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ANALYTICAL TECHNOLOGIES IN FOOD SAFETY AND NANOTECHNOLOGY

INTRODUCTION

A far reach from local greengrocers and butchers, food provision is now a global industry. The foods we buy in the supermarket may now be harvested, processed and packaged in several different countries often thousands of miles apart. Aside from the purported benefits, such as cheaper products and wider selection, this poses greater challenges to ensuring food quality and safety. It is now much more difficult for food manufacturers to know the true origins and quality of the ingredients they buy from suppliers. Furthermore, there is greater opportunity for food fraud. Some unscrupulous food manufacturers are intentionally deceiving their customers by replacing the labelled product with cheaper alternatives to achieve higher profit margins.

There has always been the risk of products becoming contaminated during preparation and so regular food analysis has long been a routine part of food manufacture. However, today there are even greater challenges to ensuring food quality.

In addition to the presence of unwanted microbes, the increasing use of chemicals to increase yields has introduced the risk of pesticides and veterinary drugs entering food supplies, and fraudsters are using more and more ingenious ways to cover their deception.

Monitoring the safety and quality of food thus requires a battery of sophisticated analytical methodologies capable of discerning the presence of very similar yet inappropriate components, identifying the presence of unexpected unwanted ingredients and detecting pathogens at the lowest concentrations. Since current legislation holds food manufacturers responsible for the safety and quality of the goods they sell, there is growing demand for cost-effective, reliable food testing methods that can be incorporated into production lines. Scientists have responded to these growing challenges to food safety with the development of innovative adaptations of a range of analytical technologies.

3.1 FOOD SAFETY AT PITTCON 2018

A fundamental goal of the food industry is to provide consumers with quality food that is safe to enjoy. There has always been the risk of products becoming contaminated during preparation and so regular food analysis is a routine part of food manufacturing. However, today there are even greater challenges to ensuring food quality. With chemicals being increasingly used to improve yield in both agriculture and animal husbandry there is greater risk of foods being contaminated with pesticides and veterinary drugs that could pose a risk to human health. Furthermore, food fraud is on the rise as unscrupulous food manufacturers intentionally deceive their customers by replacing the labelled product with cheaper alternatives to achieve higher profit margins. With the food market now being a global trade, it is often very difficult to trace the true origins of purchased ingredients and to ensure their quality. Even more concerning is the realistic risk of terrorism directed at the food industry; pathogens intentionally added to food products destined for mass marketing have the potential to cause widespread devastation.

The prevalence of intentional adulteration of consumables for financial gain has been increasing in recent years. This is highlighted by the addition of the industrial chemical melamine to diluted baby formula milk in China in order to elevate apparent levels of protein, and by the discovery in 2013 that beef products marketed in Europe had been bulked up with cheaper horsemeat. Similar dishonest practices have also been uncovered for honey and for olive oil. These are either being diluted with similar cheaper sugar syrups or vegetable oils, respectively, or being incorrectly labelled as varieties that can be sold at a premium, such as manuka honey and extra virgin olive oil. Such fraudulent practices undermine genuine businesses and must be stopped in order to protect the livelihoods of honest suppliers. With the increasingly complex food supply chain, legislation regarding food safety has been amended to make food manufacturers responsible for the safety and quality of the goods they sell. Consequently, in addition to needing accurate, reliable and selective food testing methods that can confirm the safety and verify the content of marketed food products, it is important that they are easy to conduct and cost-effective. Scientists have responded to the growing challenges to food safety with the development of innovative adaptations of a range of analytical technologies. Industry is also striving to facilitate the food testing

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obligations of food manufactures by developing fully automated systems that provide results rapidly and can easily be incorporated into food processing and production lines. Furthermore, industry and food producers are now collaborating to detect and eliminate food fraud by conducting widespread authenticity testing of target products.

Pittcon includes a range of symposia, oral presentations, short courses, poster sessions and industry-sponsored demonstrations of cutting-edge technologies. The sessions on food safety described ground-breaking technological advances made to meet the challenges of ensuring that food sent to shop floors does not present a risk to consumer health and meets quality standards. The following sections of this chapter highlight moments at Pittcon 2018 which showed how researchers are constantly adapting and enhancing existing technologies and devising innovative new solutions to detect ever-more complicated attempts to defraud consumers by some unscrupulous food manufacturers.

3.1a RECENT CHALLENGES IN FOOD SAFETY

The marketing of contaminated food and water can have far-reaching, and potentially devastating consequences.

The food industry therefore has to comply with strict quality regulations in order to safeguard the public from the potential contamination or adulteration of its products. In addition, food fraud has become increasingly common, for example the intentional mislabelling of consumables for financial gain. Furthermore, in an age of widespread terrorism there is a real threat of intentional contamination of consumables. Pittcon 2018 continues the theme of food safety to present the latest developments in the analysis of food and drink.

Defending Against Food Fraud

The globalized world of today makes it increasingly easy for food to be sold in geographically distant markets, and increasingly difficult for the origins of food to be determined. A given product may be produced in one country, packaged in another and sold in yet another, often thousands of miles away.

The difficulties in fully tracking the history of imported foods has led to some manufacturers adopting unscrupulous tactics to increase their profit margins. For example, using cheap substitutes in place of more expensive products.

The challenges that regulators face is thus substantial and sophisticated technologies are needed to help protect the livelihoods of honest food suppliers. Fortunately, the concerted efforts of scientists and regulators mean that new technologies are continually being developed and improved to provide rapid means of analysis that provide the required sensitivity to detect deceitful practices.

Pittcon 2017 highlighted advances in NMR spectroscopy techniques to verify the authenticity of virgin olive oil and the origins of wine and honey. It also presented novel laser diffraction techniques to confirm the quality of coffee and chocolate and the novel application of ion chromatography to confirm the composition of dairy products. Furthermore, advances in genetic testing have facilitated a stamp-down on the mislabelling of fish.

Food authentication in complex supply chains

Food supply chains today can be incredibly complex, with different raw ingredients being obtained from, and partially processed by a range of suppliers. There are therefore many stages at which there is the potential for contamination.
To ensure consumer safety, sample testing is a key part of any food preparation protocol to ensure that the final marketed product is of suitable quality and poses no threat to the health of customers. Ideally, there would be testing at every stage along the supply chain, and this is often the case among smaller businesses. However, larger manufactures may import their ingredients from a range of different suppliers and producers around the world, including countries with differing food safety legislation. In such cases, sampling at every point in the supply chain is not feasible and may not even be possible. There is therefore the need for an element of trust that the suppliers are conducting adequate quality checks. Reduced sample testing is acceptable as long as the company has shown due diligence in ensuring the safety of their end product, which may be in the form of frequent random testing to monitor suppliers.

In addition to deciding at which points sample testing should be implemented, there is a range of potential analyses available from which manufacturers need to select the most appropriate, for example, shelf-life, microbiological testing, allergen analysis, nutritional evaluation. To help ensure that best food safety practices are adopted, food safety regulations have been introduced to protect the consumer.

A variety of technologies are now available to food processing and manufacturing businesses to help them meet food safety requirements. One such advance to facilitate rapid, easy-to-use, on-site analytical evaluations is the development of the miniaturized mass spectrometer. A miniaturized mass spectrometer used in combination with three ambient ionization methods differentiated different milk types and fish species with 100% accuracy; similar success was achieved with real-time food authentication.

Manufacturers of compact mini spectrometers, including Edinburgh instruments and Hamamatsu, attended Pittcon 2018. Edinburgh Instruments produce the StellarNet BLUE-Wave range of miniature spectrometers for measurements in 200-1150nm wavelength ranges. Hamamatsu provides more than 20 types of mini-spectrometers that cover the spectral range from UV to near infrared.

The miniaturisation of mass spectrometry

instrumentation also opens up the potential for immediate analysis of clinical samples, for examples in a clinician's consultation room while the patient is present. Further potential biomedical uses are discussed in Miniature Mass Spectrometry Instruments for Biomedical Applications.

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Governing bodies and regulations to ensure food safety

The UK Food Standards Agency recently published plans to amend food regulation in England, Wales and Northern Ireland. The changes are centred on an enhanced system of registration for all food businesses, which will be used by the department to apply proportionate, risk-based controls. They also include improved inspections process and greater support to help businesses meet the stringent and robust standards needed to ensure food safety. In addition, the Government Chemist Programme was introduced in 2017, which comprises quarterly updates on food safety legislation to ensure consistent and accurate interpretation of chemical measurement data, and labelling of products. Current legislation requires that the methods used for sampling and for laboratory analyses should meet scientific standards, satisfy the specific analytical, testing and diagnostic need of the laboratory concerned, and offer sound and reliable analytical, test and diagnostic results.

Similarly, in the US, the FDA is responsible for enforcing legislation to ensure food safety. Following the introduction of the Food Safety Modernization Act (FSMA) , by President Obama in 2011, federal law focuses on preventing contamination. The FDA uses sound analytical practices and methodologies, details of which are publically available, to routinely analyze commercially available food and food supplements to ensure that they are

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in compliance with applicable regulations. This includes determination of the elements present and the resultant data are used to evaluate the extent and significance of these analytes in the food supply. The FDA produces Small Entity Compliance Guides (SECGs) to help small businesses meet federal standards and ensure compliance with FSMA requirements.

The FDA Center for Food Safety and Applied Nutrition (CFSAN) undergoes ongoing research to identify the best technologies and methodologies for the analysis of foodstuffs to ensure they pose no harm to consumers and are accurately labelled. It also undertakes research to better understand factors that impact food safety and nutrition.

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3.1b ADULTERATION AND AUTHENTICATION

The majority of the population are dependent on the food industry for at least some of their nutritional needs. Consequently, should a manufacturer market contaminated products, the effects would be wide-reaching and have potentially devastating consequences. The food industry has always striven to ensure that its products have not become contaminated during preparation. However, with the increase in global trade ensuring food quality poses an even greater challenge. It is now often very difficult to trace the true origins of purchased ingredients and some unscrupulous manufacturers are taking advantage of this for financial gain. This is achieved by bulking out, or even replacing entirely, the product described on the label with similar cheaper alternatives. Where this occurs in a source material supplied to many different food industries, the deception is often perpetuated unwittingly. Furthermore, with rising levels of terrorism across the world, there is a real risk of products destined for mass marketing being intentionally contaminated with toxic substances or pathogens to cause widespread damage.

New legislations have thus been imposed on the food industry to minimise the risk of contaminated or adulterated foodstuffs reaching the consumer. Food manufacturers are now responsible for ensuring the safety and quality of the goods they sell. Consequently, they need access to effective and cost-effective means for routine testing of their products.

Impact of the horsemeat scandal on food legislation

In 2013, it was discovered, through routine proactive monitoring activities, that some beef products contained horse DNA. Consumers across Europe who thought they had bought beef products may therefore have unknowingly been consuming horse meat. Beef mixed

with cheaper horse meat was sold as pure beef to several manufacturers of processed meat products, who proceeded to sell it in the form of frozen beef burgers, minced beef and ready meals. The detection of the fraudulent adulteration of beef with horsemeat resulted in widespread recalls of such processed beef products.

The scandal highlighted the complexity of food supply chains and the difficulty manufacturers faced in verifying the origins of foods obtained from their suppliers. Consequently, a range of investigations were instigated in the European Union across both retail and food service markets. An independent review into the integrity and assurance of food supply networks commissioned by the UK government recommended eight pillars of food integrity: consumers first, zero tolerance, intelligence gathering, laboratory services, audit, government support, leadership and crisis management.

Four years after the recommendations were published, industry attitudes have changed substantially. Food testing and surveillance systems are now integrated into normal practice within the food industry. In addition, the UK government has establishment of the National Food Crime Unit to help protect consumers against similar incidents occurring in the future.

Honey may be incorrectly labelled as being harvested from countries or areas with particular floral varieties for which consumers are willing to pay a premium.

Detection of honey adulteration

Honey has become a prime target for food fraud and the adoption of deceitful practices to boost profit margins. The types of adulteration being practiced include mixing honey with cheap sugar syrups to artificially increase the volume of honey that can be marketed, and intentionally mis-labelling the geographic origin of the honey. Honey may be incorrectly labelled as being harvested from countries or areas with particular floral varieties for which consumers are willing to pay a premium. The criminals

implementing such dishonest marketing filter pollen out of the honey to try and prevent detection of its true origins.

The sugar syrups most commonly used to adulterate honey are corn syrup, industrial glucose and fructose. A range of analytical methodologies, both existing and novel, have been developed to verify the authenticity of honey and bring an end to such unscrupulous practices. Various chromatography techniques can be used, but these often involve complex and time-consuming methodologies. Consequently, spectroscopy techniques, such as nuclear magnetic resonance, are increasingly being used to screen for honey adulteration.

Most recently, isotope ratio mass spectrometry (IRMS) has been shown to be a particularly powerful tool in the analysis of honey. In his presentation at Pittcon 2018 entitled 'The Use of Stable Carbon Isotope Ratio Measurement to Detect the Adulteration of Honey with Alternative Sweeteners', Richard Anderson from Siratech Inc explained how it can be used to detect the presence of corn syrups in honey. IRMS analysis measures the ratio of a rare isotope to a common isotope. Honey is derived from C3 plants whereas corn syrups are derived from C4 plants. Unfortunately, the natural variability of honey from different regions and floral sources makes it difficult to conclusively determine whether C4 sugars have been added. However, authentic honey contains enzymes produced by the bees to catalyse the inversion of sucrose to fructose and glucose. These proteins provide an internal standard, representing the value close to that of the unadulterated honey.

An European Union report on detecting honey adulteration recommends liquid chromatography-IRMS for adoption as the standard methodology for the analysis of honey. They also propose the establishment of a centralised repository of authentic honey samples to develop purity criteria for EU honeys. In the honey authenticity testing conducted as part of the EU Coordinated Control Plan, 14% of the 893 honeys analysed were suspected of containing sugar syrups from non-honey origins. The analytical methodologies employed included IRMS using a Ultimate 3000 HPLC system linked to a ThermoFisher Scientific LC-ISOLINK.

Representatives from ThermoFisher were available at Pittcon 2018 to discuss their Ultimate 3000 HPLC series, IRMS high resolution spectrometers and IRMS data processing software.

Wine Authentication

Spectroscopic technologies are also used to verify the grape variety content of a wine and to validate its vintage and country of origin.

At Pittcon 2017, Bruker presented the addition of wine-profiling module to its NMR FoodScreener which is able to assign origin for the major wine-producing countries and can also assign region for several parts of France, Italy and Spain. It is also able to detect 22 different grape varieties and a more recent feature is the addition of vintage validation.

Rapid ultraviolet/visible/near infrared spectroscopy methodologies have also been developed to facilitate the authentication of wine. Spectral fingerprints obtained from genuine wines are used to quickly check that the protected designation of origin stated on the label accurately describes the contents. Using this technique and linear discriminate analysis correctly classified Galicia wines with 100% accuracy in a recent case study.

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3.2 QUALITY CONTROL OF FOOD AND WATER

The increasing globalization of food trade has raised new issues for ensuring food safety. Food manufacturers now commonly use ingredients and partly processed products sourced from suppliers all around the world.

It is therefore becoming more difficult to be sure of the origins and purity of many food products. Recent scandals, such as the melamine in milk and horsemeat in beef incidents, show how easily food contamination and fraud can go undetected and be perpetuated across wide geographical areas.

Although the technologies historically used to confirm food safety effectively identified the presence of selected known potential contaminants, they did not highlight the presence of unexpected compounds. Consequently, the addition of melamine to milk was not highlighted in tests. It thus became apparent that in order to have confidence in the safety and authenticity of manufactured food products non-targeted screening methods were also needed.

Pittcon 2018 featured presentations and exhibits from industry leaders, who are working to create novel food safety technologies to provide enhanced protection for food producers and consumers. These include the presentation entitled “Uncovering Economic Adulteration of Foods: A Forensic Approach”, in which Catherine Dasenbrock of FDA will discuss the challenges of determining the presence of a hidden adulterants in foodstuffs and detail the multi-faceted analytical approach used in their laboratory to ensure food safety.

FDA laboratories perform a wide range of sample analyses and they describe sound analytical practices in the Elemental Analysis Manual for Food and Related Products (EAM), which provides a useful reference document when selecting an appropriate analytical methodology.

NMR in food analysis

The inclusion of non-targeted screening into routine quality assurance testing called for cost-effective, broad-scope, easy-to-use analytical technologies. Advances in nuclear magnetic resonance (NMR) spectroscopy has proved particularly valuable in this respect. NMR can combine detection, identification, and quantification of both key known ingredients and unanticipated contaminants and adulterants.

NMR provides non-destructive screening that can identify even trace quantities of adulterants or contaminants. Furthermore, it allows combination of targeted and non-targeted analyses enabling confirmation of the presence of expected ingredients and the absence of undesirable components in a single process. NMR screening of food is now fully automated, operated by the push of a button, and standardized and validated procedures ensure consistency and compliance between different sites.
At Pittcon 2018, Diedrich Harms of Intertek Food Services gave a presentation highlighting the value of both non-targeted and targeted analysis authenticity testing—"State-of-the-art Techniques For The Authenticity Testing of Honey, Agave Syrup and Beeswax".

NMR spectroscopy has been used effectively to analyze olive oil, beer, wine, dairy products and honey.

NMR authentication of honey

Bruker's FoodScreener® platform analyzes food and drink authenticity using 1H-NMR.

The Honey Profiling Consortium has used the FoodScreener platform to comprehensively profile thousands of different honey varieties and geographic origins. The consortium has also profiled honeys with known levels of adulterant by mixing honeys with various sugar syrups. Data from analyses of honey using the FoodScreener are analysed on Bruker's server and a report sent that flags any violations against the product's labelling, such as honey variety, region and country of origin, and glucose and fructose concentrations.

NMR Analysis for Process and Production

NMR is also a valuable tool for determining the precise amounts of a specific component in the final product. This is needed to provide nutritional values to the consumer, such as the fat content of milk and processed foods, and to ensure that the alcohol content of beers and wines is labelled correctly.

Analysis by NMR requires minimal sample preparation with no hazardous solvents or chemicals and does not damage the sample in anyway so allows for repeat measurements to be made or for re-analysis using other techniques.

Furthermore, NMR analysis provides data on the whole sample and not just the surface, even if the sample is opaque or comprises mixed consistencies. It is therefore ideally suited for determination of the composition of foodstuffs.

The MQC+® benchtop NMR analyser provides fast, easy measurement of oil, water, fluorine and solid fat in a wide range of foodstuffs. It can determine fat content accurately with almost no effect from sample matrix, granularity or additives such as spices, flavourings, colorants and salt. Oxford Instruments were present at Pittcon 2018 to discuss the application of the MQC+ benchtop NMR analyser in quality assurance and quality control. Similarly, the Spinsolve® benchtop NMR spectrometer provides an easy and cost-effective means of analysing commercial liquid food samples. It

can be used to quantify the different lipids in dairy products and cooking oils and the alcohol content of beverages, irrespective of whether the sample is cloudy, coloured or bubbly. The samples can be taken straight from their containers and scanned without any further purification, dilution or other treatment. There will be opportunity to explore the Spinsolve

range at Pittcon 2018 where representatives from Magritek Ltd will be available to describe their capabilities.

Chromatography techniques for Food Safety

In an attempt to ensure the safety and nutritional quality of our food in an era of increasingly complicated food production chains, a variety of regulations have been introduced that stipulate acceptable levels for individual chemical additives, residues and contaminants in food products. Food manufacturers are responsible for verifying that their products meet the legal requirements. In addition, the packaging of many foods is now required to display the nutritional value, such as the proportions of unsaturated and saturated fat.

Chromatography methodologies can be used at various stages during the production of foodstuffs to either confirm the quality of a product or to detect the presence of adulterants. High-performance liquid chromatography (HPLC), in particular has proven to be an optimal technology for detecting and/or quantifying the vast majority of food analytes. Chromatographic analysis, with boundless options for analytical separation, continues to be developed and adapted to meet a range of detection and quantification needs within the food industry and Pittcon 2018 will be presented the latest innovations.

An important measure of quality used throughout food processing is vitamin C content. This nutrient is particularly susceptible to the negative effects of food processing. Since it is often added to food to increase their nutritional value, it is commonly used as an indicator for depletion of other important nutrients. To facilitate such analysis in a commercial environment, Bio-Rad, on-site at Pittcon 2018, have developed a fast acid analysis column with electrochemical detection. The Aminex® column provides precise evaluation of vitamin C contents in food and beverages within 3 minutes. It can quantify vitamin C in fresh and frozen fruits and vegetables, fresh drinks and juices, and powdered drinks. Similarly, the Aminex® column effectively quantifies pyruvic acid, an indicator of spoilage, in milk products.

Liquid Chromatography Mass Spectrometry for Food Safety

Liquid chromatography mass spectrometry (LC-MS) is a rapidly developing technology being adapted for a wide range of applications in food safety and quality assessments.

This technique combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry to provide very high sensitivity, high selectivity and mass accuracy.

It therefore offers a powerful alternative to tandem MS for analysing complex foodstuffs, which can pose sensitivity and selectivity issues as well as the potential for interference from

other components within the product being analysed. LC-MS allows rapid screening for a wide range of food contaminants, such as pesticides, mycotoxins, veterinary drugs and plastics, as well as intentional adulteration in food fraud, such as the presence of cheaper meats in beef products.
The use of LC-MS to detect the presence of Bisphenol A (BPA) in commercially packaged ready-to-consume beverages were described at Pittcon 2018 by Siheng Li in a presentation entitled "Analysis of Endocrine Disrupting Chemicals in Various Food Matrices by LC-MS/MS".

The Orbitrap mass analyzers, which provide high-resolution, accurate-mass analysis, can be connected to liquid chromatography equipment to enhance the separation of unknown compounds and enable high-throughput workflows. Such systems can be designed with various ion traps and quadrupole mass filters, making them suitable for a myriad of food analysis applications. Thermo Scientific provided further insight into the capabilities of their wide portfolio of Orbitrap-based LC-MS systems and the ISQ™ QD Single Quadrupole GC-MS System at Pittcon 2018.

Spectroscopy

There are many spectroscopic methodologies available to food and drinks manufacturers who need to perform a range of analyses to comply with current regulations.

Ultraviolet to visible (UV-VIS) spectroscopy is one of the most commonly used analytical techniques used in the testing of food as it can provide rapid real-time data. The electromagnetic spectrum includes an array of radiation types differing in wavelength and frequency. Spectroscopy therefore has broad-reaching applications for the identification and quantification of the components of food or potential contaminants. It can be applied to a wide range of sample types, from gases to immiscible liquid mixtures to solid powders and chunks. Spectroscopy can also be used in combination with other analytical methodologies to authenticate food origins.

UV-VIS spectroscopy is most commonly used in the analysis of oils to determine the proportion of saturated and polyunsaturated fats and to confirm that quality oils, such as virgin olive oil, have not been diluted with cheaper alternatives.

A significant development is the use of Raman spectroscopy to rapidly detect pathogens in food samples. It is now possible to obtain high-specificity spectra of single cells within seconds without damaging the sample.

Leading manufacturers of analytical instrumentation tailored to meet the screening and quality testing needs of the food industry attended Pittcon 2018 to discuss their products ranges.

Ocean Optics produce spectrometers and accessories for conducting a wide range of spectroscopic analyses in food and beverage processing, authentication and packaging. Ocean Optics were at Pittcon 2018 to discuss their modular spectroscopy systems, which encompass absorbance, reflectance, fluorescence and Raman spectroscopy, for the effective authentication and safety testing of foods.

Pittcon also provides the opportunity to explore the range of chromatography and spectroscopy analytical instrumentation available from Shimadzu, who will have representatives in attendance. The Shimadzu LCMS-8030 LC-MS-MS system used in combination with the high-speed Nexera HPLC makes it possible to rapidly identify aflatoxins in food

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3.2a CAN GAC BE USED TO CONTROL PRIORITY UNREGULATED DBPS IN DRINKING WATER?

In this interview, Prof. Susan Richardson discusses the use of GAC to control disinfection by-products, which she presented at Pittcon 2018.

Your presentation at Pittcon focused on GAC for controlling priority unregulated disinfection by-products (DBPs) in drinking water. What are the current challenges associated with unregulated DBPs in drinking water?

The US EPA currently regulates only 11 disinfection by-products, DBPs, in drinking water, but we have identified, more than 700. Many scientists, myself included, believe that the human health effects that we see in epidemiologic studies, may be related to some of the more toxic, unregulated DBPs that are not controlled currently through drinking water regulations.

Why has granular activated carbon (GAC) received renewed interest compared to other methods?

We have known about GAC for around 30 years, and there has been a lot of promising research on it, but despite this, in many cases it has not been put in place as many people think that it would be too expensive to switch to GAC. Instead, a number of drinking water treatment plants have switched disinfectants, for example from using chlorine to chloramine, to lower the levels of regulated DBPs. By switching disinfectants in this way, plants that previously struggled to meet regulations can become compliant.

However, we have noticed that potentially hazardous DBPs can occur as a result of the switch, including NDMA, nitrosodimethylamine, a very potent carcinogen. So now, the U.S. EPA and the research community are thinking of how to reach a suitable solution by going back to square one and asking the initial question – are there ways we can remove the precursor material better to prevent DBP formation, and ultimately lower the level of DBPs?

There has been some indication that brominated species may increase in formation when using GAC. What research have you done to investigate the ability of GAC to remove unregulated DBPs?

Earlier studies indicated that two regulated brominated trihalomethanes increased with the use of GAC. However, no research had been done beyond that to look at other brominated DBPs, ones that are more toxic than regulated DBPs.

That's where we came in - we took about 60 unregulated priority DBPs, developed analytical methods for them, measured them with and without GAC, and with different types of GAC. We also experimented with different disinfectants, with and without prechlorination, and even using chloramination.
We're investigating, for the first time, a really broad sweep of DBPs, including the really toxic brominated ones, to understand if GAC will work for them.

What analytical techniques have you used to investigate these DBPs?

We use gas chromatography with mass spectrometry, GC-MS, and also GC-MS/MS, tandem mass spectrometry. Another tool that we use is a total organic halogen (TOX) analyzer. With the TOX analyzer, we can measure not only the DBPs that we know are in the drinking water, but it also accounts for the chlorinated, brominated, and iodinated material that we don't about and can't measure yet.
In general, the brominated and iodinated DBPs are much more toxic than the chlorinated ones, so the total organic halogen analysis gives us an idea of what's there (beyond the things we can measure). And, with the 60 DBPs that we are quantifying, we're able to get a very comprehensive measurement of the DBPs.

> "Ultimately, our aim is to make drinking water safer, and so we want to find out if GAC can do this."
>
> *Prof. Susan Richardson*

What impact does the age of GAC and types of GAC have on filtering DBPs in drinking water?

The aging of GAC is much like how we expect the material in our home water filters to age – after a while, you need to change it. GAC at a drinking water treatment plant is like having a huge Brita filter.

Sites within the filter get filled up with material as it sorbs and removes unwanted materials from your water, to the point where they stop removing DBP precursors as effectively - then it's time to regenerate that GAC.

Does GAC offer a long-term solution for reducing levels of unregulated DBPs in drinking water?

I would say so, especially as we saw such good results with it. Some of the plants we looked at were reducing the DBP levels by as much as 80% with a young GAC filter.

It is worth noting that in some cases however, we did see an increase in some brominated DBPs that were toxic, just like the early work that saw two of the brominated trihalomethanes increase. But overall, when we looked at it across the board, it's still a beneficial route to take in producing safer water.

What are the next steps in your research?

Although we were able to measure the DBPs and the total organic halogen under all kinds of scenarios in our research, we were limited by our funding in that we were not able to get real toxicology testing – instead, we calculated the in vitro cytotoxicity using the measured DBPs that we have, and using the cytotoxicity potencies that we know of from other studies.

Therefore, our next steps are to have real toxicity involved in our work, combining the chemistry and comprehensive toxicology.

What did you gain from attending Pittcon 2018 and discussing your research?

I love sharing my research with others, it's good to inform others on the work we are doing.

If I'm able to educate people with my talk at Pittcon, that GAC is a good way to go, then maybe others will promote that in their utilities and share their new knowledge with people they know in the field.

I also attend Pittcon to learn, too. I learned about new analytical techniques, new developments, new findings. It's always exciting to come to conferences and learn new things. That's a big part of it!

3.3 NANOTECHNOLOGY IN FOOD SAFETY

Nanotechnology is science, engineering, and technology involving individual atoms and molecules. The types of material produced on the nanoscale can be one-dimensional very thin coatings, two-dimensional wires or tubes, or three-dimensional nanoparticles.

Although it is challenging, since the materials used cannot be seen with the naked eye, nanotechnology is becoming big business. The micro scale of nanomaterials gives them enhanced properties such as higher strength, lighter weight, increased control of the light spectrum, and greater chemical reactivity compared with their larger-scale counterparts. They have already been successfully employed in a range of novel applications, including medicine where they have improved the diagnosis and treatment of cancer.

The latest advances in nanotechnology relating to food safety were presented at Pittcon 2018 in the session "IAEAC – Nanobiosensors for Food Safety".

The small size and versatility of nanomaterials also offers great potential in the food industry. With the increasing responsibility of food manufacturers to ensure the quality and safety of their products, the demand for quick, accurate and cost-effective screening and analytical tools is greater than ever. Custom hybrid bio-inorganic nanomaterials offer potential new tools to address the ever-changing challenges facing food safety.

Microsystems allow the sensitive and cost-effective detection of many biological and chemical cues. As such they could greatly enhance food safety by facilitating more convenient routine food testing. Indeed, the FoodMicro-Systems Project has been established to develop strategies that make analytical microsystems more widely available to the food sector. Representatives of Fraunhofer Institute for Molecular Biology and Applied Ecology IME, who develop state-of-the-art analyses for the detection of challenging contaminants in food, attended Pittcon 2018 to discuss analytical microtechnologies designed to tackle food safety concerns.

Nanomaterials in food

Nanotechnology is one of eight sustainable innovations identified as a key area of research and development for meeting the world's food needs. Nanomaterials occur naturally; many proteins, polysaccharides and lipids are within the nano range and casein micelle nanoparticle have been identified in raw milk. However, there is increasing interest in the addition of engineered nanomaterials to foodstuffs to improve their properties. This may be to incorporate supplements, such as the antioxidant lycopene, increase shelf-life, lower lipid content, or enhance flavour and colour.

Nanotechnology also provides the potential to positively impact food safety in numerous ways. Incorporation of nanomaterials tags to food when harvested will allow the entire journey of a foodstuff to be tracked from farm to fork. Nanotechnology is also providing novel means of food analysis to ensure that the final product is free from contaminants and adulterants and poses no risk to consumer health.

It is anticipated that the incorporation of nanomaterials, with their enhanced properties, into the food industry will reduce the need for natural resources and enable food to be produced, processed and transported more efficiently.

Nanotechnology in food analysis

Various new nanomaterials have been developed in order to facilitate food analysis and purification. Magnetic nanoparticles have proved particularly valuable as they obviate the need for centrifugation and filtration steps to extract minority food components. Their magnetic properties means that once they bind to the target component, they can be separated from the main food matrix using a magnet. A range of magnetic nanoparticles have been designed with a variety of functionalities tailored to the analysis of food for contaminants, such as pesticides, and adulterants.

3.3a DESIGNING FIRST GENERATION NANOBOTS FOR FOOD SAFETY VIA PHAGE ENGINEERING

At Pittcon 2018, in a presentation titled "Designing First Generation Nanobots for Food Safety via Phage Engineering" Sam Nugen of Cornell University described how genetically engineered viruses conjugated to magnetic nanoparticles can be used to provide early detection of pathogens in food stuffs. This is summarized below:

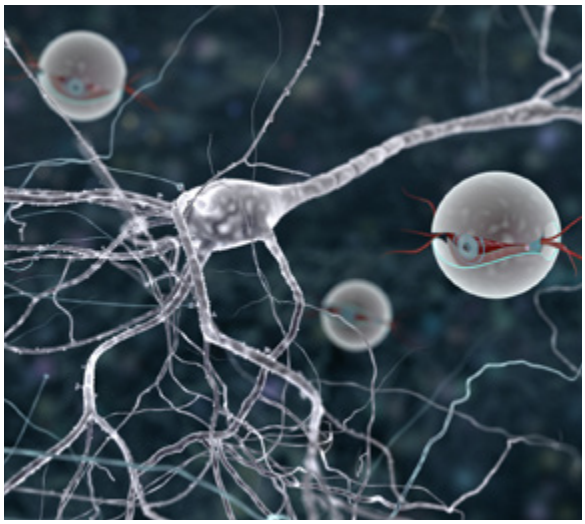
Introduction

We're interested in food safety, specifically in biosensors that will try to make it easier, faster and more accurate in the detections of not only pathogens but also indicators in food and water samples.

In our research, we wanted to be able to look at a virus to help us detect potential pathogens or indicate organisms. We concentrated on food and water testing, and we noticed that if you look at a resource limited setting, where you don't have traditional diagnostics that would work to be able to see if this water is safe, such as labs for example, there are a lot of constraints such as cost, ease of use and robustness, that you need to consider when in a remote setting.

We then realized that it is not too different from some settings in the United States, for example in a farm. Farms cannot afford expensive diagnostics, they don't have highly trained personnel to be able to use the equipment, it has to be fairly robust and they don't have a lab on the farm to be able to do their diagnostics.

50% of our outbreaks in the United States



come from produce. When people think about outbreaks, there is a greater concern for produce because we eat these products raw and so they don't have a kill. However, produce is not normally the thing we're most concerned about.

The FDA has recognized that water is a very significant source of contamination. Part of the Imperative Food Safety Modernization Act looks at water and the sorts of contamination associated with it. Water is not only being sprayed on crops growing, but after we've harvested the produce, we're also rinsing it with water. At any of those steps, where

we're potentially adding contamination, we're causing a problem – so we need to be sure that the water is safe. Water now must be tested periodically according to the FSMA, to make sure that it has low counts of bacteria, such as generic E. coli. We start off with a 100 mL sample and we want to test it for the presence of E. coli.

We began by looking at the current method that farmers are using to do this. To test an irrigation source, you take a 100 mL sample and mail it out to a laboratory, where an MPN will be carried out to see how much generic E. coli is in the sample. A few days later, you'll receive your results and it may say that the water was contaminated.

The method is not the most helpful, as the produce may have already been harvested, sold and consumed by the time you get the results. Therefore, we need to find a way to get faster results, and one of the best ways to do is to have a test that can be taken on location. When looking at the water sample, we're looking for generic E. coli as an indicator. A positive result of generic E. coli doesn't necessarily mean that you have pathogens in your food, it means that there was a route where pathogens could have entered. It means that there's some fecal contamination in the water - so an E. coli cell which might be floating around inside our water source.

Phages and their application in food safety

If we could have something that could identify the E. coli and lets us know whether or not it was there, like nanobots (technology that doesn't currently exist), that would be the ideal scenario. Bacteria phages are viruses that specifically infect bacteria. They're harmless to humans, but they are present all over the planet and if you collected them all, it would be the largest biomass on the planet.

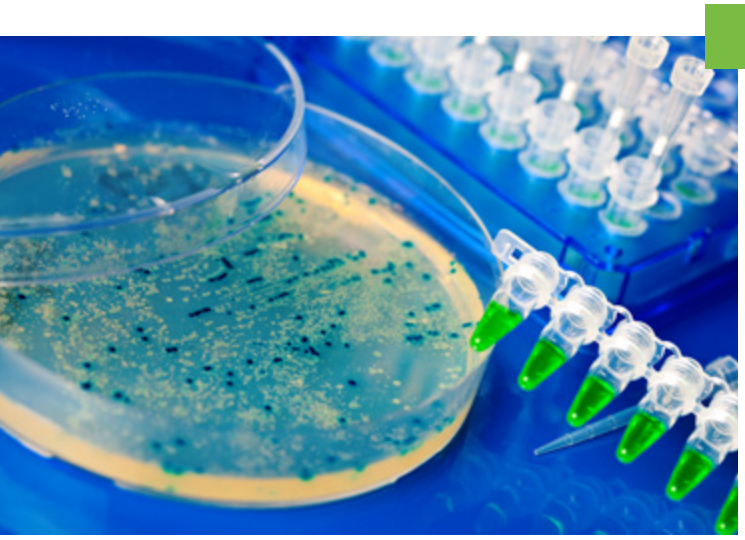
Phages specifically recognize E. coli - some broad and some more specific as far as hosts range, but they are unique because they've evolved to be an almost perfect predator of the bacteria. Phages inject their DNA into the bacteria, which replicates inside, and then enzymes are expressed in the bacteria. You can release hundreds, to possibly thousands of these phages back into your sample, making them a unique tool for food safety. We can engineer these phages to contain the DNA within the head component, also known as the capsule.

The phages also have a tail, which is used as the delivery and recognition mechanism, so the phage can deliver the DNA to the host. They have long tail fibers and the distal end is made up of about 100 amino acids, which recognizes the surface of the E. coli.

As DNA injects, it's inserting a code into that bacterium. That DNA can be used to program a particular virus. To engineer a phage, typically we would use PCR to amplify the entire genome, then remove the part we want to engineer and substitute it with synthetic part of the DNA. We then add an artificial yeast chromosome, and we transform all of this into yeast.

A lot of homologous recombination happens inside yeast, and it starts to assemble our phage again, but it's using this engineered portion of the DNA. If we transform this now into E. coli we start getting all these new engineered phages coming out of our E. coli.

If we think about these phages as being able to bind E. coli very specifically, we can use that to help concentrate our bacteria. Looking at the stage of absorption is highly efficient, and others have modelled how well phage results in bacteria. They found with each collision between a phage and a bacterium, you get a binding event. These events can even be irreversible, depending on the tail fiber, making it fairly robust.



Immunomagnetic separation is when we put magnetic beads with antibodies on them into a solution to grab our bacteria, such as E. coli, and separate them magnetically. In our research, we wanted to see if we swapped this with phages would this be just as, if not more, efficient. To do this, we looked at primary hangings on the phage. We conjugated our phage to nano or micron scale magnetic particles, and we coded a phage on them to separate them efficiently.

We compared them to Dynabeads, which are commercially available immunomagnetic separation and when comparing capture efficiency versus the amount of E. coli, you get a decrease of your capture efficiency, as you increase your number of E. coli.

We saw a small advance in the low concentration range when using phages, but not significant. Then we looked at anything outside of biological conditions, which most food testing is, that's when we saw a real advantage to using this new method. The antivirus have evolved to work in biological conditions, phages have evolved to work in many places in the environment. You find them in the ocean and even in sewage. It's no surprise that we see a peak, per capture efficiency on temperature of 37 for Dynabeads.

Phages are better on the extremes versus the antibodies in temperature and pH. When you look at salinity (PBS concentration), the Dynabeads reduce in efficiency rapidly and therefore have some advantage. If we can use these for magnetic separation, there's been a lot of studies that have shown that nanoparticles will be more efficient at separating than larger scale particles.

Although there are a lot of advantages here, there are also some disadvantages. For example, nanoparticles typically move quite slow and they don't magnetically separate as quickly as large particles. We're somewhat of a time constraint, because if we're putting these on our phage, infection is going to start as soon as it binds. If the phage isn't separated from the sample, within half an hour for example, we will lose the thing we're trying to look for.

We want to prevent our particles from moving too slowly, and so we designed our own particles rather than using the traditional iron oxide nanoparticles. We designed ours using cobalt, so whereas iron oxide is a 50 nanometer, cobalt is only 10 nanometers and we were able to increase the speed at which they separate. To keep them stable we put silicon shells on top of the cobalt particles. We ended up with a core shell nanoparticle, which we can then easily functionalize.

Antibodies vs phages

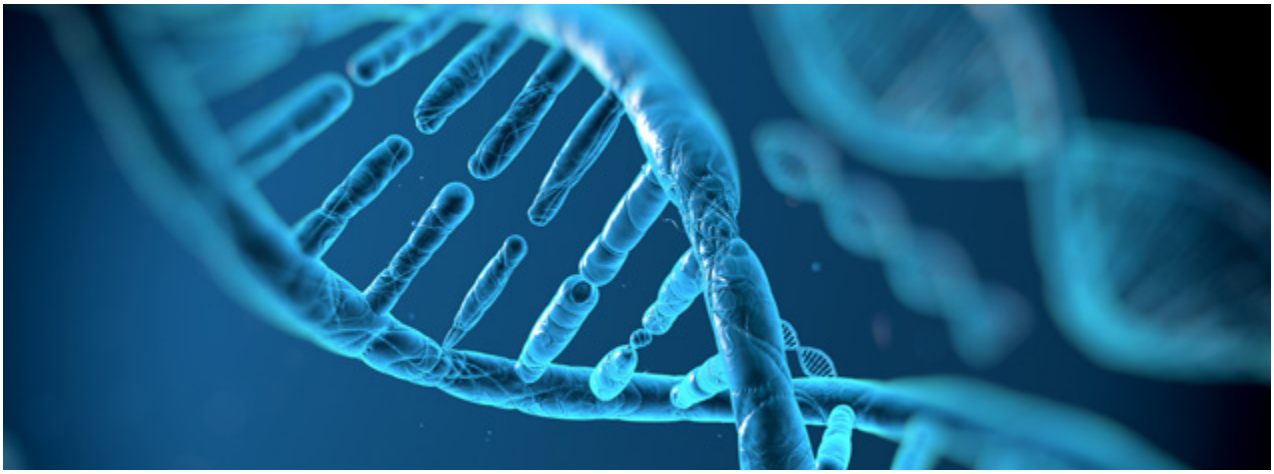
What we looked at first with phages, and then antibodies, on nanoparticles, is that we saw phages and antibody had similar capture efficiency with E. coli concentrations. We noticed that for every nanoparticle we put in, they can be completely coated with antibodies, but for every nanoparticle put with the phage, you have multiple nanoparticles per phage. You end up having a lot less discrete elements binding then with the antibody. When we normalize for this, it shows a more distinct advantage for phages, and it's most obvious with the micron scale. When looking at the phages on micron scale particles attached using genetic engineering compared to commercially available beads with antibodies on them, we can see we have a distinct advantage over them, particularly in special conditions.
We can use magnetic phages to separate the bacteria from a larger sample, the next step is how we can use it to rapidly detect the bacteria. During separation, phages are binding to the E. coli and it's causing an infection. It will then release lots more of the replicated phages and so when it does, it releases all the guts that's inside and we have the opportunity to do some additional engineering.

To give an example, we take a bacteriophage and infect bacterium like E. coli. We have them bind and cause the infection, then it would release all these enzymes out into the solution. As we've been able to then concentrate them, as these enzymes are separated, more phages are also coming out along with that. We're able to then use the enzyme which comes out and perform reaction experiments to see if it was there or not. As we can pick the enzyme we want to use, as long as it can be expressed in that host i.e. before working with E. coli, we have to make sure that the enzyme can be made in E. coli. Then we get drinking water and do our separation from here. We can add our magnet, and have the magnet separate out our E. coli and get a reaction on the phage.
What we found was that depending on the number of hours we pre-enriched for, the limited detection for our colorimetric assay would change. Therefore, these phages can be specific for a particular organism and species of the bacteria. When looking at our control, which had no bacteria at all, we saw no color change, but if we add E. coli the solution will turn red.

When adding the gene alkaline phosphatase, we didn't use the E. coli alkaline phosphatase, we used one that has been engineered to be more active. We can insert these genes and alkaline phosphatase is nice to work with because there's a lot of ways to be able to detect whether it has been successful, for example the engineered and E. coli alkaline phosphatase can work at very different pH levels, allowing us to easily differentiate whether this is endogenous AOP versus the engineered AOP.

Phages are only viable in bacteria because they use the bacteria's machinery to make the enzyme for itself. If that bacteria are killed by heat or chlorine etc, it can't make those enzymes. We can then use that principle to see if our organisms are antibiotic resistant, for example if I take this these lab strings of E. coli, ampicillin resistant ones will survive the antibiotic and have the phage infected in them and will color the indicator. Therefore, we can very rapidly, phenotypically determine if the E.coli. is resistant. Phenotypic is the better method to determine resistance because if you're looking for specific DNA for resistance, that can change over time.

Phages are only viable in bacteria because they use the bacteria's machinery to make the enzyme for itself.



Conclusion

Going forwards, we realized that sample prep is the key here and how can we clean up the sample and add a lot of unique steps to this to be able to make it easier to do. How can we make it faster and at a lower cost? Phages can be used well for separation, they bind extremely efficiently, phages for detection works well, and we can use genetic engineering. Where we're going in the future is increasing expression and activity of our enzyme, so we're doing some enzyme engineering as well. Custom report probes and engineering host range in which these enzymes or phages will go into and this allows us to make it more broad or less broad, enabling us to target the desired specific bacteria.

3.3b THE DETECTION OF FOOD-BORNE PATHOGENS

Microorganisms have long been identified as the primary cause of food spoilage and food-borne illness. Their detection is thus fundamental to the food industry. However, despite the importance of not marketing infected food, the detection of pathogenic organisms and their toxins in foodstuffs remains a challenge. Researchers strive to improve such detection in order to ensure the safety and quality of our food supplies.

It appears that nanotechnology may represent the long-awaited breakthrough in pathogen and toxin detection in foods.

Conjugation of antibodies to a nanostructure incorporating a fluorescent dye allowed the detection of one colony-forming unit of e-coli per gram of ground beef in less than 20 minutes. Similar techniques have effectively detected listeria in milk samples.

Microfluidic devices designed for conducting nanotechnology screening integrate sample handling, reagent mixing, separation and detection processes. In these systems of sub-millimeter scale, surface tension and fluidic resistance dominate meaning that laminar flow can be used to efficiently separate fluids and cells. Typically, microfluidic systems achieve a higher degree of integration than is usually possible, and this reduces cost and increases reaction efficiency.

Microfluidic biosensor modules for the detection of pathogens in food analysis have also been incorporated onto a plastic platform, which essentially condenses all the functions of an analytical laboratory in a micro format. A single chip can be enabled to detect multiple pathogens and toxins. It has been reported that 30 different pathogens were detected in less than an hour using such a system. The so-called Lab-on-a-Chip-system can thus be employed directly on the food production line (obviating the need for samples to be transported to a central laboratory) to rapidly screen for harmful bacteria in food at considerably reduced cost.

This nanotechnology was described in more detail at Pittcon 2018 by Antje Baeumner of University of Regensburg in a presentation entitled “Novel Nanomaterials for Microanalytical Systems”.

This interview with Prof. Antje J. Bauemner, describes her research into novel nanomaterials for improved food safety:

What will be the focus of your talk at Pittcon 2018?

My main focus is going to be on two or three different types of nanomaterials that we explore for specific applications in the analytical sciences.

One of the nanomaterials I will discuss will be nanofibers produced using electrospinning. These fibers are only 200 nanometers in diameter and approximately 500 to 1000 times smaller than a human hair. We use these fibers because they have a very high surface-to-volume ratio, which means that we can do efficient analytical reactions for the detection of an unknown analyte.

We explore these nanofibers on multiple levels using different types of starting polymer. We dope the polymer and implement it into different types of sensing strategies regardless of whether it’s a paper-based platform, a microchip, or a lab-on-a chip type of platform. The other nanomaterial I will discuss are liposomes. They are hollow spheres that are surrounded by a phospholipid bilayer. We place marker molecules that we would like to detect inside the spheres. On the outside, we bind bio recognition elements. Using the markers on the inside we can generate a significant signal enhancement for our analytical assay. The third material I will discuss is a laser-scribed graphene material that we’re studying for electroanalytical purposes

Why are Nano bio systems in this form important for food safety and why is this such a wide area of research?

Nanomaterials can be very important for food safety applications. The nanomaterial we are researching provides us with new features that cannot be achieved using other materials. Those additional characteristics might provide lower limits of detection or they might allow for elevated levels of tolerance for very complex sample matrices. In food safety, you always have a complex matrix and you always need to try to detect very low concentrations.

What are the main problems currently associated with food safety?

One problem currently facing the industry is food that is naturally contaminated during the food processing chain. Whether it's contamination with a pathogen, or whether there are toxins present, or there are certain allergens present in the food. Some of these contaminants can have a big impact on human health if consumed, which is why it is important to identify them before they make their way into the food processing chain.

The other aspect covers both food safety and food fraud issues. Fraudulent food is a global issue. How do you determine if the origin of a food product is true or false?
Another issue to consider is the adulteration of products like olive oil or milk mixed with cheaper substances.

"These food products may claim to be 100% olive oil or 100% milk, but if mixed with cheaper substances can cause huge problems such as allergies or food related poisoning on a wide scale."

Prof. Antje J. Bauemner

What do you hope to achieve with the different Nano materials that you're developing?

Our aim is to detect lower concentrations of a toxin, a contaminant, or a pathogen than is currently possible.

For example, we would use the nanofibers as an extension of our transducer. We would use a normal electrode onto which we place the conductive nanofibers and these conductive nanofibers can then help filter the analyte out of the solution by expanding much further into the bulk of the solution. So, we would have essentially a pre-concentration of the sample poured through the nanofibers plus are able to actually detect it at the same time.

We use the liposomes as a strict signal enhancement. We bind them specifically to an analyte, which results in a dramatic signal increase. This is because of hundreds of thousands of marker molecules that we have trapped within the liposome.
We use the laser-scribed graphene electrode to provide an inexpensive, easy, manufacturable transducer for our micro analytical systems.

Please can you elaborate on the liposomes for increasing signal output?

We have used the liposomes for quite some time for signal enhancement. For example, we have used them in lateral flow assay for visual detection. We have also used them in micro analytical systems for electrochemical detection. Our most recent advancement has involved putting a marker into the liposome, which is a luminol derivative that can provide us with an electrochemiluminescence detection.

Using this electrochemiluminescence detection, we can lower the limit of detection by about 150 times compared to a traditional fluorescence approach. Thus, by simply changing the detection technology, we can lower the limit of detection much further eventually resulting in rapid, very highly sensitive detection.

Where do you see this these new materials being applied?

I think these could be used wherever fluorescent or colorimetric markers are currently used. Liposomes could be used in place of colloidal gold to provide a significantly lower limit of detection. Colloidal gold is the current standard in lateral flow assay for visual detection.

While liposomes can provide visual detection, the current research is driving towards lateral flow assay technology becoming more sensitive. This would mean that not only can you detect abundant analytes like pregnancy hormones, but you can also detect very low concentrations, for example a pathogens in an apple juice.

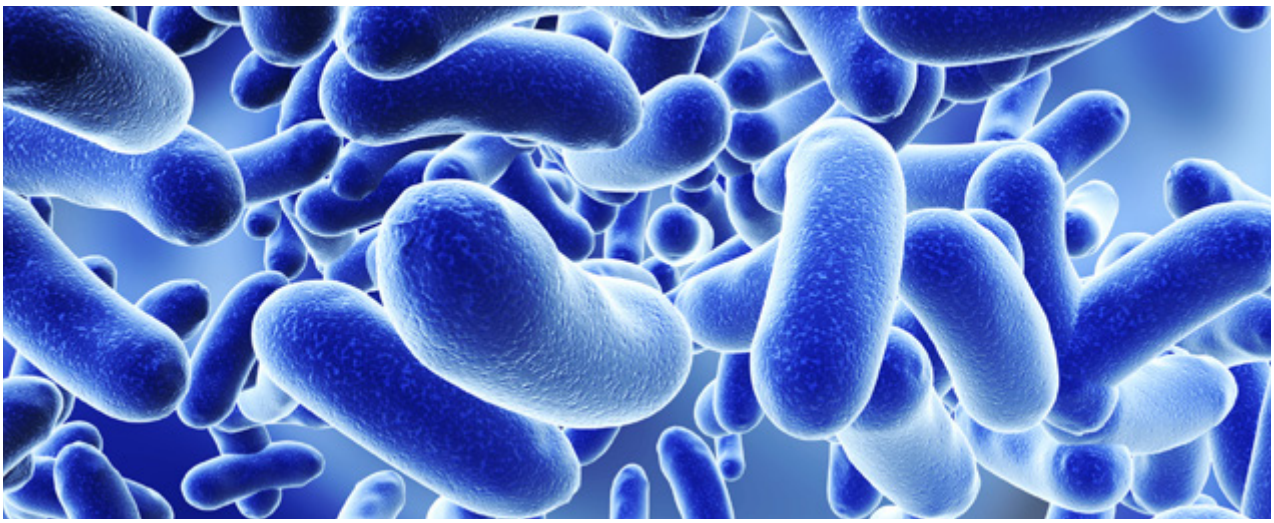
If we use liposomes with the electrochemiluminescence detection, I think we will dramatically increase sensitivity.

How does this compare to current methods that you can see on the market today?

If you look at pathogen detection for food safety for example, the gold standard is microbiological detection. In microbiological detection, you take your sample and you plate it out on agar dishes and then wait 24 to 48 hours and you see what type of bacteria grow. The next step-up are molecular biological tests using something like the polymerase chain reaction or similar approaches.

These molecular biological tests can perform the same detection much faster. and take typically four to six hours. However, they are complicated and can't be performed in the field. It also requires intensive training for personnel unless you package them in an automated device that can do the analysis for you. They are also very expensive and require a lot of sample preparation.

The niche that I see for using these Nano materials I described is detection with minimal sample pre-treatment, minimal sample preparation, analysis completed in under one hour and done in a cost-effective and simple manner.



What does the future hold for your research?

The next steps are to consider multi-analyte detection approaches using our liposomes so that we simultaneously detect more than just one analyte. In the case of the nanofibers, we're looking into using them for detection and sample preparation so that with the same type of technology you can actually solve two problems at the same time.

With regard to the laser-scribed graphene electrode, we would like to demonstrate that these are viable transducers that can compete with currently mass-produced electrodes. We believe these electrodes can be produced in a simpler fashion while having either better or similar detection capabilities.

"If you look at pathogen detection for food safety for example, the gold standard is microbiological detection."

Prof. Antje J. Bauemner

Why is Pittcon so important?

I really like Pittcon because you get the entire breadth of the analytical sciences. It's not only a conference where you are highly focused on one audience. It allows you to meet people across the analytical field and make new connections. It also means you can learn more by listening to experts from adjoining fields and expand on your own research. That's what I take away from the conference. I always feel I can start ten more research projects afterwards. It's also a fantastic opportunity for us to share our research and inspire other experts to use nanomaterials in the same way.

I also like the exhibition. It allows researchers like myself to see the direct applications and consider not only doing fundamental research, but also looking directly into what that could mean for improving analytical applications on a more societal basis.

3.3c NANOTECHNOLOGY IN FOOD PACKAGING AND TRANSPORT

The speed with which nanotechnology directed at the food industry is developing has raised concerns about regulation of the use of nanoparticles in foodstuffs. The possibility of adding manufactured nanoparticles to food, either as variants of existing ingredients or as completely novel chemical structures, is becoming reality.

Currently there is no legislation relating to the use of nanomaterials in foodstuffs and a lack of regulation regarding the inclusion of added nanoparticles to the labelling of foodstuffs. One of the obstacles to introducing such legislation is that a clear definition of nanotechnology has yet to be agreed and the distinction between natural and engineered nanomaterials remains to be clarified. There is the potential for toxicity and bioaccumulation of nanoparticles ingested in food. With the steady increase in the uptake of nanotechnology by the food industry, these are factors that must be explored. There is a need for risk assessment of engineered nanomaterials to gain consumer confidence and safeguard human and environmental health.

The European Food Safety Authority and the FDA have stated that nanomaterials proposed for inclusion in food stuffs will be assessed individually on a case-by-case basis.

The Food Standards Agency in the UK, has commissioned a range of research projects investigating the impact of adding nanomaterials to food. These include investigating what happens to nanomaterials once they enter the human body and characterizing, detecting and measuring nanoparticles in food.

Nanotechnology can also help improve the storage and transport of food. Inclusion of nanoparticles in the packaging of food could help reduce food spoilage whilst maintaining food safety. For example, they could strengthen the barrier with the environment and control the release of gases. Furthermore, they could incorporate antioxidant and antimicrobial activity. It is also feasible that nanomaterials included in food packaging could monitor the gas levels, microbe count and temperature and change colour if any level that could affect the quality of the food is reached. The consumer would then instantly know how fresh the food was and whether it was safe to be eaten. Finally, improving the composition of food packaging could also increase its biodegradability.

It appears that nanotechnology has the potential to benefit the food industry at every stage; production, processing, packaging, transport, storage and disposal.

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CONCLUSION

The increasing globalization of the food industry and the great lengths to which fraudsters will go in an attempt to cover their deception mean that increasingly sophisticated analytical methodologies and equipment are needed to ensure the safety and quality of food. Pittcon 2018 featured exhibitions from many of the companies currently providing analytical instruments designed for use in food production, and presentations from experts in the field describing the latest techniques developed to support food manufactures in their ongoing battle against contamination and adulteration of their products.

It is evident that scientists have responded to the growing challenges to food safety with a continuous cycle of innovation to meet requirements. Once again Pittcon will be communicating more novel techniques and technologies developed to enhance the detection of food contamination or adulteration or to provide evidence of mis-selling.

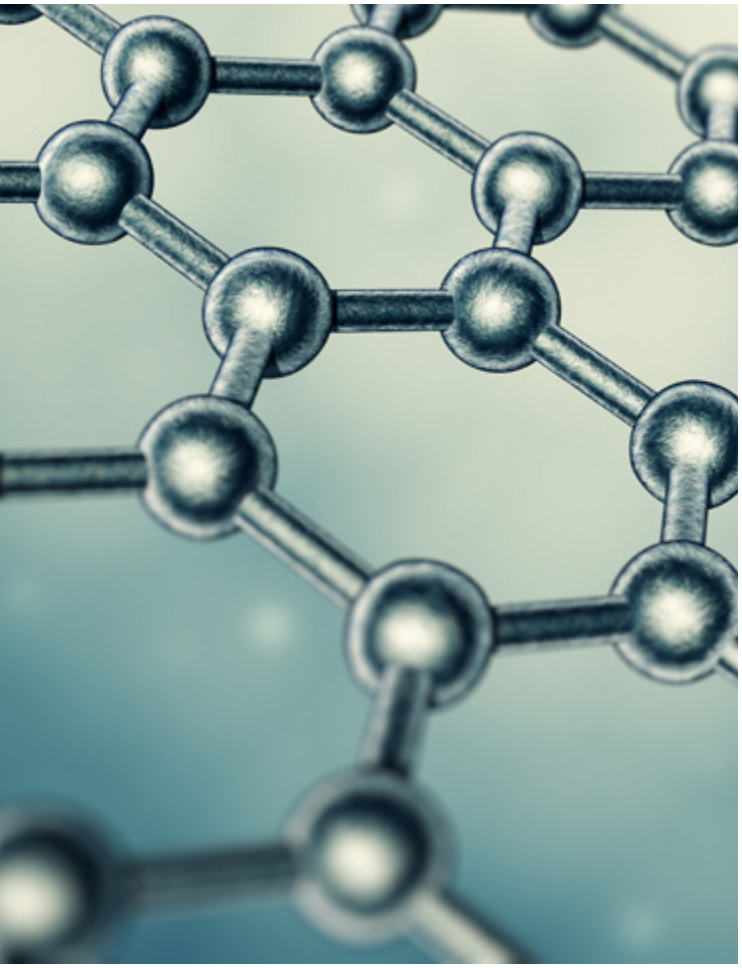
Recent food scandals exposed failings in traditional food testing protocols. Although they effectively identified the presence of known contaminants, the targeted analyses did not highlight the presence of unexpected adulterants. Consequently, there is now a greater emphasis on broad-scoped, non-targeted food screening methods.
Nuclear magnetic resonance allows for non-destructive screening and quantification of both known ingredients and unanticipated contaminants and adulterants. Fully automated NMR systems along with standardized procedures permit the routine use of such sophisticated analyses in the food industry.
At Pittcon 2018 we heard how NMR has been effectively applied to determine the authenticity of honey, agave syrup and beeswax.

The combination of spectroscopy and chromatography also provides a powerful tool for rapid screening for a wide range of food contaminants as well as intentional adulteration. Presentations at Pittcon 2018 illustrated the efficacy of liquid chromatography mass spectrometry in detecting contamination of beverages with bisphenol A from their containers and the presence of disinfectant residues in lettuce and spinach.

Spoilage of foodstuffs by pathogens is costly to the food industry, yet detection of such contamination has always been challenging. Nanotechnology has provided a long-awaited breakthrough in detecting pathogens in foodstuffs, allowing the rapid and sensitive detection of bacteria, including e-coli and listeria.

The highlights from the Pittcon 2018 food safety symposium reported here clearly indicate that researchers continue to develop new methodologies and novel uses of existing technologies to meet the growing challenges in food safety. This together with the continuing sophistication of instrumentation provide an impressive armament in the battle to ensure food quality and stamp out food fraud. Pittcon 2018 provided an informative food safety symposium that will communicate the very latest developments to enhance food screening and monitoring procedures and raise consumer confidence in food safety and quality.

3.5 THE LATEST ADVANCES IN NANOTECHNOLOGY AT PITTCON 2018



INTRODUCTION

Pittcon is an ideal place for researchers to learn about the latest trends in nanotechnology and nanomaterial characterization.

Pittcon has evolved to encompass all laboratory-based testing and the analysis of chemical/biological properties of compounds or molecules including techniques and applications relevant to the field of nanotechnology.

This year Pittcon hosted a symposium dedicated to nanotechnology. This article outlines some of the areas that were covered.

The Challenges Facing Nanotechnology

Scientists around the globe are harnessing the power of nanotechnology to create and modify materials and technologies, producing products and technologies with properties that were not previously possible.

Nanomaterials are already used in over 1300 commercial applications from sunscreen to medicines and food packaging. The power of nanotechnology stems from exotic quantum effects that are present at such a small scale, combined with properties that are not found in large particles due to the increased surface area to volume ratios and increased active surface areas.

However, the same qualities that make nanotechnology so powerful can also create risks to human health and the environment. A major challenge faced by the field of nanotechnology is assessing the impacts nanomaterials have on human health and the environment, and creating or modifying existing product regulations to ensure that nanotechnology is used responsibly.

The Challenge of Regulating Nanomaterials

To this date, regulatory bodies like the United States Environmental Protection Agency (EPA), the United States Food and Drug Administration (FDA), and the European Food Safety Administration (EFSA) have not created any specific regulations for products containing nanomaterials.

In a recent report, The Scientific Committee on Emerging and Newly Identified Health Risks of the European Commission concluded that current methods for assessing the risks of nanomaterials may not be sufficient and that existing methodologies may need to be changed or new methodologies developed. They stressed that the current lack of knowledge regarding the characterization of nanoparticles and their effects does not allow for satisfactory risk assessments to be performed.

Scientists at the EPA and around the world are researching the effects of the most widely used nanomaterials on the environment and human health. They intend to develop research protocols for characterizing engineered nanomaterials and evaluating their toxicity in biological and environmental systems.

Fully understanding the environmental and health effects of nanoparticles and developing reliable testing methodologies is a multi-stage process.

First of all, it is essential to understand how the composition, shape, size, and morphology of nanomaterials affect their physical and chemical properties. Second, it's essential to understand how nanomaterials with varying properties interact with biological systems like cells, tissues, and organs. Finally, it's important to characterize and understand the full life cycle of nanomaterials. This is achieved using a process called environmental mapping, which describes how nanomaterials move through the environment, how they change with time, and how people, animals, plants, and other organisms are exposed to them.

How Nanomaterials Can Harm Human Health

Nanoparticles are the size are viruses, and are therefore small enough to travel through the body's natural defenses via the lungs, intestinal wall, and skin.

Nanoparticles can make their way into the circulatory systems of human and animals, ultimately reaching all the tissues and organs in the body. Exposure to nanoparticles can influence cellular processes and cause cellular dysfunction, which in turn can lead to a variety of disorders. For example, cancer is caused by uncontrolled cellular proliferation, and neurodegenerative diseases are caused by premature cellular death.

Although nanomaterials have not been directly linked to specific diseases, studies in animals have suggested that exposure to nanoparticles could cause lung injuries. Other studies indicate

that nanoparticle exposure could increase the risk of cancer. However, the full effects of nanomaterials on human and animal health are currently largely unknown and the lack of knowledge of the effects of nanomaterials on human health is a major challenge for the field of nanotechnology.

The powerful and potentially unknown nature of nanomaterials means that great care must be taken to protect people, animals, and the environment from unnecessary exposure to potential harm. This begins by determining which nanoparticles are dangerous, and which are benign. Nanotoxicology is a new field of nanoscience that aims to create an understanding of the properties and toxicity of nanomaterials.

Not all nanoparticles are toxic; toxicity is dependent on the chemical composition of particles, along with their shape, size, crystallinity, and particle age. While free nanoparticles can be particularly toxic to humans and animals due to their ability to penetrate the bodily defenses, fixed nanostructured materials such as those used as thin film coatings and microchip electronics are generally benign.

Some nanoparticles can even have positive effects on human health, and even nanomaterials that do display toxicity could be used to fight diseases like cancer on a cellular level. However, to regulate nanoparticle use and ensure that nanoparticles are used in an effective, responsible manner, it is important to understand the toxicology of each material, and how factors such as morphology, and particle age can affect the human body. The right methods for characterizing and assessing the toxicology of nanomaterials are, therefore, vital.

Tools such as single particle ICPMS can be used to count metal-containing nanoparticles and measure their mass, allowing nanoparticles to be tracked as they move through the environment.

Advanced analysis techniques are required for understanding the physical and chemical properties of nanomaterials and establishing correlations with their biological and environmental effects.

Robust knowledge of the relationships between nanoparticle properties and their impacts on biological systems allows us to predict which nanomaterials may pose the greatest risk to human health and the environment based on their physical properties, rather than requiring us to study the toxicity of each new nanomaterial individually. Such relationships could, in future, form the basis for nanotechnology-specific regulations.

Environmental mapping of nanomaterials requires advanced nanomaterial characterization techniques and adequate testing protocols. Measuring the concentration and size distribution of nanomaterials is vital for environmental mapping and studying environmental behavior. Tools such as single particle ICPMS can be used to count metal-containing nanoparticles and measure their mass, allowing nanoparticles to be tracked as they move through the environment. Diane Beauchemin from Queens University gave a talk at Pittcon 2018's nanotechnology symposium on the latest advances in single particle ICPMS.

Advances in Raman spectroscopy relevant to nanomaterial characterization will be subject of talks from Eric Potma of the University of California and Bin Ren of Xiamen University. Pittcon 2018 was the ideal setting to learn more about nanomaterial characterization techniques, testing methodologies, and mapping protocols for nanotechnology regulation.

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3.6 NANOPARTICLES IN BIOLOGICAL ENVIRONMENTS

Nanomaterials have found applications in biological environments from drug delivery to imaging.

However, to fully understand both the efficacy and potential toxicity of nanoparticles, it's important to understand how they behave in complex biological solutions, tissues, organs, and bodily systems. A lack of adequate characterization techniques means that determining the behavior of nanoparticles in biological systems remains challenging. Pittcon 2018 covered the latest advances and cutting-edge technology for the characterization nanoparticles in biological environments.

Nanoparticles Demonstrate Complex Biological Behavior

Nanoparticles are precisely designed with specific physical and chemical properties that enable them to carry out their functions. For example, nanoparticles that actively target tumors are designed to be small and stable enough to travel through the body's systems to the tumor, they must bind preferentially to receptors in the tumor, and they must release the drugs within them in response to specific changes in their environment. These demands result in specific chemical and physical requirements for nanoparticles depending on the application they are to be used in.

Nanoparticles which have been designed to exhibit certain properties in a simple solution may display entirely different behavior when they are exposed to complex biological solutions and systems. Understanding the behavior of nanoparticles in biological solutions, tissues, organs, and bodily systems is key to understanding both their efficacy and toxicity, and designing nanoparticles that fulfill their functions without adverse side effects.

Analytical methods for the detection and characterization of nanoparticles in biological systems are currently underdeveloped. The small size of nanoparticles, combined with their low concentrations and the highly complex nature of biological mixtures makes nanoparticle detection and characterization a challenging area of analytical chemistry. Lack of adequate characterization techniques for the detection and quantification of nanomaterials in biological media has prevented advances in understanding the environmental fate, transport, and potentially toxic effects of nanoparticles and nanomaterials.

The behavior and toxicity of nanoparticles are influenced by a range of physical and chemical properties including composition, surface chemistry, particle size, surface area, crystallinity and solubility. It would be impossible to characterize every nanoparticle property for each experiment, so researchers and regulatory authorities must select appropriate characterization techniques for the sample and decide which properties are the most important.
For example, in a study of gold nanoparticles

As nanoparticle systems are so complex, multiple techniques are often required to detect and characterize nanoparticles in complex biological systems.

ingested by mice, which aimed to determine where the nanoparticles would accumulate, researchers had to use a combination of autometallography, ICPMS, and neutron activation analysis to determine the nanoparticle in fact accumulated in the liver.

Detecting Nanoparticles in Biological Samples

One of the first challenges of tracking nanoparticles in biological systems is detecting their presence. Elemental analysis techniques such as ICPMS, X-ray absorption, and X-ray fluorescence (XRF) can be used to detect the presence of nanoparticles, depending on their compositions. For example, gold nanoparticles would be easily detected using elemental analysis.

The Pittcon Expo will feature technology such as the M4 TORNADO from Bruker, a Micro XRF tool that provides information on composition and elemental distribution to identify the presence and distribution of nanoparticles in biological samples.

Analyzing Nanoparticle Properties in Biological Milieu

There are many well-established techniques for the characterization of nanoparticles in solutions including calorimetry, dynamic light scattering, and nanoparticle tracking analysis. Malvern Instruments, top suppliers of nanoparticle characterization technology, were present at the 2018 Pittcon Expo offering a range of solutions for analyzing nanoparticle number, size, zeta potential, and aggregate formation in biological solutions.

To fully understand the behavior of nanoparticles in biological environments, it's important to understand their interaction with biological milieu, which are complex biological solutions. As nanoparticles exist as colloids in solutions, analytical techniques from colloid science can be used as a starting point for analyzing the properties of nanoparticles in biological milieu. However, they must be adapted to address both the chemical and physical properties of nanoparticles, and to ensure they do not measure biogenic colloids.

Interactions between nanoparticles and other substances present in biological solutions infer a biological identity onto the nanoparticle, which effects how it behaves in the solution and interacts with receptors, cells, and tissues. Flow cytometry-based methods can be used to detect molecular motifs on the surfaces of the nanoparticles that enable biological recognition, allowing researchers to characterize the biological identity of nanoparticles and predict how they will interact with cells.

Characterizing Nanoparticles in Tissues, Organs and Bodily Systems

It's important to monitor the absorption, distribution, metabolism and excretion from tissues, organs and the body of nanoparticles as a whole to fully understand the efficacy and toxicity. Tracking nanoparticles within the body can be extremely challenging as nanoparticles due to their tiny size compared to the vast and complex bodily networks they are present in.

Various imaging techniques allow the biodistribution of nanoparticles in tissues and organs to be studied including super-resolution optical imaging, magnetic particle imaging, and nuclear imaging. The majority of imaging techniques require nanoparticles to be tagged with dyes or other labeling molecules. There are a few imaging techniques that enable imaging of unlabeled nanomaterials in tissues and organs without tagging. The majority are elemental imaging techniques including TEM-EDX, SXRF, and LIBS. LIBS offers particular advantages for imaging biological tissues including speed of operation, ease of use and full compatibility with optical microscopy. In a

recent proof-of-concept study, LIBS was used to obtain 3D images of nanoparticles in organs.

Nanobiotechnology

The new and improved properties of nanomaterials have led nanotechnology to find a wide range of applications, particularly in biological systems. The application areas for nanobiotechnology are vast and include nanoscopy, subcellular fractionation, drug delivery, biosensors, cancer therapy, tissue engineering, artificial organ generation, cell tracking, bioimaging, and 'omics' data generation.

The type of nanomaterial used in biological systems depends on the desired application. Whilst the applications of nanobiotechnology are vast, the number of nanomaterials that have been created for biological applications is even greater, and growing every day. Nanomaterials that have found biological applications include polymeric nanomaterials such as drug conjugates, micelles, and dendrimers; quantum dots which are used as luminescent nanoprobes; carbon nanotubes which have been used in drug delivery; and metallic nanoparticles which find applications as contrast agents and drug delivery agents.

Nanomaterials for biological applications can be further varied in their precise compositions, structures, dimensions, and surface modifications. This large variability results in an array of physical and chemical properties, which can be tailored to a required application. Silicon nanostructures have been gaining interest as analytical probes as they are well-defined materials with predictable properties and the potential to undergo surface modification.

The 2018 Pittcon nanotechnology symposium featured two talks on silicon nanostructures by Jeffrey Coffer of Texas Christian University and Jonathan Veinot of the University of Alberta.

Using Nanotechnology to Characterize Cells

Characterizing cells at a molecular level is an important step towards drug screening and personalized medicine. The characterization and quantification of the biological molecules that are responsible for the structure, function, and dynamics of an organism is referred to as 'omics', encompassing fields such as genomics, proteomics, and metabolomics. The 2018 Pittcon Expo featured Bruker, who offers a range of unique analytical methods and technological systems in omics fields. The Expo also sawe AMSBio who supply cell systems

and tissues ideal for researchers focusing on nanobiotechnology.

A talk at Pittcon 2017 by Dr Chad Mirkin of Northwestern University discussed how spherical nucleic acids can be used to characterize the genetic content of single cells. Spherical nucleic acids consist of strands of DNA or RNA arranged on a nanoparticle surface, enabling them to be recognized by cells and quickly internalized. Once inside the cellular environment they bind to complementary DNA or RNA and map the genetic content of live cells, acting as a diagnostic probe.

This behavior means they can be used to diagnose medical conditions such as sepsis and identify tumor cells. Spherical nucleic acids have also found a number of other pharmaceutical applications including as gene regulation agents, and for cancer vaccinations.

Another approach of characterizing the contents of single cells is subcellular compartment analysis. Surface modified superparamagnetic nanoparticles have been used for subcellular compartment isolation. The nanoparticles enter the cells and bind to their target; and their magnetic properties allow them to be moved, taking their target with them and resulting in subcellular compartment isolation. The composition of the isolated compartments can then be analyzed independently.

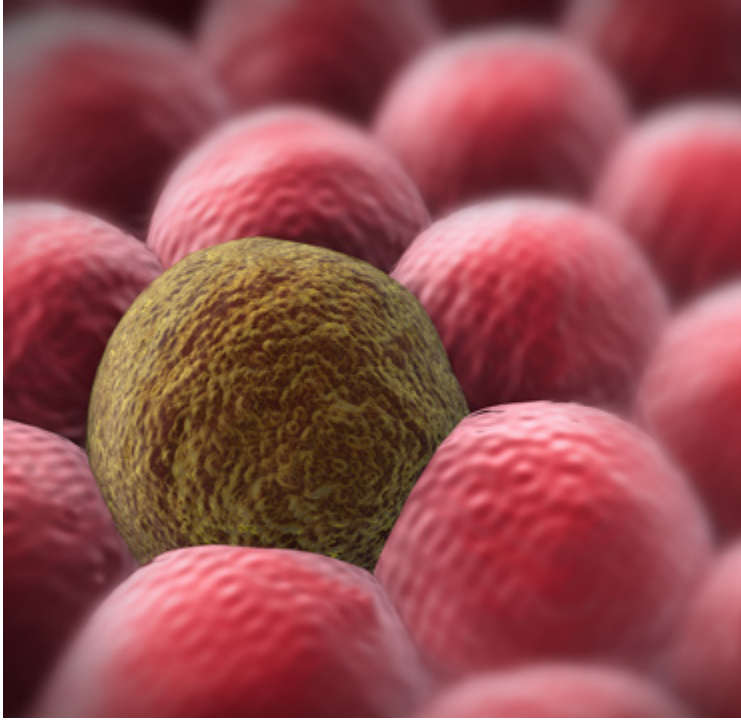
In-Vivo Imaging Systems using Nanobiotechnology

Traditionally information pertaining to the biomolecular makeup of cells, tissues, blood, organs has been based on obtaining ex-vivo samples followed by biochemical analysis and/or microscopic imaging. However, these approaches lose information such as the exact 3D mapping of molecules within a living body, and how this changes as a function of time. The In-vivo molecular imaging of cells and tissues allows biological processes and cellular functions to be visualized at the molecular and cellular levels, creating opportunities for diagnosis without any invasion of the living organism.

Positron emission tomography (PET), and single photon emission computed tomography (SPECT) are nuclear molecular imaging tools that are frequently used in clinical practice. Radionuclide-labelled substances are introduced into the body and used as contrast agents, 3D images can then be obtained with the use of a CT X-ray scan. The

enhanced permeability and retention (EPR) of nanoparticles in tumors, combined with their potential to undergo surface modifications, and specific target binding makes them ideal contrast agents for nuclear molecular imaging. Gold, carbon and lipid nanostructures have all been utilized in this way. FEI's Amira software for preclinical imaging, which will be featured at the 2018 Pittcon Expo, combines structural information from micro-CT or MRI systems with functional data from PET, SPECT, or optical imaging to provide 3D images of tissues and organs.

Nanoprobes have also found applications in other molecular imaging techniques including optical imaging, magnetic particle imaging, and photoacoustic imaging. Magnetic particle imaging is an imaging technique that detects the magnetic properties of iron-oxide nanoparticles injected into the bloodstream. Photoacoustic



imaging produces images of organs and tissues using the photoacoustic effect, which refers to the generation of acoustic waves by the absorption of electromagnetic energy. Raol Kopelman of the University of Michigan gave a talk at the Pittcon 2018 nanotechnology symposium on the use of nanoprobes in 4D molecular tumor photoacoustic imaging.

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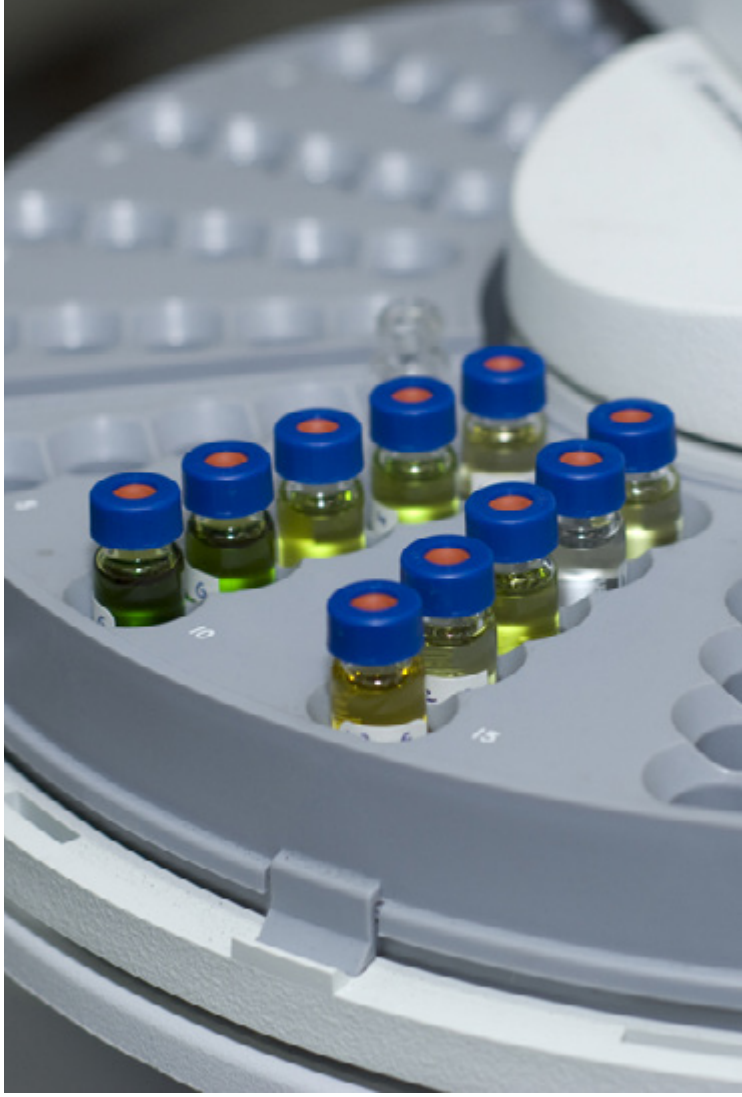
3.6a NANOSENSORS



Nanosensors offer increased specificity and sensitivity compared with traditional sensors and have therefore found a wide range of applications from medicine to food safety.

Nanosensors and related technologies will be featured at the Pittcon 2018 nanotechnology symposium and a variety of relevant companies will be present at the expo.

The term nanosensors include all sensors that use active nanomaterials. The increased sensitivity of nanosensors stems from the ability to finely tune the chemical and physical properties of nanomaterials, meaning they can be designed to only interact with the target molecule, even in complex solutions. The high surface area to volume ratios of nanomaterials and the ability to create nanostructured surfaces further enhances the sensitivity of nanosensors. The unique properties of nanosensors enable them to find a wide range of applications including disease testing, biomarker detection, contaminant detection, pollution monitoring and manufacturing monitoring.

Nanosensors can work in a number of different ways. One of the most widely applied types of nanosensors are electrochemical nanosensors, which detect changes in resistance when an analyte binds with the nanomaterial of the sensor. Electrochemical sensors have historically been an attractive option, due to the high sensitivity that can be obtained using relatively inexpensive equipment. However, previous electrochemical sensors have been limited by sensitivity in highly complex, real-world samples. Nanomaterials have high surface area to volume ratios and can therefore sample large volumes of solution, enabling them to detect target molecules even when they are present in very low concentrations. Other types of nanosensors include electromagnetic or plasmonic nanosensors, spectroscopic nanosensors, magnetoelectric or spintronic nanosensors, and mechanical nanosensors.

Nanosensors offer increased specificity and sensitivity compared with traditional sensors and have therefore found a wide range of applications from medicine to food safety.

Electrochemical Nanosensors for Biomarker Analysis

At Pittcon 2016 Dr Shana Kelly gave a talk on nanostructured microelectrodes for biomarker analysis. She described how electrochemical sensors with nanostructured surfaces allow rapid turnaround in biomarker detection, allowing infectious diseases to be diagnosed within 20 minutes. She also discussed how electrochemical nanosensors could impact transplantation medicine; rapid assessment of donated tissues and organs at a molecular level allows surgeons to quickly assess whether an organ is a good match for the recipient.

In 2018, the Pittcon nanotechnology symposium featured a talk by Heather Clark of Northeastern University on using an array of tunable nanosensors to detect small molecules and build images of neurotransmitter release in the brain. Paul Bohn of the University of Notre Dame presented the use of nanomaterials in electrochemical arrays and the use of electrochemical zero-mode waveguide arrays for imaging single reaction events. The food safety symposium at Pittcon 2018 will feature discussions on nanosensors for food safety including talks from Antje Baeumner of the University of Regensburg on nanomaterials for microanalytical systems, and Sam Nugen of Cornell University on nanobots for food safety.

Numerous applications for nanosensors have been reported, particularly in medicine. In one example, a group of researchers from China designed an electrochemical nanosensor for the quantitative detection of Brucella melitensis, a bacterium that causes brucellosis. The World Health Organization recommends preventing the spread of brucellosis among humans by monitoring and eliminating the infection in animals. However, methods for detecting Brucellae bacteria are often complex, expensive and time-consuming. As brucellosis is mainly an issue in the developing world, rapid, inexpensive, and easy-to-use detection methodologies for Brucellae must be developed.

The team from China were able to quantitatively detect low levels of Brucellae antibodies, which indicate the presence of Brucellae bacteria in animal milk within 1.5 hours. Gold nanoparticles were used to increase the electrode surface area and aid antibody binding to the sensor. The method utilized a cheap, disposable electrode and could provide the basis for an easy-to-use, hand-held device to allow the rapid detection of Brucellae bacteria

Chemiluminescence and fluorescence nanosensors

Chemiluminescence sensors can also be enhanced using nanoparticles. Chemiluminescence sensors detect the emission of light as a result of a chemical reaction involving the target molecule. For example, in a recently reported cholesterol chemiluminescence sensor, the cholesterol reacts with oxygen to form hydrogen peroxide, the hydrogen peroxide then reacts with luminol, producing a light emitting excited molecule. The light produced by the reaction is detected and measured to determine to concentration of cholesterol in the sample. Nanoparticles and other nanomaterials can catalyze chemiluminescence reactions. Furthermore, they can concentrate and localize the light produced, thereby increasing the sensitivity of the sensor.

Fluorescence nanosensors use an external light source to excite a target or product molecule, the light is then emitted at a different wavelength as the molecule relaxes, allowing the presence of the molecule to be detected. Surface modified nanoparticles can also be used to bind to the target molecule, producing fluorescent nanoparticles. Chemiluminescence and fluorescence nanosensors both require instrumentation that can accurately detect and measure light emissions, while fluorescence nanosensors also require an external excitation source.

Industry leaders including PerkinElmer, Malvern Instruments and Oriel were present at the 2018 Pittcon Expo displaying a range of optical meters and sensors, spectrometers, laser light sources, and fluorescence detection systems.

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3.7 NANOSCALE OPTICAL IMAGING: BREAKING THE DIFFRACTION BARRIER

Optical imaging of nanoscale structures has previously been limited by the 'diffraction barrier.'

Near-field techniques and the use of nanoparticles to enhance optical imaging techniques have previously pushed the boundaries of the traditional diffraction barrier. Super-resolution fluorescence microscopy has recently broken the diffraction barrier leading to a 'resolution revolution' in light microscopy.

For centuries, optical microscopy and other optical techniques have been vital components of the analytical toolbox. Light microscopes have provided many important discoveries, particularly within the fields of cell biology and microbiology. The resolution of light microscopes has traditionally been limited by the fact that light cannot be focused more sharply than diffraction allows, resulting in a 'diffraction barrier' at a resolution of approximately 200 nm, preventing light microscopes from resolving nanoscale objects. As many sub-cellular features are smaller than the resolution limit of optical microscopy, they cannot be observed using traditional light microscopy and other optical techniques.

Nanoscale imaging is an important and rapidly developing area of analytical science. To adequately understand cells and the effects that nanoparticles have on them, it is important to observe and characterize nanoscale structures inside cells, including both natural sub-cellular features, and engineered nanomaterials.
Until recently, observing nanoscale structures has relied on imaging techniques such as atomic force microscopy (AFM), X-ray based techniques, and electron microscopy. These techniques are often combined to provide information about complex biological

structures. For example, a recent study by Yangquanwei et al., at the University of Guelph, combined atomic force microscopy, scanning electron microscopy, and scanning transmission X-ray microscopy to study the nanoscale morphology, composition, and biochemical properties of quinoa chromosomes.

Pushing the Limit of the Diffraction Barrier

As the resolution diffraction limit of optical techniques depends on the incident wavelength and the objective lens of the microscope used, using near-field techniques enables imaging resolutions to move beyond the traditional far-field diffraction limit. Near-field techniques place the light source or the detection probe close to the sample (i.e., in the near-field). Such techniques include tip-enhanced Raman Spectroscopy (TERS), which provides resolutions below 25 nm. TERS can be used for the investigation of complex surfaces such as those of heterogeneous catalysts, as Bin Ren of Xiamen University will discuss at Pittcon 2018's nanotechnology symposium.

Combining Raman spectroscopy and other forms of AFM can provide additional information. For example, a study published in 2012 by researchers from Chemnitz University of Technology combined Raman spectroscopy imaging and current sensing AFM (CS-AFM), to investigate the properties of carbon nanotubes and find out whether the defect concentration in carbon nanotubes increased at the CNT/electrode interface.

Another technique that utilizes the near-field is near-field scanning optical microscopy (NSOM/SNOM), a form of scanning probe microscopy, which can achieve resolutions up to 20 nm. However, the requirement to place the excitation source or detection probe close to the target object makes it difficult to look 'into' a live cell or tissue, limiting the applications of near-field techniques in biology.

Using Nanoparticles to Improve Optical Imaging

Multispectral imaging captures image data at frequencies across the electromagnetic spectrum from near infrared to ultraviolet light, providing an array of analytical information. However, multispectral imaging technology is often expensive and bulky.

Metallic nanoparticles can act as optical antennas and concentrate and localize incident light, allowing them to act as near-field optical probes and interact locally with the sample. Nanoparticles can, therefore, be used to improve fluorescence imaging by increasing fluorescence efficiency.

Metallic nanoparticles have been found to enhance the intensity of Raman scattering by

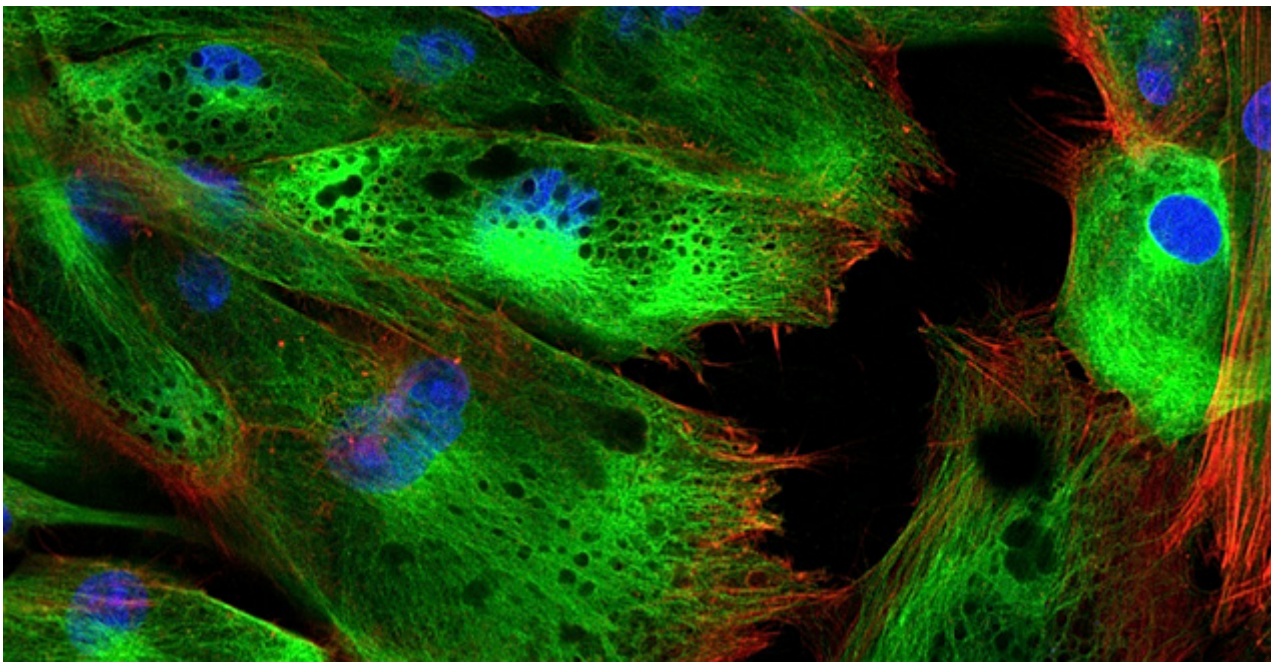
103-108 times, in a phenomenon that has been named surface-enhanced Raman scattering (SERS). SERS has been used in various studies to detect the presence of molecules at low concentrations, and provide spatially resolved nanoscale composition analysis.

Breaking the Diffraction Limit with Fluorescence Microscopy

Fluorescence microscopy is an optical technique that utilizes fluorescence tagging to image proteins, structures and cells. The ability to detect specific molecules in live cells makes fluorescence microscopy one of the most widely applicable techniques in cell biology. However, until recently the applications of fluorescence microscopy in biology were limited by the resolution diffraction limit.

Recent advances in fluorescence microscopy have led to the development of a number of super-resolution microscopy techniques that are able to break the 'diffraction barrier.' Super-resolution fluorescence microscopy provides 'diffraction unlimited' images. The diffraction barrier was broken in 1994 by Dr Stefan W. Hell and his team using a technique is known as stimulated emission depletion microscopy (STED).
In STED the physical or chemical properties of the fluorescent species are used to maintain neighboring molecules in different fluorescent states, turning fluorescence on and off and allowing them to be distinguished from each other. STED resolutions under 3nm have been reported, although 30-80 nm is more typical. Dr Hell received the Nobel Prize in Chemistry in 2014 'for the development of super-resolved fluorescence microscopy,' together with Eric Betzig and William Moerner.

Dr Hell's team has since developed a new fluorescence microscope called MINIFLUX, which provides a resolution of 1 nm, the ultimate resolution limit for fluorescence microscopy. Overcoming the diffraction barrier allows researchers to obtain images of living cells and tissues at nanoscale. Dr Hell, who is the Director of the Max Planck Institute for Biophysical Chemistry in Göttingen, will give the Plenary Lecture at Pittcon 2018 on the subject of breaking the diffraction barrier in fluoresce microscopy and MINIFLUX nanoscopy.



A variety of STED microscopes are now commercially available and instrumentation is becoming more compact, reliable and economical. Other super-resolution fluorescence microscopy techniques include stochastic optical reconstruction microscopy (STORM), photo-activated localization microscopy (PALM).

Dr Hell's team has since developed a new fluorescence microscope called MINIFLUX, which provides a resolution of 1 nm, the ultimate resolution limit for fluorescence microscopy.

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3.7a OPTICAL MICROSCOPY RESOLUTION REVOLUTION

Nanoscale optical imaging was covered in detail at Pittcon 2018, where 2014 Nobel Laureate Dr Stefan W. Hell gave the Plenary lecture on "Optical Microscopy: The Resolution Revolution."

as seen in Chapter 1. Alongside this, Dr Hell describes his discoveries in the below interview:

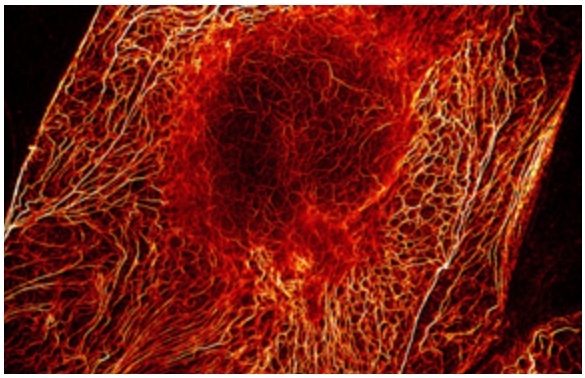
What will be the main focus of your lecture?

I will talk about the simple but fundamental idea that allowed breaking the diffraction barrier in fluorescence microscopy as well as about MINFLUX nanoscopy, the latest development in the field that for the first time provides true molecular resolution with visible light and standard objective lenses.

What was the key to breaking the diffraction barrier in a fluorescent life science microscope? How was the resolution-limiting role of diffraction overcome?

In the 20th century, light microscopes relied solely on focusing light in space for the separation (=resolution) of adjacent tiny features. Either they focused the illumination light as sharply as possible on the sample and/or the fluorescence light as sharply as possible on the detector.

As one cannot focus the light more sharply than to an extent given by diffraction, the resolution was limited to 200 nanometers. The key idea was to separate fluorescent features or molecules not by focusing but by transiently turning their fluorescence on and off so that features residing closer together than 200 nm could be distinguished by their sequential emission.



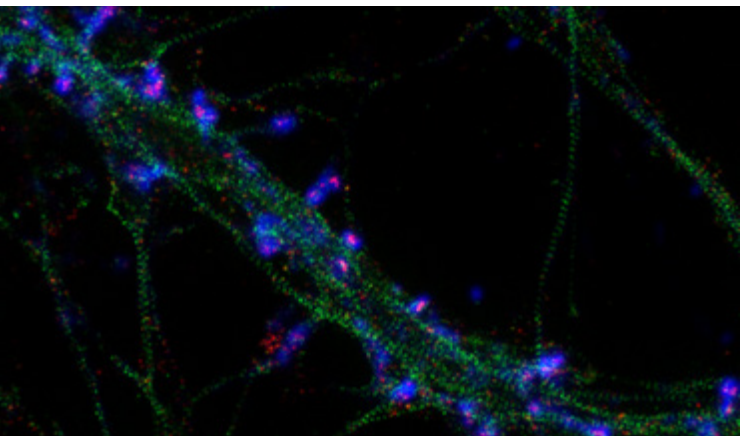
What impact has the ability to image the interior of transparent samples, such as living cells and tissues, at the nanoscale had on life sciences?

Scores of scientific studies in neurobiology, cell biology, and in many other areas of science have been carried out with fluorescence nanoscopy alias superresolution microscopy.

As the microscopes are becoming compact, easy-to-use, and less expensive, virtually all life science laboratories around the world will have to take advantage of the superior resolution in order to keep up in their respective fields.
In my view, every (single) beam scanning confocal microscope should have a STED option. Alternatively, any epifluorescence microscope can be easily upgraded to a confocal STED system.

What improvements in instrumentation would you like to see in the future?

Instrumentation will become rugged, reliable, and economic. As a matter of fact, the latest versions of STED microscopy provide state-of-the-art multicolor resolution < 30 nm with a compact module smaller than the size of shoe box. The price tag is less than a fifth of early systems. Moreover, the module fits onto literally any modern epifluorescence microscope, no matter if upright or inverted.



"As a matter of fact, the latest versions of STED microscopy provide state-of-the-art multicolor resolution < 30 nm with a compact module smaller than the size of shoe box."

Dr Stefan W. Hell

Have we reached the absolute limit yet? Do you think it will be possible to see features of the molecules, such as symmetries?

The latest edition of fluorescence nanoscopy, MINFLUX, has indeed attained molecular scale (~1 nm) spatial resolution. This is the ultimate limit, because in fluorescence microscopy the ultimate resolution limit is ultimately given by the fluorescent tags themselves, which act as proxies for the biomolecules to be seen.
Yes, I can imagine that one should see molecular symmetries using STED and related techniques, however, that will be an entirely different area of application.

What are the main challenges that still need to be overcome?

Given that MINFLUX has attained the ultimate limit, I believe that increasing the imaging speed, i.e. minimize the time to resolve molecules with highest resolution will be a major research thrust.

Read more about Dr. Stefan W. Hell's Plenary Lecture at Pittcon 2018

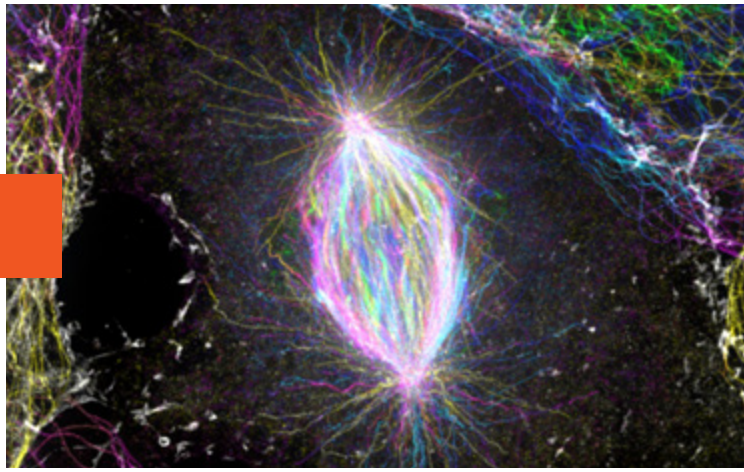
Other aspects will be sample compatibility and the quest for improved labelling techniques. Labels need to be small and minimally disturbing. Lots of progress has been made in these fields, but clearly more work needs to be done.

How do you think the imaging speed can be improved?

Concepts that require fewer emitted photons to attain the same resolution, such as MINFLUX, can be optimized further in order to attain maximum i.e. true molecular (1 nm) resolution with a minimal number of detected fluorescence photons, say < 30 detection events. Such an effective use of fluorescence photons will speed up nanoscale imaging enormously.

What impact do you think super resolution microscopy will have on neuroscience?

Just imagine a microscope that provides molecular scale resolution with minimal invasion. I think the impact of such a tool will be enormous. For example, we should be able to fully unravel the distribution of proteins at the synapse and also see many of the relevant molecules in action.



CONCLUSION

Nanoparticles have unique, tunable properties. As a result, nanomaterials have found applications in a vast array of fields including medicine, food technology, cell biology, and analytical chemistry.

The rapidly advancing field of nanotechnology presents new challenges for analytical chemistry. Understanding the behavior of nanomaterials in biological systems is particularly challenging, but vital for biological and medical applications of nanobiotechnology. Often, multiple techniques are required to adequately characterize the properties of nanoparticles and how they interact with complex chemical and biological systems.

The Pittcon Expo will always feature many industry-leading equipment manufacturers that supply the latest instruments for characterizing nanomaterials over a wide range of applications and environments. For researchers that are unsure of the best way to characterize their nanomaterial system, Pittcon is the place to find the answer.