



02

**BIOMEDICAL AND
REAL-WORLD
APPLICATIONS OF
BIOANALYTICAL
TECHNIQUES**



INTRODUCTION

Biomedical study of focused, personalized therapy has expanded in recent years as research continues to mount on the benefits of molecular-based approaches to treating disease.

Molecular spectroscopy has been increasingly utilized in this research, with the hope that this advancing technology will aid in disease diagnostics as well as in the development of novel therapies.

Some of the previous challenges with spectroscopy technology include isomeric indistinguishability and inadequate throughput of measurements. Tissue analyses can also be considerably time-consuming, particularly during multi-target sample monitoring.

As such, there is a need for analytical sampling strategies that can provide rapid performance. At Pittcon, researchers cover their latest investigations on how they've been able to overcome these analytical roadblocks with the latest spectroscopy innovations.

2.1 ANALYTICAL SCIENCE IN PRECISION MEDICINE

As biomedical research focused on personalized therapy continues to grow, molecular spectroscopy also continues to expand, with the hope that this growth will contribute to the development of new diagnostics and novel therapies for hard-to-treat diseases.

In this interview, Professor Jeremy K. Nicholson, Head of the Department of Surgery and Cancer and Director of the MRC NIHR National Phenome Centre Faculty of Medicine, introduces analytical science in precision medicine:

What will be the main focus of your Wallace H. Coulter lecture?

Pittcon is an analytical conference, so naturally my talk will be about analytical chemistry and how analytical chemistry will become increasingly important in delivering healthcare solutions, not only for rich people, but also, hopefully, for poor people across the world.

The challenges that we face in 21st century medicine will be illustrated, particularly the diversity in terms of the emergent diseases that are occurring and the genetic and environmental drivers that change disease patterns and prevalence in whole populations. For instance, the obesity epidemic is a driver for a whole range of things including cancer, diabetes and Alzheimer's disease.

“The important factor here is that we generally think about solving the problems we have currently and what technology or chemistry we need now.”

Prof. Jeremy K. Nicholson

The world is changing very quickly, so we also need to address problems that will be coming over the horizon, or are already on the horizon, that we are going to face seriously in the next 20, 30, 40 years.

Those are problems such as anti-microbial resistance; the convolution of global warming with healthcare, which changes the way that

parasites and infectious diseases operate on a global scale and the fact that our populations in western countries are living for much longer, which gives us a whole range of diseases that were not so common in the population before.

The idea is that we have to study chemistry and develop technology that is future-proofed, because, in the future, we won't have time to generate the technology. We have got a lot of work to do very quickly. It is a big challenge and everybody needs to pull together.



What are the main challenges of 21st century healthcare?

There are what we call emergent threats, which are partly due to there being a lot more people living on the planet than ever before and therefore more things that can go wrong with more people.

Emergent threats include things such as diseases that may have been previously isolated in the tropics, but now are not because of modern transportation. There are things like Ebola and many others. We are seeing diseases in the West that we haven't faced before, but are going to see more and more.

There are also diseases that have emerged due to changes in the biology of the organisms that live on or within us. Over the last 30 or 40 years, about 30 completely new emergent diseases have popped up and we should expect to see more of those.

There is also the incredible interaction of the human body with the microbes that live inside us. We are supraorganisms with symbiotic microbes and we have changed our own chemistry, physiology and also our disease risk. We have realized that we are not alone. We are not just looking at our own genetics, but at our own biology.

We have to look at ourselves as a complex ecosystem that can get a disease as a systemic disease, a disease that affects the whole ecology of the body, which seems to be increasingly the case for things like gut cancers, liver cancers and possibly a whole range of what we would normally call immunologically-related diseases.

There's a lot more than we thought that has to be sorted out analytically. We have to be able to measure it all to understand it, which is where analytical chemistry comes in, in all its various forms.

We are trying to apply analytical technologies to define human populations, human variability and how that maps onto ethnicity, diet, the environment and how all those things combine to create an individual's disease. We also want to understand it at the population level.

The other thing I will emphasize is how personalized healthcare and public healthcare are just flip sides of the same coin. Populations are made of individuals. We want to improve the therapies and the diagnostics for individuals, but we also want to prevent disease in those individuals, which means also understanding the chemistry and biochemistry of populations.



How can analytical science in precision medicine help overcome some of these challenges?

When you think about all aspects of human biology that we measure, they are all based on analytical chemistry. Even genetics and genomics are carried out on a DNA analyzer, which is an analytical device that has sensitivity, reproducibility, reliability and all the other things that we normally think of as analytical chemists.

Analytical chemistry underpins every part of our understanding of biological function. Proteomics is based on a variety of different technologies for measuring proteins for example. The different technologies put different bricks in the wall of our understanding of total biology.

What we understand much less, is how those different blocks and units work together. We understand it quite well from the point-of-view of individual cells; how a cell works in terms of its DNA, RNA, protein production, metabolism, transport and so on. A huge amount is known about how the basic engine works.

When you start to put lots of different sorts of cells together, however, we understand much less. We know much less about how cells communicate locally and at long-range and how chemical signaling enables cells to talk to each other. When we start thinking about humans as supra-organisms, we also need to understand how our cells and bacterial cells talk to each other.

One of the great challenges is not only getting the analytical chemistry right for measuring the individual parts, but having the appropriate informatics to link the analytical technologies in ways that give information that is what we would call “clinically actionable”.

It is very easy to measure something in a patient, by taking blood and measuring a chemical in it, for example. However, to understand what that measurement really means, it has to be placed in a framework of knowledge that allows a doctor to decide what to do next based on that piece of information. In almost all analytical technologies and despite all the fancy new things, whichever “omics” you are interested in, the ability to inform a doctor to the point that they can do something is lacking.

One of the greatest challenges we face is not just using the new technologies, which have got to be reliable, reproducible, rugged and all the other things you need when you’re making decisions about humans. It is also about visualizing data in ways that are good for doctors, biologists and epidemiologists to understand, so that they can help provide advice about healthcare policy in the future.

Aside from the challenges faced, one of the points that I would like to make in my lecture, is the deep thinking that needs to surround any technological development for it to be useful in the real world. That’s ultimately where all our fates lie.





Will you be outlining any specific examples or case studies in your talk at Pittcon?

I will describe the challenges and big issues in the first five or ten minutes and then we'll start to look at what creates complexity. I will give the supra-organism examples and since I am mainly a metabolic scientist, I will show how, particularly from a metabolic point of view, microorganisms influence biochemical phenotypes in humans and how those relate to things like obesity risk, cancer risk and so on.

I will deliver it in a way that provides a more general understanding of the complexity and how that affects us as human beings. Then I will talk about more specific examples about how to ensure a technology makes a difference to the patient. How you study the human biology, how biology is complex and what you need to study about it analytically is the first part and then I will give some examples where it is on a clinical timescale.

If you are thinking about understanding population health and population biology, which is what epidemiologists do, it does not really matter how long it takes to get the answers, so long as they get the right answer, because

not one individual patient is dependent on their thinking. What you are trying to do, is understand where diseases come from so that in the future, you can make a better world by actioning the knowledge.

When somebody is ill, there is a timescale. There are different timescales, with each presenting their own different analytical challenges. If somebody has gut cancer, for example, they are going to need hospitalizing and to have some diagnostics performed. Physicians will decide exactly what sort of cancer it is, if the physicians understand it, and then there will either be surgery or some sort of chemotherapy. There will be a physical or chemical intervention of some sort.

Therefore, any analytical chemistry performed has got to be done within the timescale of the decision-making that the doctors make. In this example, it would have to be done within a day or two and the answer has to be interpretable by a doctor in that timescale.

For instance, there is no point doing a huge genomic screen on somebody; it would take three months to analyze the data, the doctors would have moved on and the patient may well be dead. All analytical technology has got to fit the constraints and the timescales in which the physicians operate.

The most extreme example is surgery, which involves real-time decision-making. The surgeon will cut a bit, have a look, cut another bit and then, based on a whole range of different background information, knowledge and potentially spectroscopic information, they will make decisions about whether to cut or not to cut.

The iKnife technology developed by Professor Zoltán Takáts, which I had a part in at Imperial, connects a surgical diathermy knife to a mass spectrometer, which means the chemistry of the tissue can be read 3 times per second and based on an annotated knowledge data set, we can know exactly what the surgeon is cutting through.

“That is an example of an analytical technology that gives a surgeon unprecedented molecular knowledge in real time and it is a real game-changer.”

Prof. Jeremy K. Nicholson

Then there are other things, one of which, is on an intermediate timescale. If somebody is in critical care, for example, then by definition, they are seriously ill. They change very quickly over minutes to hours, so any analytical technology that is informing on them has to be very, very fast and the output has to be very quickly readable and interpretable by a doctor.

I think this is also very interesting from an analytical chemistry point-of-view. It's not just about what you are measuring; the whole protocol you construct has to be fit for purpose within the timescale that is relevant to the medical decision-making, which is a huge challenge. A lot of the people, when they develop these technologies, do not necessarily think about it from the point-of-view of the doctor who is on the ward.

A few years ago, I used to give talks where I put forward an idea that I called the “Minute-Man challenge.” The Minute Men were the Americans who wanted things ready within a minute, to fight off the British. There are Minute-Man missiles that the Americans made, which are ready to launch at any enemy within one minute of the go-code being given. The Minute-Man challenge for analytical chemistry is to get from a sample being provided to complete diagnosis within a minute or less.

Nothing exists that does that yet, but we are working on solutions. I thought NMR spectroscopy would be the most likely to provide that because it is a technology where you don't have to do anything to the sample; it is all based on radiophysics. Obviously, making samples and getting them ready for a machine takes time, so whatever the winner of the Minute-Man challenge is, it is going to be something that basically operates on the raw fluid or something incredibly close to that. It will almost certainly have to be directly injected into some sort of measuring device, so of course, in mass spectrometry, we start to



think about direct injection mass spectrometry. There is a whole range of other atmospheric pressure methods such as desorption electrospray ionization and so on.

There is the technology invented by Professor Zoltán Takáts, which can also give you two- and three-dimensional tissue information. I am going to discuss the challenges in time, the challenges in actionability, as well as the challenge in the idea of providing complete diagnosis very rapidly, as something that is effectively a one-shot diagnosis.

Other things will come along; whether there will be one methodology that answers all possible biological or medical questions is, to me, extremely doubtful, but I think technologies are available now, probably mainly revolving around mass spectrometry, that will allow that sort of diagnostic fire power.

Also, if you are using a zero reagent and direct injection methodology, the cost comes down. The time and the cost go together, so, ideally, if you want to study large populations of people, you need to be able to test millions of samples at a relatively low cost. You want something that can measure 10,000 things at once in less than a minute and cost you a dollar. That would be the dream.

I think we might not be that far way from being able to do that, so what I'm going to try to do in my talk is to juxtapose the big challenge ideas against the big analytical challenges and hopefully paint a picture that isn't entirely black.



Which analytical techniques have been most important to your work to date?

I am a spectroscopist by training and I am well-known for NMR spectroscopy, but over the last 15 or 20 years, I've been doing mass spectrometry just as much as NMR. We have 13 high-field NMR machines in my department and about 60 mass spectrometers, all analyzing metabolism, which is quite a collection. I never thought there would be a day when I had more mass spectrometers than NMR spectrometers, but there you go!

Historically, I also worked on X-ray spectroscopy and analytical electron microscopy and atomic spectroscopy, but it was NMR that really made my life come alive from the point-of-view of biology. When I started doing this as a post-doc, I realized that NMR could make the sort of measurements we are talking about - the Minute-Man type measurement - on a load of different things and extremely quickly, so I have been toying with that for over 30 years.



Certainly, when I first started working with NMR body fluids in the early 1980s, people thought I was completely mad. High-field NMR machines were for putting pure chemicals in, to get certain structures out and the idea of putting horse urine in an NMR machine would drive some of my colleagues completely crazy.

A side from being an abnormal use of a highly advanced spectroscopic technology, people thought it would be too complex to work out what all the thousands of components are. In fact, it was complex and we still haven't worked out what all of them are after 30 years and a thousand man years of working in my research group. However, we have sorted out thousands of signals and what they mean biologically. NMR is the most important technique for me personally because it made me the scientist that I am, but the other thing I love about NMR is the fact that it does not destroy anything. It is non-invasive and non-destructive. You can study living cells and look at molecular interactions, as well as just concentration. The binding of small molecules

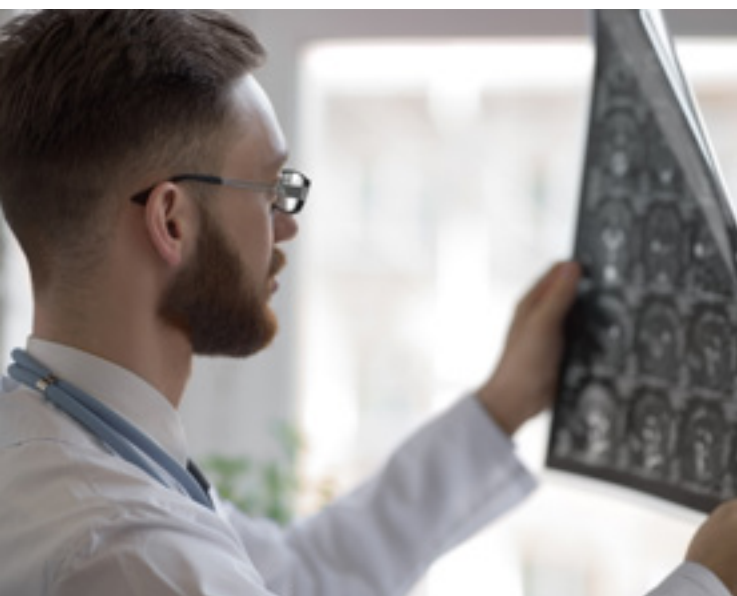
to large molecules can be studied, and those have diagnostic value as well, which I think, is underappreciated in the metabolic community. Most people think mass spec must be better than NMR because it is more sensitive, which it is for most things, but NMR provides a whole set of information that mass spec could never obtain. The two together is the ideal combination if you want to study the structure, dynamics and diversity of biomolecules.

How do you think advances in technology will impact the field?

“Technology advances in all ways, all the time and the advance in analytical technology is accelerating.”

Prof. Jeremy K. Nicholson

There are things that we can do now in mass spec and NMR, for instance, that we would have thought impossible five or ten years ago. The drivers in analytical chemistry are always the improvements in sensitivity, specificity, reliability, accuracy, precision, reproducibility; but for clinical applications, and also for very large-scale applications that you need to study populations, it is robustness, reliability and reproducibility that are the most important things. The ability to harmonize data sets is also very important; irrespective of where you are working in the world, others should be able to access and interpret your data.





It's not just the advances and individual pieces of instrumentation that are important; it's how you use them to create harmonizable pictures of biology, that also has an informatic court. It is not just the analytical chemistry or technology itself, but how you use the data.

Let's just compare NMR and mass spec. One of the things about NMR is that it uses radiophysics and is based on the ability to measure the exact frequencies of spin quantum transitions in the atomic nucleus and the frequencies of those, which are characteristic of the particular molecular moiety that's being looked at.

One of the beauties of NMR, is that if you take, for example a 600 or 900 mega Hertz or giga Hertz NMR spectrum now, the same analysis of it would be still be possible in a thousand or a million years because it's a physical statement about the chemical properties of that fluid that will never change. Even if NMR spectrometers were to get more sensitive, the basic structures of the data will be identical in a thousand or a million years. In mass spectrometry, we're changing the technology all the time. The ion

optics change, the ionization modes change and all of these things affect how the molecules or the fragments fly through the mass spectrometer and how they are detected.

One of the greatest challenges in mass spectrometry, for instance, is therefore time-proofing the data. You do not want to have to analyze a million samples now and then find that in five years the technology is out of date and you have to analyze them all again.

Some technologies such as NMR are in effect much more intrinsically time-proofed than mass spectrometry and a whole range of other technologies that are changing all the time. One of the ways forward is the informatic use for extracting principal features of spectroscopic data, which will be preserved irrespective of how those spectroscopic data were originally provided. Therefore, again, there's a different sort of informatic challenge that has to do with time-proofing, which I think is very interesting.

What other challenges still need to be overcome?

“It is mainly about making analysis faster, cheaper and more reliable. I think the biggest challenge is to do with data sharing and the way that humans work or don't work together. “

Prof. Jeremy K. Nicholson

If you pick up a copy of “Analytical Chemistry” any week, you would find half the papers describe a new, better method for measuring X, Y, or Z. It is always going to be a better, superior method, since otherwise, it would not get published in the journal. Analytical chemists who use current methods always think they can do better. That is the way they think, but when you are being clinical, you’ve got to draw the line somewhere and decide to harmonize and understand that you may have to wait some time until something significantly better comes along

An interesting problem going forward is going to be settling on analytical protocols that everybody can accept, despite the fact you know they are not perfect. It brings us back to the three Rs: ruggedness, reliability and reproducibility. When studying humans, populations or clinical situations using analytical chemistry, the three Rs are always going to be more important than absolute sensitivity and specificity (although these are always important), which is often what motivates most analytical chemists. The three Rs require very strict adherence to harmonized protocols that are sharable. I think that is going to be a challenge to people’s egos.

What do you think the future holds for analytical science in precision medicine?

Precision medicine is about getting an optimized interventional strategy for a patient, based on a detailed knowledge of that patient’s biology. That biology is reflected in the chemistry of the body at all the different organizational levels, whether it is genes, proteins, metabolites, or pollutants for that matter.

All the analytical chemistry technologies that inform on a patient or individual complexity will be ones that are important in the future. With respect to the future healthcare challenges, analytical chemistry is absolutely key; it is core to solving all of the emergent problems, as well as the ones we already face.

How do you hope the National Phenome Centre will contribute?

We have set up The National Phenome Centre to look at big populations and personalize healthcare challenges. We are in our fifth year of operation now and we are running lots of projects that are generating some tremendous findings.

We have now created an International Phenome Centre Network, where there is a series of laboratories built with core instrumentation that is either identical or extremely similar to ours. This means we can harmonize and exchange data, methodologies and therefore biology.



Imperial College was the first in the world, the National Phenome Center, and then the Medical Research Council funded the Birmingham Phenome Center about two years ago. We have transferred all our technologies and methods over and they have an NMR and a mass spectrometry core, which is effectively the same as ours, so we have completely interoperable data. In fact, we've just finished a huge trial, which involved Birmingham University as well.

There is also one in Singapore now, the Singapore Phenome Centre. The Australian Phenome Centre has just been funded by the second largest grant ever given in Australia and then there is a whole series lined up. However, we've already formed the International Phenome Centre Network, which was formally announced by Sally Davies, Chief Medical Officer of England in November last year. We can carry out international harmonization of diabetes biology for the first time ever, so we are putting our money where our mouth is. To me, this is the most exciting thing that has come out of our work; the fact that there are now groups around the world that agree that harmonization is the way forward to get the best international biology hit and also to create massive data sets that are unprecedented in size and complexity to describe human biology.

This is another informatic challenge, but that's the future. There are a lot of dark emergent problems in human disease and we are going to go through some tough times over the next 30 or 40 years. However, we are starting to get our act together with things like the Phenome Centre Network and if it is not the network

itself, it will be groupings like it that will rise to these great challenges facing humanity in the 21st century.

“This year, we will start our first joint project, which is going to be stratification of diabetes between the phenome centers that are up and running and using the same technology.”

Prof. Jeremy K. Nicholson



Further to this, Jeremy Nicholson also gave the Wallace H. Coulter lecture at Pittcon 2018.

Here, Prof Nicholson described his passion to achieve more efficient and effective healthcare solutions in the 21st century by using analytical science in precision medicine.

For the last 25 years, I have been interested in the development of technology that can analyze human metabolism in great detail. I have been trying to understand how differences in metabolism impact human health and how this enables the body to move from a health condition to a pathological condition.

My research group has a very broad range of metabolic interests, from the development of technology, right through to the mathematical modeling of data. We then present this data in ways that doctors can understand and use to make clinical decisions.

I am going to discuss the ways in which we are bridging the gap between chemical experiments in the laboratory and patients, and the importance of this, as well as the challenges that we face that are somewhat greater than those just faced in the laboratory. I will describe the challenges and problems that we face as humans in modern society, and the complexity of today's world.

The modern world changes quickly, and we tend to try and give up technologies, medicines, for things that we see in front of us. The problem is, the world changes so quickly that we now have to future-proof everything we develop and be mindful of what it will be like in 15-50 years' time.

What is precision medicine?

First, I am going to discuss the challenges that come with personalized and public healthcare and the complexity of human interaction.

The things that can be measured are vital to precision medicine, and therefore precision analytical chemistry. They allow us to make precise measurements of tissues and of body fluids, and give systems-level readouts on human biochemistry.

Precision medicine, by definition, is about taking measurements of your body to inform the treating clinician about your current physiological state.

However, precision medicine is about far more than just making measurements. It has to be regulated and it has to involve pharmaceutical companies. It also requires the involvement of educators because we need to train new doctors to be able to understand measurements given by new technologies. Ultimately, we want clinically actionable information that can be used by the doctor to make informed choices about what to do next in a patient's care.





The importance of precision medicine

To illustrate how important precision medicine is, I will describe an example of non-precision medicine.

There are at least 10 different sub phenotypes of diabetes and at least 17 different treatments. The treatments are very rarely matched to the sub-phenotypes of the disease and a patient is typically started on Metformin when they're in the pre-diabetic stage.

This is successful for a lot of people and stops them from developing full-blown diabetes. The problem arises when the drug fails and it becomes a lottery, like we're playing a diabetes roulette, where doctors are placing bets on a drug that they know and are hoping for a good outcome.

What we need to do is match the biology and metrics that we have of those different sub-phenotypes to the efficacy of those drugs. From here, we can build molecular models that could

be used to by doctors to work out the best therapeutic point of action immediately.

In precision medicine, people often talk about genetics as being very important, but it's also important to consider how precise genomics is, as it is mountable to the existing technologies. This is something slightly idiosyncratic.

For example, identical triplets took the same DNA kits received results with different ancestry. One's showed 11% French and German, whilst another one showed 17% French and German, and the third showed 18% French and German, which is biologically impossible. They've all got to have the same ancestor.

What this is really telling us is the imprecision of genomics. When we're talking about using precision medicine to decide which therapy is going to work for a patient, we need is to bring those variations to less than 1%. We're a long way from this and deployable genomic medicine.

Metabolic phenotype

Genes aren't the most important factors contributing to ill-health. 95% of the world's population die from environmental causes, which may not be exacerbated by their genomic background.

The study of genomics is only part of the story. Understanding how genes contribute to human disease is important, but we must also study the impact of environmental factors.

The environment has an enormous part to play in the metabolism of molecules and drugs. For example, all drugs must face an enormous array of environmental microbes before entering a patient's body.

Microbes are the interface between you and the environment and have their own micro-genomic environment influencing drug metabolism. All of these things together create phenome, which is an umbrella term encompassing a number of different things that can be measured about your body, physical

and chemical. This includes cholesterol, urine, height and eye color, etc. These are all phenomic properties that come from genes and the environment.

Your metabolic phenotype is an important part of that, and is a representation of the gene environment interactions.

Gene-environment interactions determine your risk of contracting a disease as an individual, and your response to therapeutic intervention.

Your metabolic phenotype is therefore a window into your personal health. That's why we've spent a great deal of time and money over the years trying to develop technologies that can analyse gene-environment interactions and the metabolic phenotype.

It's useful to remind ourselves that the conditions people are dying of now are dramatically different from the 19th century and early 20th century. This is partly due to big changes in sanitation, and the introduction of antibiotics after the Second World War that reduced the number of people contracting and dying from infectious diseases.

It used to be very common for people to die of pneumonia, malaria, or sepsis, etc. but now it is the opposite. We are now dying of diseases associated with old age, such as strokes, but also because of antimicrobial resistance.





The cost to the Health Service is huge, and it's only going to get worse going forward. One of the biggest challenges is to find new antibiotics, or new ways of fighting bacteria that don't necessarily involve chemical substances.

Non-infectious (or non-communicable) diseases have seen the biggest change. One example of this is Type 2 diabetes, a newly emerging threat to global health.

We know that the increase in BMI (body mass index) has driven diabetes over the last 20 to 25 years. What's interesting is that some of the countries are falling off at an enormous rate with increasing diabetes, on metabolic human basis. The lifestyles of the next generation will have an enormous influence on disease patterns globally.

A recent paper showed that if children are given antibiotics in the first year of life, especially boys, they're much more likely to develop obesity or be diabetes in later life. Antibiotics change the biology of a person through changing the microbiome, which in turn influences our metabolism and risk of metabolic disease.

Fifty percent of children don't get enough exercise, and thirty percent under the age of 16 get none, and 10% of children under the age of 16 smoke. That's absolutely terrifying.

These behaviors have been linked to the biggest killers in today's society; cancers, cardiovascular related disease, and stroke, etc. It is down to politicians, television, and the marketing campaigns of fast-food chains to initiate the change.

We can't improve the behaviors of children unless we as scientists speak out and say we need help from the people with the greatest influence. Otherwise, we face a tsunami of ill health, which will be unavoidable in the next 20 to 30 years.

The microbiome

There are around 20,500 coded in genes in the human body, but there are ten million genes in the microbiome, and about a hundred trillion organisms in your gut. That's ten times the number of cells in the human body!

The composition of your microbiome changes every two days. During your lifetime, you'll empty around 20 tons of bacteria down the toilet alone.

Microbiome-related disorders have been associated with multiple non-infectious diseases, such as ectopic diseases, asthma, cardiovascular diseases, and cancer. This is because the microbiome is a major regulator of human health. If you have a healthy microbial community in your body, you have a healthy immune system, which influences your likelihood of contracting lots of nasty diseases.

Your genes interact with the microbiome, as well as each of your cells, protein transcripts and metabolites. Metabolites are made in our bodies by microbes. Microbes are natural product factories, they make compounds which are absorbed and metabolized by us.

Microbes are important moderators of drug responses.

It has been shown that they can modify the chemicals we put into our body, influencing their efficacy. One particular study a few years ago showed that the genomic environment changed the outcome of a patient taking an anticancer drug.

Now we're thinking in terms of complexity, it's not just genomic variation, it's also microbiological variation that determines how effective a drug is.

Advanced analytical technology

The most important techniques in microphenotypes are NMR and mass spectrometry. A complex system may contain many thousands of metabolites and currently, there is no technology that can cope with this amount of data and sample everything at the same time.

Using current techniques, everybody can get different results from the same sample, which will all be correct, due to under-sampling of the complex system.

Something that is of interest is trying to find signatures that are associated with increased disease risk. This requires us to look at many thousands of results, which is a very big data challenge. However, having the technology to study and understand diseases in this way could help inform future healthcare policy.

We want to do to make sure that we absolutely believe the data that's come in, we'd be interested in harmonization 15 years ago.

As part of the Phenome Center we establish a network that people to find standardized protocols. This works to improve operational compatibility and future compatibility and harmonization of technologies. Now, the challenge of several of these centers around the world that are now built is to tackle global attacks on the world in medicine e.g. anti-microbial resistance and healthcare issues such as dementia, autism and obesity. Together, with multiple centers working on the same kind of technology and informatics, we can start statistically powerful studies and look at graphical distributions of the environment and fractions without variations in the data getting in the way. It's a big task and it's in infancy.



Human proteins play integral roles in cellular processes involved in health, and gaining insight into the proteins' presence, structure, function, and variance is essential for understanding disease. Subsequently, the direct insight gained with imaging spectrometry plays an indirect role in advancing the state of patient care via developing targeted therapies that improve quality of life and survival.

Proteomic research involving imaging mass spectrometry is aimed at helping to identify gene expression and complexity while allowing for identification of proteoforms produced by post-translational functions.

Measurement of proteomes shows potential for translational applications, considering that most normal and modified cellular processes depend upon protein expression and regulation.

At Pittcon 2018, Professor Xiaowei Zhuang of Harvard, awardee of the Pittsburgh Analytical Chemistry Award Symposium, discussed the level at which we are now able to study biology using bioimaging techniques, in his presentation titled "Illuminating Biology at the Nanoscale and Systems Scale by Imaging".

In biomedical clinical research, measurements using broad quantitative imaging mass spectrometry can help identify peptides and proteins that vary in number among patients receiving either intervention or control. A benefit of this strategy is the unbiased ability to identify and characterize an entire proteome in one measurement. Additionally, tandem mass spectrometry may be helpful for obtaining additional information about unknown peptides in bottom-up mass spectrometry biomedical studies.

Unfortunately, many challenges exist with current spectroscopy applications, including isomeric indistinguishability and inadequate throughput of measurements. Additionally, some tissue analytical techniques can take considerable time spent in the lab, especially when investigators are performing multi-target monitoring of samples. Also, due to the extensive number of extractions necessary for capturing small molecules in complex environments and biofluid samples, a need exists for analytical sampling techniques that can be rapidly performed without sacrificing quality of molecule characterization and quantification.

The use of molecular spectroscopy in the biomedical industry has expanded in recent years, partially driven by the emergence of low-cost, compact, and portable systems that have enabled faster and greater depth in molecular analyses. Investigators are currently using spectrometers in biomedical research to examine antioxidants, carcinogenic tumors,

the pathways involved in cardiovascular disease, bone tissue, compounds associated with neurological deficits, and viruses. As medical research focused on personalized therapy continues to grow in relation to the evolutionary speed of analytical technology, molecular spectroscopy will expand and may contribute to the development of new therapies for numerous hard-to-treat diseases. Using this strategy, investigators are able to detect and identify unknown compounds from unique Raman spectra fingerprints.

At Pittcon 2018, Paul Champion will be discussed a study which describes the use of Raman spectroscopy for the study of hemoglobin and the related vibrational mode-specific relaxation processes directly following photoexcitation. Additionally, Champion will discuss the role of the time-dependent line shape function for extracting mode-specific vibrational temperatures from Raman resonance data. This talk will also highlight the main factors involved in temperature dependence of resonance Raman scattering. Also at Pittcon 2018, X Sunney Xie, one of the worlds foremost figure in Raman, presented a talk titled “Single Cell Genomics: When Stochasticity Meets Precision”, as part of the Pittsburgh Analytical Chemistry Award Symposium.

Many Raman imaging systems, like the RA802 Pharmaceutical Analyser by Renishaw, are compact benchtop instruments that are used primarily for analyses in the clinical space. Raman instruments with a high spectral resolution across the Raman range provides greater chemical specificity, therefore facilitating enhanced differentiation between



In Raman spectroscopy, both structural and chemical data of a studied substance are provided to help researchers obtain greater detail about the analyzed material.

numerous compounds and materials. Typically, investigators comparing materials to their Raman spectra may ultimately have the ability to differentiate materials of similar polymorphic forms derived from the same chemical.

Some Raman microscopes, like the DXR Raman microscope offered by ThermoFisher Scientific, are able to provide 2-micron depth resolution and a high level of sensitivity and repeatability for medical use. Both Renishaw and Thermo Fisher were available at Pittcon 2018 to talk about their Raman technology.

In addition, using Raman spectroscopy allows analyzation of changes in various spectrum details, such as the position, width, and height of Raman bands. This enables researchers

to identify the proportion of material, layer thickness, temperature, and variation in stress state or crystallinity. The portability, compactness, and high-resolution of handheld systems may help improve efficiency in obtaining direct findings of materials. B&W Tek, another exhibitor at Pittcon 2018, provides handheld and portable Raman spectrometers, such as the NanoRam® and i-Raman®, with accompanying analytical software suited for operators at multiple levels of technological experience.

Imaging mass spectrometry is a relatively new spectroscopy approach which offers in-depth observation, monitoring, and detection of molecular processes within the tissue spatial domains. Thus, the use of this technology has practical benefits in both biology and medical research. Imaging mass spectrometry quantifies tissue molecules without having to rely on target-specific antibodies or other reagents. Richard Caprioli discussed the application of mass spectrometry in the study of biology and medicine at Pittcon 2018. Specifically, Caprioli will highlight the utility of using imaging mass spectrometry for detecting and monitoring molecular processes found in tissues' spatial domains for medical and

Promising research areas for applying the Raman approach include cell biology, pharmacology, and tissue engineering.

biological study and will describe how recent advances in sample preparation and instrument performance have enabled higher spatial resolution imaging (1-10 microns) at higher speeds. Additionally, the talk will discuss the use of the Bruker 15T solarix FTICR MS for small animal research, particularly as it pertains to imaging tissues for potential identification of compounds associated with disease. This technology directly measures molecular compounds in tissues without the use of target-specific reagents such as antibodies, is applicable to a wide variety of analytes, and can provide spatial resolutions below the single cell level.

Also, imaging mass spectrometry can be applied to several different types of analytes. Ionization methods used in imaging spectroscopy applications include matrix assisted laser desorption/ionization (MALDI), desorption electrospray ionization (DESI), and Fourier-transform ion cyclotron resonance (FTICR). According to the literature, MALDI imaging mass spectrometry is perhaps the most applicable and effective for imaging biological and medical samples. MALDI offers the ability to directly monitor lipids, proteins, peptides, and metabolites found in tissues. The MALDI Biotyper from Bruker represents one of the MALDI imaging systems which aid in the taxonomical classification of yeasts, bacteria, and fungi through fingerprinting methods.





The use of ion mobility spectrometry is an additional analytical technique used in clinical research for the separation and characterization of ionized molecules found in the gas phase. Overall, ion mobility spectrometry can enhance workflows for imaging mass spectrometry and improve efficiency of studies which require evaluation of specific molecules. At Pittcon 2018, Erin

Baker provided an overview on the study of biofluids as well as environmental samples using an automated solid phase extraction method before ionmobility spectrometry. Using this method, researchers may minimize ionization suppression, detect exogenous and endogenous metabolites, and remove salts more efficiently. In addition, Baker will discuss how ion mobility separations as well as mass spectrometry may improve isomeric distinguishability by providing high throughput analyses.

Bruker's RAID instruments are ion-specific spectrometry tools that may offer utility for some clinical applications. Additionally, the SYNAPT and Vion instruments from Waters offer ion mobility separations necessary for a range of study types, including for the biomedical field.

References

- » Whiteaker JR, Lin C, Kennedy J, et al. A targeted proteomics-based pipeline for verification of biomarkers in plasma. *Nat Biotechnol.* 2011;29(7):625-634
- » Bendall SC, Simonds EF, Qiu P, et al. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science.* 2011;332(6030):687-696
- » Addona TA, Shi X, Keshishian H, et al. A pipeline that integrates the discovery and verification of plasma protein biomarkers reveals candidate markers for cardiovascular disease. *Nat Biotechnol.* 2011;29(7):635-643
- » Kayat M. Biomedical Applications of Molecular Spectroscopy. Presentation from: B&W Tek. http://www.lahat.com/images/stories/laser_seminar_27.3.12/Biophotonics/biomedical_applications_of_molecular_spectroscopy2.pdf
- » Camp CH Jr, Lee YJ, Heddleston JM, et al. High-Speed Coherent Raman Fingerprint Imaging of Biological Tissues. *Nat Photonics.* 2014;8:627-634
- » Deschaines T, Henson P. The DXR Raman Microscope for High-Performance Raman Microscopy. Thermo Fisher Scientific. <https://tools.thermofisher.com/content/sfs/brochures/D10298~.pdf>
- » B&W Tek. Handheld & Portable Raman Spectrometers. <http://bwtek.com/technology/raman/>
- » MALDI Biotyper. Bruker. <https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html>
- » Guevremont R, Siu KW, Wang J, Ding L. Combined Ion Mobility/Time-of-Flight Mass Spectrometry Study of Electrospray-Generated Ions. *Anal Chem.* 1997;69(19):3959-3965
- » Tang K, Shvartsburg AA, Lee HN, et al. High-sensitivity ion mobility spectrometry/mass spectrometry using electrodynamic ion funnel interfaces. *Anal Chem.* 2005;77(10):3330-3339
- » Ion Mobility Spectrometry. Bruker. <https://www.bruker.com/products/cbrne-detection/ims.html>
- » Ion Mobility Mass Spectrometry. Waters. http://www.waters.com/waters/en_US/Ion-Mobility-Mass-Spectrometry/nav.htm?cid=134656158&locale=en_US
- » Ye X, Demidov A, Rosca F, et al. Investigations of Heme Protein Absorption Line Shapes, Vibrational Relaxation, and Resonance Raman Scattering on Ultrafast Time Scales. *J Phys Chem A.* 2003;107(40):8156-8165
- » Caprioli RM. Imaging mass spectrometry: molecular microscopy for enabling a new age of discovery. *Proteomics.* 2014;14(7-8):807-809
- » Liebeke M, Fuchser J, Kellersberger KA, Fearn S, McPhail D, Bundy JG. Complimentary Use of MALDI FTICR-MS and TOF-SIMS Imaging: Approaches in an Invertebrate. Poster presented at: ASMS 2014. https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/Separations_MassSpectrometry/Literature/literature/Poster/Poster_ASMS2013_earthworm_JFU.pdf
- » Baker ES, Clowers BH, Li F, et al. Ion mobility spectrometry-mass spectrometry performance using electrodynamic ion funnels and elevated drift gas pressures. *J Am Soc Mass Spectrom.* 2007;18(7):1176-1187

Bioimaging of enzymes, nanoparticles, and cells may play an integral role in the development of new, more effective, and targeted therapies for disease.

At Pittcon 2018, investigators in the field of bioimaging presented their cutting-edge research, highlighting the advantages of using various bioimaging modalities for improving disease identification and patient care.

Direct imaging of molecules, specifically metals, features important relevance to biological research. Numerous metals are essential for life, often playing roles in protein synthesis and the transcription and translation of nucleic acids. Gaining a deeper understanding of how these metals work in the body relies on high-quality bioimaging analysis. Fluorescent probes, for example, are often used for the detection, identification, and study of transition metals and their signaling actions in biological systems.

Christopher Chang of the University of California, Berkeley, presented key research on activity-based sensing (ABS) strategies for interpreting signaling of transition metals. A focal point of his research—ABS—is centered primarily on using chemical reactivity in lieu of standard “lock-and-key molecular recognition” for modifying biological molecules and systems.



In his presentation, Chang demonstrated how an ABS approach can be used to discover new chemical signals in sulfur, carbon species, reactive oxygen, and transition metals. The findings of his research is aimed at helping other researchers in uncovering the biological functions of a variety of molecules.

Observations of natural phenomenon has helped researchers understand how nanoparticles can be modified to produce complex intracellular structures featuring diverse biological functions. One study, using in vivo whole animal and ex vivo super resolution fluorescence imaging, examined the construction of nanostructures from nanoparticles in tumors under enzyme control.



Considering that enzymes play pivotal roles in catalyzing numerous biological reactions within the body, imaging research of how enzymes and nanoparticles interact in cancer represents an important step for understanding carcinoma development and progression. Additional research has examined the use of nanoparticles as acting as enzyme-directed morphological transformations in response to specific stimuli, notably targeted therapies. The investigators of this research suggest that modification of nanoparticle responses to disease related enzymes may provide clinical utility for therapeutic delivery as well as disease detection.

Liquid Cell TEM

Recently, there has been a surge of interest in liquid cell transmission electron microscopy (TEM) for imaging seemingly invisible materials through liquids. Recent studies have utilized liquid cell TEM for evaluating the growth, arrangement, tracking, and manipulation of nanoparticles.

Liquid cell TEM facilitates in situ imaging of precipitation using high-quality spatial resolution and has been applied to physics, chemistry, and materials science research.

There has been a growth of interest in using nanomaterials as medical and diagnostic carriers in a variety of disease states. Typically, nanoparticles are the most heavily used nanomaterials in biological study as they are small enough to be injected directly into the bloodstream while still being large enough to act as effective targeted carriers.

Imaging these nanoparticles and the therapies they carry is an essential step in understanding targeted therapy, which may ultimately translate into improved medical care for individuals with a myriad of health issues. Microspectroscopy systems, such as the Thermo Scientific™ Nicolet™ or Thermo Scientific™ DXR™ systems are an excellent candidate for nanoparticle research. Additionally, these imaging platforms hold utility for detecting unknown materials via a single point measurement and/or spatial spectroscopy.

During Pittcon 2018, Nathan Gianneschi of Northeastern University presented research regarding the use of a multimodal imaging platform for use in the study of targeted therapeutics and molecular diagnostics.



Specifically, Gianneschi and colleagues explained how they went about preparing highly functionalized polymer scaffolds and their subsequent production of these scaffolds as in vivo probes. In targeted therapy, the goal is to gather drugs or probes at the disease

site in substantial quantities “relative to other locations in the body.” An important area of Gianneschi et al’s research is the utility of in vivo probes as an imaging platform as well as drug carriers in targeted therapy.

References

- » Aron AT, Ramos-Torres KM, Cotruvo JA Jr, Chang CJ. Recognition- and reactivity-based fluorescent probes for studying transition metal signaling in living systems. *Acc Chem Res.* 2015;48(8):2434-2442
- » Chien MP, Carlini AS, Hu D, et al. Enzyme-directed assembly of nanoparticles in tumors monitored by in vivo whole animal imaging and ex vivo super-resolution fluorescence imaging. *J Am Chem Soc.* 2013;135(50):18710-18713
- » Ku TH, Chien MP, Thompson MP, et al. Controlling and switching the morphology of micellar nanoparticles with enzymes. *J Am Chem Soc.* 2011;133(22):8392-8395
- » Liao HG, Zheng H. Liquid Cell Transmission Electron Microscopy. *Annu Rev Phys Chem.* 2016;67:719-747
- » Liquid Cell TEM. University of York. <https://www.york.ac.uk/physics/research/cmp/new-characterisation-methods/liquid-cell-tem/>
- » Microspectroscopy Selection Guide: FTIR and Raman Microscopes and Microsampling Solutions ThermoFisher. <https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/microspectroscopy-selection-guide>.

Numerous bioanalytical techniques have been introduced in recent years for the identification of analytes as well as for diagnostic purposes. Due to the high sensitivity of detection, it is recently common to see Raman molecular imaging being developed for clinical uses, such as for endoscopy and in intraoperative imaging for the guidance of surgical resection. At Pittcon 2018, researchers experienced in Raman spectroscopy and diffuse resonance Raman spectroscopy (DRRS) shared how they use these platforms for a variety of diagnostic and quantitative analyses.

Raman spectroscopy holds important value in the biomedical space, with applications ranging from in vitro biofluid assays and pharmaceutical study to breast cancer diagnosis based on chemical composition. One study has shown that Raman spectroscopy may even be helpful for the rapid identification of ricin, a naturally derived toxin that can produce potential harm and even death to exposed individuals. Thus, Raman spectroscopy has the potential to save lives if used early.

Richard Dluhy, PhD, from the University of Alabama at Birmingham, presented a talk at Pittcon 2018 on the utilization of Raman spectroscopy for the biochemical characterization of stored red blood cells (RBCs) used for transfusion.

In his talk, Dr. Dluhy outlined how Raman spectroscopy can streamline the process of assessing RBCs for quality prior to transfusion, thus avoiding the often time-consuming process associated with standard methods.

Doing so, according to Dr. Dluhy, may ultimately improve therapeutic efficacy and reduce transfusion-related toxicity. In addition, Dr. Dluhy discussed a recent study which aimed to develop DRRS for rapidly obtaining depth-sensitive Raman spectra for RBC screening.

At Pittcon 2018, DRRS was another topic introduced, and researchers will discuss its application to a variety of scientific fields. The use of DRRS in bioanalytical techniques is important for researchers wishing to obtain depth-sensitive Raman spectra in a timely and less-invasive manner compared with typical strategies. Contrary to excitation via near-infrared wavelengths, the use of DRRS requires up to 10-fold less power and time for acquiring a similar signal-to-noise ratio.



Instead of using a focused beam, DRRS relies on illumination via a diffuse beam, thereby decreasing photon density and providing greater penetration of the sample. In an effort to augment detection of diffuse photons, DRRS includes additional pixels from a charged coupled device detector that align with the pixels in the optical line of the micro-spectrometer.



Some research suggests that DRRS amplified by surface-enhanced Raman scattering nanoparticles may provide high sensitivity and rapid acquisition time for in vivo and in situ detection of heme proteins, carotenoids, and multiplexed nanoparticles. Additionally, DRRS may enable mapping of a larger sample area in small animal models than other commonly used microscopy platforms.

ThermoFisher's DXR2 and DXR2xi Raman imaging microscopes, for example, are helpful for analytical experiments measuring

multi-layer composites while providing real-time on-screen data acquisition. Renishaw's RA802 Pharmaceutical Analyser, a benchtop Raman imaging system, provides API/excipient domain statistics and analyzation of sample preparation specifically for pharmaceutical study and development.

Horiba's XploRA ONE™ and LabRAM, which provides advanced confocal 2D and 3D imaging for micro and macro measurements, represents some of the additional available DRRS solutions for the pharma and biomedical fields.

References

-
- » CURE Spotlight: Cesar Castro, M.D. Develops Smartphone Technology for Cancer Detection in Underserved Communities. NIH: National Cancer Institute. <https://www.cancer.gov/about-nci/organization/crchd/blog/2015/castro-spotlight>
 - » Campos AR, Gao Z, Blaber MG, et al. Surface-Enhanced Raman Spectroscopy Detection of Ricin B Chain in Human Blood. *J Phys Chem.* 2016;120(37):20961–20969
 - » Bohndiek SE, Wagadarikar A, Zavaleta CL, et al. A small animal Raman instrument for rapid, wide-area, spectroscopic imaging. *Proc Natl Acad Sci U S A.* 2013;110(30):12408–12413
 - » ThermoFisher. Microspectroscopy Selection Guide: FTIR and Raman Microscopes and Microsampling Solutions. https://www.thermofisher.com/uk/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/microspectroscopy-selection-guide.html?gclid=EALalQobChM18Zaxy_yQ1wIVyxbTCh3zPAZuEAAYASAAEgLJR_D_BwE&cid=msd_mol_Microspectroscopy_adwords&s_kwid=AL!3652!3!227286618173!b!!g!!raman%20spectra%20analysis&ef_id=WD1DVwAAAHofudeq:20171027140603:s
 - » Renishaw. Resonance Raman spectroscopy for redox biology research. <http://www.renishaw.com/en/webinar-resonance-raman-spectroscopy-for-redox-biology-research-36628>
 - » Horiba Scientific. What is resonance Raman spectroscopy? <http://www.horiba.com/us/en/scientific/products/raman-spectroscopy/raman-academy/raman-faqs/what-is-resonance-raman-spectroscopy/>
 - » Bio-Rad. Electrophoresis Products. http://www.bio-rad.com/LifeScience/pdf/Bulletin_1955.pdf
 - » Heintz R, Wall M, Ramirez J, Woods S. Complementary Use of Raman and FT-IR Imaging for the Analysis of Multi-Layer Polymer Composites [Abstract]. ThermoFisher. <https://assets.thermofisher.com/TFS-Assets/CAD/posters/POS2690-IR-Raman-Multilayer-Polymer2015.pdf>
-

USING SMARTPHONES FOR CANCER DIAGNOSIS AND MANAGEMENT

In a talk at Pittcon 2018, Cesar Castro of the Massachusetts General Hospital described his research regarding the utility of smartphone technology for the rapid identification of cancer using marker-specific microbeads. In his talk “Highlighting Cancers Through Shadows and Smartphones,” Castro explained how he and his colleagues produced a 2-step labeling system that can streamline preparation of reagents via removal of antibody modification.

Castro discussed the development of the Digital Diffraction Diagnosis System (D3) device, which attaches to a smartphone. Simply, the device offers an imaging module and LED light which produces and records high-resolution pictures using the camera from the individual’s mobile phone. Overall, the D3 device has a larger field of view than standard microscopy systems and can record data on >100,000 cells using a prick of blood or tissue sample. The preliminary data presented by Castro demonstrates higher diagnostic accuracy than some other tests currently being offered.



During his talk, Castro also discussed the importance of smartphone technology in cancer screening and care in rural or resource-limited communities. In this interview, the synergistic role of future technology with holographic approaches, such as wearable sensors and deep learning, is discussed:

Please outline the current global cancer challenges.

There have been significant inroads in our understanding of what drives cancer development, spread, and treatment resistance. Sophisticated diagnostic and profiling tools along with increasingly robust bioinformatics have adequately equipped cancer researchers and providers to start regarding cancers as heterogeneous entities where one size does not fit all.

Unfortunately, these advances have only benefited resource rich areas. There exist vast regions throughout our globe where geographical, structural, and human capital constraints preclude even the basic cancer diagnostic and profiling processes we take for granted.

These are not merely inconveniences; incorrect or delayed diagnoses can lead to fatalities. A case in point lies in cervical cancer where 80% of deaths worldwide occur in resource limited countries yet most of the advanced screening (e.g. Pap smear and/or HPV DNA testing) occurs in more affluent countries. Bridging the divide between the “haves” and “have nots” remains an unmet need.

What features make smartphones a well-suited platform for point-of-care diagnostics?

Smartphones are ubiquitous and intuitive devices with sophisticated capabilities; these merits support testing of their performance in the diagnostic space.

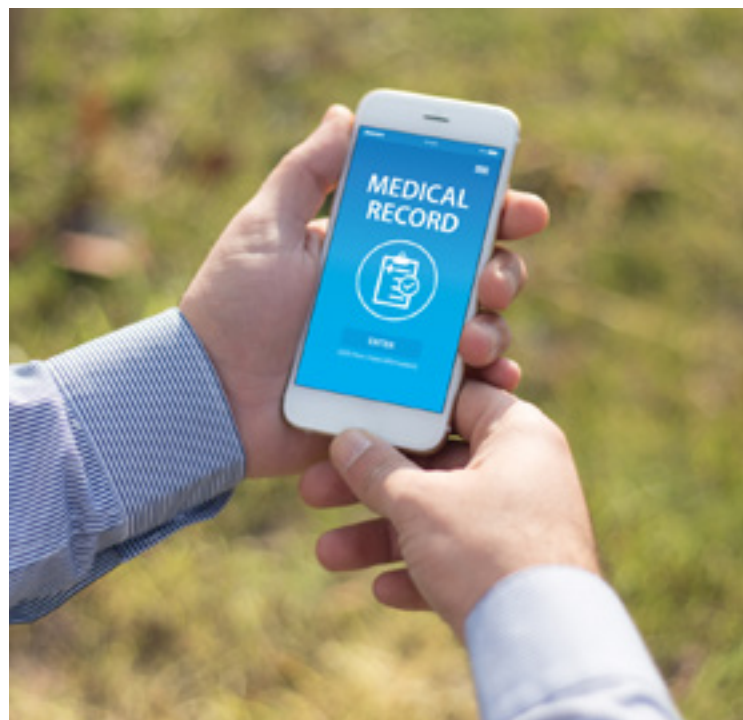
Moreover, the portability, wireless data transmission, and relative ease-of-use afforded by smartphones enable the needed decentralization of health care to expand its reach into underserved communities. In addition to direct individual patient benefits, population level benefits can be achieved.

“To put this in perspective - if you took a photo on a trip abroad, the geotagging features of the smartphone can log both the time and location. Integrating such a feature into a diagnostic approach could help with improved epidemiological understanding of disease prevalence.”

Dr. Cesar M. Castro



Beyond addressing concerns of inaccurate cataloging of cancers in resource-limited settings, better alignment of limited chemotherapy stockpiles can occur with those areas most in need.



What technology can be used on smartphones to enable molecular diagnostics?

A variety of technologies have been created by my group and other researchers world-wide to enhance smartphones for molecular diagnostics. Opportunities include fluorescence, holography, and electrochemistry among others. Each platform invariably has their pros and cons; as such, understanding the clinical need and local expertise and challenges could help identify the appropriate platforms over other less viable options for the intended application.

It has recently been announced that you will be presenting in the “Wearable and point-of-care sensor technologies for biomonitoring” symposium at Pittcon 2018. Please can you outline your research in using smartphones to detect and profile cancer cells?

“Our work focuses on holography as well as contrast enhancement microscopy to achieve the needed signal-to-noise for reliable detection of our intended targets.”

Dr. Cesar M. Castro

Early iterations of smartphones as diagnostics within the field focused on its use as a microscope to provide more cost-effective and simple setups. Practically, that would still entail highly trained pathologists as the end user. But pathologists are scarce and overburdened in resource-limited countries.

Moreover, two slides from two patients with the same cancer may look identical under the microscope, but the clinical trajectories could be quite different. We thus focused on molecular profiling tumors to better gauge potential biological behavior in addition to improving diagnostics in a more automated manner.

Our work has initially focused on lymphoma and breast cancers although other tumors can readily be analyzed. Through our smartphone approach, we regard them as vehicles for capturing data and transmitting them into the computing “cloud” where we’ve established a server that analyzes and reconstructs the data before sending a diagnostic result back to the phone within seconds. Please outline what is meant by the ‘shadows’ part of your

“Highlighting Cancers Through Shadows and Smartphones” presentation at Pittcon 2018.

The use of “shadows” is a play on the principle of holography. Much like hand “shadow puppets”, when light strikes an object, that light is redirected (or diffracted) at a particular angle. This creates a unique holographic pattern (shadow) that is inherently “noisy”. Our cloud algorithm reconstructs such noise into decipherable patterns allowing us to determine the presence or absence of cancer specific markers within a specimen.



What is a holographic assessment and how can holographic technologies be used as a cancer screening tool? What measurements do they make about a patient?

Let's say we wanted to determine if a particular specimen contained estrogen receptors on the cell surfaces. This is important information to know in the breast cancer setting, since their presence would render the patient eligible for hormonal treatment.

In sub-Saharan Africa, testing for hormonal markers is not routinely done or conducted in timely fashion in the few times it gets evaluated. In comparison, hormonal testing is invariably performed on specimens in the US. So, once our mobile platform is fully available abroad, we can place a sample of breast tissue on a small attachment that sits over the smartphone's camera.

We add small micrometer sized plastic beads that have estrogen receptor antibodies attached to their surface. If the specimen itself has estrogen receptor, the antibody (along with its attached plastic bead) would make a connection.

We then shine the smartphone light on the specimen, creating holographic patterns. These patterns are sent to the cloud and reconstructed into the original cell and bead images. If there has been target engagement, then we would see a cell along with closely attached beads at predictable distances. If there is no target engagement (i.e. no estrogen receptor present) then we would see a haphazard arrangement of beads floating near or away from cells.

The measurements can be multiplexed, meaning more markers can be evaluated to interrogate for the presence of certain markers of interest. The read outs can be achieved in an hour currently, which is a significant advance compared to conventional pathology interpretation (in resource rich regions) of a few days.

How can holographic strategies be transferred to wearable devices?

Wearable devices are indeed in their early days yet offer high promise for integration into clinical practices. In the case of a smart watch for example, a standalone device can be used to house and process specimens. The smart watch can be used to receive and store the read outs.

Artificial intelligence algorithms are being developed by us to enable analyses without the need for cloud based testing in areas with limited or absent cellular service. Such algorithms would require less resource intensive computing; programs could potentially reside in smart watches or other wearables such as glasses. With other advances such as augmented reality, future possibilities and opportunities are even more exciting!





What impact will your findings have on areas with limited medical access?

Wearable devices are indeed in their early days yet offer high promise for integration into clinical practices. In the case of a smart watch for example, a standalone device can be used to house and process specimens. The smart watch can be used to receive and store the read outs.

Artificial intelligence algorithms are being developed by us to enable analyses without the need for cloud based testing in areas with limited or absent cellular service. Such algorithms would require less resource intensive computing; programs could potentially reside in smart watches or other wearables such as glasses. With other advances such as augmented reality, future possibilities and opportunities are even more exciting!

What do you think the future holds for the Internet of Things and its role in personalized medicine?

The connected world offers patients the opportunity to leverage powerful computing for clinical analyses. The use of artificial intelligence and machine learning can only enhance such opportunities through improved precision and prediction. As medicine becomes more decentralized, the burden of care will fall more and more on the patient.

Through miniaturized devices, wearables, and sophisticated biosensors, there can be continuous interrogation of one's physiology. Data can be transmitted to primary care physicians or specialists and algorithms can be devised or used to track disease, and truly personalized medicine can be achieved.

References

» Smartphone-based device could provide rapid, low-cost molecular tumor diagnosis. Massachusetts General Hospital. <http://www.massgeneral.org/about/pressrelease.aspx?id=1801>

At Pittcon 2018, Parry Hashemi outlined her research which led to her being awarded the Pittcon Achievement Award, as described below in her talk titled “Eavesdropping on the Brain”:

We have been looking at depression, which is an important public health problem, and we’re coming at it from a basic chemistry perspective. We’ve developed tools to ask relevant questions and provide very important

Depression is a mental health issue that affects a phenomenal amount of people, and it’s on the rise globally. It’s very difficult to diagnose as there is no chemical test for depression.



information to the field.

Despite this, one in five Americans are prescribed a frontline treatment for depression, and that’s the selective serotonin reuptake inhibitors (SSRI).

The SSRIs have become under the spotlight over the past few years because they have very variable efficacy rates, and they don’t work for about 70% of patients that take them. There’s a lot of dosage problems as well. Patients must also take them for two to three weeks, sometimes longer, before they see a therapeutic efficacy. And they’re because there’s no chemical test for depression, there’s also no chemical test to screen antidepressants. So, there are a lot of issues here.

These issues persist because we don’t have a good understanding of the fundamental chemical underpinnings of depression, and there have been different theories over the years for the disease mechanism. For example, the Monoamine Theory of Depression, which has been around since the ‘70s and to summarize, the idea is that there’s a focus on the molecule serotonin.

In the brain serotonin acts as a neurotransmitter, more specifically a neuromodulator, and it’s responsible for relaying biochemical messages from a presynaptic cell to a postsynaptic cell. The serotonin is released from vesicles into the cell and it relays this biochemical message

through interaction with these postsynaptic receptors. The Monoamine Theory suggests that during depression, the individual's serotonin levels in the extracellular space of the cells are lower than normal and that administering SSRIs, which will block the reuptake of serotonin back into the cell, will raise the serotonin levels back to normal, or even higher. Not surprisingly, a theory that has been around this long has several issues with it.

The first issue is that despite this hypothesis being around for such a long time, there is consensus in the field of the actions of serotonin during depression. There is not sufficient evidence currently that supports that serotonin is lower during depression, and this is because it's extremely difficult to measure brain chemistry. The traditional analytical methods have looked at slower chemistry that's perhaps not relevant to neurotransmission time scales and research is often done with large probes that cause tissue damage. This has created inconsistencies in the data and findings, which is the first issue.

The second issue is the idea of antidepressant variability, and the delayed onset of action. The SSRIs are designed to block the serotonin transporter, thereby increasing the extracellular serotonin levels, therefore if the serotonin hypothesis is correct then they should work, and they should work for a higher percentage of patients that take them. In addition, there is the delayed onset of action, which is unexplained. Patients take the antidepressants for several weeks, and in this time the patients often feel much worse than they did, so if this was some sort of gradual raising of the serotonin levels it's



not consistent with this real-life phenomenon. The third issue is screening antidepressants. As there is no chemical test, it's difficult to screen antidepressants and have an indication of their clinical efficacy. The way that this has traditionally been done is using behavioral tests in animals, such as the forced swim test in mice. The forced swim test involves putting mice into a vessel from which they can't escape. The mice will swim to try to get out, but after a few minutes they give up trying and they go into a learned helplessness state. If you give the mice an acute injection of SSRI into their intraperitoneal cavity they'll swim for longer next time. That's the only robust behavioral shift in animals that are not depressed. When animals become depressed this behavioral shift is no longer as robust. The final thing I want to touch on is the idea of translating the information that, for the majority, has been done in mice. Most of the depression research has been performed in mouse or rat models, and, we're interested in translating that into human models.

Mouse models vs human models

In terms of etiology of depression, in mice and in humans it is very similar. Mice will become depressed if they experience stresses, chronic illnesses, as well as there being a genetic component, these are very similar in humans, but that's where the similarities end. In terms of diagnosis in a mouse there are a set of well-established battery tests, such as the forced swim test. In humans, the diagnosis is, for the most part, questionnaire based, a healthcare practitioner will ask questions and then provide a diagnosis.

In terms of treatment, there's a real disconnect, the acute intraperitoneal injection in naïve animals causes a behavioral shift in mice. If those mice are now depressed, there's much more variation in their behavioral response, and it's extremely difficult to give mice a chronic administration and it's almost impossible to mimic the human administration, which is a daily tablet.

In humans, there is chronic SSRI administration of a low dose daily tablet, which has varying effects between individuals. There has been a focus in recent years on ketamine, administered by an IV one-time dose, that has some variability in its efficacy. There are also other treatments, such as electroconvulsive therapy.

Depression diagnosis

First of all, it is important to develop a robust method to diagnose depression, and to do that we need a testable hypothesis, and we need a way to test the hypothesis. Using analytical chemistry, we want to look closely at the idea of the treatment, and for that we need to look at the timeframe issue and the delay in efficacy, as well as speeding it up. Then we want to start thinking about high throughput of robust in vitro methods for screening antidepressants that is carried out by a chemical test, as well as ensuring that it is all translatable.

Our approach to this is to look at the fundamental chemistry of the brain, the technique that we use is the carbon fiber microelectrode, and we couple it with a few other different techniques. We're interested in looking at serotonin chemistry because we want to focus on the monoamine hypothesis. Over the past few years we've created unique tools to look at serotonin chemistry.

Serotonin levels are very ambient and don't change very rapidly. They're present in the extracellular space, as well as bursts are released from the neurons during activity. This translates into two types of chemistry that we want to study, the slow chemistry and the faster, more evoked phasic responses.



Fast scan cyclic voltammetry and fast scan absorption-controlled voltammetry

Our technologies are very unique and extremely robust, they've given us some very important information. We measure fast chemistry with a technique called fast scan cyclic voltammetry, and the slow chemistry with a technique called fast scan absorption controlled voltammetry.

Fast scan cyclic voltammetry (FSCV) uses a carbon fiber microelectrode, which are hand fabricated and manually inserted directly into the brain tissue of intact, sleeping or freely moving animals. We will then apply a waveform to this, and these waveforms can differ based on the analyte that you're interested in. The waveform that we were working with is very selective for serotonin, as well as reducing electrode firing it also has other advantages.

This is then applied to the electrode, and it will capture the two-electron oxidation reduction of serotonin. We had a very fast scan rate, and with a scan rate like this we can capture this process within a couple of milliseconds, and it will give us the cyclic voltammogram.

As it only takes a couple of milliseconds to capture the cyclic voltammogram, we can present the cyclic voltammogram as current versus time, that's the two milliseconds it took to collect, and then we'll stack these next to each other (figure 1). We wait 100 milliseconds between each scan to allow pre-concentration processes on the electrode surface, and then we give this a false color, so anything in green coming up out of the graph is an oxidation and anything in blue going into the graph is a reduction, we then look at this top-down from a bird's eye view (figure 1).

Color Plots

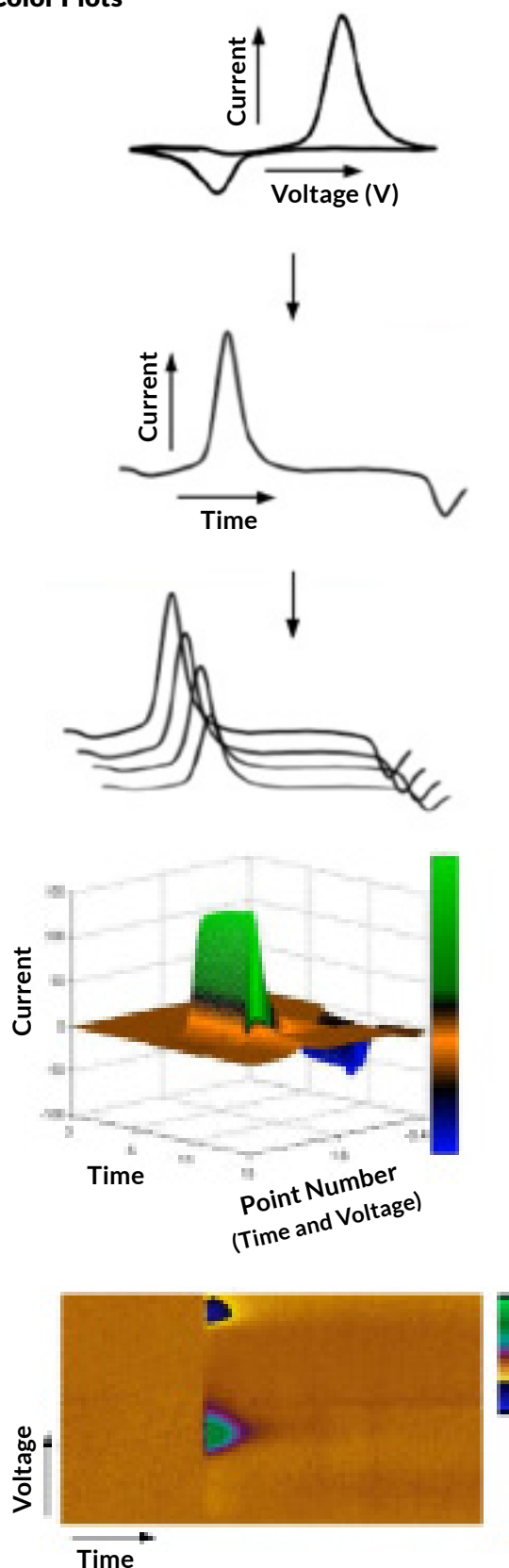


Figure 1: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018.

Figure 1 shows that there's an event occurring, if we didn't know what that event was, we could take a vertical stripe through it, reconstitute the cyclic voltammogram and know what we were looking at selectively.

More physiologically relevant would be a horizontal stripe through the peak oxidation current, that would give us the concentration versus time. FSCV is background subtracted, this means that when you scan so fast at 1,000 volts a second, if we were to take a measurement, all we would measure is a charging current on the electrode's surface. So, we have to background subtract all of the cyclic voltammograms to see the fire rate processes, these CVs under there. And so to get meaningful data we have to evoke a change.

In Vivo Model

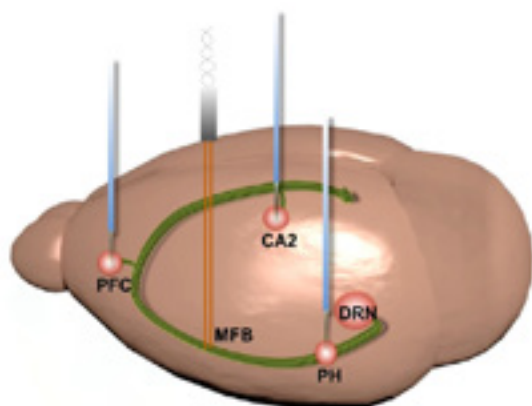


Figure 2: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018.

Figure 2 shows a representation of the in vivo model that we used, and it is of a mouse's brain. We'll stimulate an axial bundle that comes out of the cell body region, as this is an area where serotonin is made. The axons transverse to this area of the NFB and then they terminate in the terminal regions that we are interested in.

The hippocampus and in the hypothalamus

We have been working in the hippocampus and the hypothalamus, which are two very important regions for depression research.

Figure 3 gives an example of in vivo data. This figure is showing an evoked serotonin response in the hippocampus, and the cyclic voltammogram is showing the average responses of the male and female mice. You can see that the serotonin increases during the electrical stimulation, and following the stimulation, the serotonin is cleared.

In Vivo Data

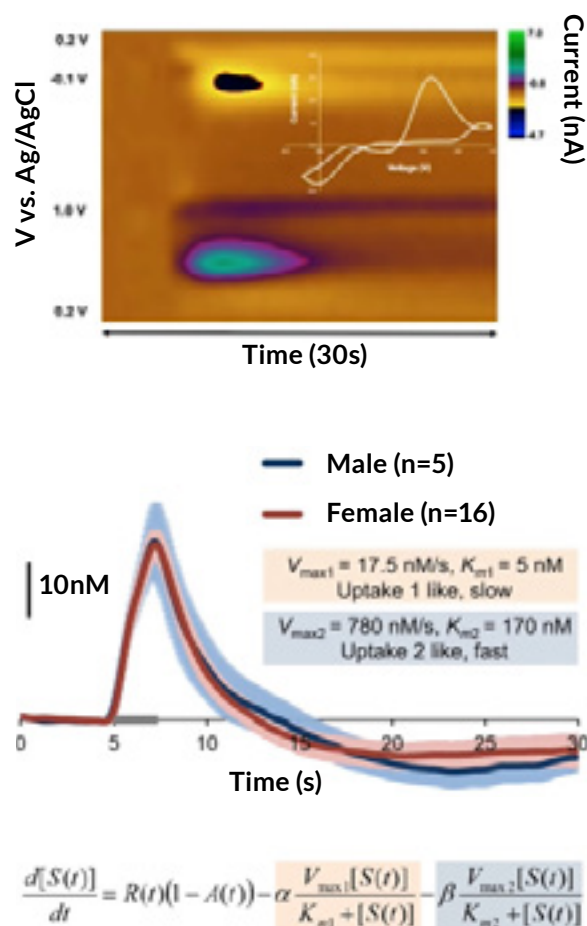


Figure 3: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018.

We tried to model this response based on the primary clearance mechanism, the serotonin transporter, which is the protein that's targeted by the SSRI's. But we were unable to model it just using that one reuptake mechanism. This meant that there had to be two reuptake mechanisms, so we have two mechanisms here in the model that describes the curve (figure 3). Other mechanisms of serotonin reuptake include monoamine transporters like the dopamine transporter, the norepinephrine transporter and the emerging class of transporters called organic cation transporters, and so will also help to clear serotonin. What is important about this is understanding that monoamine transporters are promiscuous, they'll react to each other's analytes.

Fast Scan Adsorption Controlled Cyclic Voltammetry

This technique, FSCAV (Fast Scan Adsorption Controlled Cyclic Voltammetry), measures the ambient levels of serotonin.

FSCV is used to get rid of this large charging current, which means we have to evoke a change we don't know where we started.

We developed this method where you take the same carbon fiber, you don't have to change anything, you just make a small modification to the detection scheme. If you apply the waveform at very high frequency, you can minimize the absorption to the electrodes, we assume that there's not much happening

at the electrode. Then you turn the waveform off for five to ten minutes and allow absorption to reach equilibrium with its surroundings. Then we'll apply the waveform back and now with the specific waveform to the serotonin, so everything that's on the electrode surface will become oxidized and the first few cyclic voltammograms will allow us to quantify what's on the surface, which is ambient levels (figure 4).

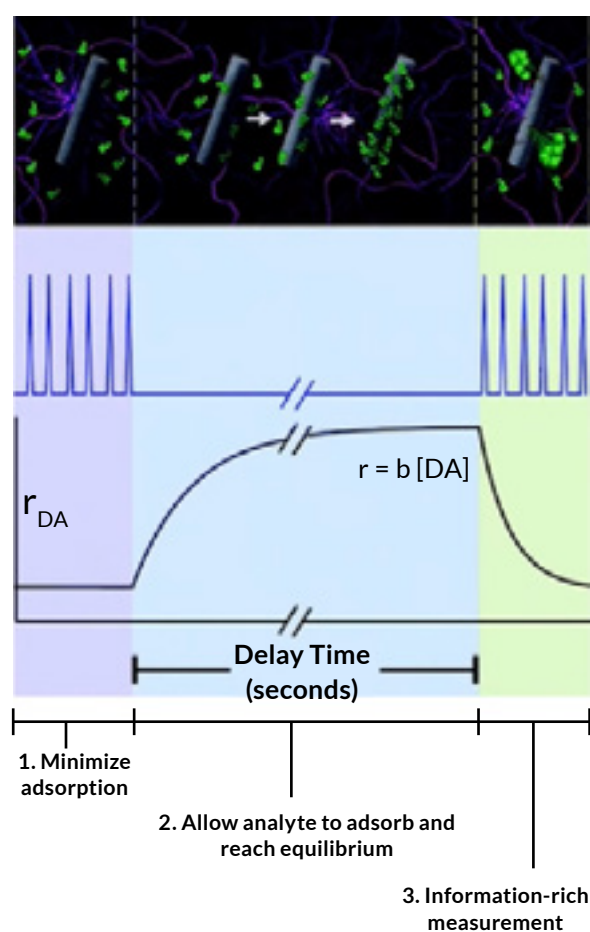


Figure 4: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018.

Figure 5 is showing you how the ambient levels of serotonin have been quantified in the hippocampus and this is a comparison of the in vivo versus in vitro responses.

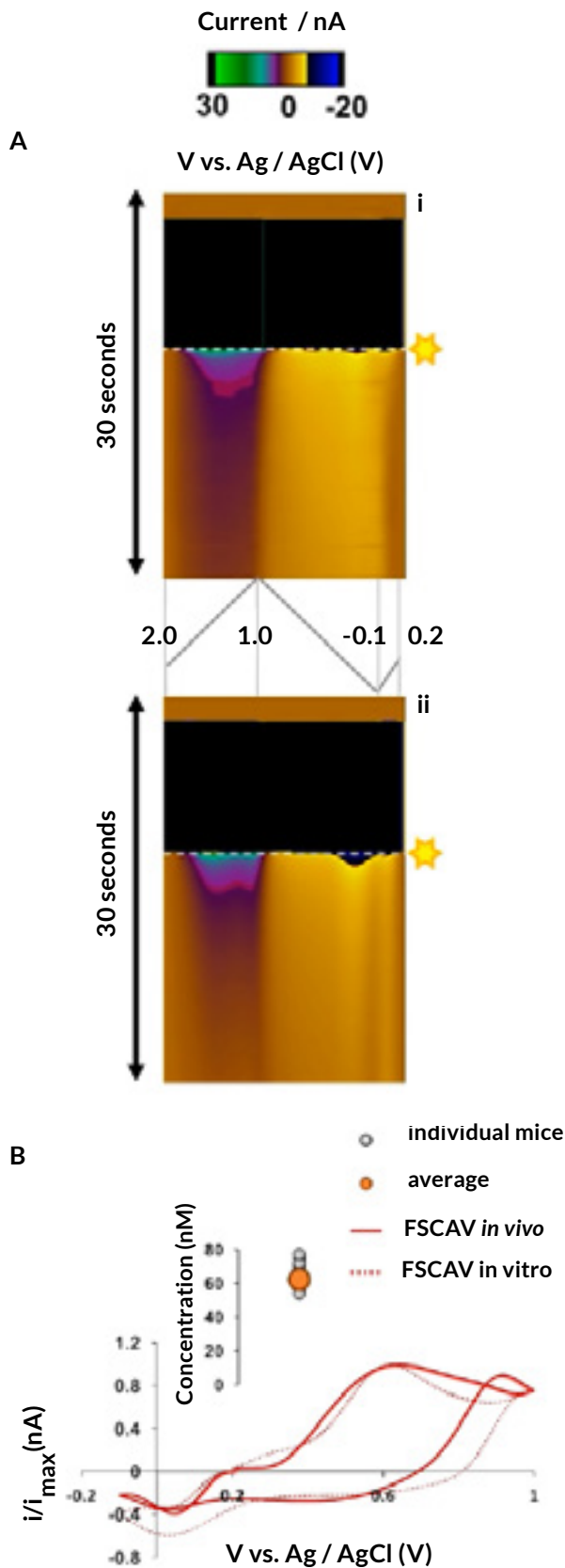


Figure 5: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

Figure 6 shows the results of the culmination of many years work and is working toward answering the question 'Does serotonin actually lower during depression?' The mouse models for this, as we can't test this in humans yet, include applying various stressors to mice in different ways in order to get them to have depression phenotypes.

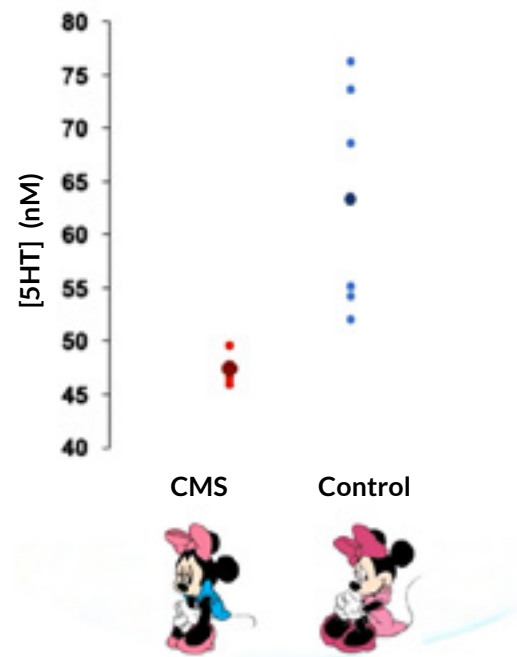


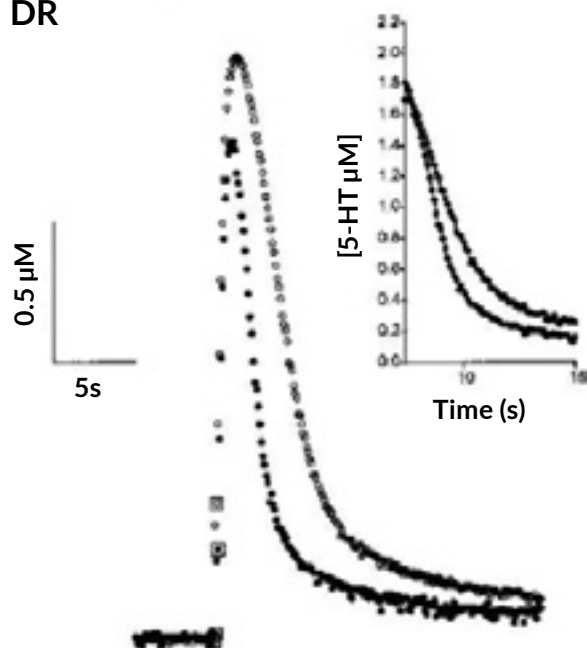
Figure 6 Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018.

One very robust behavioral model is called a chronic unpredictable mild stress. Here, mice are exposed to three unpredictable stresses daily for several weeks to several months. And after some time they start to show depression phenotypes. If we measure their ambient serotonin levels, which can be seen in figure 6, the control mice show a spread of data, which is also illustrated in figure 5, suggesting ambient serotonin levels. And you can see that in the chronic mild stress mice that have depression phenotypes, their serotonin is lower, supporting the monoamine hypothesis.

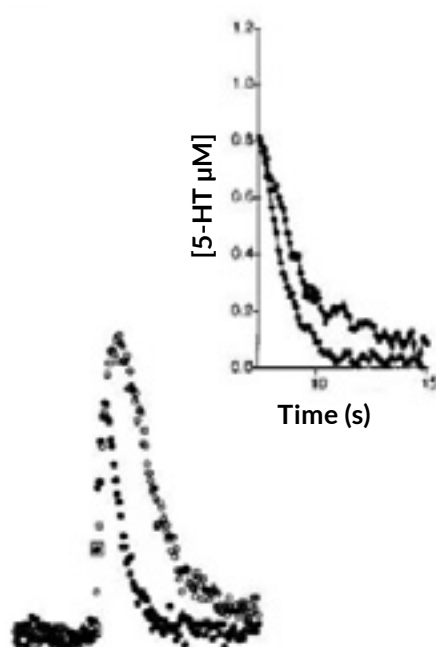
Depression treatment: Time delay and acute SSRI

We want to start thinking about SSRI treatment and understand why there is this time delay for the therapeutic effect to start. I want to highlight data from Whitman's lab from

DR



SNr



the 1990's shown in figure 7. These are results from experiments that would involve removing the brain of a mouse, slicing it up whilst keeping it alive and record the area of interest. Figure 7 shows the recordings taking from two areas where serotonin is present. They stimulate serotonin release, they perfuse an SSRI over that perforation and then they see a slower reuptake inhibition.

We tried to replicate this ourselves in vivo and we got similar results that are very robust and we wanted to understand what was happening (figure 8). What we've created here is a dose response comparison, which is when take an index of the drugs efficacy, which can be behavioural or chemical and then you'll increase that dose and plot that index of the efficacy versus the increase in dose. These are the results we're showing in figure 8.

The situation got much more complex when we gave a higher dose. Referring to figure 8, in our results you can see that at three mg/kg, which is an intermediate dose, you can immediately see there are issues here. The very first response of the brain to this intermediate dose (the five minute response) is an increase in the rate of reuptake, but this is unexpected because the drug should decrease the rate of reuptake. In theory it's binding to the serotonin transporters but all five mice have shown the same response. The response then slows down over 120 minutes, but all of these curves are slower than they were for the one mg/kg scenario. When increasing the dose even further, a steady state that is seen in the other doses is not reached and at the thirty mg/kg dose, after 120 minutes, there's almost no reuptake.

Figure 7: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

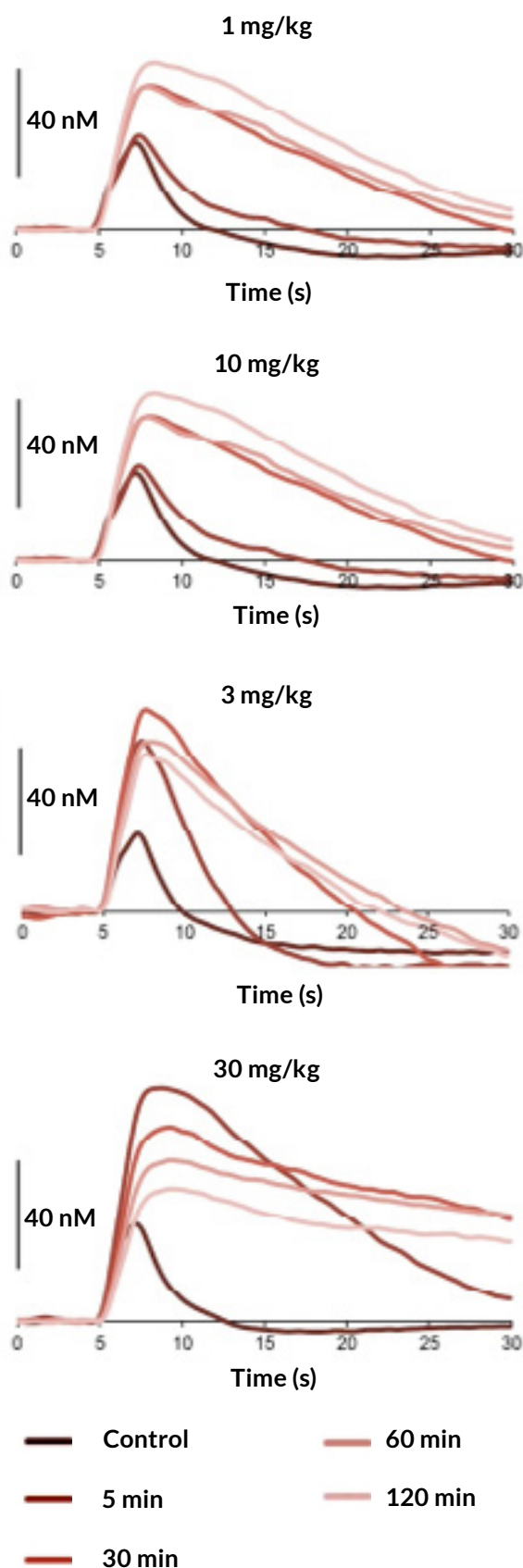


Figure 8: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

These rapid changes in the reuptake rate suggest that there are things happening to the serotonin transporters at the cell surface. So, I made some hypotheses and found a group in Germany. That have a good preparation technique.

Imaging SERTs on stem cells using confocal microscopy

A laboratory in Germany were carrying out stem cell preparations by creating cultures under a confocal microscope. They add an antibody that will target the extra cellular loop of the SERT and that antibody is florescently active under a confocal microscope. So, they look at their stem cell preparations and if there's a decrease in florescent signal, it means there's less of the serotonin transporters present and if there's an increase, it means there's more.

When perfusing their cultures with SSRI, they found that over four hours there was a decrease in florescent signal, which meant that there was less of the serotonin transporters. These results mirror the higher dose signal that we saw in our results in figure8, where there was no reuptake. They hypothesized that it was a desensitization and the internalization of the serotonin transporter over time in response to this acute dose. I then wanted to know, if we did this again but after a minute or two, that perhaps there is an over expression of these serotonin transporters and that's exactly what happened.

You have to stimulate the cells, but what you can see in figure 10 is that in the presence of an SSRI at stimulation, two minutes after SSRI administration there's an over expression of the serotonin transporters. We collected data in

several stem cell preparations and indeed you can see that when there's stimulation, the serotonin has to be released and when there's SSRI onboard there are more serotonin transporters.

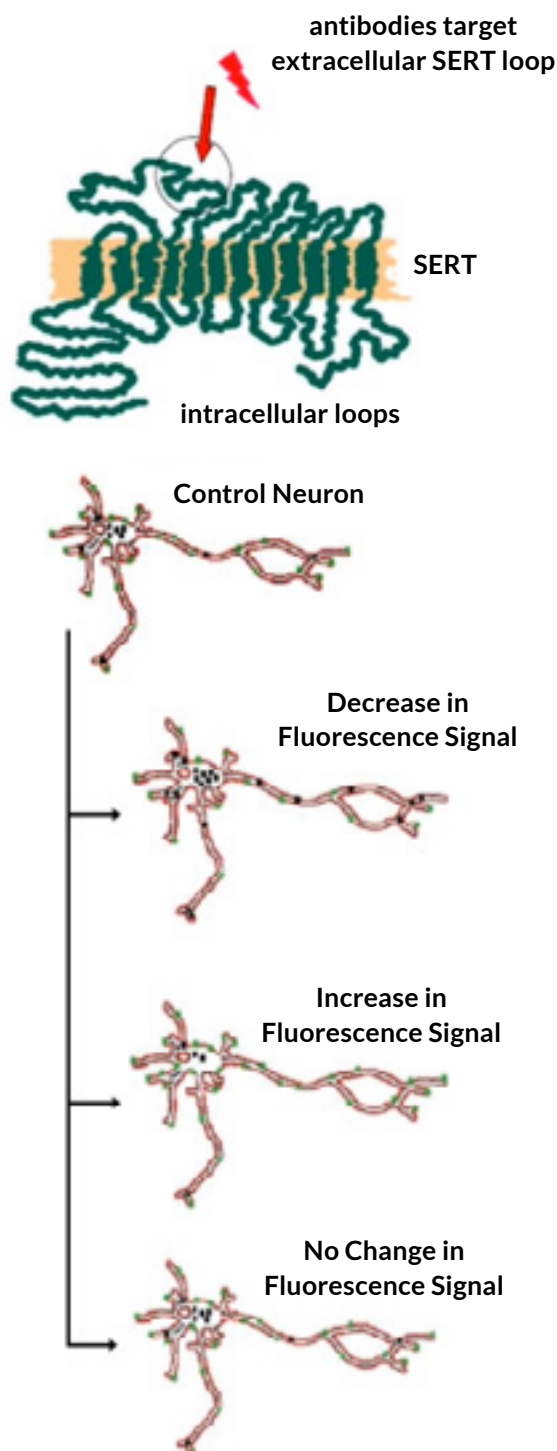


Figure 9: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

0 mM KCl (2 min) 60 mM KCl (2 min)

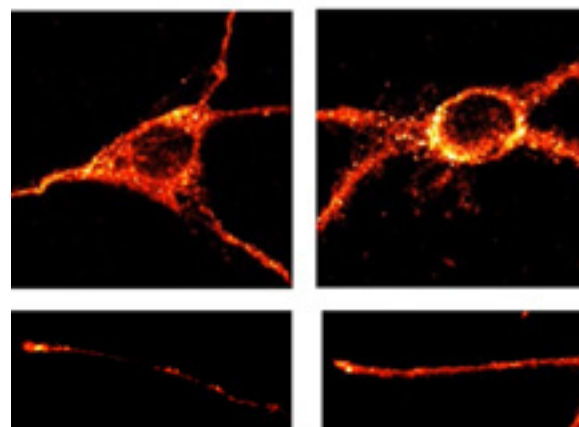


Figure 10: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

This is really significant, because I believe that this is what's happening during that low dose chronic regime in humans. I think the brain's first reaction to an increase in extra cellular serotonin levels is to express more serotonin transporters on the surface. And it's in low dose chronic regime that the brain has more opportunity to deal with that, and after three weeks they become desensitized. But in an acute scenario, the scenario that works in naïve animals that receive an acute injection, this happens quickly.

Ketamine treatment

The ketamine treatment is one acute dose in clinical conditions, so, could it be that that's what's needed for SSRI therapy? Figure 11 shows an in vivo experiment which supports this hypothesis. In red are recordings of basal levels in several mice, which were taken for an hour, then at zero we give an SSRI and you see that there's approximately a 10 minute region where there's no change in the extra cellular serotonin levels. But, after five minutes there is an increase in serotonin reuptake.

This is the brain trying to regulate its serotonin levels, but after 10-15 minutes it gives up the fight because this is acute and large and the serotonin levels start to increase because the SERT becomes desensitized.

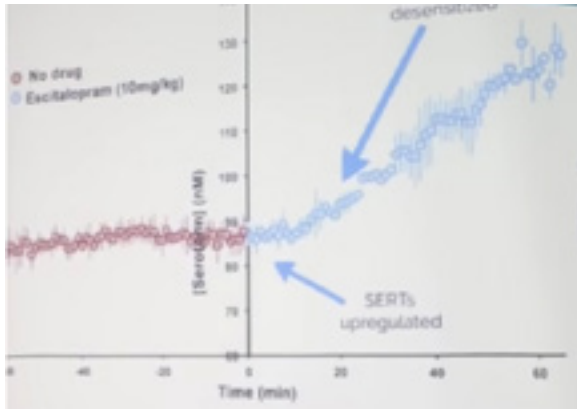


Figure 11: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

This suggests an explanation to the time frame delay of treatment, but it doesn't explain the disconnect between the variability in the SSRI treatment.

Linking this to chemistry and what we know about peripheral inflammation, it's much easier to define peripheral inflammation. Histamine is the signaling molecule of peripheral inflammation, and when it's released it sets into motion several events that can deal with that inflammation. What is not known is the role of histamine during your inflammation because it's not very easy to measure histamine.

What the research team developed is a method using a FSTV to simultaneously measure serotonin and histamine.

Priming of glial cells

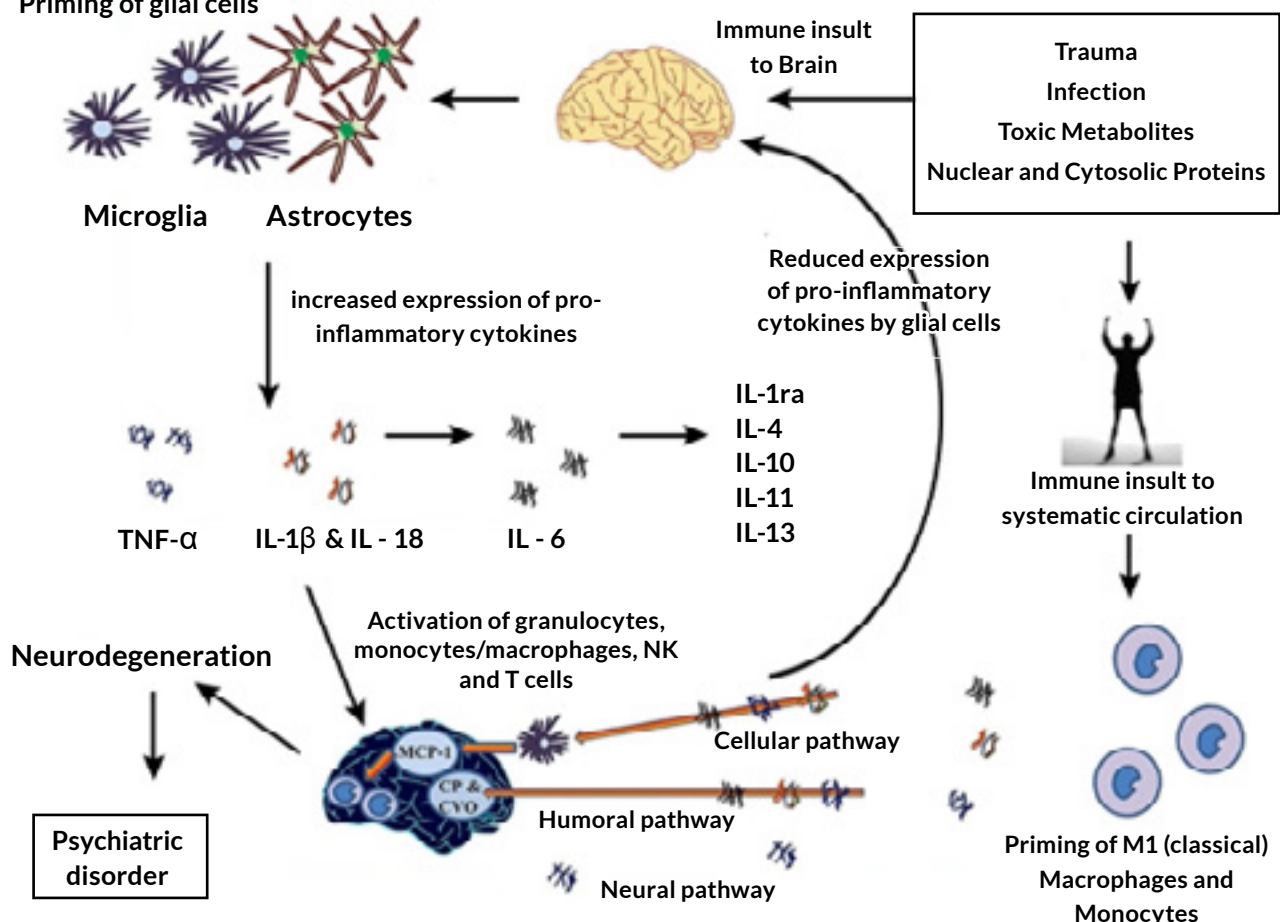


Figure 12: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

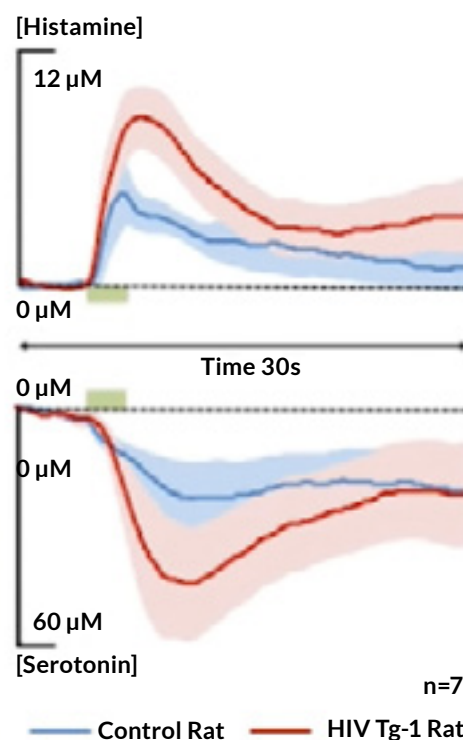
Looking at the hypothalamic region, where both neurotransmitters are present, they stimulated histamine but not serotonin release and found that histamine levels increase, then gets cleared and then in response to this, there's a decrease in the serotonin levels. We then tried to model this and also carried out lots of pharmacology. We found that this process was mediated by inhibitory H3 hetero-receptors on serotonin terminals. So, the histamine is released and acts to inhibit these serotonin receptors.

If histamine is increased during neuroinflammation and histamine is inhibiting serotonin, this could have real implications. So, we had two models of inflammation available to us and some HIV transgenic rats. We took seven of these aged rats that have a low grade chronic inflammation and we measured their histamine and serotonin profile, which can be seen in figure 13. The blue is the control and the red is the inflammation state, you can clearly see there's more histamine in the inflammation state compared to the control and as a consequence there's more serotonin. This acute neuroinflammation.

Still with reference to figure 13, we then peripherally injected LPS, a lipopolysaccharide found in the outer membrane of bacteria. When you inject it peripherally there's a violent acute immune reaction. It does not go through the blood brain barrier and we injected peripherally. Five minutes after peripheral injection, we see an effect in the brain. So, the histamine levels in the brain are increased, and as a consequence, there's more inhibition of serotonin.

Acute vs. Chronic Inflammation

Chronic Neuroinflammation



Acute Neuroinflammation

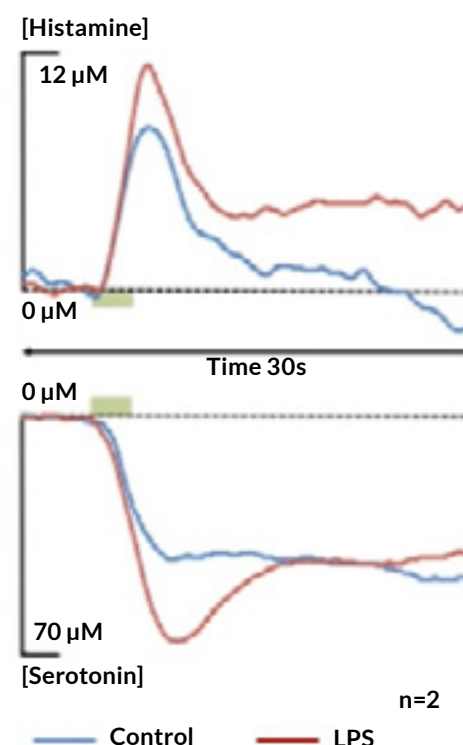


Figure 13: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

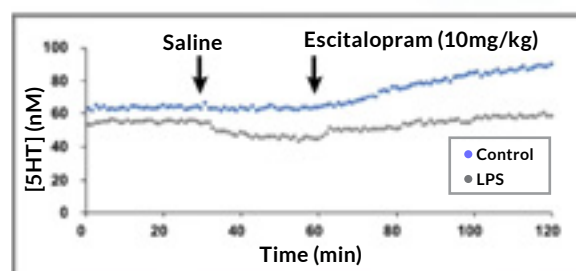
We think that there's more histamine during inflammation and that it's driving down the serotonin, but that doesn't explain the variability. but I think I'm gonna start to get to that in a second. Figure 14 is data collected by our research team that shows the basal levels of several mice. These mice data represented in blue are not experiencing inflammation and these mice are given saline and nothing much happens. Then the mice are given an SSRI, and as we saw before there's an increase in the serotonin levels. In a separate group of mice, they are given a set of saline LPS and the basal levels and serotonin drop. Then, when you give these mice the SSRI, they don't go up as much as in a non-inflammation state.

What the data is showing us is that in inflammation states, SSRI are not as effective in raising the serotonin levels. Now looking at chronic levels of inflammation, which are mice experiences chronic mild stresses and have less serotonin to start off with. If we give them saline, nothing much happens, we give them escitalopram (an SSRI), the serotonin levels go up, but not as much as the naïve mice. If you merge this data you can see the two inflammation states have the same low level of serotonin and that giving SSRIs doesn't allow the serotonin levels to come back up to normal.

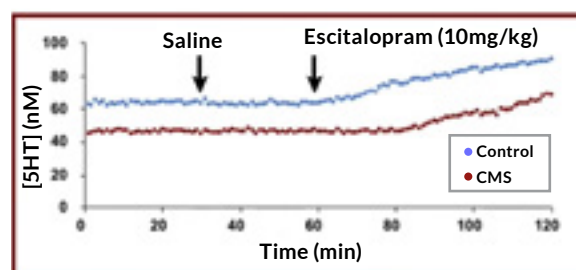
Histamine does not have an identified transporter for reuptake of histamine back into the cell. It must exist, because histamine degradation is exclusively intracellular by enzymes. Thinking about the promiscuous nature of monoamine transporters, something is reuptaking histamine. So, we inhibited everything we could think of, such as the serotonin transporter. Figure 15 shows histamine traces before and after

Acute vs. Chronic Inflammation

Acute



Chronic



Merged

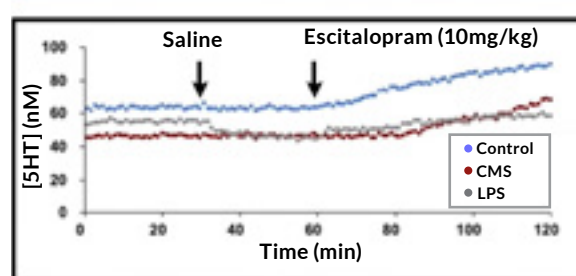


Figure 14: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

administration of the drug and what can be seen is an inhibition in the histamine reuptake. We then continued experiments with other different inhibitors and we saw the same effect but to varying degrees. The dopamine transporter inhibitor didn't show much affect, and the norepinephrine transporter we saw some effect (figure 15). We also tried the organic cation transporter (OCT) inhibitor and we saw an effect.

Histamine Reuptake

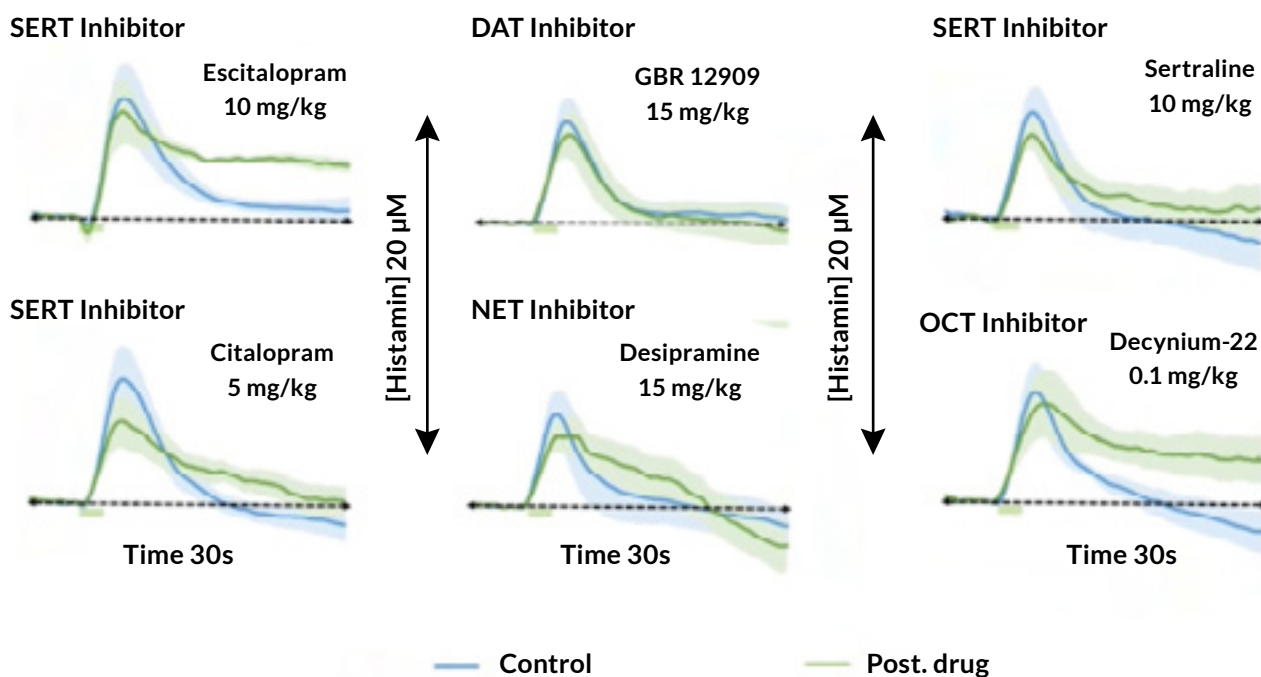


Figure 15: Slide from Parastoo Hashemi presentation 'Eavesdropping on the Brain' at Pittcon 2018,

We have yet to provide evidence but what I think is that for these four compounds that inhibit histamine reuptake all have antidepressant activity. This is phenomenally important because if we are saying that serotonin levels are lower during depression

and the front line treatment to increasing serotonin levels is also increasing histamine levels which is inhibiting the serotonin levels, you can start to see how this speaks to the variability and the response.

Conclusion

We think that we can diagnose depression by measuring serotonin levels and that we have some ideas about how to improve current treatments of SSRIs. The time frame idea is the over expression and under expression, which can probably be sped up with acute doses. The variability of responses to SSRIs is explained when taking into account the effects of histamine on serotonin reuptake. Some challenges we face are the translatability of these findings into clinical practice, as all the results so far have been obtained from mice.





PROF. HASHEMI GOES INTO MORE DETAIL ABOUT HER WORK IN THE BELOW INTERVIEW:

In your talk at Pittcon 2018, you mentioned that depression is difficult to diagnose. Please can you outline the current issues with the serotonin hypothesis for diagnosing depression?

It's very difficult to diagnose depression. To explain this, I like to use the analogy of what you do if you suspect that you have a condition such as diabetes - you visit your doctor who takes blood tests, from which they measure your glucose and insulin levels to deliver a definitive diagnosis. With depression, you can't open the brain to find answers, and the chemistry of the brain is separate from the chemistry of the body.

Over the past few decades, there have been theories forwarded about brain activity during depression, and one of the major hypotheses is that serotonin is lower in the brains of people with depression. This is known as monoamine hypothesis of depression.

This fuelled the development of modern anti-depressants, and as a result, front line treatment for depression mostly consists of selective serotonin reuptake inhibitors, SSRIs, intended to increase serotonin levels.

However, without an effective method for measuring serotonin, it's difficult to test that hypothesis.

Please give an overview of the research that you have been working on which led to you winning the Pittcon Achievement Award.

We had many questions regarding serotonin, and it became clear that to answer them we'd need new technologies. We worked on creating microelectrodes made from carbon that we could implant into brain tissue. Carbon is a really good material for making biological measurements because our bodies are full of it, meaning the body doesn't have a negative immune reaction to an implanted carbon electrode, as it would to say gold or platinum, materials that aren't present in our bodies.

We made them really small, measuring at about 1/100th or 1/1000th the diameter of a human hair. We implanted them directly into brain tissue and spent many years trying to develop different detection schemes, modifications, electrochemical parameters and physiological parameters that would allow us to measure serotonin.

“Having acquired the serotonin measurements, we spent the past few years trying to figure out how to apply those to depression research, from different animal models, biochemical assays, and collaborative work with mathematicians.”

Prof. Parastoo Hashemi

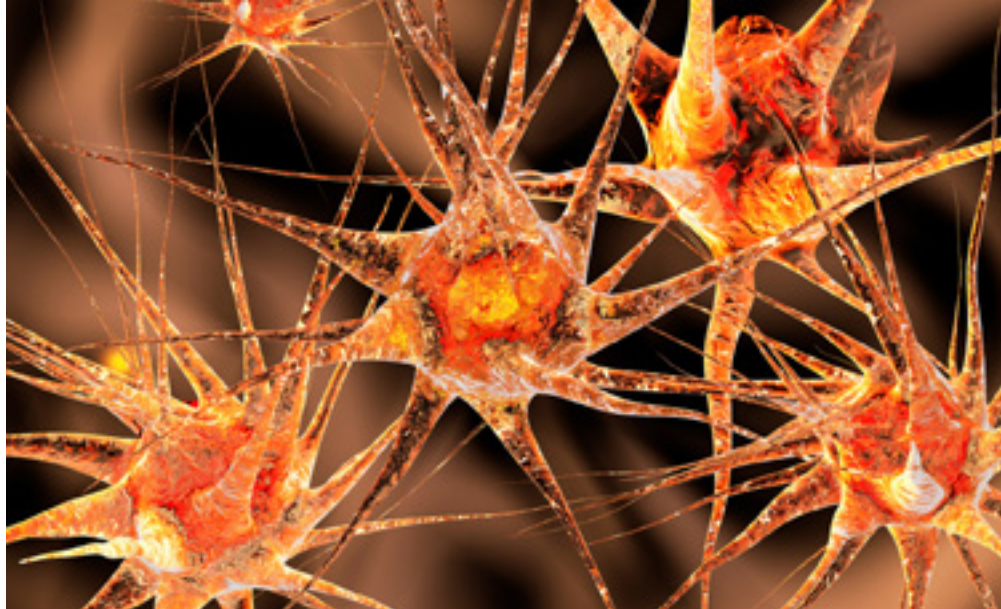
What does it mean to you to win the Pittsburgh Conference Achievement Award?

I've been coming to this conference and attending the Pittcon Achievement Symposium for about 12 years, so it's really fantastic to be recognised amongst the other winners.

It's a huge honor, on both a personal and professional level, and really a milestone in my career. Research is such an unforgiving career choice sometimes. You can work hard for a long time and yet see very little result.

We've worked hard, and are finally making an impact in the field, which is wonderful to be recognized for. It also means so much to my mentors, and of course, my students, who are honored to have their work recognized.





In your talk, you also discussed how in inflamed synapses, we see increased histamine levels and a decrease of serotonin levels. How can these findings be used to validate the serotonin hypothesis?

It's not the synapse that's inflamed, rather inflammation in general. To put it in perspective, when you're sick or some kind of bacteria enters your body, your body will have an immune reaction to it, and that's called inflammation. The same thing happens in your brain and is called neuroinflammation. While a lot of knowledge exists on certain markers for neuroinflammation, how neuroinflammation affects neurotransmission remains a grey area.

We found that during neuroinflammation, which is very prevalent in depression and other psychiatric diseases, serotonin levels are lower. We link this to greater levels of histamine, which inversely modulates serotonin.

For the first time we could verify the serotonin hypothesis of depression and conclude that the serotonin levels were lower. We know why they're lower, and are now examining what impact treatment really has, not just with serotonin but histamine too.

What neuroanalysis technology did you use to study the brain under healthy and disease conditions?

These were the tiny carbon fibers, and because it's new research technology, we actually made them by hand, and it's a real art. When people join my lab for a full year, they learn how to make these electrodes.

You take a tiny glass capillary and aspirate a tiny fiber, which are barely visible with the eye. You pull it apart under heat, so the glass forms a seal, then the hardest part is cutting that to a specific length under a microscope with a scalpel.

Once you become accomplished at that, then there's a whole bunch of instrumentation to learn how to make and troubleshoot, since they can't be bought. Next you have to learn about anaesthesia and brain surgery.



How translatable are your findings to the physiological response to depression seen in humans?

“Most depression research occurs in mice and rats, and the big question in the field is how translatable is that to humans.”

Prof. Parastoo Hashemi

There are very well-established behaviour paradigms in animals that will lead to depression phenotypes. But of course, it's a big jump to try to compare that with a human condition that's extremely complex.

To explore this, we've started working with stem cells taken from human skin, that we apply various different conditions and growth factors to, which turns them into any type of bodily cell. We work with people that can turn these into serotonin neurons, which is really cool.

The idea is that if these serotonin neurons are functioning the way that our brain serotonin neurons are, which we have some good evidence for, then we can make our measurements in a Petri dish from these cells.

From these we will know by proxy what's happening in your brain, so these are our efforts towards making what we've seen in the mice translatable to humans.

How would you like to see your findings on histamine inhibition influence antidepressant drug development in the future?

So right now, in terms of antidepressant therapies and drug discovery, there's been a loss of interest in SSRI research in developing new drugs. The major reason is because it's very difficult to screen these drugs. There's no chemical test for depression. There's no chemical test for antidepressant efficacy. Drug developers have traditionally relied on behavioural tests in mice, and it turns out they're not very reliable.

In recent years, we've seen quite a decline in drug development. Antidepressants currently work for about 30% of patients who take them, with the other 70% experiencing extremely variable efficacy rates. With this novel focus on histamine, perhaps we can reinvigorate the drug discovery efforts, and get that efficacy rate up.

What have you learned from Pittcon 2018? And what have you most enjoyed?

Pittcon is the forum for the most cutting edge analytical research, and what I love is the focus on real world, real life problems. You can attend sessions on environmental chemistry, food chemistry, and biological chemistry and more, so for students and for myself, it's always nice to see a real application behind this cutting-edge technology. This year's been a great example of that; there've been so many interesting talks about the development of chemical systems and applying it to a problem that would affect ordinary people in their every day lives.

Forensic science now makes regular TV appearances in a range of crime dramas and in factual reporting of ongoing court cases. The importance of forensic analysis in solving crimes is thus widely accepted.

The most publicized forensic technique is DNA fingerprinting, which can be used to eliminate or further incriminate potential suspects from a police enquiry. The truth is that this represents just the tip of the iceberg.

Forensic laboratories have a wealth of analytical methodologies at their disposal and, with ongoing technological advances, the capabilities of forensic analysis continues to increase. However, with so many next-generation technologies being developed, people would be forgiven for believing that some of the techniques are solely the premise of fictional dramas rather than being scientific realities.

Forensic evidence is now a fundamental aspect of the criminal justice system, being used to establish the guilt or innocence of potential suspects, determine the origins of drugs and other chemicals and detect links between separate crimes.



As criminal activity can arise in many forms and take place in a host of different environments, the nature of the evidence available can vary widely. It may be impressions in materials at the crime scene, such as footprints and markings on bullet casings or perhaps traces of body fluids, drugs or explosives.

In addition, forensic science has become an important tool in the fight against terrorism, through the detection of traces of explosives, for example in improvised devices intended to cause mass devastation, or the identification of devices and their likely origin in the aftermath of an act of terrorism.

Since potential evidence can arise in numerous guises, it follows that a wide range of different techniques are needed to analyze them and uncover the information they contain that could assist in solving the crime and bringing the perpetrator to justice. in forensic analyses.



The techniques used forensic science actually draw on knowledge from all the sciences, including physics, chemistry, biology, computer science and engineering. The resulting array of analytical methodologies include various forms of imaging and optical sensing.

Continuing technological advances are achieving ever-greater analytical capabilities, in terms of sensitivity, specificity, accuracy, efficiency, throughput, or data quality and it is important that the latest technologies are made available to forensic laboratories in a timely manner.

This is a key objective of the National Institute of Justice (NIJ) that is dedicated to advancing scientific research in order to create or enhance tools and techniques to identify, collect, analyze, interpret and preserve forensic evidence.

In 2017, NIJ funded a range of projects aimed at developing forensic science capabilities. These included studies of novel spectroscopic techniques, improved analysis of fingerprints, identification of body fluids, and addition of entries in the national powder database to facilitate the identification of gunpowders found at crime scenes.

Several novel analytical technologies, such as laser-induced breakdown spectroscopy, deep ultraviolet spectroscopy, hyperspectral imaging, and ultraviolet Raman spectroscopy, have already found applications in forensic science.

Analysis of trace evidence plays an essential role in the identification and prosecution of the perpetrators of crime. Information obtained from materials and body fluids collected from a crime scene can provide a picture of events surrounding a crime and link potential suspects to the area where the crime was committed. Improvements in analytical technologies to enhance their selectivity or sensitivity can allow more reliable or specific information to be obtained from evidence, whereby increasing the efficiency with which guilty parties can be identified and convicted.

For example, MinION sketching has provided a more rapid and cost-effective means of matching DNA samples using comparison of single nucleotide polymorphisms. Furthermore, using Raman Spectroscopy it is now possible to identify the nature of a fluid stain, be it saliva, semen or blood, with a single test since each type of body fluid has its own characteristic Raman spectral signature.

Analysis of microbiomes, the bacterial colonies present on human skin, also holds promise for predicting the physical traits and lifestyle of an individual and associating them with evidence and environments.

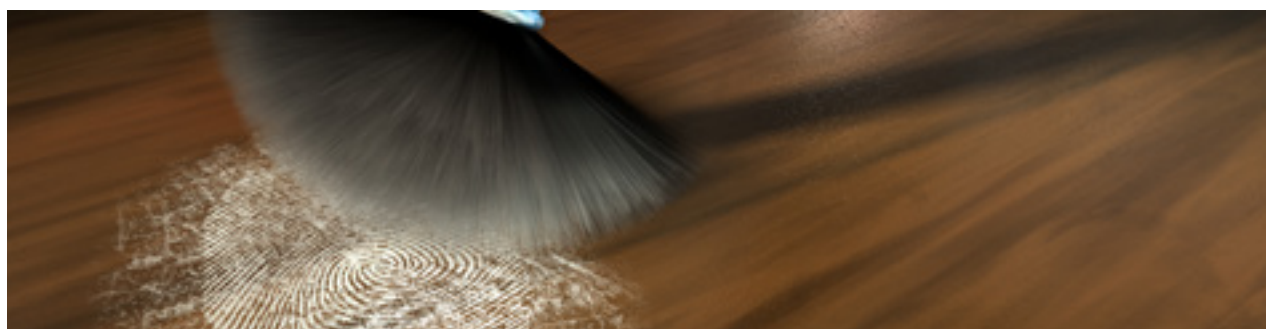
In gun crimes, the trace evidence may be in the form of gunshot residue or spent bullet casings. Developments in vibrational spectroscopy have enabled forensic scientists to discern the calibre and type of weapon from traces of gunpowder. In addition, 3D scanning technologies have facilitated the analysis of the specific marking left on a bullet by the issuing weapon.

Such toolmark analysis can identify from which weapon the bullet was fired. Now that digital

images of the toolmarks can be created, it is easier to catalogue them, share them and compare them between crimes.

Deep ultraviolet resonance Raman spectroscopy and hyperspectral imaging technologies, both of which are possible in portable handheld devices, are showing much promise for improving the stand-off detection of traces of explosives.

Progress has even been made in detecting explosive in improvised devices, which is more challenging since the presence of multiple additional components that can interfere with imaging. Portable laser-induced breakdown spectroscopy (LIBS) and quantum cascade laser (QCL) instrumentation are also proving beneficial for a variety of forensic analyses.



2.4a

THE HUMAN MICROBIOME – A NEW POTENTIAL FINGER PRINT IN FORENSIC EVIDENCE?

Each individual is home to around 100 trillion microbial cells, on the skin, and in the eyes, mouth and gut, which comprise their microbiome. A microbiome can include more than 10,000 different microbial species, the precise combination of which is determined by the environments visited and the foods eaten. Consequently, the composition

of microbial organisms associated with skin is unique to an individual because each individual has a unique range of experiences.

NIJ have been funding microbiome research for many years, but more recently research has included the investigation of forensic applications of microbiomes.

These are categorized into three main areas: the necrobiome — the community of organisms found on or around decomposing remains — which provides an indication of the time-since-death; soil microbiome, which can link a victim, suspect, or evidence to a particular outdoor environment; and the trace human microbiome — microbes on our skin and the surfaces and objects we interact with — which provides the potential for associating people with evidence and environments.

A unique study assessing whether a person's traits and lifestyle can be predicted from their skin microbiome was described at Pittcon 2018 by Jack Gilbert of the University of Chicago in a presentation entitled 'The Burglary Microbiome Project: Detecting Personal Microbiome Signatures at Artificial Crime Scenes'. Here, Prof. describes his work in the below interview:



What is a microbiome and why are microbiomes unique to an individual?

“A microbiome is the sum total of the microbes of bacteria, fungi, and viruses that live inside your body. They are all over your skin and in your gut. Bacteria alone accounts for 2-3 lbs of your body mass.”

Prof. Jack Gilbert

What's interesting is that when you're born, you're mostly sterile. You acquire microbes from your mother and the environment. Then, the way you live your life and the things you interact with shape the kind of microbes that colonize and live inside you. This results in your own unique profile, and even identical twins have different profiles.

Please outline the Burglary Microbiome Project that you discussed at Pittcon 2018.

We've been trying to understand if our bacterial signature can act like a fingerprint, and be used as some kind of forensic tool, like trace evidence.

We emit around 36 million bacterial cells into our immediate environment every single hour – so if a burglar in a room for 10 minutes touches objects and leaves behind some their bacteria, can we detect their personal bacterial signature by obtaining some of the bacteria present in the room?



It could then be possible to sequence the information, as we do with human DNA at a crime scene, and therefore identify that individual. It's an interesting idea, and at the moment it's a very early research project that we're working on with the National Institute of Justice.

How do you determine whether a microbial organism is associated with a particular trait?

We've been trying to see if the bacterial signature that lives inside us is somehow correlated with our lifestyle choices.

The signature that belongs to you is very much determined by how you've lived your life; whether you had pets growing up, whether you grew up in a city or a farm etc, are all factored in. Different working environments can determine what bacteria live inside you, how they grow and how they're distributed, so we wanted to

see if that correlates. We built up a database of over 12,000 people and we've been correlating how similar our mock burglars microbiome signatures are to people in our database. It's complicated, but we're getting really close to identifying somebody's lifestyle traits based on the microbial signature they leave behind at a crime scene.

What analytical techniques are you using to identify microbial biomarkers?

We leverage genome sequencing, especially the Illumina platforms - DNA extraction, and PCR amplification, much the same way as we would with human DNA sequences. We identify a particular gene present in all bacteria which we then sequence and use to determine which bacterial organisms are present in different subjects and what makes an individual unique.

We then produce a probability estimate that your bacteria would be found in a certain environment.

“Using advanced statistical techniques, we try and identify whether your bacteria's sequencing data can place you at that crime scene.”

Prof. Jack Gilbert



Please outline any specific biomarkers that you have already identified in this project.

The way in which we analyze the microbiota is by looking for unique strains of organisms. We all have one particular species of *E. coli* living inside our gut, and we all have *Staphylococcus epidermidis* living in our skin, but each individual has a strain with a genotype and genome unique to them. It evolves with you and mutates over time, and we call those mutations biomarkers.

We're using that information to try and identify whether we can pull out particular genotypic mutations, or biomarkers, which are unique to an individual.

If an individual's lifestyle can change throughout their lifetime, does this mean that their microbiome can too? If so, are microbiome signatures always an accurate identification tool?

We are trying to answer a very particular question; whether the microbiome profile of an individual is stable and stays with you throughout your life.

We hypothesize based on all the data we've collected that we have a unique strain based composition which is a fingerprint of our microbiota, but we haven't proved this, and there are lots of factors that could alter this theory.

Part of this project is to try and identify whether our microbial fingerprint stable, and how stable it is. Once you leave a trace of your bacteria signature on a doorknob or television set, does it decay? And if so, how rapidly?



What do you think the future holds for using an individual's unique microbiome in this way? Or for other applications?

The future is really exciting. We can potentially use the microbiome in forensic science, but that would be in the distant future. The human DNA program for forensic evidence took decades, and we're only just starting our journey.

What's most exciting is we can also use the microbiome information inside our bodies in personalized medicine, using the microbiome to predict whether an individual will respond to a particular therapy.

We could screen a cancer patient's microbiome and determine if they have the biomarkers that can indicate their response to immunotherapy, which can help health care professionals identify their ideal strategy for therapy.

“We can use this to tailor treatment to the individual rather than the average treatment of a population.”

Prof. Jack Gilbert

How can Pittcon 2018 help you with your research?

What's exciting about Pittcon is that it brings together a whole group of technological experts in areas that I wouldn't normally have access to.

Being able to access the breadth of experience and technologies that are available to me here gives me more opportunities to expand my research objectives and to do something novel and exciting, that perhaps I wouldn't normally have the chance to be involved in.



2.4b

METHODOLOGIES USED IN ANALYSIS OF TRACE EVIDENCE

Since trace evidence can comprise virtually any substance, a broad scope of very different methodologies is used by forensic scientists to solve crimes. These are based on knowledge from all branches of sciences, including biology, chemistry, physics and mathematics, and enabled by a range of sophisticated technologies.

It is this fusion of science and technology that provides forensic scientists with the power to unravel crime scenes.

There have been tremendous advances in technology in recent years. Forensic scientists now have capabilities that would have been considered pure fantasy only a matter of years ago.

Analysis of paint residue can determine the age and make of a car, gunshot particles can be distinguished from dust, dirt and other fibers, the presence and exact composition of drugs can be established, soil samples can be matched to a specific locality by determining the precise mineral and organic components – and the list continues to grow.

The technologies used to achieve these remarkable connections include spectroscopy, spectrophotometry, gas chromatography,

microcrystalline testing, and X-ray diffraction. Detection of gunshot residue can play an important role in determining where a crime took place and who pulled the trigger, for example, by establishing the presence of gunshot residue on the clothes of a suspect.

Traditionally, gunshot residue detection has relied on the presence of heavy metals, but with heavy metals becoming less common in ammunition alternative analyses were needed. Vibrational spectroscopy, such as Raman microscopy and Fourier transform infrared spectroscopy, has proved to be a particularly useful analytical tool in the detection and



characterization of gunshot residue. It requires minimal sample preparation, is nondestructive and detects both organic and inorganic constituents. Vibrational spectroscopy automatically scans large areas of the collection tape and produces a spectroscopic fingerprint detailing the various components of the gunshot residue.

A complex chemical reaction occurs when a firearm is discharged and the precise nature of this is determined by the exact chemical composition of the ammunition and the specific firearm used. The presence of gunshot residue discharged into the immediate surrounding during firing can be accurately determined using vibrational spectroscopy.

Furthermore, analysis of the gunshot residue using provides characteristic spectra that can provide information about the type of ammunition and weapon used.

In his presentation at Pittcon 2108, 'Vibrational Spectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue' Igor Lednev of the University at Albany, described how gunshot residues can have been successfully classified according to caliber based on the spectra obtained from vibrational spectroscopy.

Raman spectroscopy is often favored over infrared spectroscopy for chemical identification since it causes fewer peaks and is not swamped with water absorption lines so spectra are easier to interpret. It has the potential to detect a wealth of information from both solid and liquid samples, but the quality of information obtained



Raman spectroscopy is based on the scattering of high-intensity light. It is therefore important to collect as many photons as possible while rejecting scattered laser light efficiently.

is determined by the instrumentation used. Ocean Optics were at Pittcon 2018 to discuss the capabilities of their high-sensitivity spectrometers, including the Maya2000 Pro.

Forensic analysis is often required to ascertain whether a bullet or cartridge case found at the crime scene was fired from a particular weapon, e.g., one found in the possession of a suspect. This is achieved using toolmark analysis, which studies the specific marks made on the bullet or cartridge case by the weapon from which it was fired. The marks on a spent



bullet are unique to the weapon and so microscopic examination of these marks allows firearm examiners to assess the likelihood of it having been expelled from a given firearm.

Traditionally, a two-dimensional representation of the bullet or cartridge surface, known as The Integrated Ballistics Identification System, has been used by forensic laboratories for toolmark analysis.

However, a range of three-dimensional (3D) scanning technologies is now available to facilitate toolmark analysis, such as TopMatch produced by Cadre Forensics. These provide

a high-resolution 3D digital image of the surface topography, which directly correlates to the actual physical surface.

Examination of the digital image thus allows easy sharing of the evidence for simultaneous remote evaluation. Furthermore, the images can be automatically compared with a database and catalogued by case for future reference. A specialized viewing software that allows annotation of the areas of similarity identified during toolmark analysis was presented at Pittcon 2018 by Cadre Forensics in a session entitled '3D Surface Topography Analysis and Virtual Microscopy for Firearm Forensics'.

References

- » Bueno J, et al. Raman Spectroscopic Analysis of Gunshot Residue Offering Great Potential for Caliber Differentiation. *Anal Chem* 2012;84(10):4334–4339.
- » Bueno J and Lednev IK. Raman Microspectroscopic Chemical Mapping and Chemometric Classification for the Identification of Gunshot Residue on Adhesive Tape. *Anal Bioanal Chem* 2014;406:4595–4599.
- » Bueno J and Lednev IK. Attenuated Total Reflectance-FT-IR Imaging for Rapid and Automated Detection of Gunshot Residue. *Anal Chem* 2014;86(7):3389–3396.
- » Duez P, et al. Development and Validation of a Virtual Examination Tool for Firearm Forensics. *J Forensic Sci.* 2017; doi: 10.1111/1556-4029.13668. [Epub ahead of print]. Available at <http://onlinelibrary.wiley.com/doi/10.1111/1556-4029.13668/full>
- » Dutton GJ, et al. Cultivating the Next Generation of Forensic Scientists Through Science, Technology, Engineering, and Mathematics (STEM). *J Forensic Res* 2017, 8:4. Available at <https://www.omicsonline.org/open-access/cultivating-the-next-generation-of-forensic-scientists-through-sciencetechnology-engineering-and-mathematics-stem-2157-7145-1000384.pdf>
- » National Institute of Justice. Forensics. <https://www.nij.gov/topics/forensics/Pages/welcome.aspx>
- » Xie F, et al. Automated bullet-identification system based on surface topography techniques. *Wear* 2009;266(5–6, 15):518–522.

EXPLOSIVES IN FORENSICS: DETECTING DANGER WITH OPTICAL TECHNIQUES



In the current climate where there is a high risk of terrorist bombings and improvised explosive devices, the ability to detect traces of explosives from a distance is of increased importance.

Since there are many different explosives, which are often present in small quantities amidst numerous background components, it has proved challenging to develop an effective means of detection that provides the required selectivity and sensitivity.

Raman spectroscopy is well suited to detecting explosive traces remotely since explosives give distinct, narrow Raman spectral bands.

The selectivity and sensitivity can be further enhanced by using a deep-ultraviolet (DUV) light source.

Although visible and infrared Raman spectroscopy wavelength provides deeper sample penetration, the DUV enhancement allows for higher signal levels from traces of explosives.

DUV resonance Raman spectroscopy is this a promising potential candidate for stand-off detection of explosives. Furthermore, it is possible for the components of this technology to be produced in a compact, portable format suitable for transporting to various locations where screening is to be conducted.

A highly sensitive algorithm has also been developed for the detection of explosives under low signal-to-noise situations.

The development of prototype explosive detectors based on DUV resonance Raman spectroscopy was presented by Balakishore Yellampalle at Pittcon 2018 in a presentation entitled 'Compact Deep Ultraviolet Resonance Raman Explosive Detector'.

Ibsen Photonics, who attended Pittcon 2018, produce the FREEDOM™ HR-DUV compact deep UV spectrometer. It offers high performance whilst being compact enough to be portable and robust enough to operate in

demanding environmental conditions. Spectrometers in the FREEDOM range support many different detector systems so can be tailored to suit a specific application. Furthermore, they allow the use of existing electronics and software.

Horiba Scientific were also at Pittcon 2018 presenting their SWIFT™ ultra-fast Raman imaging system. Real-time detailed Raman images detailed can now be acquired on second/minute timescales with integration times down to 1ms. Importantly, SWIFT retains the true confocal performance of the HORIBA Scientific Raman systems, ensuring optimized spatial resolution for effective analysis of thin layers.

Vibrational spectroscopy is a label-free chemical analysis technique. We have already seen its utility in stand-off detection of gunshot residue. In order to achieve coherent signal enhancement, realizing real-time vibrational imaging, measurement of different molecular

Coherent anti-Stokes Raman scattering (CARS) was found to provide stronger signal and faster acquisition than spontaneous Raman scattering, making it especially suitable for molecular imaging.

vibrational signatures has been studied. However, the application of single-beam CARS spectroscopy has been limited by the narrow vibrational bandwidth that can be achieved with the laser sources, and the difficult extraction and representation of the Raman spectrum.

Using fiber supercontinuum (SC) as an alternative to solid-state lasers has extended the vibrational bandwidth of CARS spectroscopy. The power, the spectrum and the spectral phase of the SC showed good long-term stability. Furthermore, smooth excitation was achieved and Raman spectra were retrieved across the fingerprint region that showed good agreement with the corresponding spontaneous Raman data.

The nonlinear interaction between faint light and matter on a single atom/molecule and few-photon level is of great fundamental and practical interest. It is now possible to enhance such nonlinear interactions specifically in the highly relevant regime of weak intensities



using coherent control. Single-beam coherent CARS spectroscopy has been shown to effectively detect explosives from a 12-meter standoff distance. Single laser shot spectra were obtained with sufficient signal to noise ratio to allow molecular identification.

Coherent vibrational spectroscopy was explored further at Pittcon 2018 by Marcos Danfus of University of Michigan in his talk 'Coherent Nonlinear Vibrational Sensing'.

Ocean optics were on-site at Pittcon 2018, giving delegates the opportunity to explore the capabilities of the latest addition to their range of spectrometers. The Ocean FX can capture up to 4,500 scans per second. In addition, it has a high-sensitivity CMOS detector, onboard spectral buffering and Ethernet communications. It has potential uses in a range of industrial and screening applications where accurate data are needed rapidly, such as cavity-ringdown spectroscopy, process monitoring and control, laser induced breakdown spectroscopy, reaction kinetics monitoring, size sorting and thin film thickness measurements.

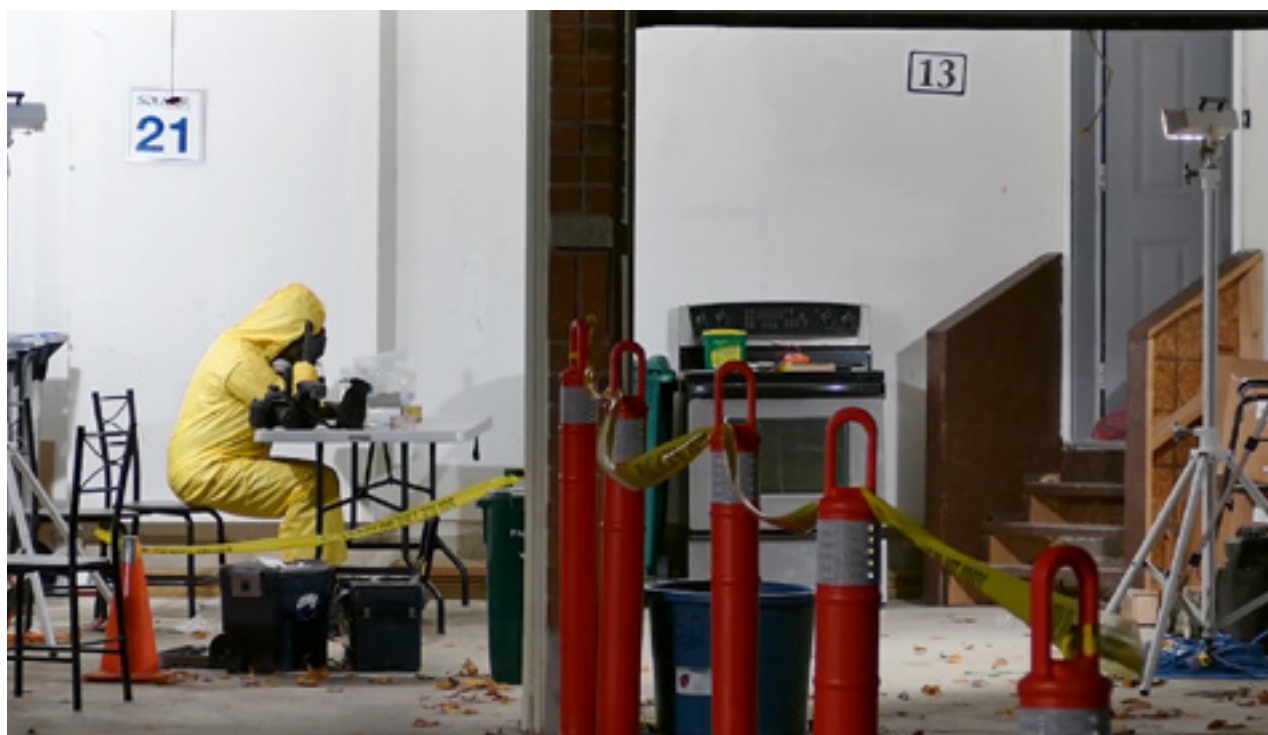
Hyperspectral imaging collects and processes information from across the electromagnetic spectrum. To date it has only been available to researchers, but many years of active research have resulted in the real potential of it being used for mainstream remote sensing applications.

Multispectral remote sensors produce images from a few relatively broad wavelength bands. In contrast hyperspectral remote sensors collect image data simultaneously across numerous narrow, adjacent spectral bands. These measurements make it possible to derive a continuous spectrum for each image cell.

Traditionally hyperspectral imaging required chemometric data processing in order to characterize and identify regions of interest within the field of view. Since this processing is only possible once the data has been collected, it precluded evaluation of the imaging in real time.

Recent development of a next-generation hyperspectral imaging technology, known as Dual Polarization-Conformal Filter (DPCF),





can simultaneously transmit multiple optical passbands allowing real-time detection. As such it represents a promising tool for the stand-off detection of explosives.

Charles Gardner of ChemImage Corporation provided further details of the application of this novel technique in the standoff detection of explosives at Pittcon 2018 in his presentation entitled 'Novel Hyperspectral Imaging Techniques for Highly Selective On-the-Move Explosives Detection'.

ChemImage provides a range of remote detection devices, including the LightGuard™ which is designed to provide reliable real-time standoff detection of explosives. It can detect multiple explosives simultaneously, including their precursors and degradation products, without the need for reagents.

Furthermore, the ability to detect explosives remotely obviates the need for contact, thereby

minimizing risk. ChemImage also supplies the HSI Examiner series of imaging systems and software to facilitate forensic analysis of hyperspectral images.

Corning, exhibitors at Pittcon 2018, produce the microHSI™ hyperspectral sensors and systems, which are small and low-weight, making them suitable for deployment in challenging applications and environments with payload and/or size constraints.

Although Raman spectroscopy can be used for standoff detection of explosives, it lacks the sensitivity required to pick up trace amounts of explosives. Sensitivity can be increased using CARS spectroscopy, but this has the downside of using lasers that have eye safety concerns. Increased selectivity and sensitivity can also be achieved using excitation in the deep ultraviolet (DUV) range, which results in resonance enhancement without interference from fluorescence.

Advances in spectrometer technologies have made possible the development of devices for standoff explosive detection based on UV resonance Raman spectroscopy. Furthermore, the DUV photochemistry for several explosive molecules is now well understood.

One such device is the Portable Raman Improvised Explosive Detector (PRIED) developed by Alakai Defense Systems. This detector system has recently been enhanced so that it can detect a wide variety of chemicals at ranges of 0.5-10m, including explosives and chemical warfare agents.

The performance of PRIED was illustrated by Robert Waterbury of Alakai Defense Systems in his presentation 'Recent Improvements in a Portable UV Raman Standoff Explosive Detection System'.

Laser induced breakdown spectrometry (LIBS) is a type of optical emission spectrometry. Unlike other forms of spectrometry, the image is formed by the excitation of atoms and ions in a plasma plume. The plume is generated when a laser pulse strikes the surface of the sample and ablates around 1 ng of material. The light generated in the plume is then quantitatively analyzed.

LIBS is suitable for the analysis of alloys. The technique rapidly measures low atomic number elements like the alkaline (Li, Na, etc.) and alkaline-earth metals (Be, Mg, etc.). Thus, it provides the ideal complement to x-ray fluorescence spectrometry, which is more suited to measuring high atomic number elements, such as the refractory elements (Nb, Mo, W, etc)

Improvised explosive devices (IEDs) are becoming increasingly sophisticated and well-concealed enabling them to effectively elude traditional explosives detection capabilities. Their detection is particularly challenging in areas where there are not specific static checkpoints. In an era of significant terrorist activity, the need to be able to detect IEDs before they are activated is becoming more acute. Such chemical 'clutter' has not been taken into account during the development of chemical sensors, which has impeded their utility for IED detection. A novel methodology for incorporating realistic trace explosive residues and background clutter into the technology development process, without the need for expensive prototype development, has been developed.

This framework predicts system performance and highlights areas where additional research is needed. Further details of this assessment framework was provided at Pittcon 2018 by Patrick Wen of MIT Lincoln Laboratory in a presentation entitled 'Standoff Explosives Detection Performance Assessment Framework'.

Quantum cascade lasers (QCL) are the first room temperature semiconductor laser source for the mid-infrared spectral region. They open up new potential for the development of new analytical methodologies, providing the high accuracy and precision associated with traditional infrared spectroscopy.

Furthermore, miniaturization of components is feasible, whereby facilitating development of portable mid-infrared instrumentation. The increased sensitivity and broad spectral source coverage with QCLs is achieved by using several lasers each with a slightly different wavelength. This obviates the need for moving parts in order to tune to the correct wavelength, making it more able to withstand movement in a portable device.

It is typically achieved using distributed feedback (DFB) semiconductor lasers; reliable, compact light sources with good capability.

Unfortunately, they have typically been expensive to produce, and so their large-scale integration was not feasible. However, lasers with different wavelengths have now been achieved simultaneously on one chip. This is promising for the mass production of QCL portable instrumentation.

The capabilities and potential future directions for GCL and DFB instrumentation was explored in more depth by Mark Wilinski of Pendar Technologies at Pittcon 2018 in a presentation entitled 'Eyesafe and Portable Standoff Detection of Hazardous Residues Using Quantum Cascade Laser Arrays'.

ChemImage Sensor Systems has developed an Eye-Safe Standoff Fusion Detection (ESFD) Sensor that combines a laser Raman standoff detector with a wide area surveillance sensor in an eye-safe configuration and is suitable for the standoff detection of explosives.

References

- » Gares KL, et al Review of explosive detection methodologies and the emergence of standoff deep UV resonance Raman. *J. Raman Spectrosc.* 2016;47:124-141.
- » Li J, et al. Experimental demonstration of distributed feedback semiconductor lasers based on reconstruction-equivalent-chirp technology. *Optics Express* 2009;17(7):5240-5245.
- » Li H, et al. Coherent mode-selective Raman excitation towards standoff detection. *Optics Express* 2008;16(8):5499-5504.
- » Liu Y, et al. Broadband nonlinear vibrational spectroscopy by shaping a coherent fiber supercontinuum. *Optics Express* 2013;21(7): 8269-8275.
- » Schlawin F and Buchleitner A. Theory of coherent control with quantum light. *New Journal of Physics* 2017;(19). Epub ahead of print. Available at <http://iopscience.iop.org/article/10.1088/1367-2630/aa55ec/meta>

NOVEL METHODS FOR BODY FLUID IDENTIFICATION AND PHENOTYPING

The detection and identification of body fluids at a crime scene can provide essential information about who was present and the events occurring around the time of the crime.

The presence of body fluids, their location and their DNA profile can significantly aid police investigations by presenting a picture of the circumstances of the crime.

The presence of body fluids at a crime scene, even if they have been wiped up or covered, can be detected using alternative light sources. Extraction and sequencing of DNA from any body fluids found at a crime scene, or on a suspect or victim, can be used for identification purposes.

However, alternative light sources will not typically allow for differentiation between different body fluids. Blood, sweat, saliva, faeces, urine, vaginal fluid and semen are all potential sources of evidence, depending on the nature of the crime.

Although blood is readily identified using color change tests, such as luminol or phenolphthalein, it is not so easy to identify the origin of other stains. Identification of urine, vaginal fluid and semen is particularly useful when ascertaining whether a sexual crime has been committed.

Historically, there was no single method for the analysis of all body fluids and identification was largely based on determining the presence of known components of a given body fluid, for



example amylase in saliva, and urea in urine. Fourier transform infrared spectroscopy and Raman spectroscopy now offer the potential to identify biological stains at a crime scene without detriment to the sample.

Each body fluid has its own characteristic spectral signature and these have been used to correctly classify stains, irrespective of whether they were fresh or dried and the color or nature of the material on which they were found. Furthermore, contamination with soaps, milk, juices, and lotions did not give rise to erroneous conclusions.



At Pittcon 2018, Igor Lednev of the University at Albany detailed the identification of traces of body fluids in his presentation 'A Universal Method for Biological Stain Characterization Using Raman Spectroscopy: From Body Fluid Identification to Phenotype Profiling'.

It was also possible to distinguish animal blood from human blood and menstrual blood from peripheral blood. Furthermore, advanced statistical analysis of the spectroscopic data enabled discrimination between Caucasian and African American donors with over 80% accuracy.

Renishaw were available at Pittcon 2018 to discuss the capabilities of their new inVia Qontor Raman microscope, which includes LiveTrack™ focus tracking technology that allows accurate analysis of samples with uneven, curved or rough surfaces.

Optimum focus is maintained without the need for time consuming manual focusing, pre-scanning or sample preparation. The highly efficient optical design provides the best Raman data from minute traces of material.

The inVia Qontor Raman microscope can be used with the Bruker Dimension Icon AFM, which provides high-performance surface characterization and the flexibility to perform nearly every atomic force microscopy measurement type at resolutions previously only obtained by extensively customized systems.

This additional pairing demonstrates the extreme flexibility of the Renishaw inVia confocal microscope, and its ability to interface to a wide range of instruments employing many analytical techniques.

References

- » Sikirzhyski V, et al. Discriminant Analysis of Raman Spectra for Body Fluid Identification for Forensic Purposes. *Sensors* 2010;10(4):2869-2884.
- » Yellampalle B, et al. Multiple-excitation-wavelength resonance Raman explosives detection. *Proc. SPIE 8018, Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XII*, 801819. June 03 2011. Available at <http://www.wvhtf.org/wp-content/uploads/2015/08/MEWRRED-DSS-11.pdf>
- » Yellampalle B, et al. Dual-Excitation-Wavelength Resonance-Raman Explosives Detector. *Proc. SPIE 8710, Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XIV*, 87100Z. May 29 2013. Available at <http://wvhtf.org/dual-excitation-wavelength-resonance-raman-explosives-detector>
- » Zapata F, et al. Differentiation of Body Fluid Stains on Fabrics Using External Reflection Fourier Transform Infrared Spectroscopy (FT-IR) and Chemometrics. *Applied Spectroscopy* 2016;70(4): 654-665.

The below article is a summary of Dr. Bruce McCord's talk at Pittcon 2018, titled: "Forensic Epigenetics, A Novel Method for Body Fluid Identification and Phenotyping"

Introduction

The project began with a court case I was working on in Michigan, in the early 90s, of a woman who was found murdered. There were traces of DNA under her fingernails that matched her ex-husband and there was a custody dispute between them at the time. The last time she was seen alive, she was handing over their child to the ex-husband and the DNA of her husband could not be excluded from the intimate mixture of DNA, and there was a presumptive test for blood, but it wasn't confirmatory.

The suspect argued that it wasn't blood and that the actual DNA was from secondary skin cells transferred from him to the child,

to the woman, and that was why DNA from the husband was found under her fingernails. Critical to the case was the determination of the DNA, was it rust or blood? Was it human or animal? And, even more importantly, since I was to testify on this case 20 years after the incident occurred, could the sample be tested many years later?

There are a number of different issues with current serological testing, based on a variety of presumptive and confirmatory tests, some of which are very useful, and others that are complex and time important. There are a variety of ways to do next generation body fluid identification, such as looking at transcriptomes, RNA, using reverse transcriptase PCR, or capillary electrophoresis.

But we've been looking at DNA methylation because methylation is at the root of the genome tree, and so we don't have to worry about differences in expression and other aspects that might have to come into play in other methods. Because we're using DNA procedures, we can use PCR, which means it can be very sensitive.



Gene expression

The epigenetic project is very compatible with existing DNA processing and the sensitivity is high, for example, we're able to achieve nanogram to sub nanograms levels of sensitivity. Our primers are human specific and the extracted DNA that we're using can be used for multiple purposes, as it's very simple to go back and take a sample of DNA that's been extracted and stored in a refrigerator or freezer and test it for body fluid specificity. However, there is one disadvantage when using this technique and that is the methylation sites must be identified prior to amplification because methylation isn't preserved by PCR.

Twins have different fingerprints, as well as over time, twins' appearances begin to change from one another. This can be explained by epigenetics.

Epigenetics is the idea that there are heritable differences in our DNA that are related to a variety of different factors that are present. We're interested in differences in methylation, which affect gene transcription and that also affects histone modifications. To summarize, epigenetics is the study of these heritable

changes. The most striking example would be the transformation from the caterpillar to the chrysalis to the butterfly.

Methylation plays an important role in the expression of genes. The methyl residues are covalently bound to the 5-prime carbon of the cytosine, which means that a covalent bond is present and so it's very stable. The turning 'on' and 'off' of genes is based on methyltransferases and control of their expression generally occurs upstream of the genes. There is great discussion of methylation of the cytosine, known as CpG islands or CpG dinucleotides. Seventy percent of CpGs are methylated in invertebrates, and distinct patterns which control gene expression can be observed.

These CpG islands are some of the areas that we're interested in and we want to find regions of gene activation based on methylation. In our example, when comparing muscle cells to epidermis cells, they are quite different. And it depends on whether or not there's a methylation pattern, which would turn on and off different aspects of the gene and the protein.

In a human body, even though all of your DNA is exactly the same, it exists in many different forms of many types of cells, e.g. skin, hair, teeth etc. but your DNA is all the same.



Forensic application

In order to exploit this forensically, we first find locations in your genes that target the expression of cellular proteins, or things that are specific to different kinds of cell formation.

So, the challenge is, finding these things, and we do this by looking at patterns. We can look at studies that other people have done with RNA, or with protein, or we can do whole genome array studies and piggyback off of studies such as for cancer. Because we don't have to start from first principles and we can look in the genome for tissue-specific methylated sites, as well as looking for differences in methylation that are dependent on cell type.

The very first thing that you can do is search the scientific literature and find that a lot of people have done whole genome array studies for cancer, or for other types of genomic studies. And if we can find particular locations that are very specific to different cell types, we can exploit that. There are arrays presently produced by Illumina that have 850,000 different sites across the whole genome. We can use these if we can target them and carry out some bioinformatics, and hopefully find some information.

To start with, we looked at a study by Eckhart, who carried out a cross-correlation of various kinds of cells, and the global methylation



differences. Those differences are displayed by color and sperm, of course, is very different from skin cells. We can then use this to look through these sites and try to find locations which are specific to one particular type of cell and not others.

There's a variety of different ways that we can help us with our forensics case. You can find enzymes that cut specifically at different methylated sites, or you can find complementary antibodies, but our preferred method is looking at bisulfite modified PCR. In bisulfite modified PCR, if the cytosine is not methylated, the addition of the bisulfite creates a change, first it sulfonates it, then it modifies the amine and you get a uracil. If the methylation is present, there's steric hindrance, and you don't get any addition. So, you can lock in place, once you do this reaction, two different forms of the same 'C'.

The 'C's are then converted, if they're not methylated, to uracils, but if it's methylated it's not going to convert, and so it gets carried on. Then following PCR, we get what looks like SNPs (single nucleotide polymorphisms), at various locations where a 'C' existed that wasn't methylated.

Now, with this knowledge, the next step is to look at specific genes or locations, and we design primers in and around those places, upstream of the genes, and we assess the candidacy produced. So the biggest challenge is finding the genes that are specific to one particular cell type, and this involves a certain amount of screening.

It also involves primer design, based around the knowledge that some of the 'C's are going to change to 'T's'. When done correctly a nice clean amplicon is done, carried out by a microfluidic chip. And then the final step, primary sequencing.

Pyrosequencing

Originally, we thought that we would use real-time PCR, because we'd had some experience with high-resolution melt, and we know that the A-T based pair, of course, has two hydrogen bonds versus the G-C one has three, so we thought there would be melt differences. However, it wasn't as simple as this and we turned our attention to pyrosequencing.

The basic idea of how it works is that the cascade reaction of enzymes, if you add a base you produce the pyrophosphate, the sulfuryase converts it to APP and the APP is then interacting with the luciferase and

The advantage of pyrosequencing is that it produces a precise signal that allows you to determine the actual quantity of difference of methylation.

converts to light. This produces a signal because you've added a 'G'. The next base you're adding, and for example, there is a series of three of them in a row, therefore the signal for these three, since we have three nucleotides, is therefore, three times as high. And a signal is produced in the same way. So, in effect, at one particular location, we're either going to have a 'C' or a 'T', and we can determine the relative percentage by what gets added.

So, our objective is to find some markers that will differentiate biofluids, design primers around them, and then perform sequencing reactions with the pyrosequencer. First of all, this involved searching the literature and finding relevant locations. Once finding a small set of markers that were very discriminatory. We then carried out the methodology, which was extracting the DNA and carrying out the bisulfite modification for a subset of those samples, and then you can either do PCR or real-time placebo.

The DNA is extracted and modified, with some of the low sites that were selected based on the literature search. One of the locations chosen was FGF7, identified as a fibroblast growth factor, which is hypermethylated in skin cells and semen.



The next one was a BRCA4 gene, which we were expecting to be hypermethylated in sperm but it wasn't, and it didn't work. But to our surprise, it was hypermethylated in saliva, and it may be related to the fact that you can use saliva for a genetic test for BRCA.

We used a marker for blood and semen, where in blood it is hypermethylated, and 95% 'C', and at the same location the semen is 2% 'C'. This is a very distinct difference between the two different types of body fluids. From this, we had a whole set of markers that our research team developed this included C20, BCS4, and Z3, where each of them are specific to a cell type: we have one for blood, saliva, and semen. In addition, we had the FGF7, which had intermediate levels for blood, semen, and saliva and is useful for multiple tests.

The next step, once we've performed all this work, was to start the process of validation, which are carried out in a very specific way in forensics. Sensitivity, age, specificity, degradation, and mixtures must be considered. One of the first things we looked at was sensitivity, and we found sensitivity varied from about 100 picograms to 10 nanograms, depending on the primer design and some

other things., for example amplifying the DNA too quickly. And we were able to drop the sensitivity, in order of magnitude, simply by slowing down our amplification.

Another thing that we saw was something called PCR bias, when we dropped the samples to really low, as well as partly being due to the effect of how fast we're amplifying and, there were also effects based on specific CPGs and primer design creating differences. We found that with the black bands, there is more air in certain CPGs than others and we had to take this into account when assessing the CPGs and collected the ones that are most specific.

It was also important to know whether our technique would work on really old samples. And to test this, we looked at a 20-year-old sample and found that when looking at both blood and semen, they're essentially indistinguishable. When doing this, we also found out that our marker was actually specific for sperm, not semen.

We then looked at a number of different samples to see if they were human-specific and found that a few of them amplified with our primers. But there's a set of three primers, ones for PCR amplification sequencing, and when you add all three, we weren't able to get any response except from humans. There was, however, one slight exception and we found that for chimps and gorillas we could obtain results. This meant that we could tell the difference between a mixture of blood and semen in a sample, there would be an intermediate level of methylation. Degraded samples could still be amplified, as well, and there wasn't a marker defect based on that, other than whether or not they could be amplified.

Vaginal epithelial cell markers

Once we'd finished the first step of analysis, the next step was something that we lacked, and this was vaginal markers, which are extremely important in sexual assault and child abuse cases.

Vaginal epithelial cells are difficult because epithelial cells found in the mouth and in the skin are very closely related. There has been some research, in terms of looking at the microfloral of the vagina, but we wanted to concentrate on epithelial cells.

We found one location, PFN3, which has a marker that is intermediate in methylation between blood saliva and semen and use it as a marker for vaginal epithelia. And again, it works well in mixtures, allowing us to look at mixtures of vaginal epithelia and semen and get intermediate levels if it is present.

Quick identification of semen based on real-time PCR

In order to increase the speed and simplicity of the analysis, we wanted to test whether we could go back to using real-time PCR, which uses differences between melt temperatures. Although it is not as specific, it could be easily done even if a lab didn't have a pyrosequencer. The challenge is, therefore, finding locations where there's a huge difference in methylation between the different types of body fluids that might be present at your crime scene. And this can be done, in this particular case with semen, because there are certain markers we have where it has very low levels of methylation.

This also meant finding more markers with specific differences and we began to categorize the virtual differences by examining a whole variety of different types of body fluids. Once we did that, we begin to find new markers, and we have a suite of new vaginal epithelial markers, and other blood markers being developed.



Determination of age

Epigenetic changes related to the environment, and not gene expression, are less robust. Up until now, we've been looking at gene expressions that are inherently more robust, but now we wanted to explore less robust changes, such as epigenetic locations which over a period of time have stochastically become methylated.

This is a gradual, slow process of increasing methylation and will affect how you look and how your body behaves, but not necessarily influenced by the environment.



We began by looking at the importance of age, because in terms of identification of people and the importance of biological versus chronological age. So we went through and looked at some specific sites and we found a number of locations which are age specific and can be identified based on plus or minus, or an average difference of about seven years. And some people have done better than that and others worse.

Interestingly we found that age specificity varied with the type of tissue being analyzed and so this opens up the avenue for further research looking at combining body fluid identification with age. We hope to find different markers for saliva, blood, semen and vaginal epithelial, for determining a person's age.

You may be aware of digital forensics, which includes cell phone forensics and computer forensics and is about using a person's devices to figure out where they've been over the past few weeks or even over the whole life of the phone.

This got us thinking about the determination of factors that aren't genetic but are related to a person's lifestyle. For example, the Dutch famine, and babies that were born in the year of 1944/45, issues that continued even after the babies were born, and babies that were born from those babies had differences in birth weight depending on whether their mother was starving the whole time or part of the time during this historic period.

Can lifestyle factors such as smoking, alcohol intake, body mass index etc. be exposure clues with markers to read in your DNA?

The first one that we've located, just recently, is an epigenetic body marker for smoking. This was identified by looking at chip arrays studies, and we found one particular marker in our gene that had a loose correlation with smoking. Our research team looked at a whole variety of different sequences around this particular gene and came up with some very specific markers in blood for the determination of smoker and a little less specific, in saliva.



Conclusion

In conclusion, we're using epigenetic methods, which are at the root of the gene expression tree, because they are very valuable in the use of forensic analysis. They can be used to discriminate between blood, semen, and vaginal epithelia. We have other loc sites that we're looking at in addition, such as sweat. The use of methylation shows great stability in samples stored for up to 20 years and mixtures of body fluids produce intermediate results. Phenotyping methods have been discriminated for age and tobacco smoking, as well as other researchers in the literature who have also found markers for body mass index and other things.

Dr. Bruce McCord goes into more detail about his work in the below interview:

Why are bioanalytical methods important for forensics?

Developing analytical methods can help the triers of fact, judges and juries, better understand the events surrounding the circumstances of a crime. In forensic analysis, we are talking about determining the identity of unknown individuals through DNA and using DNA and other chemical signatures to determine and clarify the circumstances of the crime.

The advantage of modern analytical methods is their specificity and the ability to perform statistical analysis on resultant data. Examples include using laser induced fluorescence to detect trace levels of amplified DNA at a crime

scene or using spectroscopic analysis to detect biological stains.

This improved precision and increased sensitivity that bioanalytical methods allow, mean that forensic scientists can help the jury reach a valid conclusion on what has happened.



What will be the main focus of your talk titled “Forensic Epigenetics, A Novel Method for Body Fluid Identification and Phenotyping”?

I will be presenting our work on age and tissue type. We will demonstrate how epigenetic DNA methylation permits the determination of body fluid type, suspect age, and other information from trace levels of samples left behind at crime scenes.

What is epigenetic methylation and how can it be detected?

Epigenetics involves changes in gene function that are not related to changes in the DNA sequence. It is well known that all human DNA is the same, and is comprised of the same nucleotide bases. However, it is easy to see that cells are differentiated based on their application - skin, muscle, bone, etc. These differences are the result of molecular switches which determine gene expression.

One such switch involves differential methylation of cytosine – one of the four main bases found in DNA. It is common to find locations upstream of various genes known as CpG islands (a cytosine base immediately followed by a guanine) in which clusters of methylated cytosines occur. When the methylation level of a specific tissue is diagnostic, the procedure can be used to determine the cell type. Similarly, other locations in the genome can indicate a suspects age.

The presence of methylation at these locations can be detected in various ways. A common method is to use bisulfite modified PCR. This process chemically converts unmethylated GC base pairs to AT base pairs through chemical modification and PCR amplification.

Methylated cytosines are not converted. The result of this process is then easily read using DNA sequencing methods.

Please outline the sequencing methods involved with locating epigenetic loci.

We mainly use 3 methods to read the DNA and determine whether methylation is present at specific loci. Firstly, methylation array data helps us determine potential sites of interest in the entire human genome.

We then examine and score this data and look for specific CpGs in the array data which may indicate the presence of sequences of interest. We design specific primers to target regions of interest in the genome and use pyrosequencing to determine if these locations are probative. These locations are then probed using bisulfite modified PCR and pyrosequencing.

Lastly, because of the difference in melting temperature between GC and AT basepairs, we can also target locations using real time PCR with high resolution melt capability as long as large differences in methylation are present.

How can epigenetic methylation be used in the crime scene?

We use this procedure to determine trace levels of body fluids. This can be particularly important in situations involving crimes such as sexual assault or child abuse, where the suspect is present and finding their DNA would not be unexpected.

However, the type of DNA recovered - whether it is from a body fluid, compared to from skin cells for example, might indicate if a crime had in fact occurred. Similarly,

if a number of suspects are identified, the determination of age of an unknown sample from a crime scene would be very important.

How can CpG loci identify the lifestyle choices of an individual and how valid are these assumptions?

Lifestyle choices such as smoking may alter the methylation level at certain CpGs. In many cases, just like tissue specific epigenetics, these loci are linked to specific genes which are activated by the need of the body to mediate the lifestyle effect. Validity can only be determined by determination of gene function and experiments with population samples, but this is an important area of research in many biomedical fields.

We are just beginning to examine this area, but we note previous research exists determining links between epigenetic markers and factors such as smoking.

What data will you be presenting during your talk and how does this compare to other research in the field?

While all forensic research deals with human identity, phenotyping and use in assessing the biological information left by suspects at a crime scene, our focus is different from most other research in the field as we are targeting markers of interest in forensic science. Others in this field are more concerned with biomedical applications.

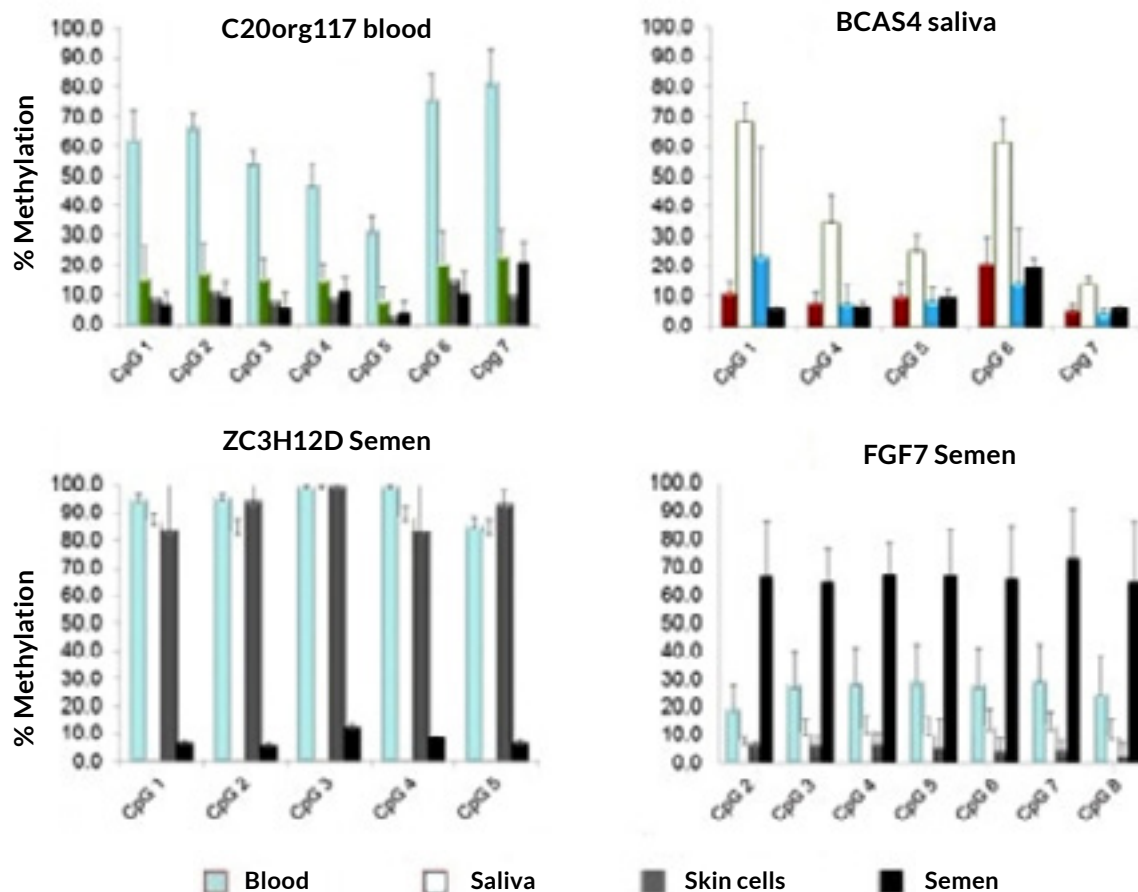
As epigenetic loci originate with DNA, we can use the power of being at the root of

the gene expression tree. By performing bisulfite modification prior to amplification, we have an advantage when it comes to DNA phenotyping as this means our process is resistant to inhibition and factors which may intervene with gene expression. Other methods using RNA or proteomics as used in

traditional forensics research, may be subject to variations in gene expression which require correction.

Thus, we envision that a portion of the forensic DNA sample can be diverted for age and tissue determination.

Body Fluid Identification by Epigenetic Pyrosequencing



Madi T, et al. 2012 Electrophoresis 33: 1736 - 1745

Are there any other advantages of using epigenetic loci in body fluid identification over other methods?

It is important to us to ensure that our interfaces work well with traditional forensic techniques, so that it is more easily implemented in a laboratory. Therefore, perhaps the biggest advantage to epigenetic

analysis is that the extraction of the sample and recovery of the DNA is the same with standard DNA typing and forensic epigenetics.

Additionally, as methylation involves covalent bonding, this method is extremely stable – samples over 20 years old can be analysed this way!

Where do you see epigenetics developing in forensic biology?

I see epigenetics developing as a tool to compliment standard forensic DNA typing, and our work is likely to contribute to this development. I think that future methods involving massively parallel sequencing (MPS), also known as next generation sequencing (NGS), will include procedures

for simultaneous genotyping and epigenetic analysis.

As far as advancing the field it is too soon to know. However, we believe that epigenetics can be an important tool in trace serology and in forensic investigations of individuals not yet in a database due to its capability to detect age and certain phenotypic information.

CONCLUSION

Forensic evidence admissible in court plays a vital role in the identification and prosecution of individuals who have committed a crime. Reliable and rapid evaluation of evidence found at a crime scene has thus come to play a pivotal role in the criminal justice system.

Analysis of materials obtained from a crime scene or the clothing of potential suspects can provide a picture of events surrounding a crime and help determine the guilt or innocence of defendants. Forensic investigations encompass the analysis of a diverse range of evidence, and novel techniques are continuously being established to enhance the reliability, sensitivity and speed with which these analyses can be performed.

Remote detection of explosives in improvised explosive devices, for example, and stand-off assessment of suspect objects with real time provision of data or high-quality images is an invaluable tool against the current widespread use of explosives across the globe.



Pittcon provides a comprehensive program of symposia, oral presentations, short courses, poster sessions, and industry-sponsored demonstrations of cutting-edge technologies. These always include sessions dedicated to scientific innovations applicable to forensic science, during which the innovations highlighted in this article are explored in more depth and many more additional advances will be detailed.

The producers of technical instrumentation used across forensic laboratories are always present at Pittcon to discuss the capabilities of their products and highlight the latest additions to their product range.