perfect analytical systems to be used in future space study programs.

Previous studies have demonstrated successful deployment of MS instruments on the Mars Viking Lander and Pioneer Venus missions. Future opportunities for MS instruments in space include studying human breath to examine microgravity's effects on respiratory function in humans.

MS instruments for space applications include time-of-flight, sector instruments, quadrupole ion traps, quadrupole arrays, and cylindrical ion trap mass spectrometers.

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CONCLUSION

The drive for developing miniaturized forms of **mass spectrometers** is multifactorial. Portability and ease-of-use are perhaps two of the most common reasons physicians wish to use miniature MS instead of the larger MS instruments.

The ability to use an instrument onsite or at the bedside are also important for retrieving data quickly. Also, the ability to use these MS products without having to learn a complicated analytical method makes miniature MS an attractive option for the clinical environment. Rapid data retrieval and analysis, two key benefits of many miniature MS methods, also play a role in facilitating the development of this technology.

To overcome the size and weight limitations in conventional MS tools, miniaturized versions continue to be developed. These tools can sit in a physician's office, and some have been developed to connect with mobile devices for quick retrieval and analysis of information.

Many miniaturized instruments can provide in-depth analysis of metabolites or disease state efficiently and reliably, and the market for these products has grown exponentially in the past few decades.

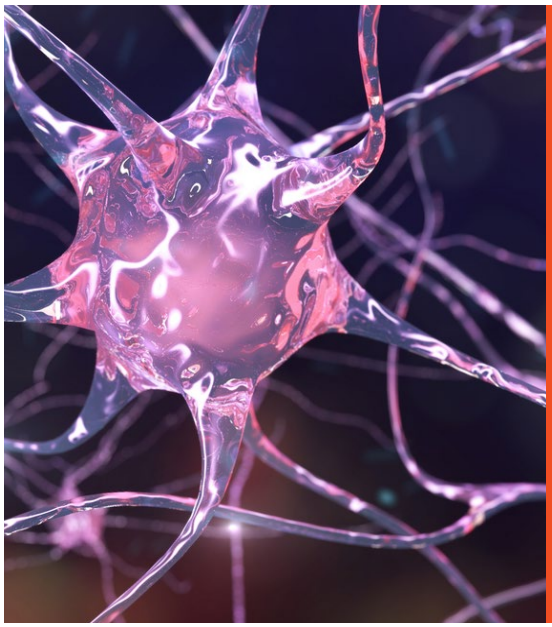
Companies such as Bruker, Photonis, Waters, Hamamatsu, and Thermo Fisher Scientific, all of whom produce instruments used in mass MS and miniature MS, were important exhibitors at Pittcon 2017. Each company not only provided visual and hands-on product demonstrations, but also gave new insights into current applications in science, forensics, agriculture, and pharmaceutical industries.

Also featured at Pittcon 2017 were leading researchers in the field of MS and spectrometer miniaturization, including **R. Graham Cooks, Daniel Austin, Christopher Brown,** and **Jingren Deng**, among others.

Speakers' presentations covered topics such as scan methods with miniature ion trap mass spectrometers, miniaturized wire ion traps, miniature field analyzers, and miniaturized electrochemistry in medicine.

More than 150 sessions were devoted specifically to mass spectrometry and covered a broad range of industries and fields, including medicine and surgery, forensics, and chemical safety. Scientists involved in every industry and environment and who are interested in new MS technology for compound identification benefited from the information provided at Pittcon 2017.

CHARACTERIZING THE BRAIN, CELL BY CELL



Professor Jonathan Sweedler is Professor of Chemistry and Director of the School of Chemical Sciences at the University of Illinois. He is highly regarded in the field of neurochemistry and neurotransmission, having conducted numerous studies on cell-to-cell signaling in learning, memory, and behavior and on the distribution and release of neurotransmitters.

Many neurotransmitters are very rare and so have been difficult to study. In the following interview, **Professor Sweedler** gives an overview of the techniques used to characterize neurotransmitters. In particular, he describes new mass spectrometry techniques used in his research and provides an overview of his talk at Pittcon 2017.

An interview with Professor Jonathan Sweedler, University of Illinois at Urbana-Champaign, conducted by April Cashin-Garbutt, MA (Cantab)

Q **Please can you give a brief introduction to your research and cell-to-cell signaling in the brain?**

A My group develops approaches to study cell-to-cell signaling in the brain – how the cells of the brain talk to each other. The brain is heterogeneous, probably more so than any other organ in our body, and many of its functions depend on the unique characteristics of these cells.

Memory, for example, by definition, arises partly because of the connections between individual cells. And so, in order to understand questions about memory, or how the brain controls behavior, or even age-old questions about consciousness, the brain must be studied at the cellular level. However, measuring the chemistry at the level of individual cells is difficult.

A microliter of brain tissue has over **100,000** cells, and this microliter will contain many different types of cells, such as astrocytes, oligodendrocytes and neurons. If one measures the proteins or metabolites in this microliter, you would learn about a hypothetical average cell. You would not gain information on the contents of neurons or other unique cell types.

What we're really after, and what many neuroscientists want to know, concerns cell heterogeneity and chemistry. We hope to determine the chemicals that neurons in the brain use to talk to each other, and how these change during learning and memory, and during different behaviors.

We could then address how these change with addiction, mental diseases, etc. Understanding the single cell level will help us answer these global questions.

Q **Why do many cell-to-cell signaling molecules in the brain remain poorly characterized?**

A Most people know about serotonin because of its role in depression and the use of serotonin reuptake inhibitors, and also they know about dopamine in terms of attention deficit disorder or Parkinson's, but there are lots of other transmitters and neuromodulators too.

Scientists have found neurotransmitters, like glutamate, serotonin and dopamine, that are used by a significant fraction of cells in the brain; however, if there are transmitters that are used by only rare cells, we may not know about them.

Consider, for example, neuropeptides and neurohormones, which are very important for changing our mood or, from a cellular perspective, changing the firing threshold of specific neuronal circuits. Sometimes a neuropeptide may only be found in a rare cell, located in a specific part of the brain where it is only present in **1 in a 1,000 cells**; this makes it easy to overlook.

Another characteristic that can make neurotransmitters difficult to characterize is that some of these molecules are formed by the actions of multiple enzymes. This means that you can't look at a particular form of a neuropeptide and point to a specific gene that makes it, because there are a lot of enzymes that help in the process.

Because of this, techniques like transcriptomics allow you to evaluate the potential neuropeptides involved in a cell, but measurements must be made to confirm whether the specific biologically active peptide form is present.

For a lot of these reasons, we're still discovering brain cell-to-cell signaling molecules. Additionally, researchers are still trying to come up with the entire parts list of the brain, so not just cell-to-cell signaling molecules, but all of the lipids and fatty acids and metabolites in the brain. These are dynamic and are not completely characterized.

Q **What bioanalytical techniques are now available to investigate individual neurons and small brain regions?**

A To make a measurement of the contents of a test tube and investigate what's in it, mass spectrometry and liquid chromatography mass spectrometry are commonly employed. We also must consider whether a technique can be downscaled to work at the single cell level.

Separation techniques, such as capillary scale liquid chromatography and capillary electrophoresis, work well at this level. Capillary electrophoresis is well suited for taking individual cells and separating what's in them, and mass spectrometry can be used to characterize the individual components.

> Accordingly, we have developed a range of capillary electrophoresis mass spectrometry approaches to sample the cytoplasm in individual cells and characterize the major metabolites within a cell.

Direct mass spectrometry is another approach, and this is perhaps the most common single cell techniques we use. In one embodiment, we scatter **10,000** cells onto a microscope slide; then we make the cells fluorescent and image the slide via fluorescence microscopy to determine their x-y locations on the slide.

We feed those locations into our mass spectrometer, and so sample only from the cells and probe what's in them.

This type of high-throughput, single cell profiling allows us to look at **10,000** cells at a time to find some of the major molecules within them. This was one of the highlights of my talk at Pittcon 2017: how we can take a sample from a brain region and use this approach to probe the neuropeptides and some of the lipids and small molecules in those cells.

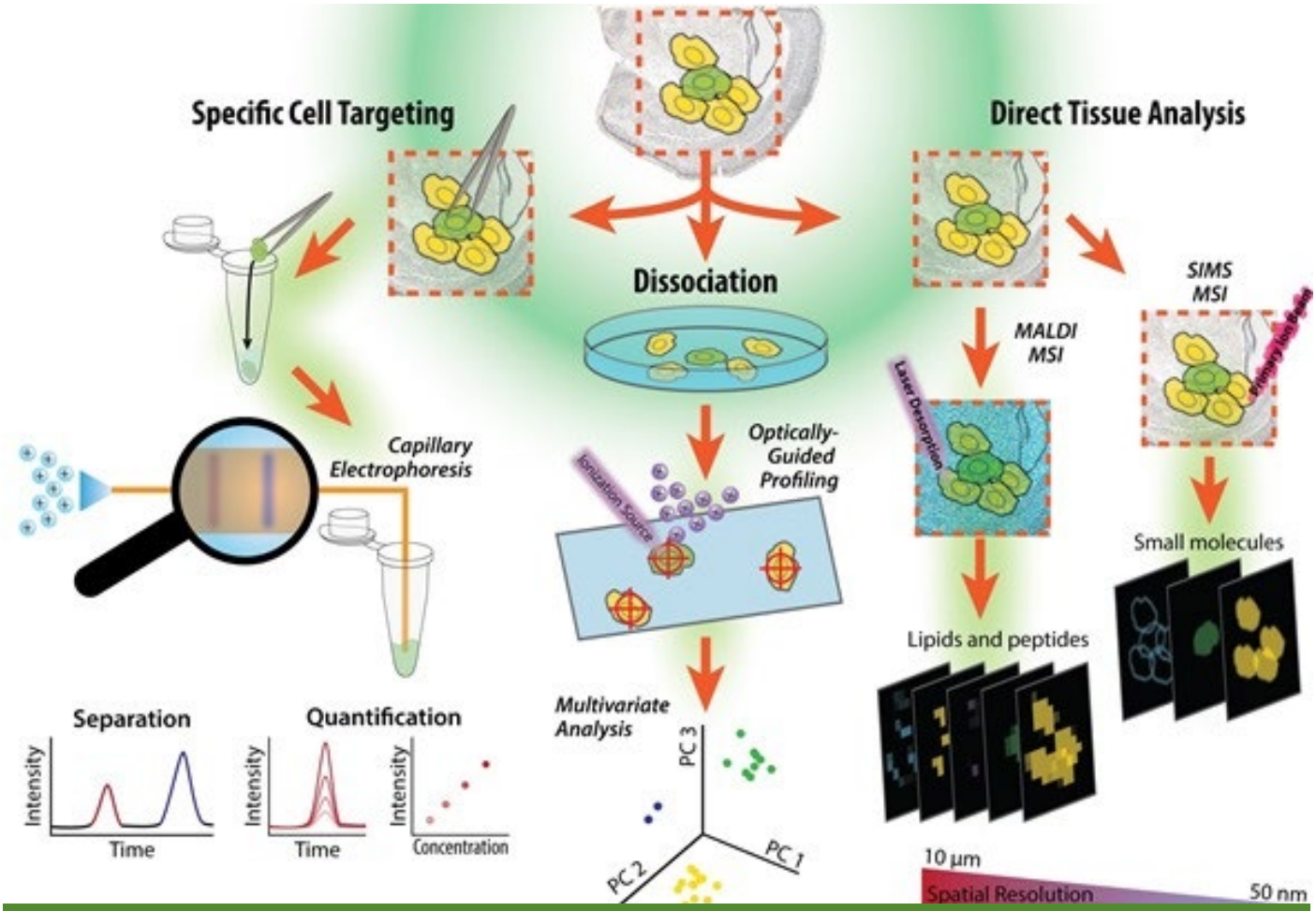
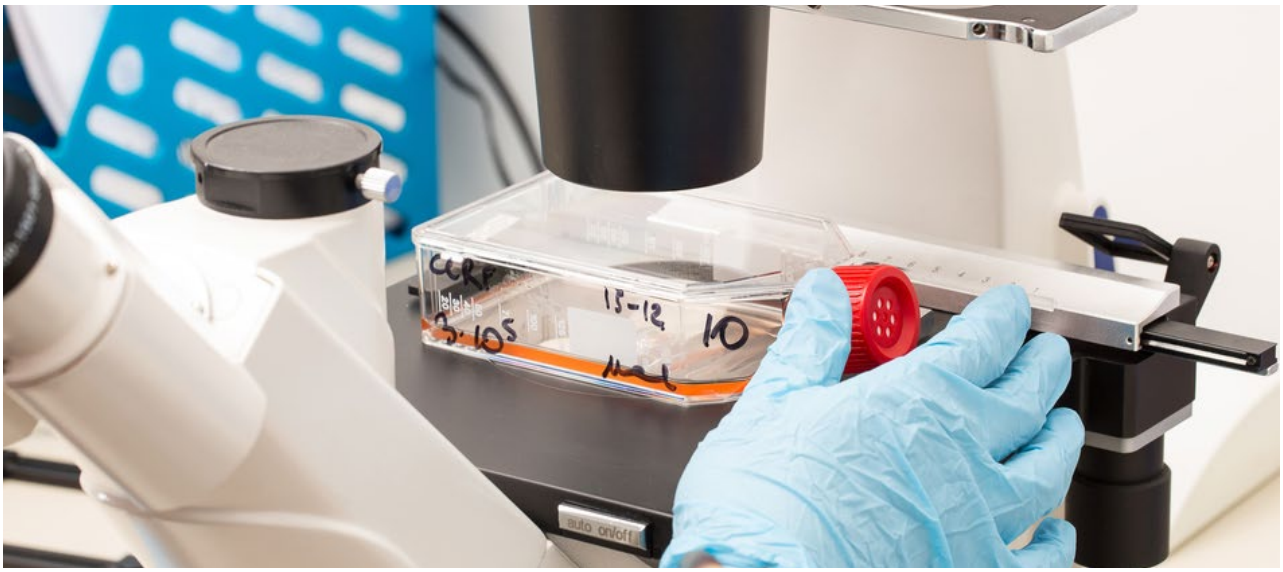


Figure illustrating workflow of a tissue sample, from cell isolation to our single cell measurements based on tissue imaging, single cell profiling and capillary electrophoresis-mass spectrometry. The cells can be dispersed onto a microscope slide and probed, or specific cells can be selected and assayed using capillary electrophoresis mass spectrometry. Figure prepared by **Troy Comi** from the Sweedler laboratory at the University of Illinois at Urbana-Champaign.

One thing that's really exciting about this technique is that it leaves about **80%** of the material behind. After the measurement, if we find that a few of the cells are particularly interesting, or have something unusual, for example, if cell number 940 has a different profile that we didn't expect, we know its exact x-y location on the slide. We can then follow up and do immunofluorescence on that specific cell, or we can even isolate the cell and investigate it with capillary electrophoresis-mass spectrometry.

This high-throughput profiling can then be followed up with other techniques in order to learn more information about the rare cells we come across.

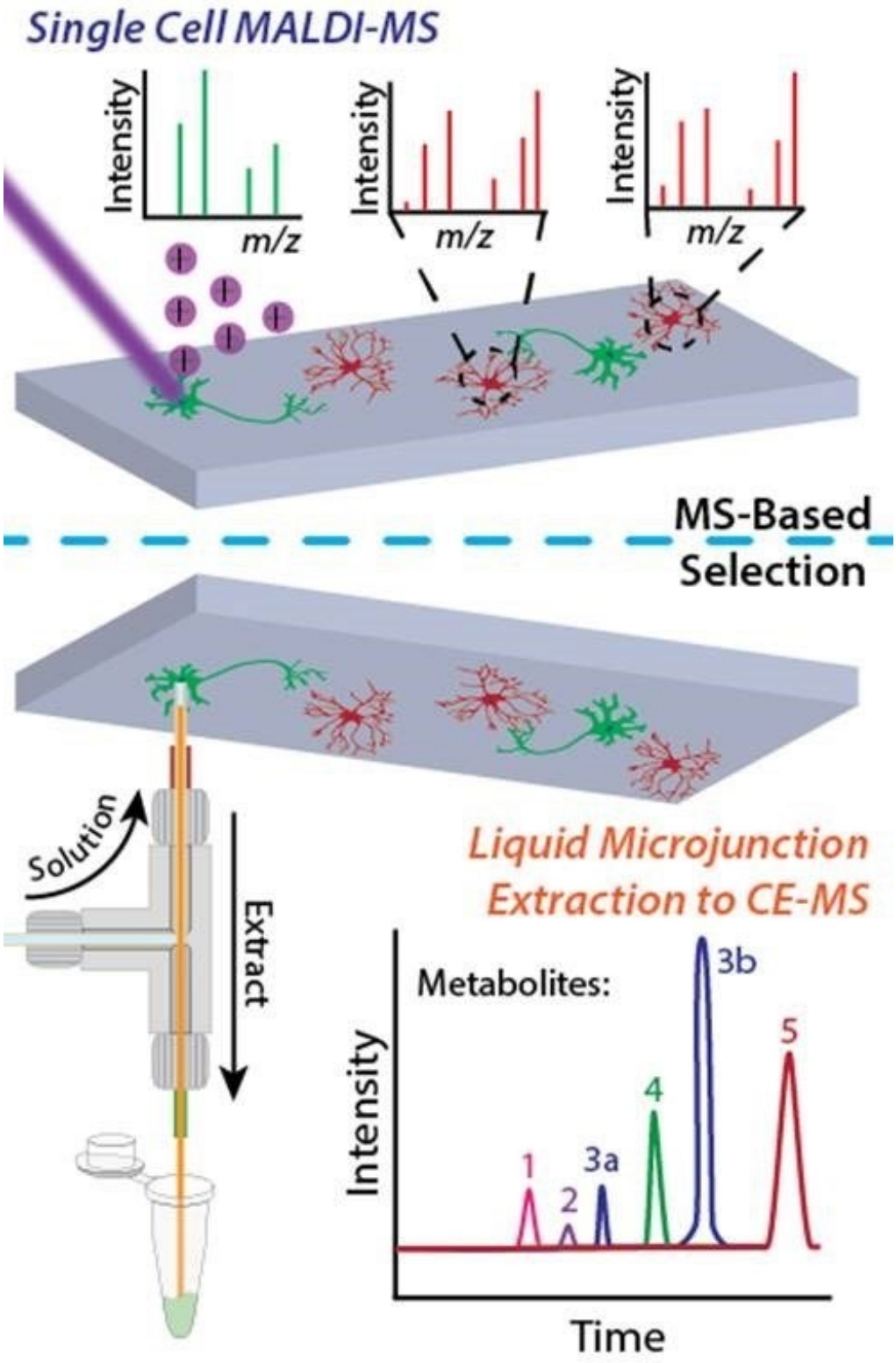


Q **Which approaches did you focus on in your talk at Pittcon 2017?**

A At Pittcon I talked about high-throughput single cell profiling using two flavors of mass spectroscopy:

- Matrix-assisted laser desorption ionization (MALDI) mass spectrometry
- Secondary Ion mass spectrometry (SIMS)

Both of these techniques can be used for profiling large cellular populations. I also talked about rare cells and using capillary electrophoresis mass spectrometry and capillary electrophoresis laser induced fluorescence to investigate the contents of these cells. Why so many flavors of mass spectrometry? One of the reasons is that each one gives us a little bit different information on the chemicals in the cells.



The Sweedler group's single cell metabolomics workflow combines direct mass spectrometry profiling and capillary electrophoresis-based mass spectrometry in a two-tiered process.

Image prepared by **Troy Comi** and **Elizabeth Neumann** from the Sweedler laboratory at the University of Illinois at Urbana-Champaign.

Q **What is the key to successful measurement?**

A The important point is that our measurements work and are robust. The most common reasons for failure involve experimental design and sampling issues. It's important to remember, I am talking about the sampling protocols and the design of complex animal experiments.

We work with animals from across the animal kingdom, from comb jellies with their simple brains, to sea slugs, and mammals such as rodents, in the effort to link neurochemistry, physiology and behavior.

> The key to successful measurements depends on how you design the experiment. If you're trying to design an experiment to assay what changes occur during drug escalation for a drug abuse study, or during disease or even during memory formation, you have to make sure that the experiment is carried out in a way that allows the dynamic chemistry in the cells to be measured.

Another thing to remember is that some of the molecules of interest, such as neuropeptides and small molecules, degrade very quickly after death, and so choosing the correct experimental protocol to retrieve the single cells is extremely important. We've had to come up with ways to preserve the cells to ensure compatibility with our measurement approaches.

> An example of a cell preservation technique, which a lot of neuroscientists would suggest, is cell fixation. However, this is a way of turning the cell into plastic, as it crosslinks many of the cell's molecules.

While it preserves the cell, it degrades the resulting mass spectrometry data. We spend a lot of time validating and designing our protocols, because if a sample is degraded, no amount of analytical measurement can rescue it.

I'm being a little general because sometimes the experiments are done locally, and sometimes they're performed at distant collaborator laboratories; factors like this will change how we go about things.

Q **How much do you vary your approach for the type of sample being assayed and metabolites being measured?**

A It varies between cases, but to give an example, when looking for rare cells, sometimes we use a sample where the cells of interest are fluorescent model. We then try to find these fluorescent cells, either in a tissue or dispersed on a slide, or even by using fluorescent cell sorting, and then we analyze them via mass spectrometry.

Moving away from the brain, we have isolated cells from pancreatic islets and investigate the peptides in the different islet cell types: alpha, beta, gamma, or delta cells.

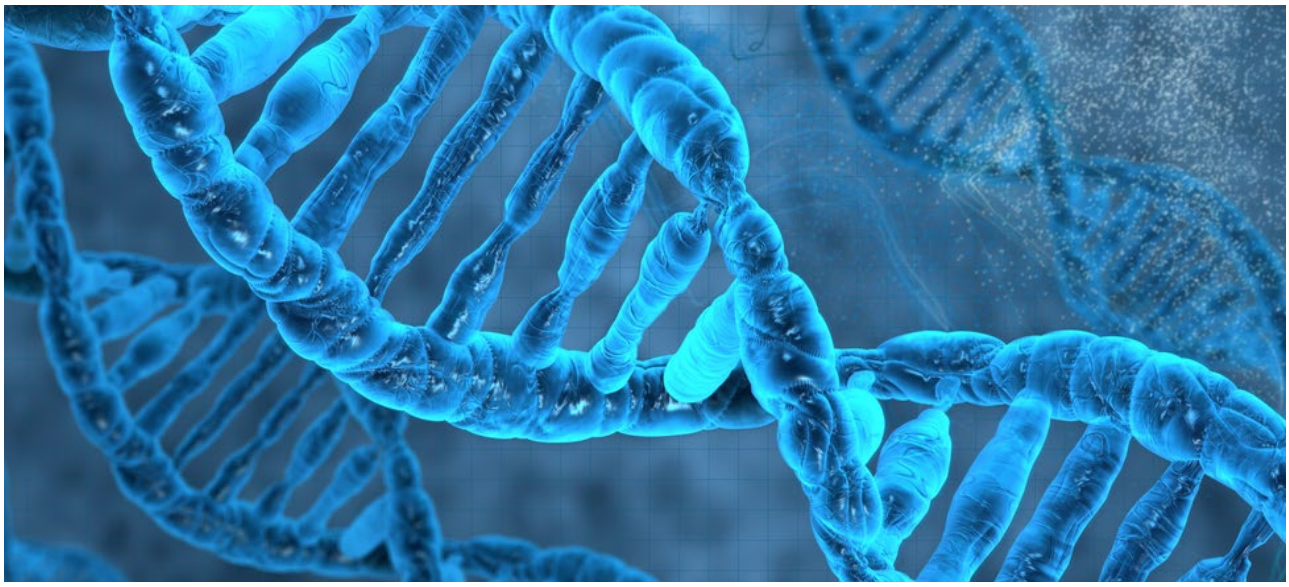
Our sampling approaches depend on whether it's a rare cell and the type of molecule we want to measure; for example, if we want to know the lipids in a cell, we make very different measurements than we do for neuropeptides.

Transcriptomics, the most common form of single cell measurement, provides a fairly complete set of the transcripts in the cell. When we do mass spectrometry on cells, we use the terms metabolomics or peptidomics, but the results are far from complete.

> It's not really "omics"-scale. For example, if there are **20,000** metabolites in the brain, and we do metabolomics in a brain region, we can measure hundreds to a few thousand metabolites.

These represent only a few percent of the metabolites. The specific metabolites (or proteins or peptides) that are characterized depend on the specific mass spec platform and operating protocols used.

If we know in advance that we're looking for a particular subset of cellular metabolites, such as the hydrophobic versus the hydrophilic, we adjust our protocols accordingly. We have to optimize our methods more frequently because unfortunately, the measurements we make are not global or omics-scale as they are with DNA and RNA.



Q **How important are single cell assays to your work?**

A My group is evenly split between technology development and neuroscience measurements. For the technology development, about **70%** is single cell measurement, and for the biological measurements it is about **30%** single cell measurements.

This is an exciting time for brain research. All around the world, in Europe, the Americas, and in Asia, there are huge initiatives to understand the brain. In America, it's the BRAIN Initiative, which is run through the National Institutes of Health (NIH), the National Science Foundation (NSF), and other organization.

One of the goals is to understand cell heterogeneity and build a cell census of the brain. Within the US, there are several BRAIN initiatives related to creating single cell measurements, and we're are involved with these efforts.

Q **What technology advances are in the pipeline and which companies are working on these?**

A We use mass spectrometry for most of our measurements. For our imaging and cell profiling work, higher acquisition speed and better detectability are important. We need new instruments that provide more information from a smaller sample.

The obvious players are: Bruker, Agilent, Sciex, Thermo Scientific, Waters, among others. They're coming up with better instruments that give you more information from smaller samples.

If you're performing single cell profiling with MALDI, for example, Bruker is creating faster mass spectrometers that provide more information from a greater number of cells. We are also trying to integrate single cell mass spectrometry with other approaches such as vibrational spectroscopy single cell transcriptomics.

The other area that is rapidly evolving involves the way we handle cells. There is an ever-increasing range of approaches for cell sampling, dispersing cells and hyphenating aspects of cell sampling with microfluidics.

A lot of these approaches are being created by academic research groups, but I also see small companies that create and offer these technologies. The combination of new mass spectrometers, better informatics and improved cell sampling suggests that the age of single cell chemical characterization has a bright future.

Q **What impact do you think these new technologies will have on your ability to uncover the complex chemical mosaic of the brain?**

A Let me highlight two areas. First, new technologies and new protocols are allowing us to look for a greater range of chemistry in the brain. These advances have been rapid and enabling.

For an example of an area outside of my research, I have been working with several groups in developing vibrational spectroscopy.

They can now image a brain slice with infrared and Raman spectroscopy at cellular resolution, getting complete vibrational spectra on thousands of cells.

> Now we can do infrared or Raman imaging on a brain slice, and then perform mass spectrometry afterwards on that same slice, which provides unprecedented chemical detail.

Another exciting advance is that these technologies are becoming more robust, so that more groups are able to use them. As an example, my group has helped teach a course on single cell measurements at the Cold Spring Harbor Laboratory.

When we teach people how to do single cell sampling and mass spectrometry, a lot of them will go back to their own local mass spec facility; in the past, the facility managers would tell potential users that single cell measurements would not work on their facility instruments. Now more of these facilities are willing to try (and succeed) with these types of measurements.

Q What do you think the future holds for pinpointing key cellular players in physiological and pathological processes in the brain?

A Two things. One, there are billions of cells and we're only measuring thousands. We need to become more global in our measurements. Second, we often find unusual peaks/masses in a cell but confirming what these unexpected compounds are remains a challenge.

Additionally, a lot of the single cell measurements have been qualitative. We need to know what's in the cell, and what's in the cell that changes based on disease and/or a physiological process. We're getting to the point where our goal is to increase the quantitative details. Adding more robust quantitative information to these types of measurements will really increase their impact.

Q Where can readers find more information?

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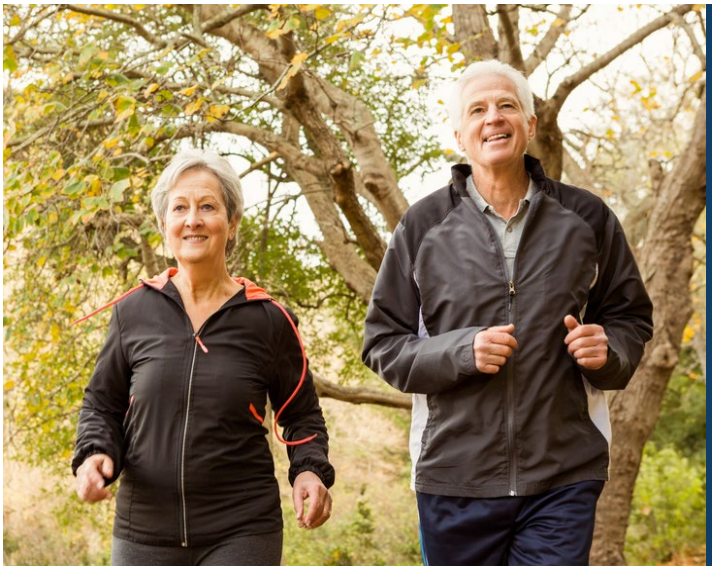
2.3 USING MASS SPECTROMETRY TO ANALYZE THE AGING POPULATION

Proteomics is the large-scale study of thousands of proteins within one experiment. It allows easy comparison between proteins in different samples. Complex physiological changes occurring during aging give rise to reduced efficiency of key systems. Understanding the changes in protein expression behind the observed impairments can help in the development of preventative or restorative strategies.

Presenters at Pittcon 2017 highlighted the value of mass spectrometry based proteomics in the study of the regulation and function of molecular complexes and pathways. This section discusses how the technique has proved particularly valuable in determining the molecular basis of impaired function associated with aging.



INTRODUCTION

The complex physiological process of aging represents many problems among rapidly aging populations. One well recognized consequence of aging is degradation of immune function, which is referred to as immunosenescence.

Although elderly people are by no means immunodeficient, their response to new or previously encountered antigens is often inefficient. Immunosenescence is partially accountable for the increased susceptibility the elderly have to infection, as well as their poor response to vaccination. Both the adaptive and innate branches of the immune system are affected, but the molecular mechanisms involved are still not clear.

At this year's Pittcon, the leading conference and exposition for laboratory science, more than **2,000** presentations were held. The topic of aging populations was discussed at PIttcon 2017, with an emphasis on how mass spectrometry is used to study age-related changes in response to infection.

PROTEOMIC ANALYSIS OF AGING

Proteomics is the large-scale study of thousands of proteins within one experiment. It involves the qualitative, quantitative and functional characterization of all proteins within a given cell, tissue and/or organism. The simultaneous study of many proteins enables a more "systems biology" approach to mapping protein content, crosstalk and activation to a particular time point, for a particular condition.

For example, it is possible to screen for proteins that are differentially expressed between healthy and diseased tissue, which can help to improve diagnosis, monitoring of therapy and drug design. Native proteins may be profiled, as well as their isoforms, splice variants, and post-translationally modified species. Interactions between proteins may also be determined.

MASS-SPECTROMETRY BASED PROTEOMICS

The past two decades have seen remarkable advances in the field of proteomics. Mass spectrometry has emerged as the method of choice for characterizing protein components found in biological systems. The technique has led to important insights into the make-up, regulation and function of molecular complexes and pathways.

Mass spectrometry-based proteomics has become a powerful tool that can be combined with molecular, cellular and pharmacological techniques to translate large sets of data and elucidate complex biological processes.

At Pittcon 2017, held in Chicago, Illinois between the 5th and 9th March. major suppliers of mass spectrometry equipment showcased their world-leading products. Among the exhibitors were Bruker, Thermo Fisher Scientific, Waters, Shimazdu and PerkinElmer who demonstrated the latest technical advances in the field of mass spectrometry.

MASS SPECTROMETRY IN AGING RESEARCH

In a 2012 proteomics study by **Lisa Staunton** and colleagues, tissue specimens derived from middle-aged (47 to 62 years) and aged (76-82 years) individuals were compared for potential changes in protein expression profiles. The study revealed age-dependent changes in the concentration of 19 protein spots.

> Mass spectrometry showed that these components were involved in muscle fiber contraction, muscle metabolism, ion handling and the cellular stress response, indicating a disrupted pattern of protein expression in senescent human muscle.

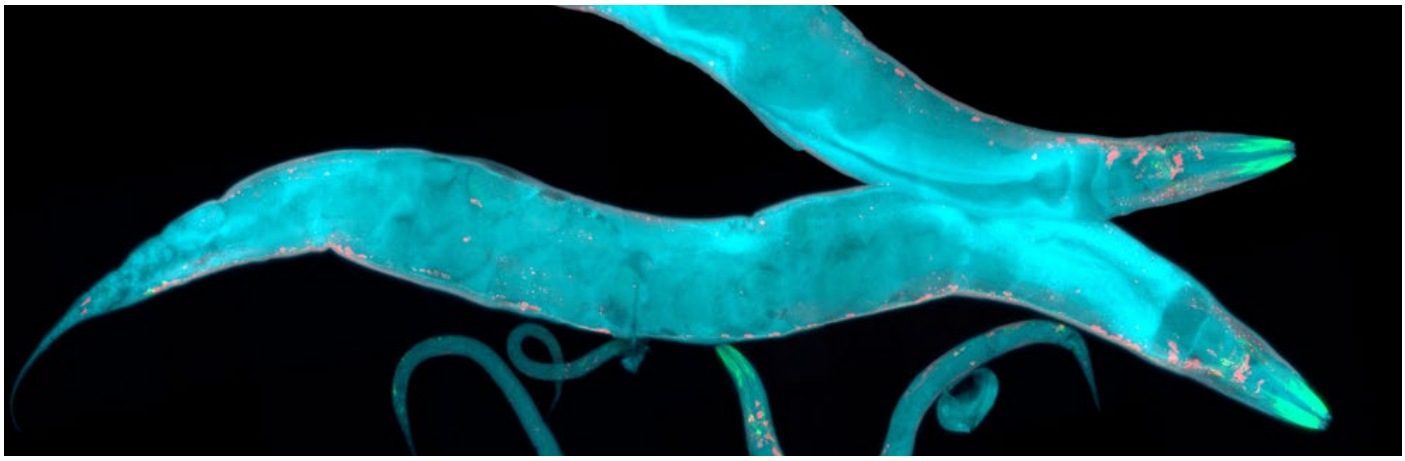
A 2014 study by **Paczek et al** examined urine proteomes derived from 37 healthy individuals, aged 19 to 90 years, to explore which metabolic processes were weakened or enhanced as people age. Protein expression analysis by liquid chromatography–tandem mass spectrometry showed the differential expression of 19 proteins between young (19 to 26 years), intermediate (45 to 54 years) and old (72 to 90) age groups.

> Importantly, protein changes in the oldest group were reflective of altered extracellular matrix turnover and deteriorating immune function, changes that were in keeping with reported alterations in cardiovascular tissue remodeling and immune conditions among the elderly.

CAENORHABDITIS ELEGANS (C. ELEGANS) AS A MODEL FOR REGULATION OF AGING AND INNATE IMMUNITY

C. elegans is a nematode that lives in the soil of temperate regions, where it feeds on bacteria. It is a major model in the study of a number of fields including developmental biology, apoptosis and aging. The worm has been shown to possess an inducible innate system and has become an important model for elucidating the mechanisms involved in innate immunity. Interestingly, it has also been shown that the regulation of aging and innate immunity in C. elegans seem to overlap.

Characterization of this nematode's innate immune system suggests that certain immune system components are conserved in metazoans. The study of immunosenescence in C. elegans is likely to provide important insights into how immunity is affected as the nematode ages. This could help researchers to understand, at the cellular and molecular level, the reciprocal effects of aging and immunity on each other and how these may be relevant to humans.



At **Pittcon**, **mass spectrometry** and proteomics expert **Dr. Renã Robinson** talked about C. elegans as a model for following age-related, proteome-wide changes that occur in response to opportunistic pathogenic infection. An overview of mass spectrometry applications to aging was given, including a demonstration of how there is a significant influence on host response due to aging in C. elegans.

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2.4 DECODING THE EXPOSOME THROUGH BREATH BIOMARKERS

Many chronic human diseases are associated with environmental factors. At Pittcon 2017 the role of the breath in providing an insight into the health of an individual was discussed. In addition to the expected gaseous components, breath contains a range of proteins, lipids, bacteria, viruses, inorganic compounds, and inflammatory molecules. These act as biomarkers that can provide valuable information on how on the environment affects the functioning of cells in the human body.

In the following interview, **Prof. Joachim D. Pleil** of the US Environmental Protection Agency explains the importance of chromatography and mass spectrometry techniques in analyzing breath to study the impact of the environment on health.

An interview with Prof. Joachim D. Pleil, US Environmental Protection Agency, conducted by April Cashin-Garbutt, MA (Cantab)

Q **What is the human exposome and how much do we know about the interaction between our genes and the environment?**

A The human exposome is probably best defined as “everything that is not the genome.” This is a bit tongue in cheek, but basically, the exposome is comprised of all of the chemicals in your body from the environment, food, consumer products, their metabolites, the endogenous “housekeeping” chemicals, the cellular wastes from energy production, as well as all of the messenger compounds and life supporting chemicals.

Unlike the genome, which is relatively stable throughout life, the exposome is a moving target depending on your current and past environment, as well as your activity, health state, nutrition, and stress, and consumption of food.

We are learning a lot about the gene-environment interaction, but honestly, it will be a long research path before we fully understand it. However, we need to remember why this is important.
It is generally accepted that 70-90 percent of long-term latency and chronic human diseases are associated with environmental factors.

> Breath analysis is at the forefront of public health research and protection – it is a non-invasive window in the human health state, and has potential for broad applications at the community level.

Q **How can biomarkers in breath be used to decode the human exposome?**

A Breath is actually an interesting biological medium that tends to be overlooked in favor of the traditional media (blood and urine), when it comes to understanding the exposome. People only think of the gas-phase when they consider breath analysis.

Sure, gas exchange with the blood is the primary function of breathing, and so we consider the breath to be a non-invasive window into understanding how respiration affects the dissolved gases in the blood.

What is not quite as recognized, is that breath also contains vapors, aerosols, and particles. By modifying purely gas-phase collection methods, we gain access to the semi- and non-volatile fraction of breath, as well as a host of dissolved water soluble chemicals otherwise missed in gas-analysis.

As such, the breath exposome is expanded beyond the volatile compounds into the realm of proteins, bacteria, viruses, inorganic compounds, inflammatory markers, and larger compounds such as fatty acids and lipids.

Q **How many different analytes can be measured from breath?**

A Traditional chemical breath analysis goes back to **Linus Pauling** who proposed that there are hundreds to thousands of different volatile organic chemicals accessible in exhaled breath. These numbers are increasing everyday as instrumentation becomes more sensitive and capable of greater specificity.

I focused the early part of my career on assessing environmental chemical exposures — examining exposure to compounds like benzene, toluene, carbon tetrachloride, chloroform, etc. that are related to adverse health outcomes.

We then started cataloguing endogenous chemicals like alcohols, ketones, and aldehydes more likely to represent current metabolism, and possibly health state.

My understanding is that tens-of-thousands (possibly more) distinct features can be identified in a single breath sample.

> "Today, the "gold standard" for gas-phase breath discovery analysis is the GCxGC–ToFMS or Orbitrap style high-resolution equipment."

Furthermore, we have access to the exhaled breath condensate fraction, which contains the particles, aerosols, and all sorts of dissolved materials.

> Here, researchers can apply all types of liquid chromatography, genetic analysis, direct Mass Spectrometry (SELDI, MALDI, PTR, SIFT) and immunochemistry to assess pretty much everything coming from the human body.

For example, in my lab, we have focused on assessing pro- and anti-inflammatory cytokines in exhaled breath condensate using second-generation robotic immunochemistry platforms.

Q **What advances in GC-MS and LC-MS have aided your research?**

A To me, the most interesting recent development is the ability to use standard (modest) **GC –MS** systems with simultaneous SIM/Scan acquisition. Much of the gas-phase breath analysis in environmental and toxicological research is based on pharmacokinetics, that is, we track the progression of compounds throughout an organism from exposure to excretion, or sometimes infer previous exposures from compounds currently being excreted.

Usually, this style of pragmatic research is conducted with specific target compounds in mind. The cool thing about simultaneous SIM/Scan is that we don't sacrifice discovery analysis, and if something out of the ordinary occurs in our breath samples, we can catch it.

Q **What further developments would you like to see from GC-MS and LC-MS instruments?**

A Personally, I think the new high-end analytical instrumentation is so advanced that the capability is often beyond our more parochial needs.

The one thing I'd like to see is further development of the standard bench top (code for inexpensive) GC-ToF-MS detector. This may be in the works, but smaller laboratories could use modest systems similar to the existing single-quads, but with higher, (maybe 0.01 to 0.001 Dalton) mass resolution for volatile compounds.

Q **Can you please outline your recent talk at Pittcon 2017?**

A I actually gave two symposium talks at Pittcon, both breath-related. The first, considered that part of breath research impacted by cellular level "respiration." Here the symposium delved into different aspects of disease/infection diagnosis, pharmaceutical development, human toxicology, and food safety based on what we now call "cell breath."

My own talk outlined cellular level research for assessing toxicity of chemicals and assessing differential contributions to exhaled breath from human cells vs. human microbiota in the gut and lungs.

The second talk I gave was part of a symposium dealing with the human metabolome. Here, I presented the basics of breath discovery analysis and current exhaled breath research for assessing fire fighters' protective gear, and the concept of using canine olfaction as a guide for developing laboratory methods for toxicology and disease diagnosis.

"I actually gave two symposium talks at Pittcon, both breath-related. The first, considered that part of breath research impacted by cellular level "respiration."...The second talk I gave was part of a symposium dealing with the human metabolome."

Q **What were the highlights for you at Pittcon 2017?**

A There were a lot of opportunities at Pittcon to explore new ideas. My biggest issue was how to split my time between the exposition floor and all the talks I wanted to attend. That said, I particularly enjoyed seeing many of my international colleagues in person.

> Every year, Pittcon has quite a good turnout of breath researchers and instrument manufacturers specializing in breath applications.

Not only did we have the two breath-related symposia, there was also a breath networking session, and a scattering of breath-related presentations.

One of the newest topics is the potential for using breath analysis for detecting marijuana impairment in drivers, like the standard police breathalyzer for drunk drivers. Though not something we would get involved with, it sounds like a really important new research area.

Q **What are your future research plans?**

A In my lab, we are working with a couple of new breath-related projects. We are collaborating with NIOSH in their evaluations of firefighters' protective gear, and we are developing new methods for assessing inflammatory conditions and other responses with human cell lines.

The cell research is especially intriguing in that it allows us to study metabolism, but avoid the expense, infrastructure needs, and ethical issues with using animals or human subjects.

Another project I hope to work on is developing methods for capturing and analyzing exhaled aerosols directly. This would be valuable for public health applications where simplicity of sampling in the field is paramount.

Q **Where can readers find more information?**

A Not surprisingly, the best source of information for all aspects of breath research is the Journal of Breath Research (JBR) published by the Institute of Physics (IoP) in the UK, an organization with roots tracing back to the "**Society of Physical Research**" established in 1873.

JBR has now reached its 10th anniversary and recently published a special issue about cellular respiration.
The web site is: **http://iopscience.iop.org/journal/1752-7163**

2.5 DIAGNOSING INFECTIONS THROUGH MOLECULES IN PATIENT BREATH

Breath has long been used to diagnose disease. Since the first breath-based test was approved (for the bacteria causing stomach ulcers), a range of technological advances has vastly expanded the possibilities for detecting specific components in breath.

Although there is the potential to diagnose a variety of diseases through breath analysis, much work is required to translate the research into clinical reality.

At Pittcon 2017, **Dr. Jane Hill** from Dartmouth College presented some of the latest research into breath analysis. In the following interview, she summarizes what has been achieved so far and the potential for the future of breath analysis.

An interview with Dr. Jane Hill, Dartmouth College, conducted by April Cashin-Garbutt, MA (Cantab)

Q **When was it first discovered that patient breath could be used to diagnose disease?**

A The breath of patients has been used to diagnose disease since was first recorded, as far as we know, by the ancient Greek physician, Hippocrates, who wrote described “fetor hepaticus” (breath of the dead) back in ~400 BC, a reference to the pungent smell of thiols on the breath of patients with liver dysfunction.

Over the past couple of decades, the analytical technology and the clinical microbiology insight needed to create a breath test for infections has seen a considerable number of advances and there are now numerous research teams focused on evaluating patient breath as a diagnostic fluid for infectious diseases. Significant challenges still need to be overcome.

> "The first, FDA-approved system using breath to diagnose an infectious agent was approved in 1996. It's a urea-based breath test for Helicobacter pylori (the stomach ulcer bacterium)."

Q **In what ways has our understanding advanced in this field over recent years and what advances in technology have been important to your research?**

A The collection of breath samples in a reliable manner is one of the ways we have increased our understanding over the last few years in this field. Components in the breath are affected by exercising, sitting down and standing up etc., and so it is vital to have a reliable baseline of breath measurement.

At Dartmouth College, we use state-of-the-art analytical equipment to analyze breath samples, this includes secondary electrospray ionization mass spectrometry on a system we modified system from AB Sciex, which enables us to carry out essentially real-time analysis of volatile.

Another instrument that we use is a two-dimensional gas chromatography time-of-flight mass spectrometry system from LECO, the profiles of which are more chemically comprehensive. With these instruments, we are able to look for molecules in the breath comprehensively.

Developing real-time sensitive detection instruments that can be translated into a clinical setting is a vital area of research that needs to be further developed.

The inclusion of medical doctors on breath research teams i.e., alongside analytical chemists, has also helped focus breath-based infectious disease research questions. Statisticians, engineers, and clinical microbiologists are also becoming increasingly more common members of breath research teams, in a bid to aid translation of research into the clinic.

Q **How can translation from laboratory to clinical setting be achieved?**

A At this moment in time, very few proof-of-concept biomarkers end in successful translation into a clinical setting. I suggest that success is more likely when the underlying biochemical hypothesis is strong and the link to disease pathogenesis well known. Without a solid understanding of the organism and the host's response during infection with the organism, as well as the clinical confounders, such as co-morbidities, or biochemical mimics, progress will founder.

Researchers need to be aware that there are several stages in the development of a breath test for infectious diseases. The process is lengthy, involving the iteration of results obtained via data discussions with chemists, medical doctors, clinical microbiologists, etc.

> "I suggest that success is more likely when the underlying biochemical hypothesis is strong and the link to disease pathogenesis well known."

Translation of research is achievable; however, it is important that assumptions are minimized and always contextualized with input from by clinicians.

Cell culture systems can and should be used when considering a developing technology, which might, for example aim to measure volatile molecules particularly quickly or sensitively. In this experimental system, evaluating the technology is an essential proving ground.

> Then, if the proof-of-concept works, a translation has to be done in conjunction with medical doctors and other people with insight into a clinical application. Although knowledge of the organism and the patient population may be well-known, other clinical confounders must be considered.

These confounders are quite different to the more discrete and well-understood problems analytical chemists typically consider in a laboratory.

For example, a patient may have a co-morbidity factor, such as an underlying heart disease issue or diabetes, which can influence the molecules in the breath as well as the control patient populations recruited to the breath study.

If the heterogeneity that occurs in a patient population is not accounted for, both in terms of the diseases that might appear similar to the one that you're trying to develop a diagnostic for, as well as those that might exist in the general population, your evaluation of putative breath biomarkers may not be effective.

> Prevention of this common error is usually obtained with the active involvement in the project, including the early planning stages, by clinical staff, such as physicians and nurses.

A common mistake when trying to translate laboratory research for clinical application for an infectious breath test is assuming that the culture volatile molecules grown in the lab will be exactly the same as the molecules detected in the human patient, which is highly unlikely. It is therefore vital that scientists remain hypothesis-driven and that research includes both in vitro and in vivo phases.

Q **What do you think the future holds for diagnosing infections through molecules in patient breath?**

A There are still some stages to overcome, getting FDA and MHRA approval is nontrivial, taking several years to make it through the approval process. However, over the next decade or so we will see more breath tests come onto the market. My team hopes to be a part of that wave.

Q **Please can you outline your talk at Pittcon 2017?**

A My talk at Pittcon covered the effective use of cell cultures to develop proof-of-concept datasets useful to the clinical context. I also talked about the design of these experiments within the bigger context of the clinical relevance.

In particular, I focused on the following three bacterial infection contexts:

Tuberculosis

Tuberculosis kills approximately one and a half million people each year making it the biggest infectious disease killer in the world each year. I presented work expanding from the lab bench to patient breath analysis.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic pathogen that particularly impacts vulnerable populations, like those with cystic fibrosis or chronic obstructive pulmonary disorder, creating a high level of morbidity and mortality for those patients. We have carried out a lot of bench and clinical work in this context and I tried and make those connections in my talk at Pittcon.

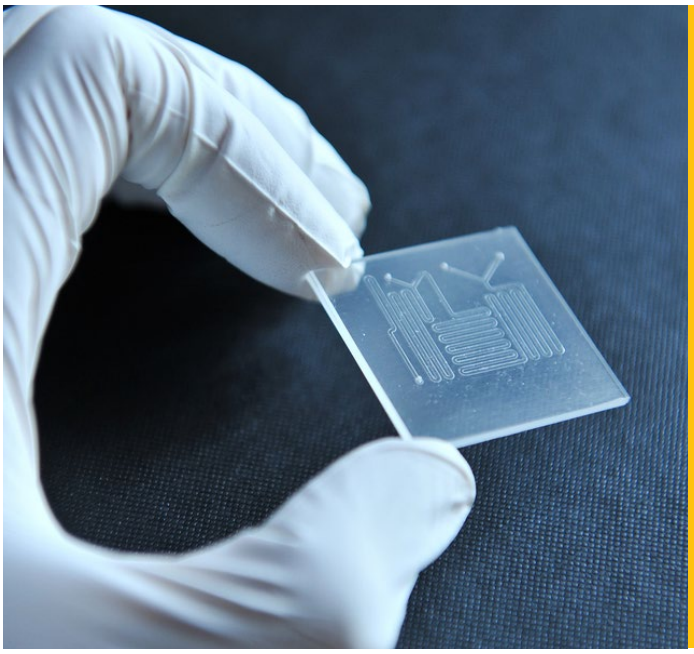
Multidrug-resistant bacteria

I shared our early work on possible strategies to target the identification of multidrug-resistant bacteria.

Q **Where can readers find more information?**

A **http://iopscience.iop.org/journal/1752-7163**
https://engineering.dartmouth.edu/people/faculty/jane-hill

2.6 ADVANCING CHEMICAL AND BIOCHEMICAL MEASUREMENTS USING MICRO- AND NANOFABRICATED DEVICES



Dr J Michael Ramsey is the Goldby Distinguished Professor of Chemistry and Director of the Center for Biomedical Microtechnologies at the University of North Carolina. He has played a key role in the development of microfluidic technologies (including founding several companies specializing in their manufacture)

In particular, he uses these technologies to create tools for the pharmaceutical and biotechnology industries. These include microfluidic point-of-care diagnostic applications and nanofluidic devices for single molecule DNA characterization.

In his talk at Pittcon 2017, which is reported in this section, he detailed the development and applications of micro- and nano- fabricated devices in chemical and biochemical measurements.

A transcript of one of Michael Ramsey's talks at Pittcon 2017.

Here I will cover the applications of micro- and nano- fabricated devices in chemical and biochemical measurements, an area we've been working in since the mid-90s. A requirement of NIH funding is showing any potential conflicts of interest. I originally trained in laser based chemical measurements in the early part of my career, late 80s and early 90s. We started thinking about using micro-machining techniques to build small fluidic devices.

We first started doing capillary electrophoresis in micro-fabricated formats using laser-induced fluorescence. Our efforts in the last decade or so have focused on integrating the nanoelectrospray and doing high performance capillary electrophoresis.

> Our group is broken up into four different parts and we have an effort in using micro-fluidics to build the point of care with CLIA-waivable types of molecular diagnostics and doing single molecule detection in a parallel array, a million assays at a time on either nucleic acids or proteins.

We have activity where we look at a single molecule of DNA, which are very large fragments to get long-range information, something that the next-generation sequencing doesn't do very well. Finally, we are also involved in a gas phase experiment that I'll talk about today, thinking about ways to make small handheld devices that incorporate mass spectrometry.

> If you're going to do a chemical measurement, our thought process is to think about how to do small package chemical measurements, like a pH electrode, or chemical sensors, both are large activities in this area. It's a fantastic way to go if you can execute your goal.

Small size, weight, power, as well as low cost is possible. They suffer from matrix effects in the accessible chemical space. If you could design a pH electrode for any molecule that you would desire, that would be a great way to go. If you want to look at lots of different compounds, you can build an array of these types of sensors. People try to do that and they'll sort through the Merck index.

There's challenges in getting orthogonality and getting an individual sensor to respond to only one compound, but it suffers from the same sorts of issues that chemical sensors themselves suffer from. DNA is one situation where you can execute this strategy very effectively in terms of having complementary orthogonality of your target molecules.

Another strategy, which is the one this session is focused, is handheld instruments, which have versatility. We can go back to the lab, have lab traceability using an assay and the results, but there's been issues with the size and the cost of these instrumentation.

There are a number of examples that are already commercially available, such as x-ray fluorescence, handheld Raman FTIR, and ion mobility. These devices range from about six-tenths of a kilogram up to like 2.2 kilograms, and these spectroscopy tools have very good specificity. They don't have great sensitivity, but Ion mobility has fantastic sensitivity. You can put packages that have a very small size, weight and power into the system, but its specificity, its chemical informing power, is relatively low.

Mass spectrometry can give us a molecular weight using a chemical formula in the tandem MS and we can get a chemical structure. There is a lot of chemical informing power in mass spectrometry. We can address gases, liquids and solids from the percent range down to parts per quadrillion and these would be very nice performance factors to have in handheld devices.

Bringing laboratory into portable systems here in commercial systems, the devices have been stuck up in the double-digit kilogram size, weight and power and creating devices that are truly in this handheld is a challenge.

We have considered ion traps to overcome this, and our analysis of different types of mass analyzers and the sort of physics of these devices is very attractive to miniaturize. For example, the resolution does not depend upon size. That's a good starting parameter. And as you shrink these devices down, the charge passed these scales in an attractive way.

Our focus has been initially on cylindrical ion traps, and we try to operate and perform mass spectrometry in a small device. Theoretically, this is achievable. At the half-millimeter radius, cylindrical ion trap operating at **6.5 MHz** using electron ionization from thermal decomposition, a hot filament or a glow discharge source of electrons.

When looking at a mustard gas simulant at mass spectra ranging from **60 millitorr,** which is a very high pressure to do ion trap mass spectrometry, up to **1.2 torr**, there's some loss of resolution and you get the same type of mass spectrum as we move up into this very high pressure region for doing mass spectrometry. If we zoom in on mass **75 ion**, you can see an increase in peak width as a function of time. Think of it like a damped harmonic oscillator is a good way to describe this sort of phenomenon.

If you shrink these devices down, charge is an issue, and so we came up with a different sort of architecture for an ion trap that mimics a linear type of ion trap. This is instead of having a single point trap where we're trapping ions, we have a line similar to a linear flagpole ion trap structure.

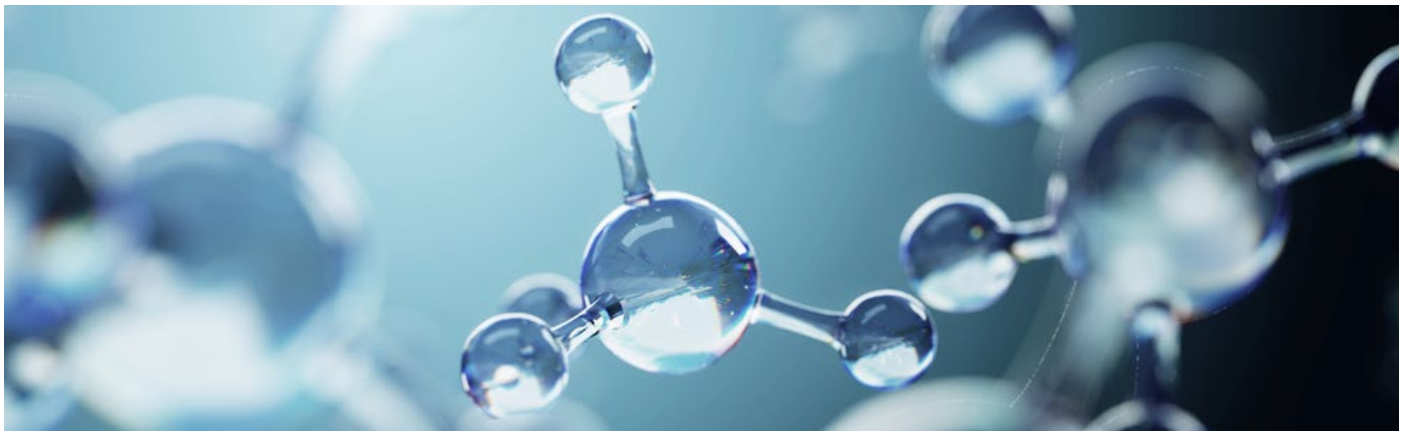
But again, a very simple sort of structure is the way we think about this, for the ring electrode, you have a piece of sheet metal, and to make the CIT, you drill through a cheap metal and form a slender hole. Then you move to a milling machine and punch that hole. Then, you translate along one axis and make a slit in that piece of metal for your ring electrode. We're typically using grids here for our end caps, which seem to be efficient.

Mass spectral performance has the same sort of resolution in a stick spectrum for a xenon. The half-millimeter CIT and the half-millimeter by five-millimeter slit, and the slit spectrum looks a little better because we have better signal to noise ratio.

When moving to higher pressures using air buffer gas, the focus is not consumables, and so we just want to use ambient air as our buffer gas in these experiments. The slit structure moving from **9 milliTorr** up to **1 Torr** for acetylene, you can see there's significant band broadening here, but you still get signatures that are characteristic of this particular molecule.

We've shown that the way to combat that band broadening is to use the physics, which says that if we increase the RF frequency, we have to get the resolution back. This is a demonstration that we do get that recovery of resolution moving from **6 MHz** here up to **60 MHz**. At **1 Torr** of air, we're down below **1 AMU** resolution here with this particular experiment. Some of the spectra looked different because of low mass cut off, found in these types of experiments.

The other characteristic that we see as we move to higher pressure is the abundance of the various ions, which is different than it is at low pressure.



I'm also involved in 908 activities, as the science founder of that company. The initial product that we put out was this M908, it's a 2-kilogram box that people can carry around, and it allows you to sample the atmosphere and detect materials. It also has a chemical desorption capability, where you would use a swab and insert it in a thermal desorber.

There is no helium, it's just using air, but it is a sensitive device, using glow discharge as a source of electron for electron ionization. It's about one PPM, the compounds will maybe be 100 parts per billion or some you'll be multiple PPM. It's not competitive with ion mobility spectrometry in terms of sensitivity. So, we looked for a different ion source.

We looked at **atmospheric pressure chemical ionization (APCI)** and coupling that to high pressure mass spectrometry, which seemed like an obvious direction to go.

If ionization occurs at atmospheric pressure, lots of collisions occur on the discharge with the energetic source of energy here to produce protonated water clusters. They do proton transfer and preferentially form these protonated anolyte apparent molecules.

For most of the experiments that we do, we don't operate at the entire system at high pressure. We tend to use electron multipliers that don't like to operate at one-fourth types of pressure. This is a differentially pumped system, so we can characterize the system.

We also do experiments in the 908 technologies entirely using Faraday cup protectors, therefore the entire system is operating at these high pressures. Instead of having a glow discharge up front, we have a chamber that has a needle that has a few kilovolts of voltage applied to it to form a grounded discharge.

There's also a sampling orifice, making this region at atmospheric pressure. A sampling orifice that delivers those ions into the mass analyzer region, there's an ion trap that's operating in the vicinity of **1 Torr**, and then also a leak through the end cap of the cylindrical ion trap to an electron multiplier, and so there's a turbo pump and a rough pump.

This device is configured in our laboratory with a grounded discharge the top. The Ion trap with the radio RF and the axial RF is applied here, high voltages for the electron multiplier in this experiment, so you see can a 1-centimeter light scale if you peer in from the sides.

These experiments were performed with traps that are slightly smaller than most of the work we've done, 200-micron ID single CIT in these experiments rather than arrays of CITs. Operating frequencies for the radio were around **16 to 18 MHz** and variable for the axial RF. Usually, we're applying somewhere in the range of 5 to 10 volts in these experiments, so we're operating at **1 Torr** of air as the background buffer gas here.

We were also controlling the gating ions access to the mass analyzer region by controlling the voltage on the aperture, which were experimentally determined. **17.5 volts** were used for the on voltage and minus **6 volts** to turn the ions off. We also included a cooling time and ramp time, so we're doing a double residence type of ejection from the ion trap to generate the mass spectrum, and then also an emptying and clear-out time.

The effect of voltage on this particular system, the chamber was about 1 centimeter, 2 centimeters in diameter with the grounded needle sitting in it, and about 1 centimeter axial extent. The needle was in the center of that chamber and looking at the signal as a function of voltage.

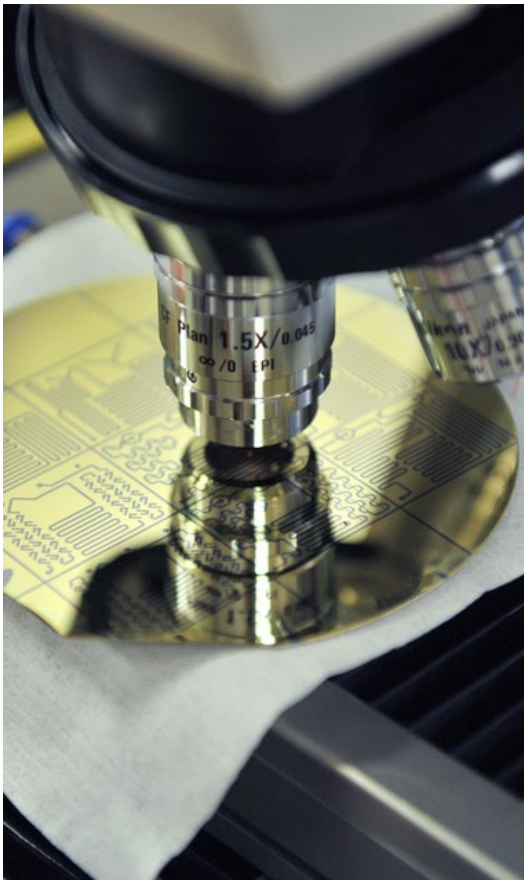
We've chosen for most of our experiments to operate at **4 kilovolts**. The spatial position of that needle from previous data was generated at a particular point, 3-millimeter separation away from the aperture for the needle in the center of the chamber. There's greater sensitivity, almost an order of magnitude, by moving it away from the center and back further from the aperture.

Just looking at the intensity of our signal for BMMP, nerve gas stimulant provided from a permeation tube, the concentration is about one part per million from the system, and we're varying the time that we have the gate open from a few milliseconds out to 20 milliseconds, with fairly a linear response.

Without the conversion to a mass spectral scale, the ramp time and the concentration is changing not in a quantitative way, but we're changing the time at which an open vial is presented to the open aperture to the sampling source. As we move to higher concentrations, you can start seeing the dimer for BMMP.

> The resolution that we would expect for this size of trap at the pressure and frequency that we're operating is **2 to 3 AMU**, although this is not super high mass accuracy type of mass spectrometry it is a lot more informative than ion mobility spectrometry.

When looking closer at the sensitivities that we could achieve here, using BMMP again, using the same CIT that I've been talking about, **200-micron** radius, about 17 MHz operation RF radio frequency. We're now using the same KinTech device to dilute down this 1 part per million sample lower. We're down to 20 PPB here, the lowest concentration we tried, and close to the detection limit.

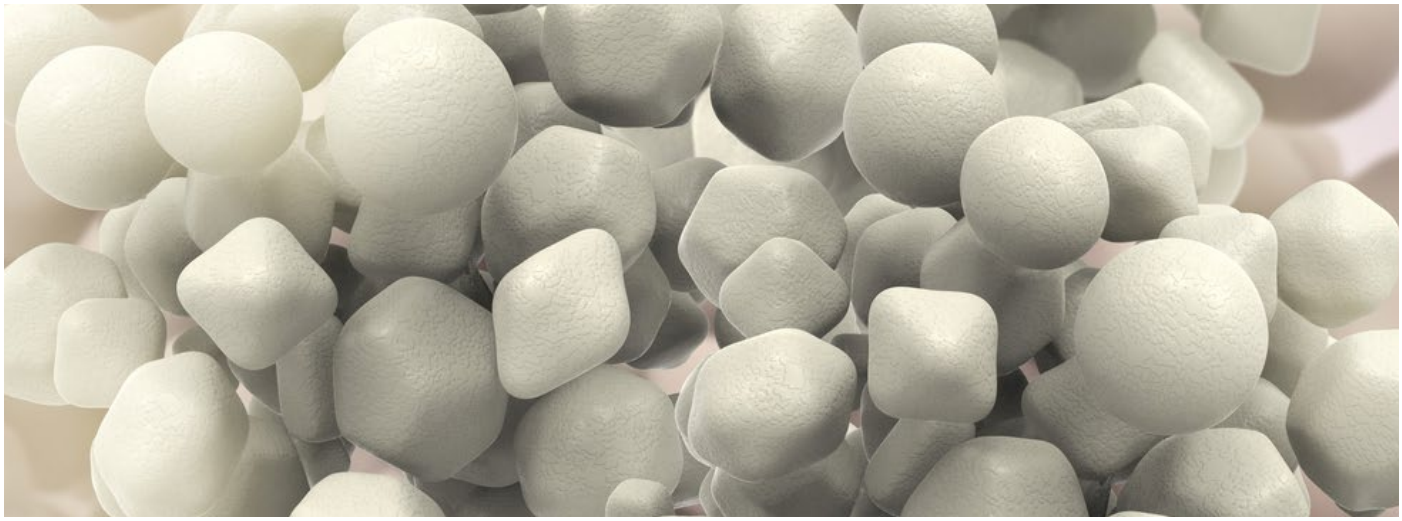


We're down a factor of 500 below what we would see with the one-millimeter type of trap, using electron impact magnetization. This is starting to get interesting and competitive with ion mobility spectrometry.

Can we do this with a slip trap? When looking at a **250-micron** slip trap the axial extent of this particular trap would likely be a few millimeters, but we can get a similar resolution spectra here from these slip traps as well.

The M908 came out a few years ago, it is 2 kilograms, with 1 part per billion sensitivity. It senses the vapor through a snorkel and thermal desorption underneath the lid. The new version, MX908, perforates APCI, has a slightly larger form factor, making it 3.6 kilograms. It has a slightly different arrangement with the front end, the inlet and is a bayonet sort of adapter similar to an SLR camera that you'd mount a lens on.

You're either looking at a snorkel to look at vapor materials or this is an adaptor to do some thermal desorption, with swabs or tickets that you insert into a slot and press it against the heater. This device has a Faraday detector, operating at **1.5 Torr**.



In an experiment, not carried out in our laboratory, looking at GA a live nerve agent, they take both negative and positive ions simultaneously. The response time was two to three seconds for this PPB GA response. It was seen that the clear-down time is on the order of a minute to a minute and a half, which is a good performance characteristic. VX, a low vapor pressure nerve agent was applied in nanograms to a swab, and doing thermal desorption, and the positive and negative ion response.

We have interest in doing those types of materials here, for example fentanyl, which is an extremely potent opioid and various variants of that material, so detection limits for the fentanyl is on the order of 25 milligrams by thermal desorption in this device.

MSMS for chemical information is extremely important and the ability to do MSMS at **1 Torr** pressure. Stability diagram with various ways to isolate different ions. A typical way would be to do apex isolation, and change the AC and DC potential to isolate a particular ion or you could do boundary isolation for the parent ion.

Can we do that with the slip trap? We did an experimental determination of a stability diagram for a slip trap at a **1 milliTorr** here and **1 milliTorr** there as you increase pressure, and it extends the boundaries, the trapping boundary of these devices. We published a paper about that a decade or two ago, but we're typically operating in these sorts of conditions.

We used a slip trap of about half millimeter, aniline, dimethylaniline as a sample, and it showed our ability to do apex isolation of the lower mass aniline ion. CID of N,N-dimethylaniline, a full scan, and in a configuration where we can only see the pyrantine and fragmentation by CID.

All these fragments with the resolution that we have on these instruments gives us a lot of capability in terms of fingerprinting or identifying molecules.

There is another CID spectrum in a slip trap where we're doing boundary isolation, in this case of trichlorobenzene and using different amounts of energy to do fragmentation and identifying different types of fragments. APCI, HPMS, what we've seen is several hundred to a thousand x improvement in sensitivity over electron ionization.

That's getting us from this parts per million range down into the low part per billion, which is making us competitive with ion mobility spectrometry, but we would argue that the informing power of this type of mass spectrometer greatly exceeds what you can achieve with ion mobility spectrometry. Our feeling now is that we can put libraries of compounds on this type of instrument.

Note that we do pay a price, we're currently 3x the swathe of the smallest ion mobility spectrometer, the JCAD or the Smiths Detection LCD format. I would also argue that ion mobility has a few decades advance on us in terms of looking at how to put that sort of instrument into a small package, we're still at the stages in terms of what these miniature mass spectrometers and high pressure mass spectrometry can do and we hope to further reduce the size of these types of instruments.

2.7 HIGH-RESOLUTION CAPILLARY LC/MS-MS IN STRUCTURAL ELUCIDATION AND MEASUREMENTS OF BIOLOGICALLY IMPORTANT GLYCANS

Milos Novotny is Distinguished Professor Emeritus and Director of the Novotny Glycoscience Laboratory and the Institute for Pheromone Research at Indiana University. He is highly acclaimed for the development of analytical separation methods and the identification of the first mammalian pheromone.

Milos Novotny gave a presentation at Pittcon 2017 entitled "**High-Resolution Capillary LC/MS-MS in Structural Elucidation and Measurements of Biologically Important Glycans**" during which he discussed the benefits of high-efficiency separation of glycan mixtures obtained from biological samples prior to positive identification. He also described the latest technologies employed for comprehensive classification of glycomes of biological significance. This section provides an overview of the key points of his presentation.

An abstract from Milos Novotny's talk at Pittcon 2017 where he received the LCGC Lifetime in Achievement and Emerging Leader in Chromatography Awards.

LASERS AND CHROMATOGRAPHY

Initially, we thought that the laser was something that physical chemists and physicians were mostly interested in. But then a chapter by **Ed Young**, published in 1981, listed four papers that described a couple of uses of lasers in **high-performance-optical computing (HPOC)**.

> Lasers have a lot of power, and so they need to be supervised by the laser specialist. They were shining lasers into typically ten microliter volumes, and the detection limits are not all that wonderful. Then **Gene Jorgensen**, who was a graduate student, led uses such as, light scattering then flowmetry detection in order to detect lipids. He used a relatively budget laser at **$400** to carry out his work.

People doing papers on lasers use fluorescent detection, the system that was described in Science in 1977 used a modulated helium-cadmium laser. These had detection limits of **760 femto grams** of alpha toxin materials. He was also pointing out that if the detection cell were only where the laser was shining, it was more like **7 femto grams**.

What we were doing at the time, was something that eventually led to the successful marriage of lasers and chromatography. The first time that I reported on the viability of capillary liquid chromatography was at the Advances in Chromatography Symposium in Amsterdam.

It was during the discussion session that I had to describe some of our first experiences and it was later published about half a year later. We were using both open tubes and packed capillaries, as well a drawing glass out of polygraphic capillaries to make small diameter type columns to do chromatography.

We were getting over **100,000** plates or so, which was particularly good in 1977, however, it also stirred some controversies especially when HPOC already worked.

In 1980 the Gordon Conference, analytical chemistry was when **Gene Jorgensen** gave a very brief, informal talk as a new faculty member at the University of North Carolina on capillary zone electrophoresis. They used a conventional flowmetry detector to look at amino acids which would become a classical paper a year later.

In 1981, at the HPOC symposium in Avignon in France, Jorgensen gave a lecture. He presented an abstract, that was accepted, and people thought that this was very important work and that was only the beginning of many efforts in capillary electrophoresis.

There was one laser wavelength good for the labelling of these compounds, and help you to introduce fluorophore into the molecule, and then you can change these things.

In 1981 there was the introduction of a lot of exciting methods, such as subcritical fluid chromatography, capillary liquid chromatography and capillary electrophoresis.

There was also lots of interest in Japan at the time, and there were some Japanese scientists, post doc in my laboratories. We organized a US/Japanese joint seminar in micro separation techniques in Honolulu. Several years, later, there was a number of reports already on using LC ALF combination.

I'd like to give credit to the first man who carried out the work in capillary liquid chromatography in my lab and that's **Dr. Takao Tsuda**. This is just one of the early compounds that he developed, where we put the packing inside of those polygraphic capillaries, draw them out hang them on the thick, glass walls of the capillary. We published some of these papers in 1978 in collaboration with Japanese scientists.

In terms of improving the detection technique, concentration sensitive detectors were gained from getting into the small dimensions. We were of course dreaming at that time about the combination of LCMS, using at the time the systems which were delivering microliters and nano liters per minute rather than millimeters per minute, and therefore they have consequences for future of LCMS. We had to wait a decade or so before that happened.

This year, there was a Japanese seminar held in Honolulu that was funded by National Science the Foundation and Japanese Society for Promotion of Science. **Professor Diguchi**, **Gene Jorgensen**, [**Ed Young, Jay Hennigan, Joe Harris**, were all interested in detectors. There were also electrochemists who were interested in smaller dimensions of the capillary.

Meanwhile, there was lots going on in the technology of these columns. As we pointed out in our paper, open tubes could be used but you had to go to a very small dimension and diameters, less than ten microns. Smaller capillaries are the type of columns which are used today.

A couple of years later we are still building on this work and papers are being published. Experiments in the beginning, we used cadmium laser, but that laser is too awkward.

There were other ways to tag compounds like shooters. We didn't want the lasers to have a high power because reached problems such as optical and variability. The windows of detection cells were getting created when you used the high-power lasers.

The stability and lifetimes of the helium cadmium laser was a serious problem. A **442 nanometer** line, there was not much of any chemistry. People are showing open fibular column, one beak or two beaks, which were the fluorescent dyes that no one cared about, or maybe riboflavin for the blue line.

That's the system that we reported to the symposium in New York, we borrowed from the laboratories near to do this and connected this to the capillary column. We tried all kinds of different optical cells and a simplest approach worked well. We looked at the capillary and its appropriate angle and the fluorescent.

In New York City, in 1984, there were about six presentations about capillary connection to a laser based detector. We were looking at bio acids, and going from capillary LC to CE, which was relatively easy.

There is an optical window there and the lasers changed quite a bit, because we got tired of the helium laser and the expenses associated with it. There was a lack of stability and the chemistry was necessary to adjust the wavelengths or be regulated.

The use of fluorescent dyes were also hopeful, I talked about the sugars we first used to label with these. We were struck by how wonderful the separation was, after labelling, and the reducing end of the sugar and one of the fluorescent labels worked.

Separating the isomers of sugars is very important. What's neat about this reagent is that it's not florescent but by the time it built a reaction it made these florescent and which are right spectrally with the blue line on the laser.

First, analogies of glycoproteins were shown in an article by capillary electrophoresis, where I took an interest into the sugars and the techniques that these people them using to analyze the sugars. They were achieving significant sensitivities, which was something we were not able to do.

The combination of CNI is a good marriage, but capillaries can accept very small cycle volumes at the inlet. That has been a drawback of capillary electrophoresis overall, because the laser used in fluorescents is inherently highly sensitive, you can't compensate for these problems. Not only for the polysaccharide but also polynucleotides. Unfortunately, the same cannot be said, as yet, about CE mass spectrometry, but I have high hopes this will change in the future.

CNI has been effective interfacing with the analysis of the content of single biological cells. That's not the avenue that our group has pursued, but **Ed Young, Gene Jorgensen** and others have used their capability quite nicely. The other thing is that the procedures are reproducible, automatable, current applications to buy pharmaceuticals and to more increasingly take samples. You can multiplex capillary separations.

Capillary separations easily translate into work with microchips. CE is both complementary and competitive to capillary analysis. These are just some of the conclusions. There has also been an impact of the technology as a result of the DNA sequencing at the Human Genome Project.

I've chosen here to give some examples by **Norm Davickey** and there have been many others that used it. He nicely demonstrated in this application from the Netherlands that it can be used for both biotechnology as well as biomedical applications, as shown for example in an article where they were looking at the differences of glycans in the plasma during the pregnancy.

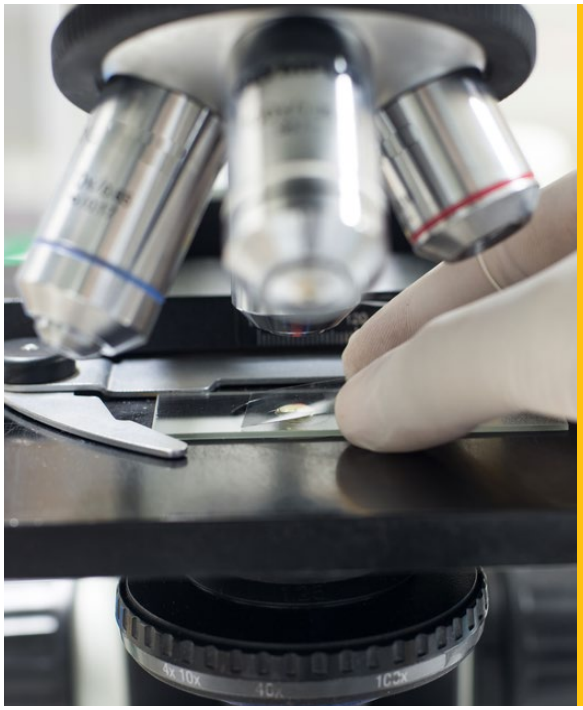
Another example is the separation of isomers and this is quite sensitive, as demonstrated many years ago, when an unnamed pharmaceutical company was given four micrograms of protein. Then it was that antibody, molecules and we were able to profile from that the composition of the sugars.

I mentioned that things can be isolated into microchips and I certainly enjoyed around ten years of common work with my colleague **Steve Jacobsen**, who is a micro fabrication expert. We looked at the cancer patients and their profiles.

This is a more modern version of the microchip that is micro fabricated, which generates about **800,000** plates. This is another application that we recently published last year, and that is again a demonstration that you can separate isomers. It is done after dying the acid by my annotation, which helps the acids to slow down and then to attach the fluorophore glycols.

I hope that I have been able to convince you that there has been a lot of time spent developing the tools over many years, and that the tool is very useful and certainly Andraj has made significant contributions through his work on the unique type of reagent, that we all use and has many further applications.

CONCLUSION



The reduction in size, and consequently increased portability, of mass spectrometry units has opened the way for a virtually limitless range of potential biomedical applications. Portable mass spectrometry instruments, with smart technology that prepares the sample for analysis so the user needs no specialist knowledge, make it possible for samples to be analyzed rapidly during a consultation.

It is possible that physicians may soon have a mass spectrometer in their consulting room to allow immediate, evidence-based diagnosis and individual tailoring of treatment.

There are already several mass spectroscopy-based diagnostic tests and numerous others are currently being researched or developed. Mass spectrometry may even find its way into the operating theatre to evaluate the extent of diseased tissue before it is removed, whereby avoiding the risk of leaving some in situ necessitating further surgery.

In addition, miniature mass spectrometers are proving valuable tools in security and safety screenings, forensic science and in on-site environmental analyses. The possibility of mass spectrometers being taken on space missions to analyze the atmosphere of other planets is even being explored.

Presenters at Pittcon 2017 described some of the latest additions to the vast array of potential applications of miniaturized mass spectrometers. The possibilities within the biomedical arena are particularly impressive. In addition to speeding up conventional diagnostic testing, mass spectrometry makes it possible for some diagnoses to be made using less invasive testing —breath analysis.

It is commonly known that sulfurous (thiol) breath is an indication of liver disease and that sweet (ketone) breath is a warning sign for diabetes. Breath also contains wide selection of proteins, lipids, bacteria, viruses, inorganic compounds, and inflammatory molecules that can be identified using the high sensitivity of mass spectrometry.

Signature breath compositions are being correlated with disease processes so that the precise composition of breath can be used to diagnose certain conditions.

Research in underway to design tests for diagnosing tuberculosis and infection with the opportunistic Pseudomonas aeruginosa or bacteria with multi-drug resistance from the breath.

Breath analysis is also being investigated for evaluating the impact of the environment on health, testing for illicit substances in drivers and evaluating the effectiveness of the protective gear used by fire-fighters.

Mass spectrometry-based proteomics has become a powerful tool for studying the changes in protein expression during the aging process that give rise to impaired function. It has already been shown that there are age-dependent changes in the concentration of 19 proteins associated with muscle function. Researchers are now using mass spectrometry techniques to studying why immune responses decline with age.

New mass spectrometry technologies may also lead to the development of novel treatments for addiction and other mental health issues. In his talk at PIttcon 2017, **Prof Sweedler** explained how new mass spectrometry protocols are allowing us to identify rare neurotransmitters, define the precise composition of the brain and study neurosignaling pathways.

Advances in mass spectrometry technologies, and in particular the miniaturization of equipment, has broadened research horizons hugely and we are likely to learn of more exciting discoveries, facilitated by mass spectrometry, at Pittcon for many years to come.

3 FOOD SAFETY

Food and water safety is an essential part of everyday life. Marketing of contaminated food and water can have far-reaching, and potentially devastating, consequences.

INTRODUCTION

To safeguard against such tragedies, the food industry has thus been subject to particularly strict regulations for many years in order to ensure confidence in the quality and safety of its products.

In recent years, the risk of intentional adulteration of consumables for financial gain has increased.

This is highlighted by the case in China of the industrial chemical melamine being added to baby formula milk in order to elevate apparent levels of protein and mask dilution, and by the discovery in 2013 that beef products marketed in Europe had been bulked up with cheaper horsemeat.

Similar scams are now being reported for honey and for olive oil in which products are being diluted with similar products that are cheaper than those indicated on the label.

Although the latter are not detrimental to health, unlike the addition of melamine that made **300,000** babies ill, they are fraudulent practices that need to be stopped.

As honey is becoming more scarce, some products are being diluted with cheap sugar syrups to increased profit margins. Similarly, as a result of preferences, due to improved taste or associated health benefits, for honey from particular regions or from beehives in areas with a high concentration of a particular plant, these honey varieties are retailing at higher prices. A trend for intentional mislabeling of the origin of honey in order to increase retail value has thus ensued.

Quality virgin olive oil can also command a higher price than lower grade olive oil or oils from other sources, such as hazelnut, sunflower or soybean. A recent analysis of five leading brands of extra-virgin olive oil sold in the USA revealed that almost three quarters of the samples were of poor quality or had been diluted with cheaper oils.

Alarmingly, there is also the risk of food being intentionally contaminated for more sinister reasons. Current high levels of terrorist activity in both Europe and the USA have raised concerns that toxic substances or deadly pathogens could be added to foodstuffs in acts of bioterrorism.

Alarmingly, there is also the risk of food being intentionally contaminated for more sinister reasons. Current high levels of terrorist activity in both Europe and the USA have raised concerns that toxic substances or deadly pathogens could be added to foodstuffs in acts of bioterrorism.

Within weeks of the 9/11 tragedy, the Center for Food Safety and Applied Nutrition (CFSAN) had redirected **30%** of its intramural research program and most of its extramural program to food security.

As has been described for honey, it is also important that the origins of meat and fish products are confirmed by scientific testing. For example, some unscrupulous fishermen are netting fish from areas where fishing has been restricted to ensure the sustainability of fish stocks. It is important that such deception is stopped to protect both the environment and the rights of honest fishermen.

In order to guarantee the food industry's strict quality and safety standards and stamp out dishonest and deceitful practices, reliable laboratory instruments and methodologies are needed.

The tests must be able to verify that food and drink products are not contaminated and are indeed what they are purported to be. In addition, the analyses have to be easy to run and provide results rapidly so they can be used routinely.

This chapter highlights the concerted efforts of scientists and testing companies to enhance food safety, assure security from terrorist interference and protect the livelihoods of honest food suppliers.

3.1 LATEST ADVANCES IN FOOD SAFETY: AN INDUSTRY GUIDE

Increases in legislation to protect against contamination and mis-selling across the food industry have posed scientists with the challenge of creating a battery of effective tests that are easy to execute and provide results in a timely manner.

Presenters at Pittcon 2017 described several emerging technologies that have been developed in order to help regulators enforce food industry legislation. Collaboration of testing companies has further helped eradicate unscrupulous practices in the preparation/ labeling of foodstuffs.

This section provides an overview of the actions that have been taken and research that is underway in order to ensure the quality and safety of the food and drink we buy.

INTRODUCTION

The deliberate or accidental contamination of food products is a prominent issue with wide-ranging consequences in the 21st century. It can encompass multi-billion-dollar fraud, hazards to public health and even deliberate attempts to harm human life.

The Chinese melamine scandal of 2008 and the European horsemeat scandal of 2013 made international headlines. Also in addition to food fraud, international governments are confronting the prospects of food contamination being used as a biological weapon for terrorism.

In order to combat these potential threats, regulatory agencies are taking measures to protect public health. For example, in the USA, the FDA has recently introduced the first US legislation against deliberate food adulteration, requiring manufacturers to put much more stringent protections in place throughout the food production pipeline.

Meanwhile, in Europe, recent legislation has seen a measurable impact on levels of fish fraud, an activity that poses a threat to consumer health, as well as threatening fish sustainability and the livelihoods of fish producers.

In this guide, we will look at food safety issues in depth and explore the emerging technologies being used to assist regulators in fighting these modern-day challenges. Through case studies we will cover adulteration and contamination of food and drinks, and also consider how food analysis assists the industry in quality control.

Advances include the use of NMR spectroscopy to analyze olive oil, wine and honey, laser diffraction to ensure coffee and chocolate quality, and ion chromatography to assess drinking water safety.

The very latest research and advances in this area were presented at this year's Pittcon, which took place in Chicago March 5-7 2017. The Pittcon symposium on food analysis looked at innovative analytical methods, including novel applications of NMR, wireless sensors and DNA barcoding.

Presentations were given by manufacturers at the forefront of advancing these technologies, as well as by the analysts and regulators who are putting them to use in real-life scenarios.

For example, the symposium "Food Analysis – Looking Beyond Mass Spectroscopy", organized by Katherine Carlos and Lowri deJager of the US FDA, featured a presentation from GE on "Multivariable RF Based Sensors for Food Quality and Safety" which outlined the development of wireless technology in food quality control.

In another presentation, "Food Authenticity: DNA Barcoding and Genomics", **Rachel Gloyer** from the UK-government affiliate FERA Science Ltd, showed how recent developments in DNA sequencing are being applied to detecting and tracking food pathogens.

Furthermore, all of the major spectroscopy and analytic science companies were in attendance at this year's exposition, including **Perkin Elmer, Bruker, Renishaw, Metrohm and Wyatt Technology**, making Pittcon a great opportunity to see and hear the latest trends and advances in food analysis.

RECENT CHALLENGES IN FOOD SAFETY

The issues of food safety and adulteration are not new, but they have taken on different dimensions and guises in the 21st century. We now live in a globalized world where food is frequently produced in one country and served up on plates thousands of miles away.

Imported food poses a challenge for food safety due to both legal and logistical restrictions on inspections and standards. And with access to global markets and increased competition, food manufacturers may have additional incentives to cut corners and flout the law, sometimes at the expense of the consumer's pocket and their health.

We also live in a post-9/11 era where the threat of terrorism is a presence in our lives. Bioterrorism, including through food-supply contamination, is a legitimate concern that governments have had to take significant steps against in recent years.

This year's Pittcon featured presentations and exhibits from industry leaders, who are working to create the next generation of food safety technologies. The challenges that face regulators are substantial, but these advances are delivering new ways to tackle them and enhance the protection of producers, consumers and public health.

FOOD SAFETY AND FOOD FRAUD IN THE 21ST CENTURY

In recent years, there have been food safety incidents that made international headlines, which highlight the need for ongoing vigilance and continued innovation.

For example, in 2008, around **300,000** babies in China were made ill by formula milk that had been contaminated with the industrial chemical melamine and six of them died from kidney damage.

The melamine, which is dense in nitrogen, was thought to have been added to artificially inflate tests for protein content, allowing the product to be watered down. The Chinese government made several prosecutions in relation to the contamination, which also resulted in the collapse of the milk production firm Sanlu.

Even more recently, in 2013, consumers in Europe were shocked to discover that they may have unknowingly been consuming horsemeat, when it was found that processed meat products, such as frozen beef burgers, mince and ready meals, contained horse DNA.

The scandal was thought to have been through economically motivated intentional food fraud – horse meat is much cheaper in some European countries than other types of meat. The discovery also highlighted the complex chains of food supply stretching across the European continent, which can make keeping track of foods origins and suppliers extremely tricky.

BIOTERRORISM

In 2011 an outbreak of E. coli in Northern Germany affected over 3000 people, leading to the deaths of over 50. The episode was traced back to fenugreek sprouts, which it is believed may have been grown from contaminated seeds imported from Egypt.

However, in a report published earlier this year, researchers who modelled the spread of the outbreak said that the speed at which it emerged and the virulence of the particular strain of E. coli suggest the possibility it was deliberately caused.

WHAT IS BEING DONE?

In response to cases like these, governments and regulatory authorities have been taking action to attempt to reduce the risk of food adulteration through improved prevention and detection.

The USA responds to the threat of bioterrorism

In 2006, the US government introduced a new ruling that is intended to help safeguard the food supply against acts of intentional adulteration.

Previously, under the Bioterrorism Act, introduced in 2002, food businesses were required to register with the US **Food and Drug Administration** and provide them with shipment information and other records.

Now the **Food Defense** rule, which is the first specific US regulation against intentional adulteration, takes this further. Large food companies (smaller companies are excluded from the regulations) must now have a written food defense plan. This involves assessing vulnerability to attack at each stage of the food manufacturing process. Once identified, manufacturers must then put in place mitigation strategies to reduce the risks including monitoring procedures and corrective actions.

EU rules drastically cut rate of fish mislabeling

Another type of food fraud that affects both the USA and Europe is seafood mislabeling. Many types of fish are indistinguishable from each other, particularly when delivered in fillets or slices meaning they are easy to substitute or mislabel.

This practice poses harms for conservation and sustainability of fish species, as well as for consumers who are being defrauded and potentially exposed to unknown toxins. It is also detrimental to fish producers who may be economically disadvantaged against competitors who are operating illegally.

Recent analyses in Europe that looked at the most commonly bought types of fish found that species, such as cod, tuna, hake and plaice, were mislabeled in up to **40%** of cases. But the advent of widespread genetic testing has allowed the rate of mislabeling to be tracked and assessed in light of ever-tightening EU rules on food labeling, tracing, and standardization, as well as increased public awareness of fish fraud in recent years.

Data published in 2015 suggested that the effect of these factors has been a marked turnaround in rates of mislabeling, which averaged **4.9%** in a cross-Europe survey. By contrast, the fight against fish fraud has made less of an impact in the USA, perhaps due to less stringent, and often non-binding, regulations.

THE LATEST ADVANCES UNDER ONE ROOF

New developments in technology are emerging to assist regulators in meeting the challenges of food safety. This year's Pittcon offered the opportunity to hear from leading experts on the expanding armamentarium of tools and techniques available to identify food fraud and keep our food safe.

As well as more familiar analysis techniques such as NMR and MS, the symposium "**Food Analysis – Looking Beyond Mass Spectrometry**" also highlighted the advantages of Raman microspectroscopy, a non-destructive approach to characterize the composition of complex food products and the use of wireless sensor technologies and DNA barcoding in the food industry.

> Delegates heard directly from the US Food and Drug Administration and the UK government-affiliated FERA Science, how lesser known technological applications are being put to use to protect citizens right now.

Furthermore, there were exhibits from companies including Perkin Elmer, Metrohm, Bruker, Renishaw and Wyatt. These included technologies such as the Perkin Elmer AxION 2 Time-of-flight mass spectrometer, a device that provides high-speed analysis of food contaminants, and Metrohm's beverage analysis devices that harness Raman spectroscopy and ion chromatography.

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3.1b CASE STUDY: ADULTERATION AND AUTHENTICATION OF OLIVE OIL

Olive oil is one of the world's most adulterated products. What are the reasons for this? Firstly, it's a product that is growing in popularity – consumption in the US has grown by **50%** over the last 10 years. It's also one that's able to command a premium price in comparison with other types of oil, as consumers are willing to pay for the product's unique health and sensory qualities. This is especially true for extra-virgin varieties. Hence, **olive oil is prime for economically motivated adulteration**.

A recent study of extra-virgin olive oils on sale in the USA – the world's dominant consumer of olive oil – found that **73%** of samples taken from the five leading brands failed to meet international sensory standards, indicating that they had been oxidized, were of poor-quality or had been adulterated with cheaper refined oils.

And the US Food Fraud database contains over **260** incidents of oil adulteration. Examples of adulteration include dilution or substitution with other types of oil, such as hazelnut, sunflower or soybean oils. Additionally, fraudsters may add other chemicals that allow the substances to pass routine screening tests.

DEVELOPMENTS IN OLIVE OIL ANALYSIS

Fortunately, there are a number of advances in food analysis that are helping to tackle this particular variety of food fraud, and many of the companies at the forefront of this will be present at this year's **Pittcon**.

These technologies are intended to provide rapid, reliable and cost-effective approaches to detect olive oil adulteration and overcome the drawbacks of other more time-consuming and labor-intensive methods such as gas chromatography/mass spectrometry and high-performance liquid chromatography.

For example, in a recent study, researchers showed how the Perkin Elmer AxION 2 time-of-flight (TOF) mass spectrometer (MS) integrated with the company's AxION Direct Sample Analysis (DSA) system could be used to distinguish soybean oil contamination in olive oil samples.Their approach was to look at the relative quantities of fatty acids, which are present in characteristic proportions in pure olive oil.

The researchers purchased olive oil and soybean oils from a local supermarket and diluted them to 1% in iso-propanol with ammonium acetate. They first characterized the fatty acid composition of the two oils separately, which showed that soybean oil had a higher response ratio for linoleic and linolenic acid to oleic acid, compared with olive oil.

They then mixed to the two oil types in various concentrations ranging from percentages of **5 to 50%** soybean oil. They showed that, as the concentration of soybean oil increased, so too did the response ratio for linoleic acid and linolenic acid to oleic acid. For example, at 10% concentration of soybean oil, the response ratios were almost double compared with pure olive oil.

The team say the findings show the potential for DSA/TOF to rapidly detect olive oil adulteration with soybean oil. The samples required minimal preparation and took only 30 seconds to analyze, which will improve lab productivity and cut operating costs.

A NEW TAKE ON FAMILIAR TECHNOLOGY

An alternative and well-established technique for analyzing oil samples is infrared (IR) spectroscopy. This method can be used to check if a substance has been adulterated and, if the adulterant is known, to quantify it.

In a study using attenuated total reflectance fourier-transformed IR on the Perkin Elmer Spectrum Two, researchers showed how an Adulterant Screen algorithm could accurately and sensitively detect and identify adulterants in olive oil samples. The approach works by creating two libraries of spectra: one of unadulterated reference samples and one of pure adulterants.

When a sample spectrum is scanned, the algorithm compares it with a model generated from the reference materials, before adding each adulterant in turn to the model. If an adulterant improves the fit of the model to the sample spectrum, it indicates a likelihood that it can be found in the sample.

The researchers explored the method by first creating a reference library of spectra from 24 unadulterated olive oils. They then obtained and saved spectra of sunflower and rapeseed oils, which were the adulterants in question in the study. After this, the team tested samples of olive oil adulterated with different concentrations of these two oils.

They found that all of the adulterated samples were classified as failing the screen by the algorithm; the only one to pass was a pure sample of unadulterated olive oil.

Furthermore, the algorithm is also able to give an estimate of the level of the adulterant by calculating its relative contribution to the total spectrum. This can save further time by removing the need to generate extensive quantitative models, the researchers say.

Bruker, who were also exhibiting at this year's Pittcon, produce a line of FT-NIR spectrometers that can perform non-destructive analysis of food samples for adulterant screening. The method can be applied by comparing a single spectrum from the sample in question to spectra from un-adulterated samples of known quality, therefore providing a non-targeted screening solution. Bruker have also developed automated software that can perform each step of sample analysis in sequence with minimal operation required.

RF-BASED SENSORS IN FOOD QUALITY MONITORING

At the Pittcon symposium on food analysis **Cheryl Surman** and **Nandini Nagraj** of GE Global Research demonstrated how RF-based sensors can be tailored for use in food quality monitoring.

They outlined how sensor components used alongside chemical or biological films can be configured for measuring multiple parameters simultaneously, providing an opportunity for multivariate analysis. The team also provided an update on the latest developments in RF readers including state-of-the-art technology created by KemSense.

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CASE STUDIES: HONEY AND CHOCOLATE

Scientists have recently been applying nuclear magnetic resonance (NMR) profiling to the detection of fraud in the honey trade.

Honey is another product apt for economically motivated adulteration. It is both in high demand, and in short supply, due to a number of reasons including pressures on bee colonies. Additionally, consumers are willing to pay a premium for products originating from certain countries or for particular floral varieties. As a result, adulteration has been on the increase.

DETECTING HONEY ADULTERATION USING NMR

Types of adulteration include mixing honey with cheap-to-produce sugar syrups and attempting to disguise the geographic origin by filtering out pollen. These types of food fraud can be difficult to detect, but NMR provides a non-targeted and sensitive solution.

This has recently been exploited in a project called the **Honey Profiling Consortium** carried out in association with Bruker BioSpin, who were exhibiting at this year's Pittcon.

The project uses Bruker's FoodScreener platform, which analyzes food and drink authenticity using 1H-NMR. Multiple labs using the technology collaborated to share results from their honey analyzes in order to create a comprehensive profile of **1000s** of honeys of different varieties and geographic origins. The consortium also profiled honeys with known levels of adulterant by mixing honeys with various sugar syrups.

Based on this wealth of data, the consortium were able to generate statistical models that will indicate when a newly tested honey differs significantly from the established patterns recorded in the database. Any deviation could be a sign of adulteration.

Bruker operates the system by conducting remote data analysis; testing is carried out at local labs but the statistical testing is done on Bruker's own servers. Bruker say this helps to ensure the comparability and robustness of results and also allows new statistical models and targeted parameters to be implemented quicker.

The Bruker FoodScreener itself is also designed to be highly automated to reduce the influence of any operator factors. For example, the system can perform tuning, temperature matching, pulse-width calibration and spectral processing without the need for operator input.

When a honey is tested in a third-party lab, Bruker delivers them an automatically generated "traffic light" report. By systematically comparing the NMR signals generated from the honey, the report will flag any violations against the product's labeling, such as honey variety, region and country of origin, and glucose and fructose concentrations. Because of the non-targeted abilities of NMR, the report will also include the absence or presence of any additional signals that would be expected in the authentic honey.

At this year's Pittcon, **Clark Ridge** from the US FDA explored the use of NMR in food analysis. The presentation included an overview of the technology, as well the advantages and disadvantages of some specific applications including quantitative NMR, "iso-tagging" and chemometric "fingerprinting".

MEASURING PARTICLE SIZE DISTRIBUTION OF CHOCOLATE

Also present at Pittcon was Malvern. Their particle sizer, the Mastersizer 3000, accurately measures particle size using laser diffraction in wet and dry dispersed particulate samples. This method is useful in food analysis due to the influence of particle size on product characteristics such as color, taste and solubility.

For example, particle size has a major role in determining texture and mouthfeel of chocolate. Research has shown that when a significant number of particles exceed **30µm**, consumers report that the chocolate is of lower quality. Assessing and controlling particle size during manufacture can help ensure product quality control and also lower costs for manufacturers.

In an experiment by Malvern researchers, the Mastersizer **3000** was used to analyze three different types of chocolate – dark, milk and white – all of which contain different combinations of particulate ingredients. The method was able to identify three clear separate profiles of particle size for the different types of chocolate.

It showed that dark chocolate had a greater density of smaller sized particles compared with the other two chocolate types, indicating that it should have a smoother and luxurious texture. The analysis was able to provide the proportion of particles above **30µm** in each type of chocolate – only **2.13%** in dark chocolate, compared with **12.56%** and **17.32%** in white chocolate.

ION CHROMATOGRAPHY ANALYSIS OF DAIRY PRODUCTS

Another method that is a standard in food analysis is ion chromatography. Industry leader Metrohm exhibited at this year's **Pittcon**. Their ion analysis products are capable of inline ultrafiltration and dialysis to automate sample preparation, helping to reduce the number of manual steps and improve reproducibility.

Ion chromatography as a technique has a number of advantages. The method is able to determine and quantify anions, cations, amines, carbohydrates, organic acids and polar substances in a range of food and drink. Effective at even low concentrations, it is able to detect from minor ingredients and trace contaminants to major components. Importantly, it allows multiple analytes to be determined in the same run, with little or no sample pre-treatment.

A key application of the technique is in the analysis of dairy products for quality control purposes. For example, it can be used to confirm the lactose content of foods labeled "lactose-free" and to check for contaminants in milk such as thiocyanate, perchlorate and melamine.

Preparation of dairy products for ion chromatography analysis is normally quite time-consuming and laborious due to the need to perform a protein precipitation step with Carrez reagent. But inline dialysis, as offered by Metrohm, means that protein-containing matrices are automatically removed.

ADVANTAGES OF RAMAN SPECTROSCOPY IN DETECTING PATHOGENS

A technique that has undergone significant advances in recent years is Raman spectroscopy. In the food industry, this technique provides a rapid method to detect pathogens in food samples during food production. The current gold standard for characterizing pathogens is bacterial culture, but this can take several days, and many bacteria are not in fact culturable. Another alternative approach – polymerase chain reaction (PCR) – is fast and accurate, but is also sensitive to experimental conditions, and samples may contain substances that inhibit the PCR reaction.Raman spectroscopy offers the advantage of being able to deliver high-specificity spectra of single cells within seconds in a non-destructive manner. And, although it is possible to apply the technique to cultured bacteria, it does allow this step to be omitted.

This year at Pittcon, a presentation from **Steven Zbylut** of General Mills considered the advantages and applications of Raman spectroscopy to the food industry. Zbylut explained how the technique is particularly suited to detect food adulteration and contamination and explored how its use will continue to grow in future thanks to developments in other technologies and software.

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CASE STUDIES: BEVERAGE ANALYSIS

Beverage analysis is important from both the point of view of product quality and taste, as well as meeting the regulatory requirements in place in many countries. There are many spectroscopic and analytic options available to those needing to perform beverage analysis and many of the leading companies providing devices for these purposes were in attendance at this year's **Pittcon**.

ION CHROMATOGRAPHY FOR WATER ANALYSIS

Drinking water is subject to much regulation and must be routinely tested for contamination and impurities. Ion chromatography can accurately determine the components of water samples, including trace contaminants. One particular advantage is that it can analyze multiple components at the same time from one sample.

> Ion chromatography can be used to analyze common ions found in water, such as fluoride, chloride, and bromide, and is used in the US by the **Environmental Protection Agency** for this purpose.

One highly toxic contaminant hazardous to human health that must be detected in mineral and drinking water is hexavalent chromate, which can make its way into water via environmental pollution. The EU and World Health Organization set the limit value for chromate concentration at **50 µg/L**, but there is debate about whether this should be lowered. The state of California have chosen to lower the hexavalent chromate limit to **10 µg** in drinking water.

A team from Metrohm, one of the companies that was exhibiting at this year's Pittcon, developed a method that they showed could detect chromate at levels as low as **0.02 µg/L**, meaning the technology is able to support detection at lower limit values if they were to be imposed by regulators.

Metrohm also offer a fully automated water analysis system, which can assess up to **59 100-ml** samples at a time. The system can assess conductivity, pH value, titratable acidity, alkalinity, hardness, and several individual substances such as chloride, without the need for multiple sample preparation.

LASER DIFFRACTION TO REPLACE SIEVE ANALYSIS IN COFFEE QUALITY CONTROL?

While health and hygiene are important concerns for any drinks supplier, analytical methods are also widely used in quality control. One example of this is in the production of ground coffee, where manufacturers need to ensure that the particle size is maintained and the final product is consistent.

A team from Malvern showed that laser diffraction has the potential to replace the traditional method of doing this by sieve analysis. The company, who were on hand at this year's **Pittcon**, has produced a funnel sample feeder which they used in association with a Mastersizer **3000** dry powder disperser. This allows the dispersion and measurement of coffee samples up to **130 ml** in volume.

The team showed that the device permits laser diffraction of coffee samples with the level of reproducibility required by international standards. Using the set-up, they analyzed two different grades of coffee: one coarse ground and one finely ground. The team put through 15 samples of the two coffee grades, which they were able to do at an average interval of two minutes.

The results showed laser diffraction was able to make a clear distinction between the coarse and fine coffee. Looking at the variation between the 15 different measurements taken for each coffee, it also fell within limits stipulated by ISO13320 (within **3%** for the Dv50 and within **5%** for the Dv10 and Dv90). Malvern say that the system is easy-to-use and allows for high throughput, which could make it an ideal replacement for sieve analysis in coffee production.

WINE AUTHENTICATION THROUGH NMR

The wine industry greatly benefits from the ability to authenticate its products. Bruker has recently introduced a wine-profiling module to its NMR FoodScreener which allows a range of relevant parameters to be measured. The system is able to assign origin for the major wine-producing countries and can also assign region for several parts of France, Italy and Spain. It is also able to detect 22 different grape varieties and a more recent feature is the addition of vintage validation.

Bruker's system offers both targeted and non-targeted analysis, as the NMR delivers information on targeted parameters but the same spectra obtained for a wine can also be compared with those stored in a database of spectra from authentic wines, allowing for verification of variety, origin and vintage.

ADOPTING THE COULTER PRINCIPLE FOR BEER ANALYSIS

Another technique applied to the analysis of alcoholic drinks is the Coulter principle or electrical sensing zone method. It was originally developed in the 1940s to count blood cells but has since been co-opted for many other purposes in life sciences and industry.

One such purpose is to assess particle size during or after the brewing process for beers, in order to evaluate and correct any errors in the production steps.

Particle size plays a role in the characteristics of different beers and also affects shelf life. The method can also be used to assess the efficacy of filtration, a process used to clarify beers and make them more stable when subjected to temperature change.

Beckman Coulter, who presented their technology at Pittcon 2017, have shown how their instrument, the Multisizer 3, is able to determine particle size distribution and concentration within beer samples. The method benefits from speed and accuracy and the automated function of the Multisizer 3 also means that results are comparable over time and between locations.

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SUMMARY

Despite the growing number of challenges to food safety, a continuous process of innovation is allowing the emergence of new technologies, or new applications of existing ones, giving us increasing power to face up to these matters.

Recent examples have included the use of NMR to detect the adulteration of olive oil, a widespread problem in imported products, as well as novel applications of mass spectrometry and infrared spectroscopy. Raman spectroscopy is also emerging to have important applications for the rapid detection of foodborne bacteria. And, when it comes to that most vital substance, water, a whole wealth of technologies can be applied to assure its quality and safety, many of which can now be fully automated.

This year's Pittcon exposition featured many of the companies who are developing and delivering these devices to the food analysis industry and they were on hand to present their current lines of products. Companies featured included Bruker, Metrohm, Renishaw, Perkin Elmer and Wyatt Technology.

The most up-to-date advances in the industry were highlighted in a symposium, which included presentations from leaders in the field on food analysis methods. This year's topic was "beyond mass spectrometry", which covered some lesser known and emerging techniques using NMR, Raman spectroscopy, DNA barcoding and wireless sensor technology.

For example, a team from GE Global Research demonstrated how RF-based sensors can be adapted and applied to a variety of food quality control purposes, and outlined the latest developments in GE's KemSense technology.

In a further presentation, **Rachel Gloyer** from the UK's FERA Science Ltd showed how next-generation DNA sequencing has created a path for DNA barcoding in food fraud detection. Gloyer explained the techniques used by FERA, who work on behalf of the UK government, to track bacterial food outbreaks and test food samples using the latest DNA sequencing technology.

Pittcon 2017 provided the opportunity to hear about the most up-to-date advances, the latest instruments, and innovative technological applications in the field of food analysis.

CONCLUSION

The food industry has always striven to ensure that its products have not become contaminated during preparation. However, today there are even greater challenges to ensuring food quality as some manufacturers are intentionally deceiving their customers to obtain high profits from low-grade materials.

There is also now the ever-more-present risk of bioterrorism in which pathogens are added to food products destined for mass marketing. Furthermore, with increasing global trade, it is often very difficult to trace the true origins of purchased ingredients.

To instil greater confidence in the quality of foodstuffs that reach retail outlets, legislation has made food manufacturers responsible for the safety and quality of the goods they sell. To this end, the food industry needs access to effective and cost-effective testing of their products.

As seen in the presentations given by major spectroscopy and analytic science companies at Pittcon 2017, scientists have responded to the growing challenges for food safety with a continuous cycle of innovation to meet requirements.

Given a goal, whether it be to detect food contamination or adulteration or to provide evidence of mis-selling, a suitable technology is created.

These technologies include, to name but a few, mass spectrometry devices that provide high-speed analysis of food contaminants, radio frequency-based sensors for the simultaneous detection of multiple impurities, nuclear magnetic resonance spectrometers to determine the origin of products in the honey and wine industries, ion chromatography for quality assurance in the dairy industry and water purity assessments, laser diffraction for particle size control (which is important in the coffee and chocolate industries), and Raman spectroscopy for the detection of pathogens.

Analysis of the fatty acid profiles of many different types of oil has allowed the rapid determination of the purity of olive oil using mass spectrometry. The adulteration of olive is readily identified by assessing the profile of fatty acids in a sample and comparing it with the profile of pure olive oil. This provides a non-targeted screening solution that allows adulterated samples to be quickly spotted.

Nuclear magnetic resonance profiling has proved invaluable in the determination of the authenticity of honey. The value of this technology has been enhanced by establishment of the Honey Profiling Consortium that allows the sharing of data from honey analyzes between multiple laboratories.

The consortium has compiled a library containing the profiles of thousands of honeys of different varieties and different geographic origins. The library also includes the profiles of honey with known levels of a range sugar syrups added. Such a tool has hugely simplified the identification of fraudulent honey.

Ion chromatography can accurately determine the concentrations of multiple analytes contained within a sample in a single run, making it an ideal tool for analysis of water purity.

Improvements in ion chromatography techniques have massively reduced the detection limit for the highly toxic contaminant, hexavalent chromate. It is now many magnitudes lower than the limit current guidelines require, supporting calls for the limit to be reduced.

Significant advances have also been achieved with Raman spectroscopy, a technique that provides a structural fingerprint based on the scattering of monochromatic light, such as laser.

Raman technology is used in the food industry to rapidly detect pathogens in food samples. It is now possible to obtain high-specificity spectra of single cells within seconds without damaging the sample.

The food safety symposium at Pittcon 2017 clearly illustrated that the development of new methodologies and novel uses of existing technologies has provided an impressive armament in the battle to ensure food quality.

4 CANNABIS TESTING & PHARMACEUTICAL R&D

Until recently, cannabis use was prohibited in almost every country. With the increasing use of cannabis for medicinal purposes., e.g., to ease chemotherapy-induced nausea and vomiting, relieve neuropathic pain and spasticity in multiple sclerosis, attitudes towards cannabis use have become more accepting.

INTRODUCTION

In Chapter 3, we learnt how developments in analytical techniques were required to meet new challenges to ensuring food safety. Here we see how changes in legislation can impact the need for tests to determine purity and composition of a marketed product.

In response to such changes in societal opinion, many countries around the world are relaxing their laws on cannabis use. Although cannabis is still illegal in the USA according to federal law, more than half of US states have legalized medicinal cannabis use and eight states have introduced legislations allowing recreational cannabis use. As a consequence of restrictions on cannabis production and use, rigorous data supporting the medicinal benefits of cannabis are limited and there are no standardized procedures for control of cannabis quality.

While a legal cannabis industry is now a flourishing, consumers have no guarantee that the cannabis they purchase contains the agents purported to provide therapeutic effects and no assurance that it is free from toxic contaminants. This chapter includes a discussion of these issues and an overview of the latest technologies for the analysis of cannabis, as presented at Pittcon 2017.

The vast potential for Raman spectroscopy in analytical process technology is also described with examples of its potential for a wide range of applications in research and development and pharmaceutical production environments.

The delivery of toxic drugs to tumors without unacceptable toxicity to healthy tissue and the targeted delivery of diagnostic markers to cancer tissues have been areas of ongoing research. Similarly, introducing imaging agents and treatments to the brain has been limited by the need for them to be small enough to cross the blood-brain barrier. The solutions were found recently in the form of nanoparticles.

Nanoparticles can be made from a range of materials, including metals, polymers, dendrimers, liposomes, viruses, and carbon nanotubes. Consequently, nanoparticles can have a variety of different properties that can be exploited to develop tailored and targeted therapeutic and diagnostic solutions.

Nanoparticles are typically between **10 and 100 nm** in diameter so they are large enough to avoid rapid elimination by the kidneys and small enough not to elicit an immune response.

The latest development is the creation of nanoplatforms that can perform different functions simultaneously. Since these functions were originally diagnostic and therapeutic, the technology is commonly referred to as theranostics. This chapter summarizes the latest nanoparticle research presented at Pittcon 2017, illustrating their vast potential in various fields of medicine.

CANNABIS TESTING: AN INDUSTRY GUIDE

With the increasing move towards decriminalization or legalization of cannabis comes the need for standardization of cannabis processing and quality.

Cannabis has a complex chemical composition and the precise combination of its constituent molecules determines its potency and potential for health benefits. In addition, it is important to verify that marketed cannabis is not contaminated with chemicals that are toxic to man, such as pesticides.

Even in areas that have chosen to relax cannabis laws, there remain limits to its legal use and detection of illicit cannabis manufacture or use continues to be important, e.g., intoxication whilst driving. This section provides an overview of the variety of cannabis testing that is required and the technologies being adopted to achieve it.

INTRODUCTION

Historically, the vast majority of countries have opted for the blanket prohibition of marijuana. But, after decades of stagnant legislation, this has begun to change at pace in recent years as several countries and US states have adopted, or are poised to adopt, more liberal approaches to regulation of the drug.

These have ranged from the experimental – as seen with the nationwide legalization of cannabis in Uruguay – to the tentative – as in Canada which introduced laws permitting strictly regulated medicinal use over 15 years ago. Currently over half of all US states permit medicinal use of the drug and eight, as well as Washington DC, have opted to allow some recreational use for adults under state law.

These legislative changes have been driven by a marked shift in public attitudes towards the legal status of cannabis. For example, in the US, Gallup polls have shown an increase in support for marijuana legalization from **25%** in 1996 to **60%** in 2016. A 2016 survey of **1000** Canadians found that **70%** were either supportive or somewhat supportive of marijuana legalization. And the figures are even higher when voters are asked about medicinal cannabis use specifically.

But moving away from the strict prohibition of cannabis bound in the laws of most countries is far from straightforward. When legislators shift away from this model, there are multiple alternatives to consider and there is no simple black and white, legal or illegal, stance available.

Regulators must decide whether to opt for legalization or decriminalization, whether the laws apply to all forms of marijuana (for example dry marijuana or oils and extracts), what quantities and age limits are restricted, and how the laws apply separately to the growth, possession and sales of the drug. Even countries like Uruguay, which have opted for widespread legalization, must decide on a commercial model for legal sales of the drug.

This year's Pittcon, which took place in Chicago from 5-9 March, 2017, hosted a number of symposia that covered issues emerging from the rapidly developing cannabis industry from a medical, legal, social and analytical point-of-view. They included talks that covered the current state of research into cannabis and derivatives for medical purposes, the social stigma of cannabis and whether this is holding back medical research, as well as a wealth of advances in analytical methods for characterizing cannabis, which will play a role in quality control, regulation and pharmaceutical research.

For example, the session "**It's Legal! Now What?**" looked at the challenges facing laboratories in the era of legalized cannabis. What approaches are available for analyzing cannabis samples? How can we stay ahead in the detection of contaminants and adulterants? How can we develop and enforce laboratory standards to ensure medicinal cannabis is safe for patients?

This symposium featured speakers from the **Colorado Department of Public Health and Department of Agriculture**, one of the few US states to allow recreational cannabis use, and the **Association of Commercial Cannabis**

With an update on the use of cannabis for medical purposes, **Tracy Ryan** from Cannakids, a company that provides cannabinoid-based treatments for children with serious illness, was a speaker in the symposium "Analytical Cannabis II".

Ryan presented anecdotal and clinical trial data relating to the medical use of cannabis for children and adults, and discussed the range of conditions, such as **epilepsy, Crohn's disease and PTSD**, that could stand to benefit from cannabis treatment.

The US **Food & Drug Administration** has stated that it supports research into the medical use of marijuana, although it has not approved the drug for any indication. During this symposium, **Uma Dhanabalan** from TotalHealthCareTHC.com, discussed whether the continued stigma surrounding cannabis is holding back research in this field, and argued that greater awareness and fewer misconceptions will help realize the full potential of cannabis as a medicine.

In the following sections, we take a look at some of these topics in greater detail, including highlights from this year's Pittcon. They include analytical methods for characterizing active ingredients and contaminants in cannabis, the development of industry laboratory standards, methods of cannabis extraction, as well as cannabis detection for law enforcement purposes.

With participation of major industry players, including Sigma Aldrich, Shimadzu, PerkinElmer, Restek and CEM Analytical, Pittcon 2017 covered all the latest developments in cannabis analysis.

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ACTIVE INGREDIENTS IN CANNABIS

Tetrahydrocannabinol (THC) is the major psychoactive ingredient in cannabis and has consequently received much attention from scientists. However, it has emerged from research that cannabis actually consists in excess of **500** chemical entities and around **60** of these come from the same family as **THC** – the cannabinoids.

Another important family of compounds present in cannabis are terpenes and terpenoids which give cannabis its distinctive flavor and aroma. Both terpenes and other cannabinoids can interact with **THC** to enhance or antagonize its psychoactive effects.

POTENCY TESTING

Verifying the potency of cannabis is key for cannabis testing labs, and can be achieved through LC and GC methods.

Restek's Raptor LC columns can be used alongside any HPLC instrument, accelerating the analysis time without the need for UHPLC equipment. The company says that their high-throughput approach can complete cannabinoid analysis in **3.7** minutes. They have also shown that their Rxi®-35Sil MS column can be used alongside GC equipment to rapidly analyze cannabinoids in a matter of minutes.

Another approach that has the potential to offer cannabis producers access to on-the-spot potency testing is Fourier transform mid-infrared (FT-IR) spectroscopy.

A team from PerkinElmer tested the method on intact and ground cannabis bud samples. They showed that the method could accurately quantify the levels of tetrahydrocannabinolic acid which is converted to THC upon heating and, another important cannabinoid, cannabidiolic acid (CBDA) which is converted to cannabidiol.

The researchers also demonstrated that FT-IR could detect changes in cannabinoid concentrations according to growing time and conditions, showing that it has the potential to allow cannabis producers to monitor and optimize conditions and determining harvest time.

Steep Hill, who presented at Pittcon 2017, have developed a cannabis analyzer called the QuantaCann2 that uses near-infrared (NIR) spectroscopy. The company argues that the method, although less versatile, has advantages over other spectral approaches that are destructive, require more sample preparation, greater operator expertise, and the use of solvents.

The team that developed the device has shown that it can quantify four major cannabinoids in cannabis – CBDA, cannabidiol, THCA and THC to within **0.7%, 0.4%, 1.3%** and **0.6%** accuracy, respectively, when compared with HPLC reference spectra.

TERPENE PROFILING

Cannabis contains a complex profile of terpenes, which are thought to be responsible for some of the drug's purported health benefits. As this profile varies between strains, from crop to crop and even plant to plant, it can also be used for quality control purposes.

An increased interest in characterizing terpene profiles has followed from the wider legalization of cannabis and is required in some states by law.

Several companies have developed approaches to terpene profiling. For example, Shimadzu, who were present at this year's Pittcon, created a method using their mass spectrometer with a full evaporation headspace technique (FET) to overcome the fact that plant material does not dissolve in solvent.

The Shimadzu team has shown that using a single-phase liquid-gas system they could quantify the presence of terpenes in accordance with Nevada state law in three different strains of cannabis.

Restek have also generated a workflow for separating terpenes using a headspace gas chromatography-flame ionization detection (GC-FID). Like Shimadzu's approach, the method also uses FET with a single-phase gas system. They have demonstrated the efficacy of the method in characterizing the terpene profile of pelletized hops, as a proxy for cannabis. They used the Shimadzu Rxi®-624Sil MS column, which has a small-bore configuration, and can also be used for analyzing residual solvents in cannabis, using the same setup and technique.

CANNABIS COMPONENT ANALYSIS AT PITTCON 2017

At Pittcon 2017, **Scott Kuzdzal** from Shimadzu Scientific Instruments introduced the symposium 'Analytical Cannabis II'. In the session '**Current and Future Analytical Technologies for Cannabis Testing and Research**', Kuzdzal discussed the many different chemical compounds found in cannabis and some of their reported health benefits.

He also outlined why cannabis testing labs are so important for quality control in the era of medical cannabis, including the measurement of terpene concentrations, and the detection of contaminants such as pesticides, heavy metals and mycotoxins. Kuzdzal also considered how analytical technologies are enhancing quality control testing including in clinical settings.

In an oral session, the conference heard from **Laura McGregor** of Markes International who described how two-dimensional gas chromatography coupled with time-of-flight mass spectroscopy can aid the analysis of complex plant-based samples, such as cannabis. McGregor also outlined how the approach of tandem ionization could help researchers to keep pace with the emergence of so-called legal highs by boosting researchers' ability to identify novel structures for which no reference spectra are available.

Also in attendance at this year's conference was **Sigma-Aldrich** who offer a range of solutions for cannabis testing labs. This includes the company's Ascentis Express columns which can be coupled to any HPLC, UHPLC or LC-MS instrument allowing the characterization of a sample's active ingredients, including cannabinoids.

Advion was also presented their compact mass spectrometer at Pittcon 2017. The device, which is much smaller and priced lower than a standard MS system, is designed to increase the accessibility of MS to labs, particularly those with restricted space. Advion have shown that compact MS can be applied to the analysis of cannabinoids alongside thin layer chromatography for qualitative detection of cannabinoids and alongside HPLC for quantitative determination.

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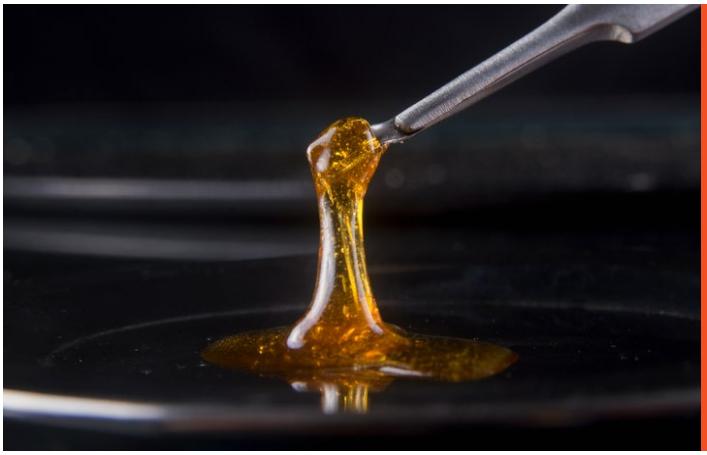
CANNABIS PREPARATION – EXTRACTION TECHNIQUES & RESIDUAL SOLVENTS



With the market for cannabis growing along with expanding legalization, there is increasing demand for cannabis extract products. This includes oils and waxes that can be used in vaping, drinks, and edibles.

For both recreational cannabis users seeking a high and for potential medicinal applications, there is also growing interest in purified forms of cannabis such as cannabinoid isolates.

METHODS OF EXTRACTION

Traditionally butane extraction has been the most commonly used method of cannabis extraction, but it is being used less and less in commercial settings these days due to the risk of explosions from the highly flammable gas. However, the technique has apparently been growing in popularity among illegal cannabis producers who use it to create high-strength butane hash oil (BHO).

In the UK, two people have died and 27 people have been injured in the process of generating BHO over the last two years, according to police reports.

In lab settings, the most popular approach is solvent extraction, which requires further purification steps to remove any residual solvents.

Another method, which is more expensive but gaining more widespread use, is supercritical fluids chromatography or CO2 extraction, which uses carbon dioxide as a solvent and therefore doesn't require as much post-processing.

At this year's Pittcon, **Xiaoning Lu** from Sigma-Aldrich presented another technique that could be applied to cannabis extraction – online solid phase extraction (SPE). This method is widely used by labs to isolate analytes from complex matrices but off-line methods are time consuming, labor-intensive and have poor reproducibility.

Lu presented research carried out on thyroid hormone samples in biological matrices using an online-SPE cartridge developed by the company, alongside liquid chromatography/mass spectrometry (LC/MS).

He showed how the online cartridge was able to significantly boost the LC/MS response and improve reproducibility, and also explained how the same cartridge can be applied in the detection of cannabis analytes in blood serum and urine samples.

REMOVING RESIDUAL SOLVENTS

When solvent-based extraction processes are used, the cannabis extract must undergo further steps to remove any residual solvents, as these can be harmful to human health. It is therefore vital to verify that the solvents have been completely removed and there are a number of chromatographic options available for doing so.

Commonly used is gas chromatography (GC) and static headspace GC can be used to concentrate volatile analytes for analysis and provide rapid identification and quantification of residual solvents. Shimadzu and Sigma-Aldrich, who both presented at Pittcon 2017, offer such solutions for residual solvent analysis.

Also at Pittcon 2017, **Robert Driscoll** from Robatel Inc., a Massachusetts-based centrifuge provider, discussed centrifugal chromatography as an approach to isolating components from organic samples.

Driscoll outlined the benefits of fast centrifugal partitioning chromatography – a method that allows components with similar molecular structures to be isolated from a sample – over other techniques available. He also discussed recent advances in the design of the technology and its use in isolation of cannabis as well as tobacco, opiate derivatives and nutraceuticals.

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CANNABIS TESTING – IDENTIFYING CHEMICALS AND CONTAMINANTS

In the United States, although recreational or medical use has been authorized by several states, cannabis is still illegal at the federal level. Consequently, the **Food & Drug Administration (FDA)** does not recognize cannabis as a regulated product and does not provide any standards for its cultivation and supply.

This raises the risk that cannabis products could be contaminated, potentially endangering consumers' health. This is particularly pertinent for those accessing medical cannabis who may have compromised immune systems.

Fortunately, there are a number of analytical techniques that have been applied in clinical, pharmaceutical, food safety and environmental settings that are equally effective for verifying the absence of chemicals and contaminants in cannabis samples.

At Pittcon 2017, **Joshua Crossney** from jCanna, Inc., a non-profit organization interested in improving cannabis analytical testing technologies, discussed how the emerging cannabis testing and research industries are benefiting from collaborating and sharing the knowledge and experience of these other more long-standing industries.

At this year's conference, details of the specific techniques and the latest methods being developed to assist the provision of safe, uncontaminated products in the new era of legalized cannabis were also presented.

PESTICIDES

Cannabis plants are susceptible to a number of bacteria, fungi, yeasts and molds but many pesticides available to try to prevent infestation can themselves be harmful to human health. And while pesticide use on other crops is federally regulated, this is not the case for cannabis.

What's more, no pesticides have even been tested or registered for use on cannabis. As a result, individual states that have legalized cannabis are having to come up with their own regulations for pesticide use and eliminating contamination from the final product.

This year's Pittcon featured discussion of a number of techniques now available to help meet the challenge of cannabis quality control. Attending the conference were Restek, Shimadzu and Sigma Aldrich, who all provide LC-MS and GC-MS solutions for pesticide analysis.

There was also a presentation from **Julie Kowalski** of Restek who outlined a modified QuEChERS technique – which is already popular for pesticide testing in food and agriculture – using LC-MS/MS to analyze pesticide residues that has so far been applied to over 200 pesticide types.

Jack Henion from Advion also discussed how mass spectrometry using the company's compact device could be used as a screening method to test the quality and purity of cannabinoid products and detect the presence of pesticides and other contaminants.

OTHER ANALYTES AND CONTAMINANTS

Another trace contaminant that can find its way into cannabis products are heavy metals, which are absorbed from the soil into the cannabis plant. Many of these are considered toxic, such as lead, arsenic and mercury.

Options for detection include Ultrasonic Nebulizer Inductively Coupled Plasma Optical Emission Spectrometry (USN-ICP-OES) and Mass Spectrometry (ICP-MS) methods, both of which are capable of rapidly analyzing all heavy metals but differ in simplicity and sensitivity.

Moisture content is also another important parameter as excessive moisture can promote mold growth, which can be established using an electronic moisture analyzer like the Shimadzu MOC63u.

Related to this is concern over the presence of mycotoxins, which are toxic metabolites produced by mold. Several of these have been shown to be especially harmful to humans and can be dangerous to immunocompromised patients even at very low concentrations. Therefore, it is critical to have sensitive methods for detecting low levels in cannabis samples. Chromatographic methods available include GC, HPLC and LC-MS/MS.

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The increasing legal status of cannabis has led in turn to a surge in demand for cannabis testing labs. But cannabis testing is very much an emerging industry and there has been considerable confusion surrounding how such labs should operate.

For example, some have been uncertain over whether they were able to provide their services legally due to the continued federal prohibition of the drug. And with no federal oversight into cannabis standards, such as cultivation conditions or acceptable levels of residue solvents and pesticides, states have been left to come up with their own rules, sometimes belatedly following legalization.

Labs have also had to contend with uncertainty because of the prospect that current standards become obsolete when state lawmakers decide to change the regulations.

At this year's Pittcon, the symposium "**It's legal! Now what?**" explored some of these emerging issues. **Heather Krug** from the Colorado Department of Public Health and Environment detailed the challenges the state has faced since legalizing recreational cannabis. They include issues such as a lack of scientific evidence over the toxicity of cannabis contaminants, systems for monitoring cannabis lab performance and an absence of industry-accepted standards for labs to work to.

The symposium also heard from **Robert Martin**, representing the Association of Commercial Cannabis Laboratories, who outlined efforts to try to establish evidence-based quality measures for cannabis testing. He discussed the issues of pesticides, residual solvents and microbiological testing, as well as the future of the cannabis testing industry.

Also at Pittcon 2017, **Autumn Karcey** from Cultivo, Inc. discussed the optimal conditions for growing cannabis indoors to facilitate medical research into its effects. This includes consideration of factors such as temperature, humidity and room pressure as well as how to minimize the presence of airborne particulates, pests and pathogens.

Barry Schumbmehl from Fritsch Milling and Sizing, Inc. discussed how milling and grinding procedures during sample preparation can help to generate uniform, representative samples suitable for analyzing commercially produced cannabis.

He discussed the variability of analytes between crops, and even within the same plant, and provided data on how potency can therefore differ between that which is stated and that determined from dried flowers or post-milling.

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CANNABIS DETECTION AND LAW ENFORCEMENT

As we have seen, analytical techniques have been assisting the regulation of legalized cannabis but the fact still remains that the substance is prohibited in most countries, and is still illegal for recreational use in the majority of US states. Detecting illicit cannabis in biological samples and contraband therefore continues to be a priority and advances in analytical science are helping this to be done with greater accessibility, speed and sensitivity.

One such development is **cantilever-enhanced photoacoustic spectroscopy, or CEPAS**. This technique was detailed at Pittcon 2017 by **Jaakko Lehtinen** from Finland-based Gasera Ltd., who were involved in developing this new measurement technique.

It can be used in gas phase for detection of **volatile organic compounds (VOCs)** from drugs or their precursors and in this talk, **Lehtinen** discussed how the use of different laser sources can lead to Ppb-level detection of VOCs. He also outlined how photoacoustic spectroscopy is able to distinguish hair samples from cannabis users and non-users, and how THC levels can be determined from cannabis samples using the same set-up.

Pittcon 2017 was also attended by a number of companies whose technologies are providing novel options for drug detection to assist law enforcement.

For example, Advion presented their compact mass spectrometry device. Their team have previously shown how the device, which is intended to make MS more accessible, particularly in labs with limited space, can be used alongside a method called **Atmospheric Solids Analysis Probe (ASAP®)** combined with **atmospheric pressure chemical ionization (APCI)** to allow for direct analysis of samples suspected of containing cannabis.

The approach was able to produce strong signals for cannabinol, THC/cannabidiolic acid and THC/cannabinol within 20 seconds. Furthermore, they showed that the method could potentially be applied to trace samples from fingertips, as when a person has rolled a cigarette containing cannabis.

Bruker, who also presented at this year's conference, offer a number of drug testing solutions, including the Toxtyper workflow which facilitates LC-MS drug screening by incorporating a library of over 830 compounds. A team of researchers showed that Toxtyper could be applied to the identification of synthetic cannabinoids.

New derivatives of these compounds frequently emerge in slightly modified varieties, sometimes as a deliberate attempt to bypass drug laws, and they can pose a risk to consumer health due to their unknown strength and toxicity.

The research team showed using a library of 46 synthetic cannabinoids and nine isotope-labelled analogs, that spiked serum samples could be detected for all substances at concentrations of **0.5 ng/mL** or lower. Bruker also offer an ion mobility spectrometer, called DE-tector, a desktop and portable device, which can detect natural, synthetic, pure and street drugs from samples when present in the low nanogram range.

The company have also shown that it's Fourier transform-infrared spectrometer, ALPHA, is effective in detecting cutting agents that have been laced into drug samples, and are themselves often illicit and harmful to consumers.

Also at this year's Pittcon was Biotage. They have created a range of supported liquid extraction plates and columns called ISOLUTE which can be used in place of traditional liquid-liquid extraction. Not only can the set-up be used to detect cannabinoids in blood and urine samples, but the company have also shown that it can be applied to saliva samples, potentially presenting a quicker and easier way to obtain samples in settings such as traffic accidents or for workplace drug testing.

Thermo Scientific offer a solution for cannabinoid detection in biological samples using their TurboFlow technology – an automated online sample preparation technique – coupled to LC-MS/MS. Their team has shown that the approach is able to quantify THC and its metabolites in spiked blood samples, with a total extraction and analytic runtime of 10.4 minutes. Thermo Scientific say the method, by cutting out sample preparation time, can provide advantages over SPE or liquid-liquid sample preparation.

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CONCLUSION

Against a background of increasingly liberal cannabis laws, regulators and scientists have been trying to keep pace with cannabis as it has gone from a near-universally illicit substance to a burgeoning industry in just a matter of years.

Fortunately, the analytical science industry, drawing on experience in the fields of food, environmental and agricultural science, is rising to the many challenges presented by this revolution.

At Pittcon 2017, companies at the forefront of innovation in cannabis analysis, regulation and detection presented their latest technologies. There was also a wealth of talks and symposia given by the experts involved in developing these technologies and methodologies, and putting them to use in the field.

The many presentations explored recent developments in cannabis research, law and ethics. The conference also looked to the future, and what it holds for the cannabis testing industry and medicinal uses of the drug.

For example, a talk by **Kevin Rosenblatt** from Integrated Biosource/ Cannabis Labs, considered how medical marijuana fits into another healthcare revolution – personalized medicine. Rosenblatt looked at the potential for genomics, metabolomics and pharmacogenomics to tailor cannabis treatment to patients on an individual basis, and how this could be assisted by analytical techniques like mass spectroscopy.

As this industry tries to make sense of legalized medical and recreational cannabis from an ethical, legal, and analytical perspective, Pittcon 2017 provided an overview of where we are now and where we could go next.

4.2 EFFECTS OF OPTICAL SCATTERING ON REPRESENTATIVE SAMPLING IN SOLIDS OR TURBID MEDIA IN PROCESS RAMAN SPECTROSCOPY

Dr. Karen Esmonde is a postdoctoral research fellow at the University of Michigan Medical School. Her research focuses on the application of Raman spectroscopy techniques.

Raman spectroscopy uses the scattering of laser light by a sample to obtain a molecular fingerprint for the sample, which can be used to identify the constituent molecules. It allows real-time and in-situ measurements to be made, making it a powerful tool for quality assurance testing in pharmaceutical production and other process environments.

In her talk at Pittcon 2017, which is reported in this section, Dr Esmonde explained the benefits of Raman spectroscopy and how it can be used in conjunction with other techniques for a wide range of applications.

A transcript of Dr. Karen Esmonde-White's talk at Pittcon 2017

Optical scattering can be harnessed or avoided to obtain representative sampling regardless of whether the sample is a liquid, turbid media, or a solid. Examples of applications include polymer and small molecule pharmaceutical processing.

RAMAN SPECTROSCOPY: A BRIEF INTRODUCTION

Raman spectroscopy is a fundamental vibrational spectroscopy technique that produces a molecular fingerprint of a sample. This fingerprint can be used to identify components in a sample and look at the changes in their molecular structure, chemical composition and concentration. This enables the Raman spectra to collect qualitative and quantitative information of chemical compounds.

The wavelength of the laser used in Raman spectroscopy is typically of visible or near-infrared light. The laser is delivered to the sample, a wavelength of light is then scattered back, focused onto a spectrograph and collected by the CCD (Charge Couple Device); this produces a fingerprint of the sample. The laser can be coupled to anything from a microscope to a fiber optic probe that's placed 200 meters away from the analyzer.

The Raman spectra has different modes, for example the transmission mode and the backscattering mode. The backscattering mode collects the signal from the same surface as the laser i.e. the wavelength is directed back in the direction it came from, and this mode is most convenient for process.

ADVANTAGES OF RAMAN SPECTROSCOPY

One of the advantages of Raman spectroscopy in a process environment is that it provides direct, real time and in-situ measurements. When directly comparing the Raman spectra with the near-infrared spectra, the difference between varying polyethylene densities can be seen more clearly in the Raman spectra than in the near-infrared spectra.

Sensitive to the chemical and physical parameters of the molecule, the method development for Raman is also more straightforward, having a simpler cross scale and so, better transferability between instruments.

Raman is compatible with classified environments with an ATEX system, as well as different process control software. Sampling flexibility and compatibility with aqueous systems makes Raman spectroscopy is a powerful tool in a process environment. This flexibility allows users to measure in cells and biological tissues, solutions, streams, and gas-liquid mixtures.

A microscope or fiber optics can be used with Raman spectroscopy, and fiber optics enables sampling outside of the laboratory. Fiber optic probes can be used to take inline measurements of liquids, turbid media, solids, as well as an offline analytical sample chamber.

Knowing what the desired sampling volume is an important consideration for in-situ Raman spectroscopy. Optical scattering occurs everywhere, because of the differences in refractive index. In a process environment, optical scattering can arise from bubbles, particulates, differences in miscibility with components etc. The optical scattering, therefore, goes from a very narrow laser beam to a more diffused beam.

There are variations in Raman spectroscopy, and a broadened laser beam can be used to measure a sample with a larger volume, this is especially important for spatially heterogeneous samples such as pharmaceutical tablets or copolymers. The most straightforward method is a single fiber in and a single fiber out that are situated close together, allowing it to catch surface information.

The broadening of the excitation laser to a large area incorporates multiple collection fibers, this is known as a large volumetric Raman. Large volumetric Raman has an advantage over backscattered Raman, because it can collect surface and sub-surface information. In addition, a reflectance material can be added underneath this to enhance the reflection and increase the sensitivity of the large volumetric Raman.

Transmission Raman uses a similar principal to large volumetric Raman except that it has a 180-degree collection as opposed to a backscattering collection. This too, is a very powerful technique for getting surface and sub-surface information.

APPLICATIONS OF OPTICAL SCATTERING

The optical scattering effects in process Raman are very important for samples that are spatially heterogeneous, for example in pharmaceutical tablets, solids processing or in spatially heterogeneous polymers.

A technology called the fat technology, which incorporates global illumination and multiple collection points, can be used to produce a sampled 3D volume as opposed to a small surface area measurement. This allows representative sampling at low laser power and means that only one measurement of a spatially heterogeneous sample needs to be taken, instead of multiple measurements.

Starting from first principles and then moving into a probe that is offset from the sample by approximately ten inches, measurements between a one millimeter and six-millimeter spot size can be collected.

With this type of global illumination or large volumetric measurement, between three to five millimeters down into the sample can be collected. This can be applied in secondary processing for small molecule pharmaceutics.

One of the first applications for the fat technology was in-situ measurements of blending and content uniformity tablet coating in a batch process. Since then the batch processes have been changed to continuous coaters.

This shows another example of how the technology is used in a blend uniformity application and in this process the probe takes measurements. This part of the spectrum was used for a PLS mile input, and even though the numbers are small, results agree with the Raman predicted API concentration versus the offline method.

Another example of using a large scale, hybrid approach, is the use of two probes by researchers. One was an emergent probe that had a small sampling volume, used to understand the micro scale chemistry. The second probe was a non-contact probe, used to get information of the macro scale morphology.

The preparation of high impact polystyrene was monitored, which starts with two miscible phases and then the phases becomes immiscible, and then finally there was a phase inversion. Following this, a spatially heterogeneous salami morphology type polymer remains. They demonstrated how the immersion probe can be used to understand polystyrene polymerization.

> The non-contact probe was shown to be more sensitive to changes in miscibility, which could be corresponded with viscosity and diffusivity increases. The researchers showed that not only can the chemistry be measured, but also some of the physical parameters of the process.

CONCLUDING COMMENTS

Raman spectroscopy is an exciting analytical process technology with wide applications in many different environments from R&D to manufacturing. In-situ, it is a powerful tool and vital to understand optical scattering in the system and its effect on how the sampling of a system can provide insight into the best sampling approach, to get the most out of this technique.

4.3 EVOLUTION OF PEAK CAPACITY IN LIQUID CHROMATOGRAPHY

Dr Pat Sandra, professor of separation sciences, founded the Research Institute for Chromatography in Belgium, a center for research and education in chromatography, mass spectrometry, and electrophoresis. He has received numerous international awards including three honorary doctor degrees.

This section presents highlights of his talk at Pittcon 2017 in which he described recent developments in 2D-liquid chromatography that allow the analysis of complex matrices. He provided examples of how the technique can be applied in the production of cosmetic ingredients, biopharmaceuticals and natural products. He also explained how the technology is suited for routine commercial use in Good Medical Practice and quality assurance testing.

An extract from Pat Sandra's talk at Pittcon 2017 where he received the Lifetime Achievement in Chromatography Award.

At the beginning of our activities, we were working in phases, but we were never going over into the GMP activities. Our customers asked us to continue to develop methods and take responsibilities for GMP, quality assurance and quality control. We recently began to do this, so we're not a company highly specialized in GMP, we started the company to be complementary to research and development and GMP.

Looking through monolithic tunnels for peptide mixes and comparing them with all different other columns in the miniaturized form. The data allows you to see depending on the gradient time, we can go from 25 centimeter columns up to four meter columns, which have relatively high resolution and key capacity around **5/600**.

> Unfortunately, in a quality assurance environment, we cannot work on those type of applications because everything must be commercially available and by different instrument manufacturers or column manufacturers.

That's the reason we were looking to key capacity, because this was what we tried to do at the highest key capacity. We, first of all, were looking to convention tunnels. How far can we go, what was "S" in the marked variable, and what can we transfer to the industry to directly apply what we developed?

> We know now that key capacity is defined by the number of peaks with resolution, and is one that can be between the first and the last eluting compound.

That worked for complex mixes and gradients and there are different equations which can be used interchangeably, this is because we came to the conclusion that whatever we do to an automatic graphic forms, the key capacity is proportional to the square root of the plate number. Then, there's a certain function and this function is always smaller than two.

In gas chromatography, the temperature programing rate and in liquid chromatography, the radiant time, already early 2006, we came up with an example of high resolution. This is what we want and this is what we need in bio market studies together with the company which was a spin-off of the University of Ghent.

Comparing such chromatogram, which is the peptides in serum after the inflation of the six most important proteins, we coupled eight columns of **25 centimeters** and eight columns with five micrometer particles, because we had to use instrumentation only with a pressure limit of **400** par. We then realized that the high key capacity in the order of **900**, took a time of about **500** minutes. If you like to have high resolution, the price is high.

Nevertheless, this was in the beginning when we published this, and it was criticized by a biomarker company who were extremely successful with that approach. Time is not that important in biomarker studies because if you find a real biomarker in one year, it's unbelievable because you have to realize that you have all those biomarkers, **90%** today are no longer biomarkers because you were going much too fast in the final decision that this was a biomarker, therefore time is not at all important. This was in different laboratories, highly in conductible with all of this being commercially available.

This was coming from the fact that we learned the chromatic blocks. The chromatic blocks told us that although we have instruments with help from the course from **1.8 micrometer** particles, the highest efficiency that we could reach was with **5 macro meters** for **100** parts.

> From the efficiency and the analysis time, we know that the analysis time will be very long. Those temperatures are important because if I take for example if I take **1.8 micrometers** from the par and I increase the temperature then the efficiency increases and I decrease my analysis time.

There was a fundamental reason why we were selecting those columns. This was successful, here for example you see that those using our technology asked us to make a column of three meters. We coupled 12 columns of **25 centimeters** and **5 macro meter** particles. But this is for example the result of identified peptides and this was **4700** identified peptides. It was highly reproducible and this was a platform that could be used in different laboratories of that group.

Nowadays we can do better because with the advent of high pressure instrumentation and also smaller particles, for example if we are using **25 centimeter, 1.7 micrometer** particles operated as 60°c, then you can use longer columns and you see that the peak capacity depends on the gradient time. So, on the same column you can create **200** peak capacity but you can also select for example a **600** peak capacity.

What is important in this comparison in gradient times is that we can easily go from **200-850** peak capacity on the same column if we change the gradient time. In regards to time, in one hour we can have a maximum **600** at peak capacity and therefore is a limiting factor.

Carl was looking to the theory of peak overlap and he came to the conclusion that in order to resolve **98%** of the compounds, but we prefer to separate **100%** of the compounds, then the peak capacity must exceed the number of compounds by a factor of **100.**

Carrying out a septic dye test, I have roughly **100** peptides. In order to separate these **100** peptides chromatographically I must have a peak capacity of **100** multiplied by **100 (10,000)**. This means that I need **100** million plates to achieve that. This means that I can eject today and my first peaks, but that's not realistic, we have to do something in order to do that.

However, at this time, we did not have at all mole spectroscopy, which is also an extreme, powerful technique to help to introduce selectivity and that selectivity can be translated at peak capacity. For example, we do routinely just based on NMN an average of **300** pesticides in fruit and vegetables. How can we do that? The selectivity from Mass spectrometry. Because all those pesticides have different fragment ions that we can exploit.

We also published a paper on the skin ceramides, a very important group of compounds found in the skin. You see immediately when you use triplicate because we like to be **100%** sure when we identify something that we can reproduce it. If we do an extraction of the skin removing the glycerol, esters and the triglycerides and you have only the ceramides.

The ceramides are very important for cosmetic industries and what we observed is that we learned from the kinetic plots, we can apply it because it is the maximum on the given column set that we could use.

> Therefore, we used chromatography on 1.7 micrometer columns and up to 80°c because the ceramides were perfectly stable, none of them will decompose. I recognize LCMS and then the final conclusion is that we identify **264** unique lipid spots.

What can we do? We know already if we need peak capacity with the tools that are commercially available there is no problem to have a peak capacity of roughly **400/500** peaks within one hour. But we also have multidimensional chromatography, and that's interesting if you look to the story of multidimensional chromatography.

> The first publication of lipid chromatography was from the group of J**im Jorgison**, who separated and moved the dimension approach from Robbins. That's what we like to do, a two-dimension liquid chromatography test applicable for everybody.

If we look to genetic, routine methods, we also develop reverse phase liquid chromatography with reverse phase liquid chromatography and very often people blame us to say these two mechanisms are the same, but this is not true. If I take two different stationary phases and two different mobile phases, I have even more autoanality, and this is what we are doing in gas chromatography.

In gas chromatography, we have paper face distribution, a little bit of selectivity, but the mobile phase is always the same, so if we combine reverse phase we're in a similar situation.

Now the first interface developed was a quite easy interface based on a valve with eight orbs and two positions and for the neofites in the field. You take a column with a small, internal diameter, one to two milometers internal diameter. If you have a high-quality column of one milometer internal diameter it's perfect because the optimal flow is in the order of **50 to 100 micrometers** per minute. In the valve, we put two loops. The loop must be in the order of volume in the volume per minute of the first mobile face.

So, suppose we have **100 micrometers** per minute, we will install two loops of **50 micrometers**. This means that every 30 minutes I change the valve and I go over from loop one to loop two, follow the first injection and first separation, I fill loop one and I analyze what is in loop one, and I turn the valve. Loop two is used now to collect the second minute or 30 seconds and I turn the valve again.

Every 30 seconds, I let the valve from **50 microliters** and use **100 microliters** per minute flow. Once we come here I have a second pump and I use columns with an internal diameter of **4.6 milometers**, a short column with a very high flows. I use flows three or four mils per minute, which means that I dilute the first due to the fact that we have to take care of solvent compatibility. Now the system is commercially available.

There are several companies introducing the system that we are using for comprehensive and I will also explain multiple, hardcutting, liquid chromatography.

Another approach is multiple hardcutting, but I will not go into too much in detail, but on the same valve we can fill **12 loops** with different fractions of a chromatogram.

Liquid chromatography is based on a two position, four-part valve. One group had a problem with one of the active pharmaceutical ingredients when they injected that, they always had those peaks which are considered impurities in a manufacturing process.

When you look in a little bit more in detail and we were are taking seven hardcuts from the main compound and here you see the seven hardcuts. We had exactly the same profile which means that's impossible, something must be formed by the main compound due to the tunnel, stationary phase or mobile phase, because it was a pH of 10.9 with sodium hydroxide.

But if we were taking it from a pharmaceutical product only with sodium hydroxide in the recipient nothing happened. So, it was a combination of the mobile phase and the stationary phase that was forming from this analine that an iso-hydro and an iso-compound.

It's a high efficiency, high resolution, high peak capacity blots and exactly the same as in one dimension, the slower the gradient, the more peak capacity you have. For example, if you have a gradient of let's say 60 minutes, you have a peak capacity of close to **2000** with every gradient, then you have much smaller peaks on the cation exchange material, the same peak capacity in the reverse phase where you go to close off in **4000** peak capacity.

But there's an advantage of that when you take a moss pack that are pure. You don't have collusion of others, you don't have ion separation etc. at the different spots. We combined the UV with mass spectrometry positive or negative when we take some spots and it made a beautiful spectrum, which can be directly identified in the peptide composition.

Unfortunately, we cannot run this on the routine and the reason for that is we are using phosphate and let's say every day we have to clean the ion optics from the spectrometer because you can really see that we have layer on the ion optics that reduce tremendously the response of the spectrometer. But let's look to a simpler application and let's come also to what we call genetic metals and comprehensive LC.

We were looking to detect things and an example we used was taxo, which is a component of the Texas plant and is anti-tumor and is a very important new drug against lung cancer, breast cancer, bronchiolus cancer and others. But the problem is, is that this product cannot be synthesized at a reasonable price, because the compound has 11 chyrocenters, which makes it extremely expensive and time consuming.

So, they still work with extracts of plants and then the extract of the plant you have other taxanes that can serve as a precursor to make the final taxol. They extract precursors, modify them slightly and then they have taxol.

If you look for example to a standard sample, we don't worry so much that the compounds are not separated. We know that we can slowly improve that if we use other conditions. But, we don't do that because what we are doing in that analysis is we use two the times reverse phase.

The reverse phase here on the C18 and reverse phase on the fellow axle, reverse phase column. Here we are using water methanol and here we are using water acidtone nitrate. So, we also change the mobile phase and you see a very nice contour blot. We have short blots that can be quantified because we have no tailing at all because we are using very compatible mobile phases.

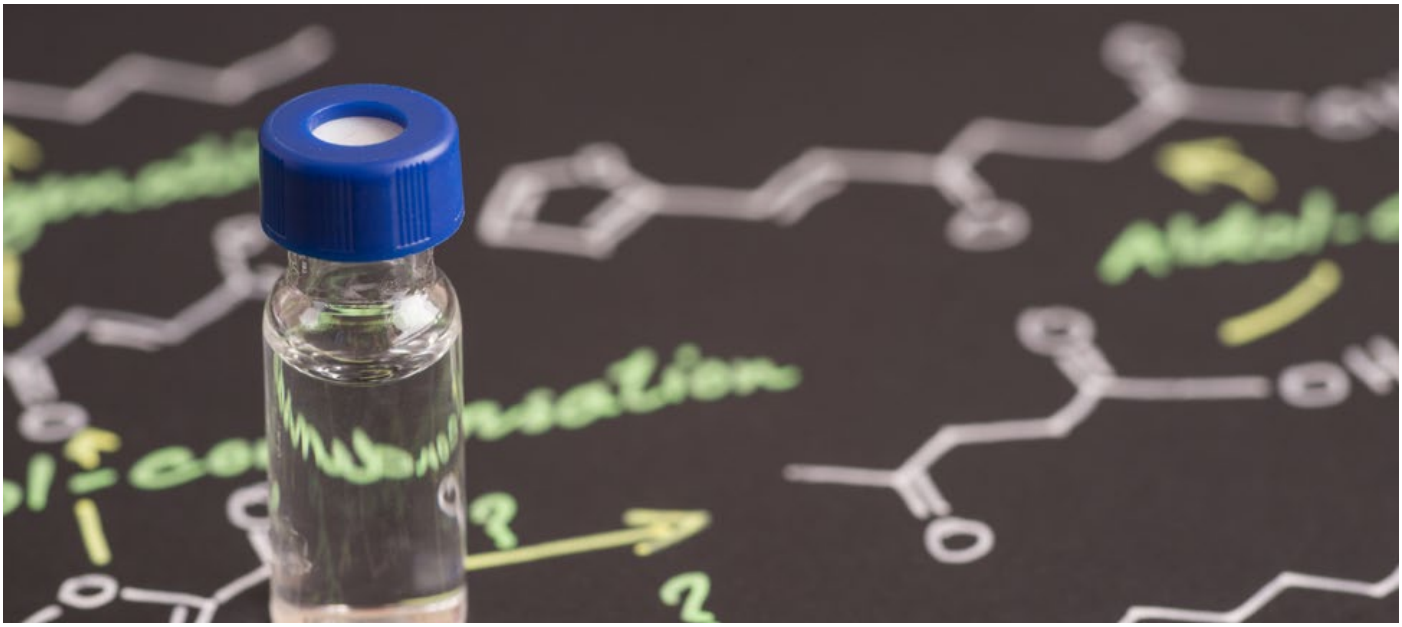
Important of course when you have that natural product extract, you'll see here the extract, the yellow ones or the precursors and this negative mode so we can easily follow exactly what's going on in a very reproducible way.

Because when we have seen the data of the quantification, this allows us to do exactly the same as a 1D analysis. The power of both the dimension LC in the analysis in biopharmaceuticals. We are not very familiar with biopharmaceuticals, for example atorvastatin, a product very well known in United States and the rate is 588.

Now I have to compare that with the monoclonal antibody. That molecular rate is 145 thousand, so it has more than 20 thousand atoms. If there is something wrong with my antibody, I must observe also the impurities so it's exactly the same problem in validation of a biopharmaceutical as you find with normal.

For example, a typical drug we use is an antibody, so the antibody enters the cancer cell and releases a compound. The chemotherapeutic agents is a direct target. So, it goes to the cancer cell, it's encapsulated and it releases the drug. Other monochrome antibodies without that chemotherapeutic agent are less effective.

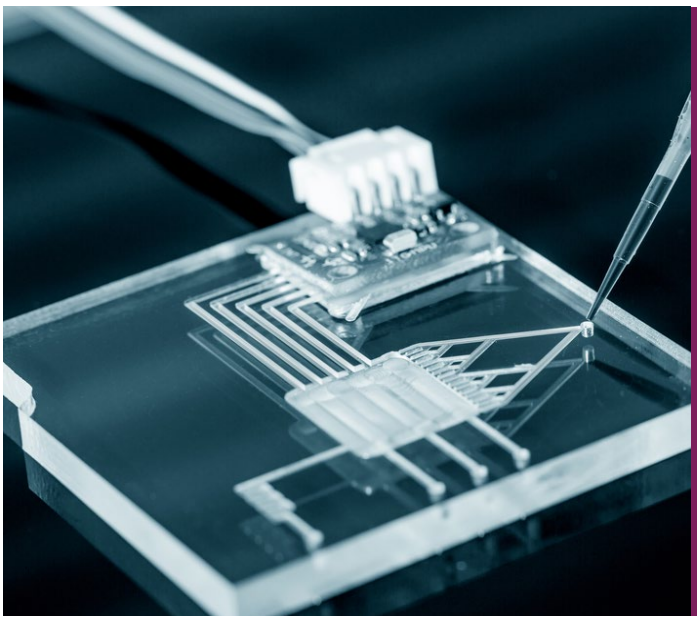
We go over and all we can identify precisely on the spots, which are highly isolated, low tailing etc. because here we also use reverse phase. The only difference is we were in the first column at the pH of two and in the second column at the pH of two and as amino acids are empathetic, they change their charge. And so we can easily identify the peptides and we can spot into the structure where the congregation size are. You can of course also do that with special spectro techniques but this is very easy technique combining liquid chromatography with mass spectroscopy.



In conclusion, peak capacity is important at peak capacity and we know that in one dimension and at a reasonable time, for example 60 minutes, we can go to 500 maximum. But, if we need more peak capacity then on 2D LC, especially now the instrumentation is available, the operational conditions are extremely simple if you can work with the 1D and you should have no problems.

I thank all my coworkers at the research institute for chromatography who are responsible for the 2D LC project.

4.4 COMBINED IMAGING AND THERANOSTIC NANOPARTICLES FOR NEUROLOGICAL DISEASES



Nanotechnology is becoming an important feature in medicine. It provides the basis for a range of diagnostic, monitoring and therapeutic techniques. In fact, the same nanodevice can now be used for all three functions. The use of such multi-function nanoparticles in medicine is referred to as theranostics.

Novel nano-platforms combine the best molecular medicines and imaging agents with highly specific, targeted drug delivery systems, and they have enabled new paradigms of therapy, diagnostics and even surgery.

At Pittcon 2017, well known leaders at the forefront of developments in this field described the latest state-of-the-art developments. This section summarizes key points from these presentations, giving an overview of current theranostic capabilities.

INTRODUCTION

Neurological diseases are some of the most challenging areas for drug discovery and development. There is significant unmet need in the marketplace, with an associated high degree of disability, lost productivity, and loss of quality of life.

Progress has been non-linear at best in the development of therapies for neurological diseases, and for many diseases there is still a need for earlier diagnosis. The field presents the added challenge of crossing the **blood brain barrier (BBB).**

Nanoparticles, that is particles between **1 nm and 100 nm** in size, offer a potential solution not only for more potent therapies that can cross the blood-brain barrier, but also improved biomarkers for neuroimaging.

A new category of product, the theranostic, combines a diagnostic biomarker with a therapeutic. In neurological disease, theranostics can allow imaging of a disease state of the brain and the ability to treat that disease at the same time.

Pittcon 2017 featured presentations on advances in the development of nanoparticles for imaging and theranostics.

CREATING NANOPARTICLES

Advanced laboratory technology is required in order to work with nanoparticle products for therapeutic applications. Field-flow fractionation is a method of separation that can be used for particles between **1 nm** and **100 micrometers** in a liquid medium.

The sample is separated without the use of any type of packing or stationary phase. Instead, separation is carried out by physical forces, such as liquid flow, centrifugal force, temperature gradients, or gravity. **Postnova Analytics Inc.**, an exhibitor at Pittcon 2017, offers a range of field-flow fractionation systems.

Light scattering is the most common method for measuring the size and zeta potential (electrokinetic potential) of nanoparticles. Light scattering analysis can be used to answer important questions in the development of a biotherapeutic nanoparticle, such as whether the nanoparticle mixture meets the intended specifications, efficacy of its targeting groups, and physical properties of the mixture.

Wyatt Technology Corp., another Pittcon 2017 exhibitor, make light scattering instruments that are suitable for development of products for imaging and theranostic applications.

ADVANCES FOR IMAGING

Magnetic resonance imaging (MRI) and positron emission tomography are the primary imaging technologies used in diagnosing brain disorders. Nanoparticles containing metals such as iron, gadolinium, and manganese are often used as contrast agents for brain imaging. **Superparamagnetic iron oxide (SPIO)** nanoparticles are of particular interest because they have a large surface area, magnetic properties, and low toxicity.

Biodegradable nanoparticles of poly(n-butyl cyanoacrylate) coated with polysorbate have also shown some potential as carriers of drug across the blood brain barrier. They can be used to deliver molecular imaging probes that are normally unable to permeate the **BBB** for imaging of amyloid plaques.

Another nanotechnology-based approach for brain imaging is the use of stem cells loaded with magnetic nanoparticles. This allows MRI monitoring of the stem cells as they migrate to injured brain and spinal cord tissue.

THERANOSTICS

Nanoparticle constructs that contain diagnostic and therapeutic functions at the same time are known as "theranostics." Theranostics go beyond imaging diagnostics and therapy, they can be used to monitor pharmacokinetics, distribution of the particle in the tissue, and accumulation of drug at the target site.

Imaging biomarkers can also be crucial in tracking progression of disease, success of therapy, and predicting outcomes. Such uses would also contribute to the trend toward personalized medicine.

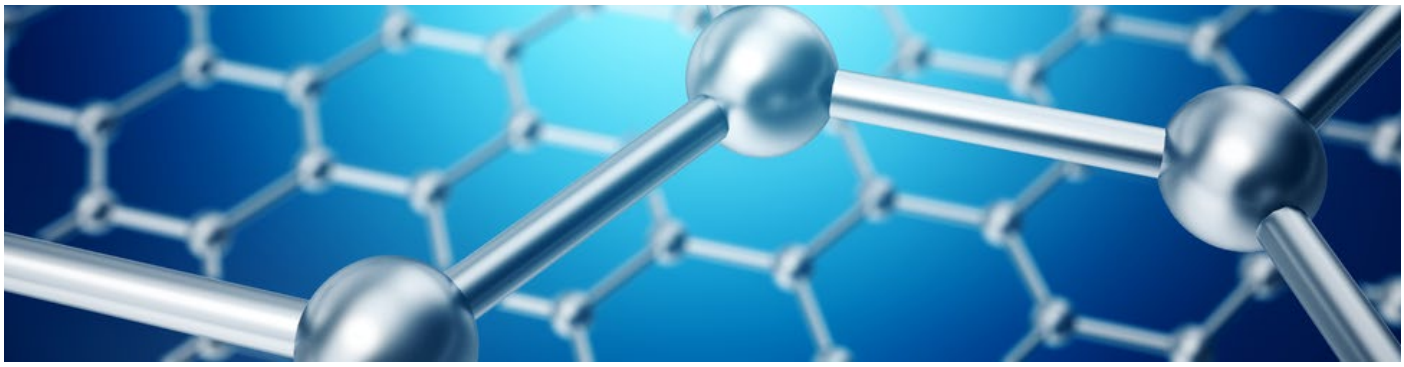
A Pittcon 2017 presentation, "**Nanomedicine for Functional Imaging and Therapy of Brain**," by **Paras Prasad** of SUNY at Buffalo illustrated how innovative design of a multifunctional nanoparticle can transcend ideas of what a drug or diagnostic can do it the brain.

The researchers used their nanoparticle platform to build an optical probe and contrast agent for photoacoustic and magnetic resonance imaging of the brain. Its targeting and payload capabilities allow it to deliver a genetic material or drug to treat a brain disease or enhance a cognitive ability.

The particles also function as optical nanotransformers to convert skull penetrating infrared light into a blue light that is absorbed by channel rhodopsin. Essentially, the nanoparticle allows optogenetic control of neuronal activities at specific sites within the brain.

The technology has therapeutic potential for brain injury and concussions, and it may even be possible to use it to enhance certain cognitive states or sensory functions to create "super human capabilities."

The ability to deliver imaging agents and therapeutics across the BBB using nanoparticles has the potential to transform the field of neurological disease, and may be the key to curing some diseases that currently have inadequate treatment options.



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4.5 NANOTECHNOLOGY ADVANCES FOR CANCER DIAGNOSTICS AND NANOTHERAPY

The previous section described how simultaneous diagnosis and therapy has been made possible by multifunctional nano-carriers. The development of nanoplatforms has also facilitated targeted delivery of combinations of therapeutic agents, allowing greater efficacy to be achieved with reduced toxicity, this has proved particularly valuable in the treatment of cancer where the treatments needed to destroy cancerous cells are also very damaging to healthy cells.

In addition, nanoparticle technology has led to the development of cancer vaccines, treatment to control arrhythmia, and enhancement of magnetic resonance imaging. This section provides an overview of the latest therapeutic applications of nanotherapy described at Pittcon 2017.

INTRODUCTION

The delivery of drugs and diagnostic markers to cancer tissues has been a major challenge in the development of cancer therapies. Typical delivery methods distribute products throughout the body, affecting healthy and cancerous cells, thereby toxic effects to healthy cells limit the scope and effectiveness of the anticancer therapy.

Targeted therapies and diagnostics provide a means of delivering more potent molecules to the cancer cells, but conventional targeted delivery methods still have many limitations.

A combination of targeted delivery methods, both active and passive, can increase the concentration of a drug or diagnostic molecule in the cell, while minimizing the effects on healthy cells. Nanoparticles, particles between **1 nm and 100 nm** in size, can be engineered to contain multiple functional groups, making use of synergistic targeting strategies.

Nanoparticles have become a promising new class of therapeutics for cancer diagnosis and treatment. A number of scientists and exhibitors at Pittcon 2017 presented advances in the use of nanoparticles for cancer diagnostics and therapy, often in combination - ”theranostics.”

NANOPARTICLE PROPERTIES

The size of the nanoparticle is an important feature for drug delivery. The optimal size for cancer applications is between **10 and 100 nm**. At less than **10 nm**, the particle will be eliminated too quickly by the kidneys and particles larger than **100 nm** tend to be cleared rapidly by the immune system.

Light scattering is the standard method for measuring the size and zeta potential (electrokinetic potential) of dispersed nanoparticles. N**anoparticle tracking analysis (NTA)** and **resonant mass measurement (RMM)** are newer techniques that can also be used to measure as well as count nanoparticles in a sample.

Malvern Instruments Ltd. supports NTA through its NanoSight range of instruments and RMM via the Archimedes product range. From **Particle Metrix Inc.**, the ZetaView carries out nanoparticle tracking analysis with micro-electrophoresis and Brownian motion analysis. Malvern and Particle Metrix were exhibitors at Pittcon 2017.

Nanoparticles can be made from a range of materials, including metals, polymers, dendrimers, liposomes, viruses, and carbon nanotubes. Different properties of the materials can be exploited for use in targeting cancer.

For example, dendrimers have been used extensively in biological applications because their highly branched, repeated, three-dimensional structures offer many options for placing functional groups with precise control of the size and shape of the particle.

Liposomes, as well, are popular for creating targeted delivery systems. They have been in use for 50 years, but a newer generation of polymer-coated liposomes have been shown to increase the duration of blood circulation of a drug from a few minutes to as much as three days.

Metal nanoparticles have attractive potential for diagnostics and enhanced forms of therapy, such as thermotherapy. Iron oxide nanoparticles have long been used as imaging agents for MRI. They are now being investigated as drug carriers that can be targeted to a desired tissue using an external magnetic field.

The unique properties and design characteristics of nanoparticles lead to some unique applications. For example, combining a cardiac-targeting peptide and a photosensitizer group in a nanoparticle enables tumor-focused targeted imaging in combination with targeted therapy or cancer. The technique overcomes the imaging limitation of light penetration in conventional optical imaging technologies.

In the presentation, "**Nanotherapy and Nanodiagnostics: From Cancer to Heart Disease,**" at Pittcon 2017, **Raoul Kopelman** detailed how this nanotechnology-based photodynamic therapy (PDT) method was effective for cardiac ablation to resolve arrhythmia. A similar approach can be used to target cancer cells in the heart or other tissues.

TARGETING NANOPARTICLES

Targeting strategies for therapeutic nanoparticles can be passive or active. Passive targeting makes use of the size of the particle and tumor vasculature. New blood vessels serving the tumor have larger gaps than blood vessels in normal tissues, allowing nanoparticles to penetrate.

Nanoparticles can also be actively targeted by incorporating a targeting group on the surface of the particle. The targeting group usually binds a tumor-specific antigen or receptor. An ideal candidate for a target should be unique to tumor cells and abundantly expressed on the therapeutic payload into the cell.

In another Pittcon 2017 presentation, "**Spherical Nucleic Acids as Potent Immunomodulation Agents for Cancer Therapy**," **Chad Mirkin** from Northwestern University described a new class of nanoparticles called spherical nucleic acids that interact with cells through toll-like receptors (TLRs) to activate the innate immune system in antigen-presenting cells (APCs). Those APCs then mature and activate effector T-cells to kill tumor cells.

SNAs, which consist of a dense shell of oligonucleotides conjugated to a spherical nanoparticle, are being used to develop vaccines against prostate cancer, melanoma, lymphoma and triple negative breast cancer.

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CONCLUSION

With more lenient attitudes towards cannabis over recent years, cannabis use is becoming more widespread. A significant proportion of this use is justified by the purported therapeutic benefits obtained from cannabis.

> Historically, cannabis was illegal globally. Today, the picture is more complicated with several countries and US states adopting various more liberal stances in their regulation of the drug.

However, despite such steps to decriminalize and legalize cannabis use no standards for its production have been introduced. This raises concerns for many since there is a not-insignificant risk of the cannabis being contaminated with toxins, which could prove particularly dangerous for individuals who are already unwell.

Furthermore, the composition of active ingredients varies considerably from pant to plant. Users of the drug therefore have no idea of the potency or therapeutic efficacy of the particular cannabis they buy.

Presenters at Pittcon 2017, illustrated the range of existing and new analytical techniques that can effectively detect contamination with pesticides or heavy metals and define the precise composition, and hence potency and therapeutic potential, of batches of cannabis.

These include infrared spectroscopy, mass spectrometry with a full evaporation headspace technique, headspace gas chromatography-flame ionization detection techniques and two-dimensional gas chromatography coupled with time-of-flight mass spectroscopy. In addition, an online solid phase extraction method was described for safely extracting cannabis oil, a popular option among consumers requiring medicinal cannabis.

However, despite the array of available technologies many of the companies able to provide quality assurance testing are unsure about where they stand with regard to the law when it comes to testing cannabis. The situation is particularly confusing in the USA where cannabis is illegal according to federal law but permitted to varying degrees by some state rulings.

With ever-increasing usage of cannabis, enforcement of standardized quality measures is needed to ensure that cannabis is safe. Since cannabis use is still illegal in the majority of countries, detecting illicit cannabis in biological samples and contraband continues to be a priority. Advances in cannabis detection technologies, such as portable spectrometry devices, are allowing law enforcement agents to detect cannabis more readily.

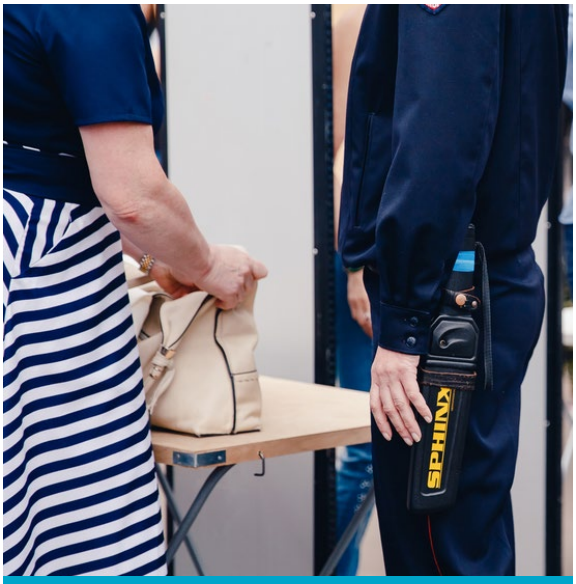
Pittcon 2017 also highlighted the advantages of Raman spectroscopy in process environments, such as research and development and pharmaceutical manufacture. It provides direct, real time and in-situ measurements allowing immediate quality assurance assessments.

The use of nanoparticles has helped solve some of the latest challenges in medicine. For example, they can be used for the targeted delivery of highly toxic chemotherapy to tumor cells, thereby minimizing damage to healthy tissues.

More recently, in a technology referred to a theranostics, nanoplatforms combines a diagnostic biomarker with a therapeutic agent. For example, in neurological disease, theranostics can deliver contrast agents to allow imaging of the brain to assess disease state whilst at the same time providing the means to treat that disease.

Pittcon 2017 symposia illustrated the powerful analytical and therapeutic technologies that are now available and highlighted the importance for legislation to follow changes in practice so that quality standards are established and adhered to.

5 HANDHELD DEVICES AND ENVIRONMENTAL CHEMISTRY



The detection of explosives is important for safeguarding well-being in a diverse range of environments, each of which poses its own challenges. In the prevention of explosions caused with malicious intent, screening of both passengers and their luggage for explosives is now routine at the majority of airports.

INTRODUCTION

Similar screening may also be implemented at access to high-security areas or key events. Identifying areas with high levels of potentially explosive chemicals is also valuable in thwarting illicit production of improvised explosive devices (IEDs).

The detection of explosive residues can also provide forensic evidence for firearms offences and illegal shipping of explosive materials. Similarly, analysis of post-explosion residues can help determine the cause of an explosion, be it intentional or unintentional -this can be particularly important in maintaining the safety of workers at high-risk sites where there is the potential for explosion. Such analysis can help determine the precise factors that caused an explosion, thereby allowing corrective procedures to be installed to reduce the risk of similar events occurring in the future.

These are just a few of the instances in which analysis or detection of explosive materials are needed, and already it is apparent that one test will not be suitable in all situations.

In some cases, such as in the airport, it is important that the act of screening does not have detrimental effects on the people or items being screened. In other cases, such as at sites with an imminent threat of explosion, the screening needs to be conducted at a safe distance from the potential explosive device or explosion site. In most cases, it is also helpful for the screening to be conducted and interpreted rapidly, often by personnel who are not specialists in analytic techniques, so that the appropriate action can be taken as soon as possible.

The identification of explosive materials and analysis of post-explosion residues is thus complex and challenging and relies upon the development of an array of analytical technologies and methodologies.

With explosives intended for malicious use being camouflaged in increasingly sophisticated ways, comes the need for increasingly sophisticated technologies to detect them. In particular, the use of seemingly harmless everyday items in the construction of IEDs has made them especially difficult to discern.

To this end, NATO developed a global counter-IED action plan, and a recent report by the Office of Naval Research highlighted the need for improved rapid detection of explosive materials in the field.

Scientists have risen to the challenge, developing increasingly sensitive and easy-to-use technologies for rapidly detecting explosive materials and the ingredients for producing them.

This is clearly evident across the various sections of this chapter, which provides an overview of relevant presentations at Pittcon 2017. It includes a discussion of the challenges posed to explosives detection in more detail and highlights the latest advances in the development of spectroscopic techniques for the identification of explosive materials and their adaptation for use in varying situations with specific requirements.

5.1 IDENTIFYING EXPLOSIVE MATERIALS AND ANALYZING POST-EXPLOSION RESIDUES – THE RISE IN HANDHELD DEVICES

With the mounting threat of terrorist activities, detection of explosives is becoming an increasingly important aspect of modern life. This is a challenge in itself, since many of the nitrate and hydrocarbon substances used in explosives are also present in chemicals routinely used for productive purposes, e.g., fertilizer. Similarly, improvised explosive devices are difficult to detect as they often comprise harmless everyday items.

Analysis of post-explosion residues can be dangerous but provides information valuable in determining the cause of an explosion so that similar incidents can be avoided in the future. As presented at Pittcon 2017, this section discusses the latest technologies developed to meet these challenges.

INTRODUCTION

Explosive detection is an application of advanced spectroscopic technologies which impacts an increasingly prominent global issue. Explosives, such as gunpowder, have been in use for centuries, but it is only very recently that technology has allowed for the rapid identification of explosive materials in a range of situations.

Detecting explosives can be a dangerous task, due to the potential presence of toxic materials, and the imminent threat of explosion, which can hamper traditional forensic investigation. It is clear, therefore, that any analysis techniques which are capable of working quickly, efficiently, and at a safe distance, will be highly desirable in this application.

Of course, explosive incidents are not always intentional. Accidental industrial explosions, for example, are a major risk factor in several sectors, and understanding how and why they occur is crucial when trying to prevent future incidents.

Civilian agencies such as the U.S. Chemical Safety and Hazard Investigations Board regulate the analysis of post-explosive residues, in order to determine the cause of accidents. In these investigations, identification of these residues is a highly important task, which can benefit from many of the same technologies.

Pittcon 2017 allowed researchers to learn about the latest trends in explosive material analysis. Pittcon began life back in the 1950's as a conference on Analytical Chemistry and Applied Spectroscopy. Over the years, it has evolved to encompass all laboratory based scientists testing or analyzing the chemical / biological properties of compounds or molecules.

There were several talks relevant to this topic at various symposia of this Pittcon 2017, such as '**Eye-Safe Near-Infrared Trace Explosives Detection and Imaging**' delivered by **Marcos Dantus** of Michigan State University, '**Discriminating Power of Volatiles from Forensic Specimens in the Field Using Innovative Sampling and Analysis**' delivered by **Kenneth G Furton** of Florida international University and '**Mass Spectral Tools for Characterization of Synthetic Phenethylamines**' delivered by **Ruth Smith** of Michigan State University.

This year's Pittcon featured several leading companies relevant to explosive material detection. Companies such as **Thermo Scientific, Metrohm and Rigaku** demonstrated their latest optical detection technologies ranging from lasers to spectrometers and optics all helping to miniaturize detection technology.

Recent technological developments have led to a rapid rise in handheld devices capable of tackling explosive detection, removing the need for extensive sample preparation in a laboratory environment and allowing quick, accurate analysis at a suitable standoff distance directly in the field.

RECENT CHALLENGES IN IDENTIFYING EXPLOSIVE MATERIALS

EXPLOSIVE MATERIALS

Explosive materials are generally composed of a hydrocarbon-based fuel component, and a nitrogen- or oxygen-based trigger, such as a nitrate or a peroxide. Explosives are classified as high or low energy, depending upon the speed of propagation of the combustion reaction – in high explosives, the reaction propagates faster than the speed of sound, creating a shockwave. It is estimated there are at least 150 separate materials in use today.

As well as the vast array of explosives available today, many have multiple uses – for example, industrial chemicals like nitric acid, or fertilizers like ammonium nitrate combined with a hydrocarbon like diesel fuel. Detection of these more common materials can be difficult, as their presence does not necessarily indicate the intent to detonate an explosive device.

RESPONSE TO THE GLOBAL IED THREAT

A notable concern in today's world is **improvised explosive devices (IEDs)**. These are particularly tricky to identify, as they take many forms, and use numerous activation methods. By definition, they are non-traditional and often contain non-military components.

They are often used indiscriminately, causing both military and civilian casualties. The adaptability of these devices is both why they are employed, and also why they can be so difficult to counter. They can be deployed in a wide range of ways, typically disguised as part of the environment or as an everyday object. Detonation methods include via trip wire, cell phone or by hand.

To tackle this ever-changing threat, NATO developed a counter IED action plan in 2010 with three focal points:

- Defeating the device
- Attacking the network
- Preparing the forces

The first point looks at how to detect and neutralize an IED, which is the primary focus in this article. The 'Defense Against Terrorism Program of Work' has several projects specifically relating to developing sensors and information technology for the detection of IEDs. The other two points of NATO's C-IED plan require organizational cooperation across multiple agencies and often across international borders.

The response to IEDs is mainly driven by various global militaries, who understandably have a large stake in developing portable sensing devices for use in the field. Homeland security maintains slightly different requirements in that detection equipment does not necessarily need to be portable, but it still needs to be adaptable to different situations, including airports, vehicle scanning, and cargo ships.

TECHNIQUES FOR PRE/POST EXPLOSION RESIDUE DETECTION

The requirements for a useful detection technique for explosive devices in the field can be summarized by three points:

- High sensitivity, with the ability to detect trace quantities of material
- Can operate from a safe distance
- High specificity for explosives, in the presence of other common materials (sometimes termed 'chemical clutter')

Unfortunately, in practice these three requirements often conflict with each other.

It can be seen that there is a need to develop a range of solutions, to cater to multiple possible situations. A recent report supported by the Office of Naval Research has suggested research is necessary in a diverse set of scientific topics including 'chemical, environmental, and electrical engineering; chemistry and analytical chemistry; applied physics; forensic science; spectroscopy; and optics'. This is to counter the threat not only from the explosive material itself but also arming and firing systems.

An important area of development is the improvement of rapid detection in the field. It is one thing being able to detect trace amounts of material in a lab environment, but entirely different in more challenging and less controlled conditions.

Hence, the report continues to recommend research in 'plume and aerosol dynamics; x-ray, microwave, infrared, and terahertz imaging and spectroscopy; neutron, gamma-ray, magnetic resonance, and magnetic-field systems; optical absorption and fluorescence; light detection and ranging (LIDAR), differential-absorption LIDAR (DIAL), and differential-reflectance LIDAR (DIRL); biosensors and biomimetic sensors; and microelectromechanical systems (MEMS). Researchers in chemical, mechanical, nuclear, and electrical engineering, bioengineering, chemistry, spectroscopy, applied physics, and optics should be involved in these efforts.'

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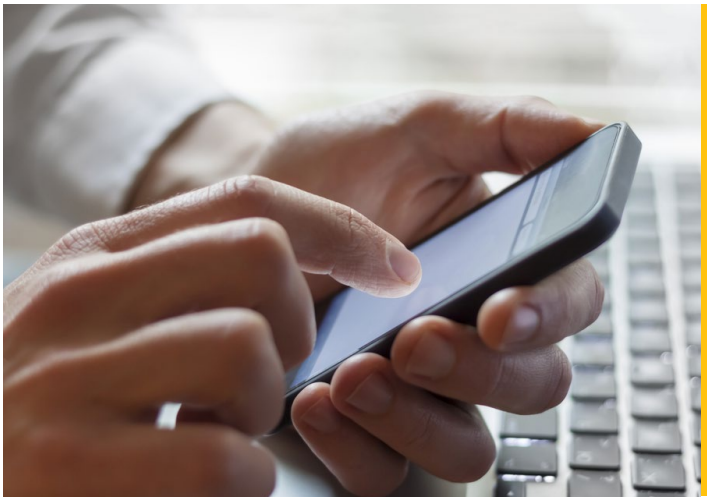
THE RISE IN HANDHELD DEVICES



The detection of explosive materials is challenging for several reasons. The need for fast identification in the field by a wide range of organizations (government agencies and military in particular) has driven a number of recent advances.

Optical spectroscopy techniques are today seen as the most promising for remote detection capability. In general, determining the amounts of oxygen and nitrogen in a sample relative to the other elements present is a good indicator of whether the compound will be energetic or non-energetic.

While the full chemical structures may be complex and difficult to distinguish by traditional chemical analysis methods, explosive materials tend to have an abundance of oxygen and nitrogen, whereas diesel, for example, is a mixture of hydrocarbon chains with no oxygen or nitrogen, so it is not an explosive material.

Optical spectroscopy techniques can exploit this by determining the ratio of hydrocarbon to nitrogen/oxygen content. Optical spectroscopy can also distinguish known explosive materials within a cluttered background signal, by looking for more specific chemical signatures from fluorescence or vibrational energy modes.

These optical methods must be simple to operate in a non-laboratory setting by a non-expert user. In field-based applications, it is unlikely that there will be a trained chemist present, especially in more hostile environments. The analysis must also be fast, as there will often be an element of time sensitivity to the situation.

There is a wide array of techniques under development for detecting bulk qualities of explosives, such as terahertz imaging or x-ray analysis, which have seen use at airports and important government buildings. However, the equipment required for these techniques is bulky, expensive, and of limited use for detecting trace quantities of material. X-rays, in particular, are a highly energetic ionizing form of radiation, making bulk x-ray analysis impractical for safe use in the field. Terahertz radiation is non-ionizing, but is susceptible to atmospheric contaminants, with a particularly unfortunate strong absorption band present for water vapor, which has a tendency to mask other signals.

Lower energy optical techniques, including Raman, Laser-Induced Breakdown Spectroscopy (LIBS), Laser-Induced Fluorescence (LIF), and Fourier Transform Infrared Spectroscopy (FTIR), have all seen advances towards handheld use in recent years, as have mass spectrometry techniques such as Ion Mobility Spectrometry (IMS) and Differential Mobility Spectroscopy (DMS).

MASS SPECTROMETRY (IMS, DMS)

These techniques make use of the ability to separate ions based on their mass and charge. In mass spectrometry, a sample is bombarded with electrons so that its molecules will break into fragments, which can be separated by their mass to charge ratio.

The specific separation technique will vary depending on the phase of the ions (solid, liquid or gas), amongst other factors. One specific technique, which has seen growing use in airports in recent years, is IMS, a mass spectrometry technique for the gas phase which relies on the mobility of ions in a carrier gas.

DMS is a slight variation on this concept, where high electric fields are used to filter out certain types of ions. In both of these techniques, the choice of carrier gas is crucial for an effective analysis.

One reason that mass spectrometry based techniques are popular for explosive detection is their speed and accuracy. They can measure trace amounts of material, as low as picograms (**10-15 per kg**), putting them on a par with optical techniques such as LIBS.

However, proximity is an issue; they require samples of materials to be inserted into the spectrometer unit. This limits the applications to more controlled situations, such as airport security, rather than broader field applications where detection at a distance is highly desirable.

OPTICAL SPECTROSCOPY (RAMAN, LIF, LIBS, FTIR)

To date, many optical techniques have been adapted into portable form factors suitable for standoff explosive material detection. Some examples include Laser-Induced Breakdown Spectroscopy (LIBS), Raman Spectroscopy, Laser-Induced Fluorescence (LIF), Cavity Ringdown Spectroscopy (CRDS), and Photofragmentation followed by Resonance-Enhanced Multiphoton Ionization (PF-REMPI), amongst many more.

We look in detail at case studies using some of these techniques later in the chapter. They each have their own advantages and disadvantages; Raman for example may be suitable at stand-off distances of over 10 m, although long integration times hamper the technique.

The progress towards advanced handheld devices with the detection capability of full-sized lab instruments is accelerating, and Pittcon 2017 showcased the latest trends. For example, Rigaku exhibited their latest X-Ray detectors and optics. There were also symposia and posters as well as several short courses relating to both mass spectroscopy and optical techniques.

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LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS)

In this first spectroscopy technique, a highly energetic laser pulse is employed to excite sample residues to the point of breakdown into their constituent parts, forming a microplasma. The light emitted from this can be compared to standard elemental and molecular spectra to determine makeup of the residue.

LIBS is a surface characterization technique, i.e. just a very small amount of material is ablated from the surface. Measurement can take place very quickly – there is an initial broad continuum of emitted radiation lasting a few milliseconds, which needs to be gated out, but the characteristic elemental spectra begin to appear very rapidly, allowing strong signals to be gathered in typically just a few seconds.

Historically, **LIBS** has been used primarily to test for the presence and relative quantity of individual elements, i.e. metals. Today however, high-resolution broadband spectrometers have enabled detection of multiple elements and compounds from the same sample.

In theory, any material may be detected; not just explosives, but any form of matter, and LIBS is now used in a diverse range of applications, from metals to plastics and even biological samples. It is envisaged, with suitable optics or a mechanized setup, LIBS could scan over large areas of a sample to create an elemental map.

The benefits of LIBS are numerous. It is a straightforward technique, with no prior preparation of samples required. This also makes the equipment relatively cheap to setup and operate. The system can be miniaturized, as there is little in the way of complex optics, making it easily adaptable to handheld instruments.

The sensitivity of LIBS is high enough to detect trace quantities in a short time (< 1 second, a single laser shot is employed with no long integration times). The small amount of ablated material means the technique is minimally destructive. A drawback associated with this is a lack of depth analysis and the technique being prone to surface contamination, however this may be mitigated by firing several 'test shots' of the laser prior to analysis.

Work by **Jennifer Gottfried** and colleagues at the Army Research Laboratory has focused on optimizing **LIBS** for detecting explosive residue. They suggest that the ability of LIBS to breakdown and analyze a sample with a single laser shot is highly beneficial for explosive detection. As it is a purely optical technique, LIBS is ideal for standoff detection, with the potential for very long distance ranges with the incorporation of telescopic optics.

Building on work by researchers for organic material detection, **Gottfried's** team took the ability to identify carbon, hydrogen, oxygen and nitrogen atomic emission lines from complex compounds and applied it to military grade explosives.

In explosive materials, the ratio of nitrogen and/or oxygen to hydrocarbons is high, which is a useful measurable characteristic to distinguish between a dangerous organic compound such as RDX or TNT and something more benign such as nylon. However, it should be noted that the contribution from the atmosphere cannot be ignored (air is largely made up of nitrogen and oxygen).

To overcome this in a lab environment, an inert atmosphere such as argon may be used, although this approach is far from ideal for standoff detection in the field. Again, several laser shots in quick succession may be the best way to overcome this issue.

A modified method known as double pulse LIBS was employed by these researchers, using collinear nanosecond pulses to first impact the material and displace the surrounding gas, creating an area of reduced pressure. By the time the second pulse hits, the contribution from atmospheric gases is minimized.

In this study, a LIBS system was utilized with a **1064 nm**, neodymium–doped yttrium aluminum garnet (Nd:YAG) laser, emitting **320 mJ**, **8 nanosecond** pulses. Power at the focal point was under **1 GW/cm2** to produce the microplasma, with the double-pulse setup allowing for a complete measurement to be taken in less than one second.

Lenses were used to defocus the resulting plasma spark onto a fiber optic bundle of seven fibers, for complete analysis by a broadband spectrometer over the **200-980 nm** spectral range. A 1.5 microsecond gating delay was used to allow the plasma to begin cooling.

A wide array of explosive materials was surveyed via standard LIBS: RDX, HMX, TNT, PETN, NC, C-4, M-43, LX-14, JA2 and A-5. Subsequently, double pulsed LIBS (each pulse **160mJ** separated by **1-10 microseconds**) analyzed RDX, mixtures of RDX and TNT as well as RDX contaminated with diesel fuel to try and distinguish these materials.

It is worth noting that the laser-induced microplasmas never initiated detonation of the materials, as the short-lived spark is an insufficient ignition source. This is something to be aware of when working with energetic materials, however, and laser intensities must be managed sensibly.

Initial results of the single-pulse LIBS analysis showed successful identification of materials such as charcoal over 95% of the time. Double pulse tested LIBS also distinguished the explosive materials well, and is perhaps the more promising technique for standoff detection, thanks to its ability to reject atmospheric background signals without the need for argon flow.

The **Army Research Laboratory** have been working on a promising advancement of this technology known as 'suitcase LIBS', with the laser and detector contained within a hand-held wand and laptop/spectrometer fitting in to a suitcase. Weighing only 9 kg (20 lbs), this shows much promise for employing LIBS for standoff detection in the field.

At Pittcon this year, there were several relevant talks, as well as live demonstrations by world leading spectrometer and optics companies. For example, **Ibsen Photonics** gave a live demonstration on '**How to select the right spectrometer**' a valuable insight especially for broadband applications such as LIBS. There was an entire symposium dedicated to LIBS, as well as a half-day course for those who wished to learn more about this technique, entitled '**Elemental Analysis via Laser Induced Breakdown Spectroscopy and X-ray fluorescence**'.

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RAMAN SPECTROSCOPY

A second optical based detection method, **Raman Spectroscopy**, works on the principle of the Raman effect, where incident light is inelastically scattered and shifted in frequency due to specific molecular vibrations. Light may be scattered in three ways: elastically (known as Rayleigh scattering), or through two inelastic methods whereby the incident photon induces a dipole within the molecule, causing a transfer of energy.

If the molecule absorbs energy, the scattered photon ends up with less energy – this is known as Stokes Raman scattering. Conversely, Anti-Stokes scattering is when the molecule loses energy to the scattered photon. Raman should not be confused with light emission from an excited energy state (fluorescence or phosphorescence). The light is merely scattered, rather than absorbed and re-emitted.

The benefits of Raman spectroscopy include the ability to measure at a safe standoff distance, and the ability to detect small concentrations of material at low vapor pressures with significant optical and chemical interference. It is non-invasive, can analyze solids, liquids or gases without sample preparation and can yield direct molecular structure information for identification of potential explosive materials.

Many Raman signatures of explosives are well known. The challenge comes in obtaining discernable spectra of trace amounts of material at a safe standoff distance of a few to hundreds of meters, quickly and in a contaminated and changing environment. Often for long standoff measurements pulsed lasers are used as an excitation source with large scale optics to detect the scattered signal.

Work by **Diana Smirnova** and colleagues at Caltech has investigated explosive materials through Raman Spectroscopy, utilizing the unique quality of these materials of decomposition during heating. Although this sounds like a dangerous prospect, the researchers were careful to select a suitable temperature range – particularly for a heat sensitive explosive like TNT, caution is always recommended!

As a bonus, any surrounding material should not interfere in the analysis, as it should be relatively unaffected by heating. This type of spectroscopy is known as 'two-dimensional correlation spectroscopy' where a variable is shifted and two dynamic Raman spectra are correlated. They proposed that during heating, Raman peaks for explosive materials would decrease, while decomposition products would increase.

Various experiments were undertaken to analyze Stokes lines (these have a much higher intensity than Anti-Stokes at room temperature as it is far more likely a molecule will be in its ground state rather than excited) of these explosive materials.

For standoff measurements, a frequency doubled **532 nm** Nd:YAG laser was used at a distance of **9 m** from the samples. This wavelength is carefully chosen; Raman can be performed in the infrared, visible or UV range, with corresponding trade-offs between sensitivity of scattered signal, spatial resolution and fluorescence suppression.

Thermal modulation is achieved by successive infrared pulses from a CO2 laser. Military grade explosive materials (RDX, HMX, PETN and TNT) were investigated, first by measuring the 1D spectra, followed by 2D analysis. To make the study realistic, a contamination study was performed, mixing samples with saliva and urine amongst other potential contaminants.

The results of this study showed that this 2D correlation spectroscopy technique is indeed successful, perturbing the spectra of the measured explosive residues, and distinguishing it from the static background contaminants, hence reducing the chance of false positive measurements in Raman analysis of explosive materials.

One of the drawbacks of Raman spectroscopy is that the signals can be relatively weak, compared to other spectroscopy techniques based on fluorescence or absorbance. The synchronous spectra from the 2D correlation spectroscopy technique proved useful to help combat this, due to the higher signal-to-noise ratio for strong peaks.

It was not expected that the asynchronous spectra would yield much interesting information – however, due to the crystalline nature of the explosive materials under study, lattice separation did produce a Raman signal which contributed to distinguishing the samples from the non-crystalline contaminants.

Further work is also being undertaken by the researchers to optimize the optics and reduce long integration times, to make this technique truly portable and rapid in the field.

Large area analysis is still a concern with the pulse repetition rate (10 Hz) not enough currently to make this practical. Further studies also aim to analyze other non-traditional explosives similar to fertilizer, which would be particularly significant as the lack of characteristic chemical structures in these materials pose a great challenge for spectroscopic techniques.

Renishaw, the manufacturer of the spectrometer used in this work, exhibited at Pittcon 2017. Also exhibiting at this year's Pittcon was Biotools, Inc, who performed a live demonstration of high sensitivity and fast results with a handheld Raman device. B&W Tek demonstrated their i-Raman Pro technology for portable real time monitoring. Metrohm USA demonstrated their Raman capabilities at Pittcon 2017.

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LASER-INDUCED FLUORESCENCE (LIF)

Laser-Induced Fluorescence (LIF) is a spectroscopic technique whereby a molecule is excited to a higher energy state by the absorption of laser light. Spontaneous light emission will follow when the molecule drops back down to its ground state.

The difference between fluorescence and Raman spectroscopy is that in LIF, light is absorbed by the molecule and a unique photon is emitted, rather than the interaction and scattering due to vibrational energy which Raman spectroscopy makes use of.

> The preparation of explosives, particularly non-professional or improvised devices, can often leave measurable quantities of residue on surfaces during preparation, transport or other forms of handling. Even if a person is not directly handling explosive material, there is enough transferred material, for example from one person to a door handle, and then a second person touching that residue, for a quantifiable trace to be identified.

Work by **Charles M. Wynn** and colleagues from MIT aims to develop a specific adapted LIF technique capable of detecting these residues rapidly and at a standoff distance of at least ten meters. They suggest that transfer mechanisms across a variety of conditions usually yield at least **1 ug/cm2** of material, and set out to detect explosive material in this quantity.

It is clearly preferable to discover explosives during the preparation of material, before there is an actual bomb in a dangerous and volatile situation. **Wynn's** team therefore aimed to develop a technique that could be used to forensically detect explosive making activities within a relatively large area, negating the need for higher-risk activity by security of law enforcement forces further down the line.

The proposed technique involves photodissociation followed by Laser-Induced Fluorescence (PD-LIF), whereby the first step of the analysis is dissociating polyatomic material into diatomic molecules. As discussed in the previous example, high explosives such as TNT and RDX tend to be nitrogen-rich molecules with similar structures, which will dissociate when illuminated with UV light.

The dissociation products are very characteristic of these materials – one key fragment being **nitric oxide (NO)** which has very well defined spectra. Although NO can be present in background quantities as an atmospheric pollutant, the difference here is that it dissociates with excess vibrational energy (rather than being in its ground state), and hence is clearly distinguishable from surrounding contaminants.

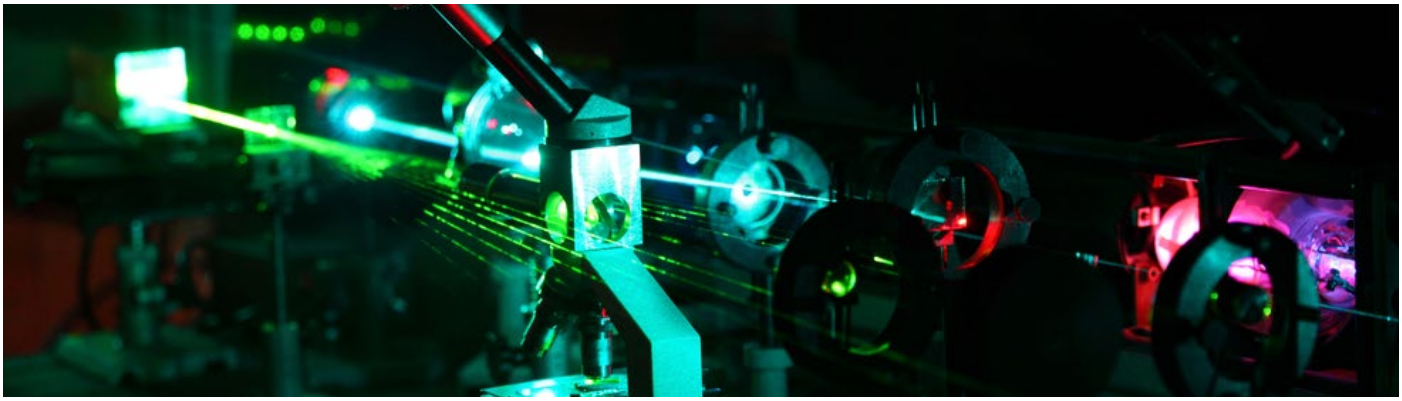
After dissociation, a second photon (from the same laser pulse) of the same wavelength is absorbed. Importantly, this happens within a few nanoseconds, within the lifetime of a vibrationally excited NO molecule. This will pump the NO to an excited electronic state.

Finally, a photo-detector is setup to detect what should be a slightly shorter wavelength (higher energy) UV beam returning from the sample (if it is indeed dissociated rather than surrounding contamination), indicating the presence of a nitrogen based explosive.

This technique differs subtly from LIBS (subject of a previous case study) in that LIBS dissociates material into constituent atoms by creating a microplasma from a high-energy laser pulse, whereas PD-LIF utilizes lower energies to break the sample into molecular components.

This provides several advantages over LIBS, in that a lower power eye-safe laser may be used. Also, LIBS is susceptible to significant contamination from nitrogen and oxygen present in samples from contaminants or simply the surface it sits on.

Other benefits of this technique include a strong returning signal and strong specificity. In this work, a laser pulse of **236.2 nm** is used, and returning light from the NO has a wavelength of **226 nm**. Even if there is surrounding contamination that absorbs in the UV range, it is extremely unlikely for it to fluoresce a precise **226 nm** photon.



It is unusual for a fluorescence process to emit higher energy radiation than the excitation energy. This means that the availability of vibrationally excited NO molecules immediately prior to further electronic excitation is extremely unlikely to occur, unless nitrogen-rich explosive materials are present.

LIF also benefits from very low 'optical clutter', as it operates in the UV spectrum below **300 nm** known as the 'solar-blind region', where absorption due to atmospheric ozone prevents light reaching earth. This is a distinct advantage over infrared spectroscopy techniques, such as Raman, where filtering of this clutter is paramount for good signal to noise. The only filter needed for LIF is an optical narrowband filter to filter reflected light of the same wavelength.

Experiments were performed on DNT, TNT, PETN and RDX (all nitrogen-based explosives). When comparing to a spectrum for NO, it is clear for all samples that the returning signal is from the vibrationally excited (and hence dissociated) NO. Further experiments were then performed on RDX and TNT dissolved in acetone to yield a concentration of **2 ug/cm2** to emulate trace level detection. This too was successful, and showed a good signal to noise ratio when compared to a control sample of a bare silicon wafer.

Work is currently being undertaken to scale up the laser and optics for large area observation. The authors estimate at least **5 mJ** laser pulses are required, with a much higher pulse repetition rate than the current 30 Hz, to enable large area scanning. Colleagues at the Lincoln Laboratory's Laser Technology and Applications group are designing a laser specifically for this purpose.

There were many companies exhibiting at Pittcon this year who make high quality lasers and spectrometers. Avantes exhibited their range of spectrometers, lasers, and other components for fluorescence analysis, and high-performance OEM laser supplier Cobolt was also an exhibitor at Pittcon 2017.

There was a specific oral session at the conference dedicated to various fluorescence and luminescence techniques as well as a poster session. A short course entitled '**Analytical Spectroscopy Methods: Absorption, Fluorescence, Raman and SERS**' allowed delegates to explore this developing technology in more depth.

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CONCLUSION

In summary, identifying explosive material is a challenging task which is not getting any easier. As soon as new techniques are developed, it seems new ways to disguise explosives from them come along. However, advances in spectroscopy techniques and the rise in handheld devices shows promise for a safer future in this field.

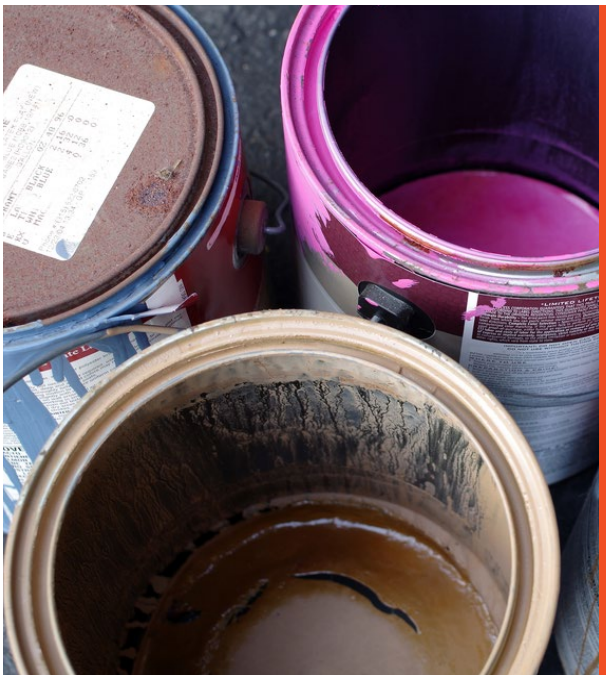
There is clearly not a 'one size fits all' approach for explosive detection. Techniques such as X-Ray imaging and mass spectrometry have found a niche in airport security, where proximal sampling is reasonably safe to carry out. Optical techniques are finding many ingenious applications in more hazardous environments, where standoff detection is required.

In the case studies above, researchers have shown it is possible to adapt and modify well known techniques to improve accuracy and portability. For example, a double pulsed LIBS system is shown to mitigate some of the traditional issues with surface contamination, Raman is enhanced using 2D analysis, and by exploiting the decomposition of explosive materials during controlled heating. Finally, LIF can be enhanced by an additional laser pulse to photodissociate molecules prior to analysis.

Work is continuing both in academic and industrial environments to miniaturize these techniques further and increase the standoff detection distances, and Pittcon 2017 featured many experts in this field.

Dozens of companies exhibited, including **Thermo Scientific, Rigaku and Metrohm**. There were many symposia and short courses enabling delegates to enhance their knowledge and network with like-minded colleagues.

5.2 ANALYZING VOLATILE ORGANIC COMPOUNDS (VOCS) IN THE ENVIRONMENT



Volatile organic compounds (VOCs) are organic compounds that easily become vapors or gases. Exposure to VOCs is common during routine daily activities since they are released from burning fuel and are present in diesel exhaust particulates. They are also included in many household products, including solvents, paints, glues, air fresheners, dry cleaning fluid.

Many VOCs are highly toxic and several are confirmed or suspected human carcinogens. VOCs are also known to contribute to air pollution. It is therefore important that their levels are monitored. This section provides an overview of the latest VOC detection technologies presented at Pittcon

INTRODUCTION

The analysis of **volatile organic compounds (VOCs)** was one of the most important topics covered at Pittcon 2017 in Chicago. The accurate measurement of VOCs enters numerous scientific arenas from the detection of VOCs as biological markers for cancer cells (health monitoring chemical fingerprint) [1] to monitoring the environment for levels of VOCs deemed harmful to human health [2, 3, 4].

The identification and quantification of both commercial (paints, coatings and fossil fuels) and naturally occurring VOCs has become an essential part of analytical chemistry. Volatile organic compounds typically occur at low levels and are many and varied and so research in this field requires detection and identification at low level requiring lower and lower limits of detection (LOD) or even 'concentration' (e.g., headspace analysis) and identification [5].

Traditionally the most useful methods have involved solid phase microextraction e.g., Tenax tubes and sensitive techniques such as GC-MS to resolve and quantify VOC mixtures [6] but there are now more sensitive techniques that can provide real-time sensing of VOC levels by direct injection mass spectrometry (DIMS) [7].

Some of these techniques [7] include MS-e-noses, atmospheric-pressure chemical ionization (APCI), proton-transfer-reaction mass spectrometry (PTR-MS), and selected ion-flow-tube mass spectrometry (SIFT-MS). All of these instrument configurations were on display with various expert vendors during Pittcon 2017.

DIRECT INJECTION

Direct injection MS techniques are regularly used for rapid detection and accurate quantification of VOCs [7] and proton transfer reaction mass spectrometry (PTR-MS) [8] is one of the methods used regularly for the on-line analysis of biogenic and anthropogenic VOCs.

> State-of-the-art PTR-TOFMS instruments based on time-of-flight mass spectrometry, such as the latest offering from Ionicon (who attended Pittcon 2017) for ultra-trace VOC analysis, have achieved detection limits of **20** parts per trillion after **100 ms** and **750** parts per quintillion after just **1 min** [9].

This type of system is ideal for the analysis of volatile organic compounds that might be obtained in a clinical environment, for example, the analysis of VOC cancer biomarkers from human breath samples.

PTR-MS is a soft ionization method that uses H3O+ ions to handover protons to all compounds that have a greater proton affinity than water. The nitrogen and oxygen in air are not ionized by the hydronium beam, but the majority of VOCs are ionized by H3O+ with little or no fragmentation.

Other molecules such as hydrogen sulfide (H2S), hydrogen cyanide (HCN) and ammonia (NH3) are also detectable by the H3O+ PTR-MS technique. The ionization occurs in a low-pressure reactor (0.1 to 2mbar) in dilute conditions to avoid any competition for charge between different analyte molecules, as could be possible with API sources.

MINIATURIZATION AND PORTABLE INSTRUMENTS

With the measurement of VOCs in the environment there has been a demand for miniature/portable mass spectrometry (MS) systems for in situ analysis. Miniature MS is now an attractive reality and the challenge to overcome the size and weight limitations of conventional MS has been realized. MS instruments are being condensed and improved to allow portability and accessibility, and some are even adapted for handheld operation.

Portability in MS is also a sign that systems are now amenable to ‘non-experts’ such as firefighters, police officers and environmental inspectors. MS miniaturization technology was discussed at Pittcon 2017 in Chicago. There were sessions covering ion traps and a symposium on the ‘Miniaturization of MS’.

Various companies provided presentations and demonstrated their mass spectrometer products across a range of applications. The Miniature Mass Spectrometry symposium included a presentation on the Portable Digital Linear Ion Trap Mass Spectrometer from the Guangzhou Hexin Instrument Co., Ltd. This portable instrument has a foot print of only **45.5cm x 42.1cm x 22.1cm**, a sensitivity of **5ppb @ 30** seconds sampling time (toluene), a scanning rate of **10000amu/sec** and weighs less than 25 kg.

ENVIRONMENTAL SAMPLING

For environmental detection in oil and gas installations or city centers handheld portability for MS systems is the best solution. In these cases, the total VOC concentration is the important figure and a photo ionization detector is the ideal solution.

The VOC-TRAQ® II from MOCON® Inc. – Baseline (exhibitor at Pittcon 2017) is a portable, hand-held, photoionization detector which is designed to evaluate total volatile organic compounds (TVOC). This highly compact instrument is ideal for air quality consultants or safety engineers, has no moving parts and with a rapid response time operates using just the simplicity of diffusion.

The analysis of air samples for VOC content over a set collection time has now been made easier with the availability of reliable automated detection systems.

Extrel (an exhibitor at **Pittcon 2017**) provide the MAX300-AIR Environmental Mass Spectrometer, which is custom designed for environmental VOC analysis. This instrument is an industrial gas analyzer that uses quadrupole mass spectrometry for the rapid detection and quantitation of a wide variety of VOC based industrial contaminants.

A single analyzer can measure unlimited compounds and be automated to monitor more than 160 sample points across a manufacturing area. Benzenes, esters, alcohols, ketones, alkanes, chloroalkanes and alkenes are the major emission components and the most frequently monitored VOCs are benzenes [10].

CONCLUSION

VOCs can be harmful to health and although the general consensus is that industries such as oil and gas, vehicle manufacture and furniture manufacture are the most prolific producers, the highest health risk from VOCs is in the home and office. Instrumentation is now achieving a level of sophistication where VOC levels can be monitored in the workplace, on-site or even in the home to protect human health.

The applications for VOC detection are many and varied and with the growing complexity of the range of VOCs that have to be monitored, organizations have to keep up with the most recent developments in analysis equipment. Miniature MS is set to be a definite advantage VOC analysis and Pittcon 2017 provided an overview of recent developments.

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SOLID PHASE MICRO EXTRACTION

University professor, **Dr. Pawliszyn** leads research into the design of highly automated and integrated instrumentation for the isolation of analytes from complex matrices and the subsequent separation, identification and determination of these species. In the following interview, he provides an overview of the presentation he gave at Pittcon 2017.

Dr. Pawliszyn explains the importance of sample preparation and details advances in solid phase micro extraction. Such techniques have a wide range of applications, including the monitoring of air pollution, quality control in the food industry and detection of explosives; they have even been used for analysis of living systems in biological and medical research.

Dr. Pawliszyn, University of Waterloo, talks to AZoM about the Solid Phase Micro Extraction and his talk at Pittcon 2017.

Q **Can you tell us a bit about how you became interested in sample preparation techniques as an area of study?**

A When I started my scientific career in the mid-1980s, there was beginning to be a lot of discussion about pollution. Agreements like the Montreal Protocol stated that we should try to address the impact on the air and on the environment – and today this is only becoming more and more important.

Sample preparation is a very important part of the practice of analytical chemistry, environmental chemistry, and many other fields – despite the fact that it is not always seen as the most crucial or the most highly "scientific" area.

> "The ability to isolate components of interest into some form which can be introduced into sophisticated instrumentation, such as chromatography or mass spectrometry or direct spectroscopic measurement, is highly important for this type of work."

The traditional method of preparing a sample was to use tens of milliliters, or even larger volumes of organic solvents per sample to extract the chemicals of interest. But then what do you do with the solvent afterwards? Usually it is evaporated, and goes straight into the atmosphere. So by using analytical chemistry to do environmental monitoring, you are contaminating the environment!

""""The reason that this had been the situation for so long was simply that there were few scientific groups working on better sample preparation or sample extraction methods.

> "I spotted that there was therefore an opportunity to make a really big impact on analytical chemistry, not by working on new, exciting analytical techniques, but by streamlining the analytical process, and improving the science of sample preparation."

So looking at this problem for analytical chemistry and environmental monitoring specifically, we realized that the most powerful sample preparation process would be one which can integrate sampling on-site with preparing the sample for convenient introduction into analytical instrument. That's actually the main area of my research – trying to develop devices which can facilitate that approach, both in the laboratory and in the field.

Such strategy involves sampling on-site, but increasingly in the future, the analysis is going to be carried out there as well, with portable instrumentation. I'm not saying there will be no labs, of course, but most of the chemical analysis will be done on-site. So these devices will help take us towards that future of on-site analysis.

Q **Tell us about Solid Phase Micro Extraction, how does it work, and how does it differ from conventional extraction methods?**

A When I introduced Solid Phase Micro Extraction in 1990, it was referred to by many people as a paradigm shift in extractions. The most popular format for an SPME device is basically a fiber about the diameter of a human hair – around **100 microns** – coated with another **100 microns** of an extraction phase, involving appropriate sorbent. About **1 cm** of the coated fiber is placed inside the needle of a syringe using piece of metal tubing – we chose the syringe format because its highly portable, and everybody understands how to handle and use it.

SPME faced considerable skepticism in its early days – it was hard for scientists experienced in conventional separations to understand how we could get the same sensitivity in measurements using microliters of sorbent volume as they could by using many milliliters of solvent! It sounds impossible, but of course this is because the principles of microextraction are somewhat different – rather than flooding the sample with an excess of solvent to be ensure you have extracted majority of the target compounds, you limit the volume of the extraction phase to a very small amount, but take full advantage of the entire capacity of that extraction phase leading to high enrichment.

> “Then, rather than concentrating those milliliters of solvent down to a 100 microliter volume and injecting a microliter into the instrument, you simply inject the entire extraction phase into analytical instrument.”

So this was certainly something of a paradigm shift in the thinking around extractions, and in the early days it was challenging to attract funding!

Q **How does SPME deal with complex, mixed-phase samples, such as soil samples, biological samples etc.?**

A Yes, this is another issue where it seems like there might be a shortcoming of the microextraction approach – if you put this small fiber directly inside a complex sample, with thousands or millions of components, it seems like there might be all sorts of things like macromolecules that could interfere with the analysis. I’s not as difficult as you might think to avoid this, however.

One approach that is traditionally used in these situations is headspace analysis – and in the early years of SPME we did use this approach, since it does give a very clean extraction and analysis. All the compounds which go into the headspace will desorb from the fiber, and of course will also go through the GC column.

The problem with that, however, is that it doesn't always give you an accurate representation of what is in the original sample. Headspace extraction is typically done from an aqueous solution, which means hydrophobic compounds are going to be expelled into the headspace more readily, so they will be overrepresented. There are more polar compounds that you might not see at all.

> "For some food samples, like mint leaves, so much of the aroma compound goes to the headspace that you can smell it all over the lab – in this case the fiber actually becomes saturated or swollen, which makes it impossible to obtain or properly quantify your target analytes. The solution in such cases is to use shorter extraction times, but this results in lower sensitivity."

To address this challenge and facilitate the direct extraction we developed an approach that adds a third component to the device – you have the fiber support, the extraction phase coating, and then a very thin layer of a biocompatible, "restricted access medium" – essentially a selective membrane which will allow small molecules to pass through, but will block macromolecules and ions which might interfere with the analysis. This "protection layer" is designed to eliminate adsorption of macromolecules as well.

If we use an extraction phase like HLB, we can extract a wide range of compounds varying in polarities in one sampling, and you get a beautiful cleanup in the process.

Q **What are the main applications that SPME has been used in?**

A Well originally, as we have discussed, it was used in environmental research and food chemistry. I did not really design the technology for a specific area of application, however – really it is applicable in many different disciplines.

> “Food analysis is probably the area where the most devices are used. It has been commercialized by a number of companies that we have been working with for a long time, primarily for use in that area.”

Devices have also been commercialized for the military, for use in identifying explosives etc. in the field. SPME is ideal for this application, since it doesn’t need qualified chemists to operate the device.

Right now, the most exciting area is using the fibers to sample living systems, for animal and medical research or clinical applications.

We’ve done lots of work on plants, animals, and fish. We are working with surgeons to try the method for monitoring organs prior to, and during, transplantation procedures. This is not an FDA-approved method yet, but Toronto General, our local hospital and the largest hospital in Canada, is working with us on these trials.

> These devices work really well for these biological applications, since we don’t actually extract any tissue, only the small molecules – we call it a chemical biopsy tool.

We have a project on sampling brain tissue, where before they were using microdialysis which heavily favors polar compounds and ions. It works well as an extraction method, but of course the samples are not compatible with certain instruments, like a mass spectrometer.

> “Using our fibers, the sample we get is so clean, it is not only compatible, but we can inject it directly into the mass spectrometer! We don’t even need to do LC beforehand.”

So what you have is a rapid diagnostic tool, where you can sample directly investigated living object and put that straight into an instrument with no further preparation – medical applications are a big area of interest for this naturally, but you can see how it is great for environmental applications, food screening, forensics, and so on.

Q How does the approach to quantification differ when using SPME rather than conventional solvent extraction?

A Typically, the traditional analytical chemist will work with a fixed volume of sample, and exhaustively extract the components into the extraction phase. This is a very limiting approach, because you need to have a very well-defined sampling process, to collect a precise volume of the sample, which in many cases can be very restrictive.

> “With simple samples, this is quite easy, but for more complex samples, this quickly becomes more difficult. It is also difficult to ensure that you perform a truly exhaustive extraction.”

So with SPME, I proposed to not bother with that at all, and simply place the extraction phases directly within the sample, and quantify it based on the amount of the components which migrated and enriched into the extraction site over a fixed length of time.

We called that kinetic extraction, and proposed calibration procedures based on timed extractions, and the diffusion coefficient and the mass transfer conditions for the sample.

Q **You have also done some work on extracting proteins from complex biological samples – SPME typically excludes macromolecules from the extraction, so how do you modify the technique to select for just proteins rather than small molecules?**

A When you want to extract the proteins, that is a completely different challenge. You need to introduce some molecular recognition, in the same way some biosensors and affinity chromatography work – we have worked on this and made some progress, but it is very difficult to perform well in practice.

First of all, you need to design a surface containing high-density molecular recognition. The surface area available on the fiber is not very high, so a high concentration of ligands is required to make sure there is sufficient sensitivity.

Then, you need sorbent which will only selectively interact with proteins and eliminates no-specific adsorption. We only leave the device in contact with the sample for a few minutes to an hour, which is an advantage over some sensor designs which work this way, which might have to keep working for days, weeks, or years. But still, the biggest challenge is to design a strategy to eliminate non-specific adsorption.

Q **How do you see direct extraction techniques like SPME developing in the future?**

A I think there is a lot of future promise in using nanoparticles as the collection medium. That gives a very fast extraction – however, it's not very convenient to operate, compared to the fiber-based devices.

The other technology which I've been working on, which has historically been used on an industrial scale, is membrane extraction. In my opinion, membrane extraction is under-developed, primarily because the membrane will work best if you interface it directly with portable instrumentation.

That way, the membrane acts as a barrier between the complex sample and the instrument, which is sensitive to contamination – only the volatile components penetrate the membrane, making it a simple, effective cleanup method.

> The main challenge in such approach is the calibration method able to account for varying convection conditions, the task we have accomplished by using calibrant in the stripping media.

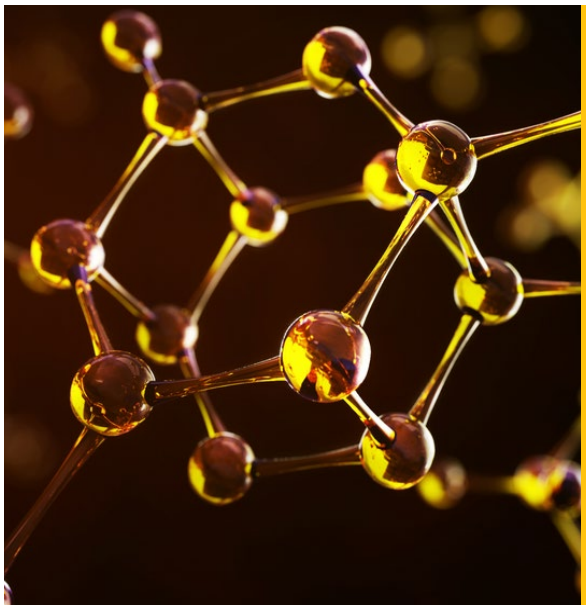
Q **What did you most enjoy at Pittcon 2017?**

A Every year, I enjoy seeing the exhibition, discussing the new developments in analytical instrumentation with my academic and industrial colleagues and interacting with the participants interested in my research area.

> “Pittcon has been the most influential analytical meeting over many decades. The conference with its comprehensive approach to analytical chemistry both during scientific sessions and on the exhibit floor helped to deepen my understanding of the area I studied as graduate student. This is the meeting where I gave my first conference talk.”

During the following decades Pittcon was integral part of education strategy for my students. I have always been admiring and impacted by the contributions made by recipients of Pittcon Awards and now I have addressed the Pittcon audience. It was a real honor.

5.4 FUNCTIONAL METAL-ORGANIC FRAMEWORK MATERIALS



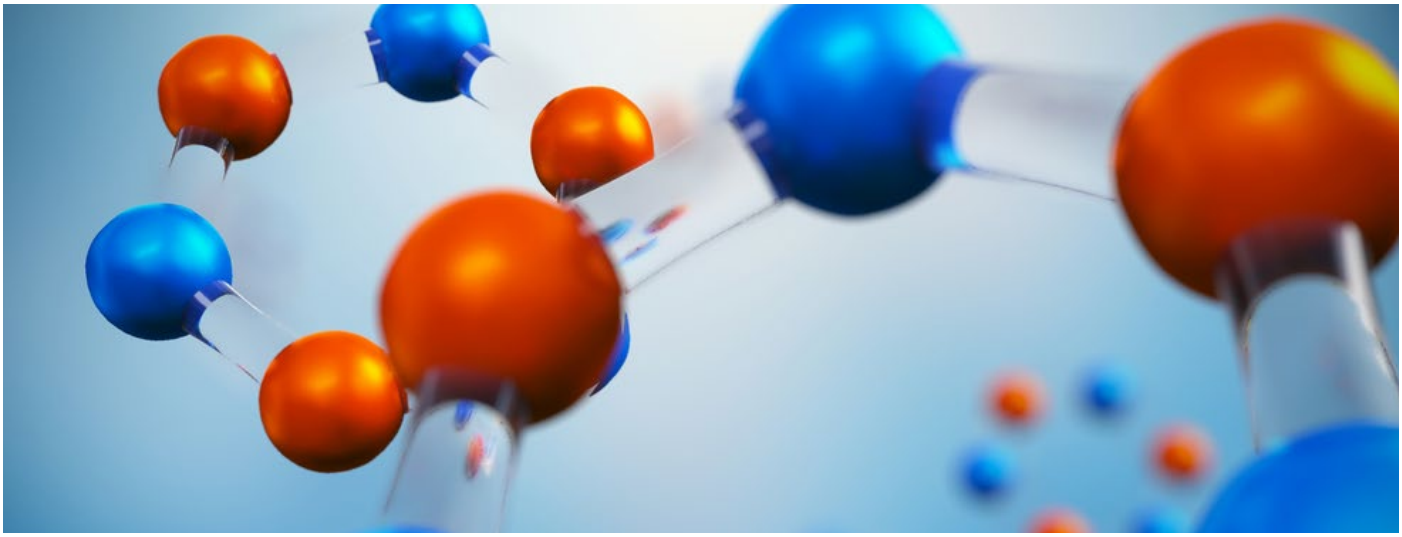
Omar K Farha is a research professor of chemistry at Northwestern University, Distinguished Adjunct Professor at King Abdulaziz University, and president of NuMat Technologies. His research focuses on the rational design of **metal–organic frameworks (MOFs)** and porous-organic polymers for separations, storage, sensing, catalysis, and light harvesting.

In his talk at Pittcon 2017, which is presented in this section, he detailed the latest advances in separation science and explains their utility across a range of disciplines, including chemistry, materials science and defense-related challenges. The precise composition and structure of MOFs can be tailored to give the precise properties needed to achieve a particular function, giving them endless potential applications.

A transcription of Omar Farha's talk at Pittcon 2017 where he received the Satinder Ahuja Award for Young Investigator in Separation Sciences

I will be discussing the chemistry of the work we did in collaboration with the department at Northwestern University, UC Berkeley, Minnesota, and with the CCBC as well. I will specifically be highlighting a lot of the work we've been doing in the last year and a half with **Professor Alex Katz** from UC Berkeley. We cannot do the work without the funding agencies so we thank them.

I believe that if you want to do new things, you need new materials and that means the only way you can come up with new chemistry is by designing new materials. Ideally, making a material that is not just one type of material, but it can be a platform and be used in many applications.



I am a trained inorganic chemist and so I am a simple-minded kind of person! We like to make 3D complex structures and we want them to do hard tests, but they also need to be made from simple building blocks, so that it can be commercialized. Therefore, you must be able to scale it up, do it at low cost and make a lot of it.

> The best thing you could do is make a material with organic and inorganic materials, but in a way that they form a 3D structure, not by stitching it through covalent bonds, but through coordination bonds or self-assembly. To do this, you must start with simple components and gradually get more complex.

We don't want to build it one piece at a time, we want to throw it together and have the right chemistry come together and self-assemble. Most importantly, it has to be programmable, that means you don't just put things together and see what it gives you, you want to have control of the end product and so we must take thermodynamics and kinetics into consideration.

NANO ORGANIC FRAMEWORKS

In nano organic frameworks, we're talking about metals and metal clusters. We have the whole periodic table to pick from, where there are many different metals with a range of properties to choose, such as cheap, expensive, soft, and hard metals.

Then there is the organic, we build them on a tool box from **200 - 300** years of organic chemistry. We're not trying to invent anything new, we can take advantage of tool boxes, coordination chemistry, inorganic and organic to make 3D molecules, and I don't mean them to be super molecular in nature. Those are the three-dimensional materials that go in the X, Y and Z.

> I particularly like a material that has a lot of metals and metal clusters because of the variety of choice, but you also have a lot of organic to choose from too. You have organics like benzene carboxylic acid, the main component in plastic bottles, that's as cheap as it gets. There are more sophisticated organics as well, it depends on your application.

Secondly, once you know how to make it, because these materials are self-assembly there is no reason for it to be simple and scalable. There is nothing special about metal organic frameworks (MOFs) that you cannot scale. MOFs are special in other ways, like the properties they have. Their cousins get scaled, e.g. zeolites and the activated carbon, and they were able to make hundreds of kilos of material.

> To our advantage, we have a lot of building blocks to choose from, however, to our disadvantage, sometimes it is difficult to decide which one to choose and for what application.

We could make all these topologies made with metal organic frameworks so they are not hypothetical, those made have different physical structures, chemical structures that allows you to tune things such as the aperture, the cavity, and the sizes etc., to your specific needs.

At the same time those materials are crystal, which means you know the structure exactly, down to the atomic level, and there are a few families of materials that we have that level of understanding of. Although they have a huge diversity, we know exactly what you make because it's a structure of materials.

If you put those two together, because we know exactly the structure, you have the diversity, that's when compositional modelling could come to fruition. With the new map technologies, in collaboration with my colleague at Northwestern University, we frequently share separation and storage applications.

> When it comes to catalysis, there is a huge drive there to predict before you make, but we are not there yet. But with storage and separation, I think the codes and the force fields are good enough to tell us which materials we could make before we go to the lab and make it.

For simplicity, I will only talk about the crystals in their smallest repeating unit and you could take that crystal and start diving it until we get to the unit cell of the smallest repeater, repeating unit of X, Y and Z. The material NU100 is made up of a lot of diverse materials based on metals and linkers. When making metal organic framework, you could make something with moderate surface area or you could make something with high surface area.

Material NU100 has a surface area of higher than **6,000** square meter per gram. In comparison, an average zeolite, which is about **1,800** to **1,000** square meter per gram, or an activated carbon, which is about **2,000 - 2,500.**

> Surface area is an important property to consider, for example for a storage application, gas and molecules like to stick to surfaces, therefore the more surface to volume ratio you have, the better the tunability of those cavities you will have.

In our group, we don't put all our eggs in one basket, we work on applications from catalysis. Recently we've been doing a lot of catalytic deactivation of nerve agents for working with DOV. That's' not what I'm going to talk about today.

We also work on storage applications, sensing, light harvesting and anything in between. To just walk you through what we do in my lab, we work on MOF synthesis. Even though there are **20,000** MOFs, it doesn't mean we stop working on what's the next new MOFs for different applications.

We have processing, if we have to map a MOF how can we decorate it and change its property. We could use different technology, like the MOF technology coupled with atomic layer that position technology and make a single sight catalyst for a lot of applications.

If we cannot make a MOF there are other options, we could come up with techniques for example 'How can we make a MOF then make sure that by the time we finish with the processing, we have a completely new MOF that we couldn't make?'.

We also have to consider the purity of the MOF and we came up with techniques to purify MOFs to make sure it's a single phase pure material. When you process them, these materials are super porous, which means that the solvent molecule sitting in the cavity has to be gone before you utilize it in the application. To maintain its ferocity, there are a lot of tricks that you must do, and we came up with some of those tricks as well.

We also work on CO2 sequestration, and hydrogen and natural gas storage. Those are the holy grail applications, but today I'm going to be talking to you about smaller niche kind of applications because sometimes high value and low volume is the way to start before you go to large scale technologies.

For example, the separation of tough molecules like propane and propene, those are not simple molecules to separate. And doing catalysis in the condensed phase with single sight. We do it in the gas phase as well. A lot of people say you cannot use MOFs in the gas phase.

Some of those catalytic reactions happening above **200** degrees Celsius, some of them with pure oxygen. And that's when it becomes neat and surprising. You have a lot of organics but they are not burning that easily.

We do a lot of egregious selective catalysis, and typology directed catalysis. For example, choosing components that are the same as the node and the linker however how you put them with respect to each other? You could stabilize the intermediate, except you couldn't do that in solution and you get reaction to go one way but not another. We hope to learn from enzymes and infiltrate that into human made materials, that could have huge amounts of potential.

SEPARATION OF LARGE MOLECULES – SUGARS AND ENZYMES

A lot of people have most likely heard of the separation of gas molecules, small molecules and how we separate them. However, separating smaller molecules is a lot more difficult than separating large molecules from each other. If you take nitrogen from oxygen, and CO from CO, those are give or take similar sizes, sometimes we rely on small changes to be able to either sift them or get them to react with material in a different way.

Larger molecules are a little easier to deal with, but I want to show a different view of where MOFs could also be implemented in the condensed stage of water based large molecule separation.

There's one thing we always get asked, 'Are those materials water stable? The answer is yes; they are water stable. If you looked at the MOFs that were made fifteen to twenty years ago, what we've been making in the field in the last seven years, there are now very stable materials available.

Today I will be talking about separating sugars and enzymes. **Alex Katz** came up with that if you have activated carbons with surface areas that have aromaticity in them, in their modelling, they show that with those apertures, cavities or channels, and activated carbon of three millimeters, they could pick up long oligomers (the dimers, trimers, or even longer) to do analysis with.

> But they were not very selective and took up also the monomers, fructose, and glucose, as well as xylose. This is because those areas are where the action happens, and there is tension in different places. It's very hard for them to know exactly where they are, and how many there are of them.

Separation in this field is a carbon neutral process and one of the things in the US that people are working on. Two ways to get glucose, you could hydrolyze starch or cellulose. You make them monomers, with some enzymes for fermentation, that gives you the ethanol. Starch are more commonly used than cellulose itself for the activity purposes, but we're talking about separation here, not the catalytic activity.

The question is, 'How can we take those materials up and not the monomers?' We want to separate only the large oligomers before they go to the fermentation. If you take these materials and dehydrate them, you will make Hydroxymethylfurfural (HMF) and Furfural. HMF and Furfural are inhibitors for the enzymes that they apply to the ethanol for fermentation.

> How can we separate those molecules from those molecules and how can we take up those inhibitors so that we don't kill our fermentation enzyme? If you have molecules that don't look that much different than this except you have one here and another one there, you fuse pyrenes together, but why can't this be taken in and we make a MOF out of it?

NU100 and its linker, pyrene petraphenyl carboxylic acid, is not a hard MOF to make and we actually make a lot of it. This MOF is made from six zirconium, therefore, we chose zirconium, because they are water stable. We need a water stable MOF to separate sugars from each other from water media.

Now we know the crystal structure, where the aromatic rings are and how uniformly they are placed. Does that help the separation and the cell activity? We have to make sure it's quite stable to purify this material from the reaction mixture, and so that we know we can torture this material and it's not going to give up on us.

Ways in which we can test this is, we could boil this material in water, or use the nitrogen isotherm, which is pressure verses uptake of nitrogen. We also modulate these crystals, because for engineering you want to know what's the crystal size effect, pressure drop, and other things and we were able to do that with this material. If you zoom in to this material, you can see the channel and how the pyrenes are lined up. We don't have an activated carbon, just little batches of six pyrenes which are lined up.

In Alex's lab, we sent this material to them and they took the monomers and dimers tested them. The monomers don't get picked up, whatsoever, and has 100 percent selectivity. Here we're doing not just as good as the carbon, but a lot better because we only take those dimers and none of the monomers.

Our hypothesis, and there has been a lot of EFT (effective field theory) modelling done from this work and there is a lot more CH interaction with those pyrenes than with the monomer. The monomers take a lot of water molecules, and interact with them more than they interact with the actual platforms.

Another question we want to know is 'Does the structure stay intact?' We looked at the structure integrity of this material by looking at the nitrogen isotherms. Two things you could do to test this is if it's half loading you could see down in pore volume mainly from the micro pore regime, but this step, for this material, did not change much. When we looked at the pore size distribution, we saw a decrease from the smaller pores than the larger pores.

What we believe is happening, is that the first few sugars are going into the smaller pore, the triangular pore, because there is more interaction with the walls than the larger pore. And once you fill that, you start filling the hexagon, and that's where we could see there is a drop in the micro regime.

Multiple components, not just having one species at a time, but putting all the species in the same reaction cause any observable difference, so if they are together or separate, you take off only the aromatic materials, but not the monomers, or the sugar monomers. Alex went ahead and decided to compare it to other absorbents because he wants to see how it does verses this material that has similar channels or cavities and those are the two carbons we're choosing.

What we saw was that the NU1000 takes up a decent amount of HMF, whereas the other two carbons take up more HMF, but they also take up a lot of other molecules. Beside taking up the HMF they take up the molecules that we want to retain for the enzyme to do the fermentation.

Why did we pick up eight million more of HMF? That's a concentration that inhibits the enzyme from working and that's where we must get to take up this HMF at this concentration. If it's a little less, the enzyme works okay but once you reach this concentration it goes back. A high amount of glucose verses HMF and we see very much the same, a good amount of selection of these materials is occurring. We see that PXRD, nitrogen, core sizes, everything looks the same. That means we're not killing the material.

This is just the first MOF we tested based on the question 'Does pyrene work if you have it in the right environment?' The good news, you could take zirconium MOFs with new topologies. It becomes important to look at the cost performance relationship. Some of those materials are cheaper than others. We're looking at different topologies and different materials.

Another advantage of MOFs, once we decide on a topology, there is no reason why we can't make isostructural material and that's the next part of it, where we're going with this project.

I wanted to make something that we could design and be able to predict MOF and a colleague challenged me, saying that everybody knows everything about transition metals and so I'm not talking about anything new. Can I make, design, predict MOF for actinides? Do the you know the coordination chemistry of actinides as well as people know the coordination chemistry of transition metals? I am naïve and took on the challenge.

Starting with actinides, we looked at the properties and the kind of properties we want in that MOF. We wanted high ferocity. There are a few uranium MOFs out there that have low ferocity, large cavities and large aperture. We wanted to separate large molecules and a uranium MOF 6 with carboxylic acid which is going to be stable in water. Let's go and do large molecule separation. I'm going to use uranium MOFs for separation.

There are a lot of topologies that you could pick from to do this chemistry, we decided to pick a topology called **TPF (Topology Processing Framework)**. It has a three cage topologies, and it meets a lot of the criteria. We wanted to stick with the linker that we knew how to deal and it's called the 3 - 4 connective topology. We decided to use this well-known uranium 3 Carboxylic acid and the uranium.

After a few trials, the postdoc produced beautiful crystals, but those crystals could be anything. From the shape of them they look like what we had designed, but we needed to be sure they were the right crystal. If you take our drawings, it was what we got from our colleagues and from the modelling and the structure, we were able to obtain from a single crystal that they were very much identical. We named this material NU13000.

We have the material that has uranium, and the right topology, but the question is, is it porous? It looks porous in the crystal structure. Can you activate it? We had a number of options to test this, we could simulate an ethanol exchange. We could process this in many ways, and after doing this, we got a nitrogen isotherm where you could see the meso porous channel.

There was about four Nano meter cages there, with apertures of about two and a half Nano meters. We've got the large windows and cavities and we have the high pore volume. That means we could load a lot of stuff into it and it has a high surface area.

Is it anionic as we predicted? We didn't see all the cations in the crystal structure, and every node should be anionic which means it should come with a cation, but with the crystal structure we did not see that. We took a cationic dice for every uranium with different size dice, and everything checks. Then we wondered what would happen if we take a cationic die, and we found that it didn't take it up, which means that all our uranium MOFs are anionic.

If we made it to make large molecules, can we start separating enzymes from each other. As I said, the whole idea is that large molecules condense things. Taking two enzymes at pH 6, with one material that is positively charged and one material that is negatively charged and you put them together. Everything is mixed at equal Molar and we follow by time and the wave length of where they take up that absorption and if we do that in two dimensionalities.

This shows you that we could make a MOF that has a large aperture top separate enzymes from each other by just modulating the pH. At the same time, we did these experiments in water and this material is stable to do that multiple times.

Today I described two stories about using MOFs in condensed water phase to separate large molecules. There are a lot of families of MOF materials. Some zinc or zeolitic images of frameworks, and a lot of those are quite stable materials. The stability has to be stable only under your application. Some materials are stable at a higher pH, some at a lower pH, for example. It is not likely that you're going to find a MOF that's stable from pH zero to pH 14, but I don't think that's necessary either. Looking at what your application is, and what's the environment and finding the right family, that's the way we do it in our group.

CONCLUSION

Pittcon 2017 included presentations from many experts in the field of explosives detection, and demonstrations from companies producing specialized detection technologies and devices.

Researchers around the world have refined and adapted current spectroscopic techniques and developed new approaches to maximize the sensitivity and efficiency of the detection of explosive materials.

> Furthermore, the technologies have in many cases been miniaturized, allowing the production of portable and even hand-held analytical devices. The availability of such devices has paved the way for explosives detection in the field.

The identification of explosive materials and their components is made particularly challenging by the fact that they are similar in composition to many harmless everyday products. The differentiation is typically achieved through determination of the ratio of nitrogen and/or oxygen to hydrocarbons, which is high in explosive materials. This characteristic can thus be used to pick out dangerous organic compounds such as RDX or TNT from more benign materials such as nylon and fertilizers.

The proportions of nitrogen and/or oxygen to hydrocarbons can be readily determined using traditional spectroscopic techniques. However, they are often time-consuming and need to be performed by a trained chemist.

Recent advances have thus focused on the development of spectroscopic methods that can identify explosive materials quickly and can be used by a non-expert in the field. They have also been adapted to allow detection of explosives from a safe distance.

A range of optical spectroscopy techniques are now available, each with its own specific advantages and disadvantages. These include **Raman spectroscopy, laser-induced breakdown spectroscopy (LIBS), laser-induced fluorescence (LIF), and cavity ringdown spectroscopy (CRDS)**.

Furthermore, compact versions of many of these technologies have been achieved so portable and handheld devices for the detection of explosive material are now feasible. LIBS and Raman spectroscopy in particular have proved amenable to adaptation to handheld instruments. Both techniques allow rapid and simple detection of explosives.

LIBS has been shown to be a prime candidate for standoff detection, having greater sensitivity for trace amounts and the potential for very long distance ranges with the incorporation of telescopic optics. In contrast, photodissociation followed by PD-LIF allows a wide area to be screened simultaneously, making it an obvious tool for the detection of explosive-making activities.

There is also huge potential for these new technologies beyond explosives detection. Environmental analysis is one area where more sensitive spectrometry techniques have proved particularly valuable. Volatile organic compounds, as found in particulate matter of diesel exhaust fumes and in a range of household products, such as glue and air freshener, pose a risk to both our health and the environment.

Advances in technologies, for example **direct injection mass spectrometry (DIMS)**, have made it possible to obtain real-time sensing of volatile organic compounds.

Furthermore, the development of portable devices providing this analytic power are now available making it accessible to fire-fighters, police officers and environmental inspectors. The technique is also being investigated in medical applications; it is so sensitive that it has been able to detect cancer biomarkers in human breath.

Pittcon 2017 illustrated the power and immense potential of spectroscopic techniques and gave a taste of further advances we are likely to see in the future.

CONCLUSION

Each year **Pittcon** is packed with innovative technological advances and developments in laboratory science across a whole range of disciplines.

Pittcon 2017 even looked to taking the laboratory into the field for on-the-spot analyses clearly illustrating that researchers and biotechnology companies continue collaborating to optimize techniques in order to further our understanding, improve the lives of patients and help protect us from harm through both accidental and intentional acts.

Bioengineering capabilities are increasing so rapidly that society cannot keep up. There are a number of ethical and financial issues that need to be addressed before they can be utilized to their full potential. For example, which uses of genetic information should be permitted and should novel gene-based diagnoses and therapies be funded as routine clinical practice?

Genomic research has progressed so fast that even the ground-breaking Human Genome Project has been eclipsed. The ease with which gene sequences can now be determined and manipulated has made possible a huge array of new research and therapeutic methodologies. The ability to rapidly obtain gene sequences has opened the way for tailored treatment strategies to maximize efficacy. The capability to accurately replace specific gene sequences means it is possible to rectify genetic disorders.

At Pittcon 2017 we learnt that it is now even possible to measure the function of individual cells that, amongst a myriad of other potential applications, enables the identification of rare aggressive cancer cells in biopsy samples before they become symptomatic. This hugely increases the chances of successful treatment.

Presenters at Pittcon 2017 described some of the latest additions to the collection of mass spectrometry techniques and their potential applications. Furthermore, miniaturization of the components of mass spectrometers has allowed the development of hand-held devices that can conduct highly accurate analyses on-site.

Demonstrations illustrated how the smart technology included in these portable mass spectrometers prepares the sample for analysis so the user needs no specialist knowledge. Such devices thus have huge potential, including saving lives through in-the-field detection of explosives, accelerating diagnoses by enabling testing of samples during consultations, providing high-speed analysis of food contaminants, and allowing constant monitoring of air quality.

Prof Sweedler also explained how new mass spectrometry protocols are allowing us to identify rare neurotransmitters, define the precise composition of the brain and study neurosignaling pathways.

One such technology, optogenetics, is facilitating research into treatments for conditions such as **Parkinson's disease** and **Alzheimer's disease** by enabling the precise firing sequences associated with particular activities to be studied.

Pittcon is proud to continue providing the greatest arena for showcasing the myriad benefits from emerging technologies that are achieved through the dedication of laboratory scientists.

The scope and quantity of the technological advances described at Pittcon is truly remarkable. It is impressive to witness how the creativity and collaboration of scientists and industry is successfully devising analytical solutions to the ever-changing challenges of life today.