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the Analytical Scientist

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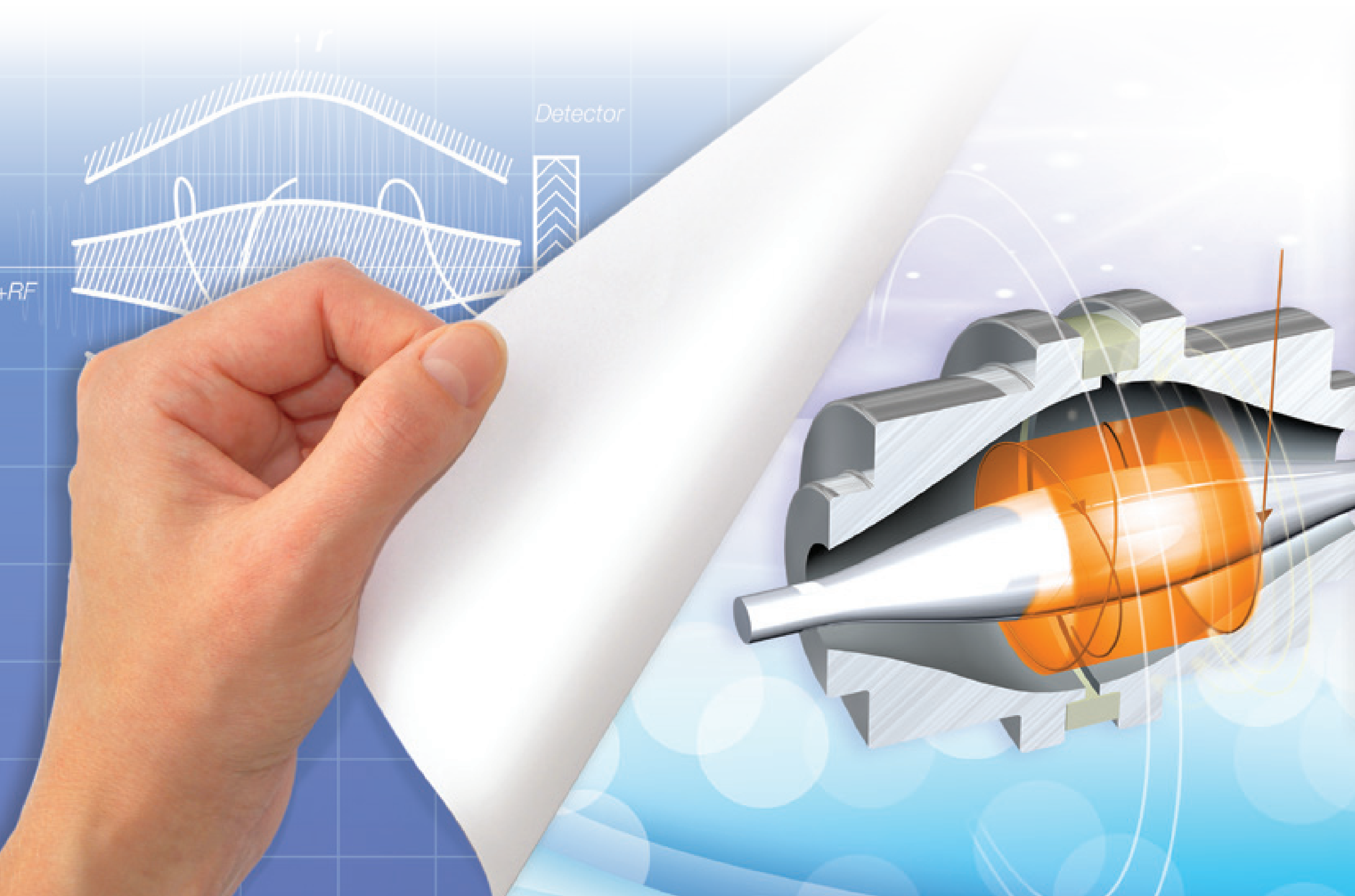

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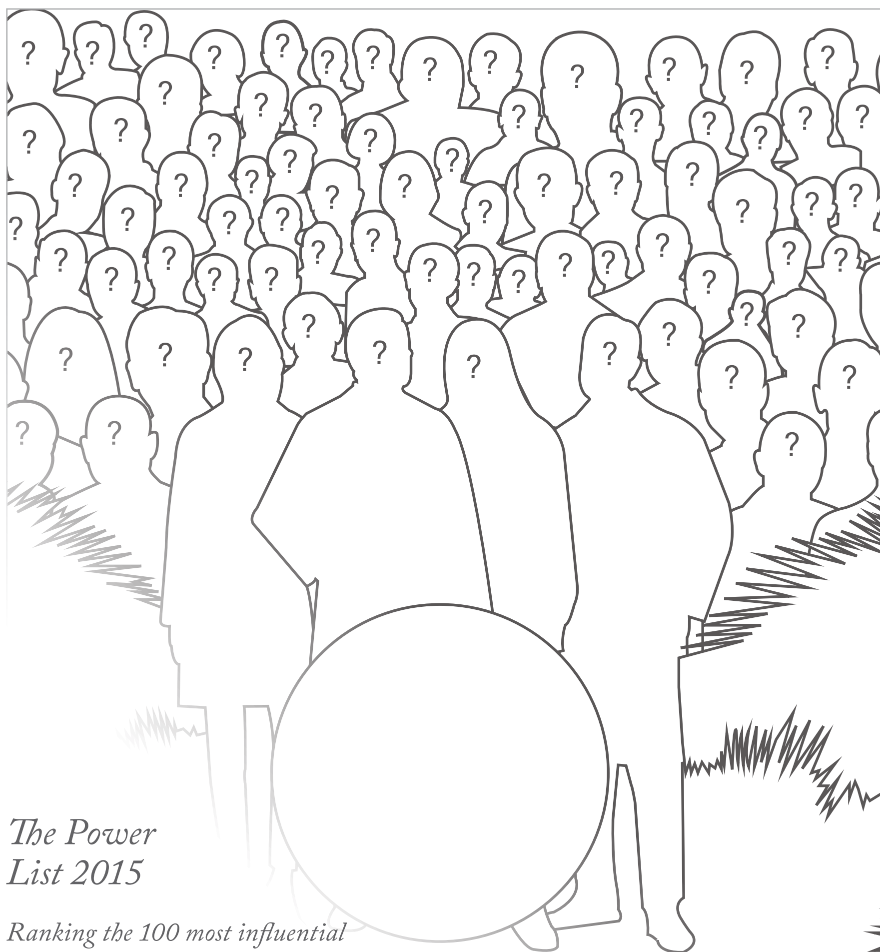

The Power List 2015

Ranking the 100 most influential people in the analytical sciences

In 2013, John Yates (page 26), Ruedi Aebersold, George Whitesides, Jonathan Sweedler, and Pat Sandra took the Top Five spots – what about 2015? Have your say today.

Nominate:
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The Art of Analysis

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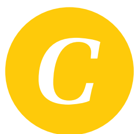

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2013 Power List Number One John Yates is joined by abstract elements of his passions.

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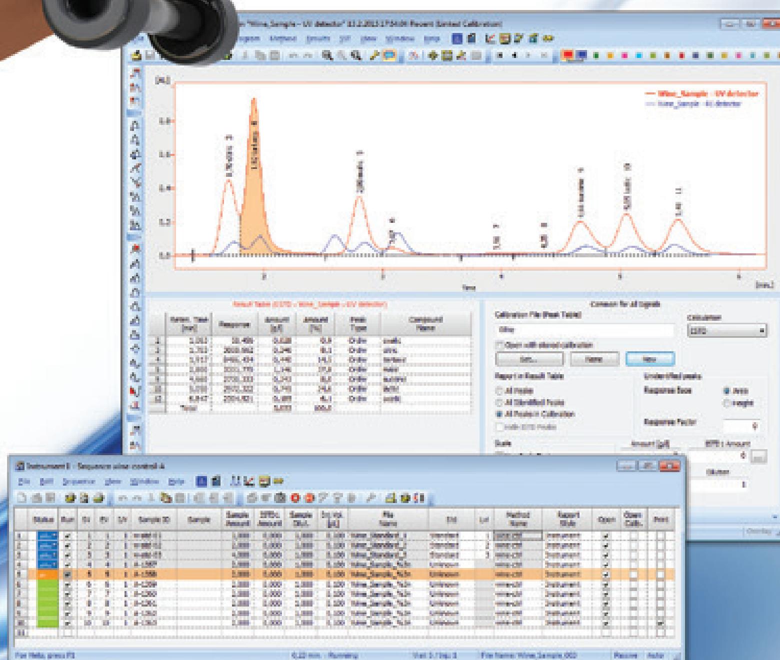

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the Analytical Scientist

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HUMANITY IN SCIENCE AWARD

&

the Analytical Scientist

Meet the Winners

Peter H. Seeberger and Andreas Seidel-Morgenstern

Peter H. Seeberger and Andreas Seidel-Morgenstern of the Max-Planck Institutes in Potsdam and Magdeburg have been chosen as the winners of the inaugural Humanity in Science Award for developing a method for the continuous flow production and purification of cheaper antimalarial medicines using plant waste, air and light.

They were awarded with a humble prize of $25,000 during an all-expenses paid trip to Pittcon 2015 and their insightful essay will be published in a future issue of The Analytical Scientist.

Could it be you in 2016?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

Has your own work had a positive impact on people's health and wellbeing? Details of the 2016 Humanity in Science Award will be announced soon.

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I’ve Got the Power

Actually, I don’t have the power. But you do, as we launch the 2015 Power List to celebrate the Top 100 most influential analytical scientists.

Back in 2013, The Analytical Scientist put its young reputation on the line by publishing a list of 100 names. The Power List drew its significant talent from your nominations and highlighted the passions, motivations and greatest achievments of gifted analytical scientists whose diverse areas of expertise covered everything from metabolomics to paper-based diagnostics to spectrochemistry.

In this month’s issue, John Yates III takes center stage. A humble guy who is forging a long-lasting legacy in proteomics and mass spectrometry, John tells his life story – why and how he got to where he is today – on page 26. Some of you will no doubt remember that John took the number one spot on our 2013 Power List – an accolade that belies his quiet and unassuming nature, but was nevertheless entirely deserved.

Therefore, it seemed entirely fitting to me that we should use this issue – and the ASMS 2015 meeting – to kick off the 2015 Power List by asking you to nominate the great and the good in analytical science.

Now, as John told me in New Orleans, “The Power List really put The Analytical Scientist on the map” – a great side-effect of throwing a positive spotlight on the field’s endeavors. Of course, he may have been politely telling me that it ruffled a few feathers... Clearly, it was not our intention to cause any friction in the community (quite the opposite), but some excellent and respected scientists did miss out. Why? Perhaps, because they weren’t even nominated. And just as about one half of the UK are reeling from the recent general election result, if you don’t vote, you can’t make your opinion heard.

Notably, the 2013 Power List disappointingly featured only eight women – none of whom made it into the Top 20. Is that really representative? And what about other inequalities? Both questions that are certainly worth bearing in mind as you make your selection.

And so, I use this month’s editorial message to urge you to nominate your beloved colleagues and respected peers – especially those who do not readily promote themselves or their own achievements – by completing the extremely short Power List nomination form at: tas.txp.to/0515/POWER or by emailing me at rich.whitworth@texerepublishing.com.

The Power List is a celebration – and I invite you to join me in cheering our wonderful field and the excellent work that you all do. You’ve got the power!

Rich Whitworth
Editor

Power List 2015 nominations now open.

Fill in the online form: tas.txp.to/0515/POWER or email: rich.whitworth@texerepublishing.com with the name, affiliation and reason for yor nomination.

Upfront

Reporting on research, personalities, policies and partnerships that are shaping analytical science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email:
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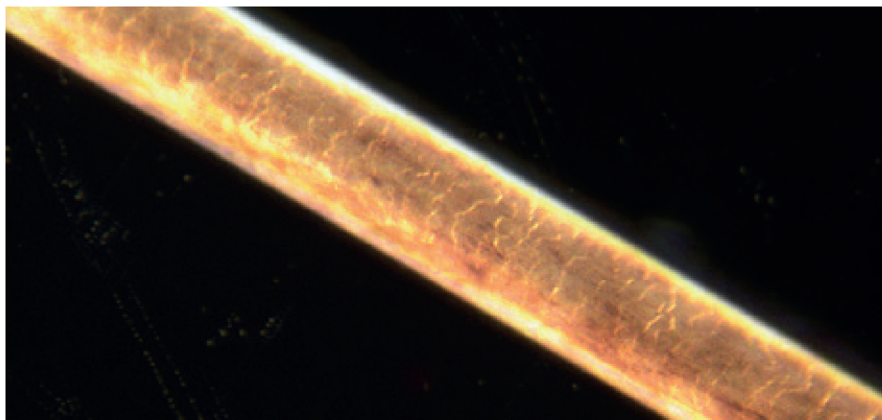

Forensic Science Under Fire

Hair analysis has been spectacularly debunked as "pseudoscience" – what now?

"FBI admits flaws in hair analysis over decades" – Washington Post (1), "FBI admits pseudoscientific hair analysis used in hundreds of cases" – Smithsonian (2), "Thirty years in jail for a single hair: the FBI's 'mass disaster' of false conviction" – The Guardian (3).

The headlines in popular media are damning to say the least. A report published on the Innocence Project (4) kick started the press frenzy – but what does it mean for the reputation of 'analytical' procedures? Jose Almirall, a professor in the Department of Chemistry and Biochemistry and Director of the International Forensic Research Institute at Florida International University, US, explores the issue.

How important is this case?

The Innocence Project report describes the errors in the reporting and testimony in around 90 percent of hair analysis and comparison cases reviewed. It is important to note that all these cases were examined prior to 2000 – the year that mitochondrial DNA analysis on hair evidence was included in the analysis protocol. It is also important to note that the FBI laboratory self reported the errors, even if this was not done as soon as it could have been. A large number of cases and individuals were impacted and these revelations will have a huge social and economic cost for years to come.

What exactly has gone wrong?

The errors were due to the overstatement of the significance of a match, when hairs were found to match in a hair comparison. Forensic scientists conduct the physical, chemical and sometimes biological analysis of evidence and then interpret the significance of the evidence as it relates to implicating an individual to a crime by associating the suspect to another person, to an event or to a location. The significance of the evidence should not be understated nor overstated, and forensic scientists balance the need to be precise in the language they use to describe the significance with the available support from the collective body of knowledge in the relevant scientific discipline. Until 2012, consensus standards focused on the interpretation of hair analysis and comparisons did not exist. It appears that some of the statements made by the examiners in their reports and in their

testimony were not supported by the available scientific literature.

Could this happen again?

The National Academy of Sciences report of 2009 (5) shed a bright light on the need to address many of the weaknesses within the forensic sciences, and so today, it is much less likely that such a systematic misinterpretation of evidence can take place. But (and it's a big but) there are still some disciplines for which there are no consensus guidelines or standards that address the significance of the evidence. The risk still remains to overstate (or understate) the value of the evidence for those laboratories not adhering to recommended standards or that conduct work in an area where there are no standards.

How about the role of analytical science and scientists?

Forensic scientists in the areas of biochemistry and molecular biology (DNA analysis) standardized both the analytical protocols and the interpretation of the significance of a matching DNA profile very early in the development of DNA analysis – and it was the FBI laboratory that took the lead in this effort. The relevant analytical community has developed scientifically rigorous guidelines and standards that are now widely used to promote precise language (including statistical descriptors) to communicate the significance of a DNA match for a particular case. DNA evidence is very reliable as a result and this high-quality evidence is also responsible for the exoneration of many wrongly convicted for other reasons (some of which do not have to do with science in the courtroom; for example, false eyewitness testimony, defense incompetence or prosecutorial misconduct).

What lessons should we learn?

The forensic science community should strive to develop interpretation standards that are supported by the scientific literature and, where the research to support interpretation guidelines does not yet exist, society should place a value on funding this research and encourage the community to develop consensus standards based on the research.

What do we do now?

One important (and ambitious) effort that was initiated this year is the creation of the Organization of Scientific Area Committees (OSAC), spearheaded by the National Institute of Standards and Technology (NIST). The aim of this effort is to bring together the top experts in diverse forensic disciplines to:

i. identify existing standards in both the analytical sciences but also the interpretation of scientific evidence in order to raise awareness of the existing standards by creating a national registry
ii. develop a strategy to create new standards in those forensic disciplines that need them.

It will then be expected that operational laboratories be required to use standard methods of analysis when such methods are warranted so that appropriate analyses are executed and the correct interpretation of the evidence is properly communicated to the court. We are making progress, but there is still a lot of work to be done.

Reference

1. *http://tas.txp.to/0515/hair1*
2. *http://tas.txp.to/0515/hair2*
3. *http://tas.txp.to/0515/hair3*
4. *http://tas.txp.to/0515/hair4*
5. *"Strengthening Forensic Science in the United State: A Path Forward" (National Research Council of the National Academies, The National Academies Press, Washington, D.C., US, 2009).*

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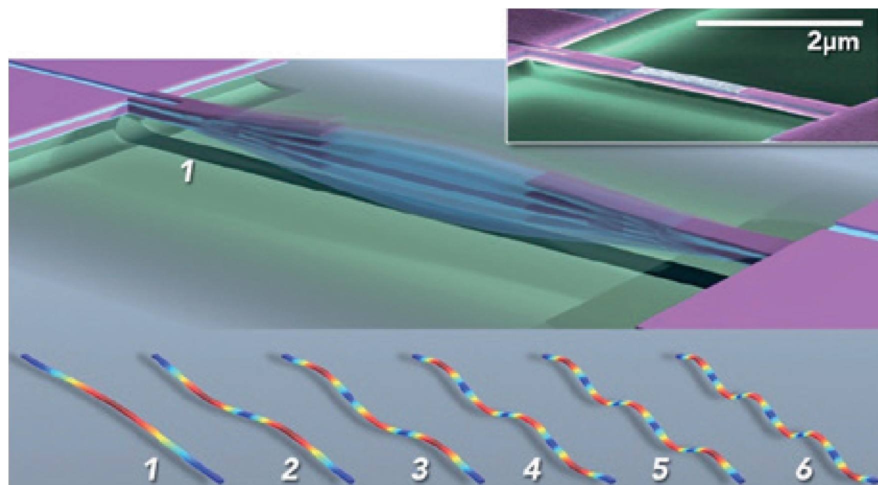
Single-Molecule Mass Spec with Inertial Imaging

Conventional mass spectrometry can only measure the mass of a molecule – what if you could image the same molecule simultaneously?



Credit: M. Matheny, L.G. Villanueva, P. Hung, J. Li and M. Roukes/Caltech

Multimode nanoelectromechanical systems (NEMS) based mass sensor; the main figure schematically depicts a doubly-clamped beam vibrating in fundamental mode (1). Conceptual "snapshots" of the first six vibrational modes are shown below (1-6), colors indicate high (red) to low (blue) strain. The inset shows a colorized electron micrograph of a piezoelectric NEMS resonator fabricated in Caltech's Kavli Nanoscience Institute.

John Sader, a professor at the University of Melbourne and one of the authors of a new study (1), says such MS imaging is now possible. Inertial imaging with nanomechanical systems (NEMS) was developed at the California Institute of Technology and could have a big impact on biomedical applications. Sader tells us more.

How did you get involved in the project?

The work arose during a one-year sabbatical at Caltech. I've been collaborating with Michael Roukes' group at Caltech since 2008, and spent the year (2012-2013, at Michael's invitation) working closely with his team as the Kavli Nanoscience Institute Distinguished Visiting Professor of Physics. It was during this period that his group asked me if I could improve on the mathematical theory they were using to interpret their NEMS mass spectrometry measurements (a technique they pioneered). After some thought, I managed to dream up a new mathematical theory that was radically different and much simpler than the existing one. Rather than using a complicating nonlinear algorithm, my new theory only required adding up the raw frequency measurements, to measure mass – at first sight it seemed crazy that this should work, but it does (and there's a theoretical proof!). It could also be trivially extended to a broader and new application: imaging the particle. This formed the basis for the inertial imaging work.

What is inertial imaging?

The idea is simple. Consider a vibrating taught string. When you pluck the string, and listen (and watch) closely, you'll notice that it actually vibrates at many different frequencies (and vibration shapes). Each of these frequencies corresponds to a different string mode. It is the combination of these modes, and the frequencies they generate, which makes a guitar string sound different to a violin string. This is what musicians normally call 'tone'.

Now, when a particle lands on the string, all of the string's frequencies drop; the musical note sounds lower and the tone changes. These frequency drops depend on where the particle lands. They are largest if the particle lands on an anti-node of the vibrating string – zero on a node. But the nodes and anti-nodes of all the modes occur at different positions along the string. So when a particle lands on the string, the frequencies of its many modes drop differently – this gives a 'frequency shift signature' for the particle. It's like a fingerprint!

The new theory interprets this signature by adding up the frequency shifts in a special way to measure the mass and shape of the particle.

What are the key benefits?

Conventional mass spectrometry only gives information about the mass of the particle or molecule. It cannot see what the particle looks like. In contrast, advanced microscopy allows the particle or molecule to be imaged, but not weighed. Our inertial imaging technology changes this paradigm by enabling an individual particle or molecule to be both weighed and imaged simultaneously. This gives a vital new piece of information that can be used for diagnostics.

For example in biomedical applications, mass spectrometry can be used to discriminate between

different proteins, molecules or pathogens in general, based on their weight. But it is entirely possible that two different molecules will have very similar weights. Inertial imaging now enables the difference between these two molecules to be distinguished, by measuring their individual shapes. This can be done molecule-by-molecule, one at a time.

How does it compare with conventional techniques?

In conventional imaging, light (for example) is fired at a sample, and the reflection or scattering of that light is measured, which generates an image. While these techniques work fantastically well, there are some well-known drawbacks. One is that it is very difficult to directly image a particle that is much smaller than the wavelength of light. This is the so-called diffraction limit. It's one of the reasons why electron microscopes are used, instead of light microscopes, to image very small nanodevices and nanoparticles – the size of these nanoscale objects is much smaller than the wavelength of light. Using electrons overcomes this barrier because their wavelength can be very small compared to that of light. But it can't simultaneously measure mass.

While inertial imaging also uses waves – in the form of standing waves of an elastic beam – the wavelength of vibration plays no role in the imaging resolution. This is completely counterintuitive. The particle can be many orders of magnitude smaller than all wavelengths of vibration used – inertial imaging will work! Rather than the vibration wavelength limiting the measurement resolution, it is the inherent noise in the frequency measurement that gives the ultimate limit. But our analysis shows that this is no issue. The noise in current NEMS devices is sufficient to image particles with atomic-scale resolution. Specifically, our work shows that the minimal resolvable size using current carbon nanotube NEMS resonators is 0.3 nm – roughly the size of a silicon atom. We are working to experimentally demonstrate the ultimate limit of this new technology.

What are the next steps?

Our next step is to fully explore the ideas we reported in our paper. For example, we want to develop new NEMS devices specifically designed to optimize the performance of inertial imaging, explore the full potential of the new mathematical theory and algorithm, understand its intricacies, experimentally demonstrate the ultimate limits of its performance, and implement inertial imaging in real time mass spectrometry amongst many other goals. My collaborators at Caltech and Melbourne and I are all very excited about the possibilities.

Reference

1. *M. S. Hanay et al., "Inertial Imaging with Nanomechanical Systems," Nat. Nanotechnol. 10, 339–344 (2015). DOI: 10.1038/nnano.2015.32*

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Unusual Analysis of the Month

Sons and daughters; love and lemur scent

If you're interested in animal behavior, you'll likely know that olfactory cues can affect how mammals interact with one another. Previously, we highlighted the "alluring" aroma of blood and its effect on carnivores (tas.txp.to/0415/bloodlust). Now, after conducting research at Duke University, Christine Drea, Professor of Evolutionary Anthropology, and her student Jeremy Chase Crawford (who has since moved to the University of California) say they can tell whether pregnant lemurs will give birth to a male or female by analyzing the scent secretions of the mother-to-be (1). Drea believes that olfactory cues are often underappreciated, and that their relationship with gestation is poorly understood.

Drea and Crawford collected secretions from ring-tailed lemurs for gas chromatography-mass spectrometry (GC-MS) analysis. They used a measure of "chemical richness" (the number of different chemical compounds) and compared results between nonpregnant and pregnant lemurs. Richness decreased with pregnancy and, importantly, dams bearing male offspring showed a greater decrement in richness than those bearing females, particularly later in gestation.

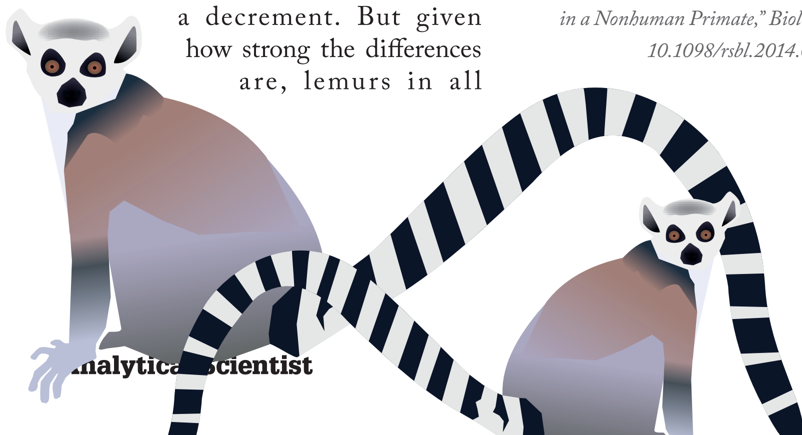
"It isn't clear to me yet why dams with sons would show such a decrement. But given how strong the differences are, lemurs in all likelihood can detect them," says Drea. "If this phenomenon evolved as an adaptation, it would have to confer a benefit to the pregnant female. In this scenario, the pregnant female may use the information about her own scent as a type of self-referent/phenotype matching mechanism, allowing her own body to act on the information provided by these signals, such as continuing to invest in a fetus or to disinvest."

For Drea's lab, the latest study was a logical next step in their work; they've been studying lemur olfactory communication for quite some time. "The main challenge when working with mammalian odors is that it is very difficult to identify the exact compounds that are responsible for signaling [...] given that each secretion contains hundreds of compounds, in varying proportions, it hasn't yet been possible to show which ones are responsible for the many pieces of information conveyed," says Drea.

Could the research have implications for other primates? In principle, Drea believes that fetal sex-differentiated patterns in a mother's scent could well be present more broadly. Certainly as a diagnostic test, the process is labor intensive, so the work is more relevant to those studying animal behavior; olfactory cues may be linked to promoting mother-infant recognition, reducing intragroup conflict, or counteracting behavioral mechanisms of paternity confusion. *SS*

Reference

1. *J. Crawford and C. Drea, "Baby on Board: Olfactory Cues Indicate Pregnancy and Fetal Sex in a Nonhuman Primate," Biology Letters, DOI: 10.1098/rsbl.2014.0831 (2015).*



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PREP Preview

Two advances in (bio) pharmaceutical manufacture that aim to democratize the gift of medicine

Andreas Seidel-Morgenstern – a PREP 2015 Scientific Advisory Committee member – won the 2015 Humanity in Science Award (www.humanityinscience.com) along with Peter Seeberger for his part in a new process to manufacture lower cost anitmalarials. By coupling flow chemistry with advanced chromatography methods, Seidel-Morgenstern and Seeberger were able to manufacture artemisinin combination therapies (ACTs) – the most effective drugs to treat malaria – from plant waste material, air and light. The new process is currently being implemented in a pilot plant in Vietnam and produces an active pharmaceutical ingredient with a purity of greater than 99.5 percent. Look out for the full story behind the project in an upcoming issue.

Seidel-Morgenstern, who is director of the Department of Physical and Chemical Foundation of Process Engineering at Max-Planck Institute in Magdeburg, highlights aspects of the work: "An efficient isolation of a continuously synthesized target component requires the development of advanced separation processes. Considering the reactor effluents generated in Peter's group as pseudo-ternary mixtures (an impurity fraction 1, the target, and an impurity fraction 2), artemisinin and artesunate could be purified with our process using several periodically operated chromatographic columns. The approach can be applied to also solve other challenging separation problems."

And though the chemistry and engineering involved in the project are both spectacular and innovative, the potential impact of the resulting complete process is spellbinding. Peter highlighted in his acceptance speech that 660,000 people die of malaria each year – and 90 percent of those are children under five. In other words, it is a disease of poverty.

Seidel-Morgenstern will give a lecture in a special keynote series at PREP 2015 to honor the late, great Georges Guiochon – a preparative chromatography visionary. Seidel-Morgenstern offers a brief sneak preview: "I will describe both my time in Knoxville with Georges and the direct connection between the courses of adsorption isotherms and the shapes of elution bands. This problem brought me to Knoxville and still keeps me busy!"

Meanwhile, researchers from the Austrian Centre of Industrial Biotechnology and the University of Natural Resources and Life Sciences Vienna have developed a continuous purification method for production of lower cost recombinant antibodies from clarified CHO cultures (1). The team converted a two-stage batch precipitation-based antibody capture step to continuous mode using tubular reactors. There is no protein A capture step; instead, the precipitation process uses calcium chloride and ethanol – both inexpensive reagents.

"In essence it is a continuous precipitation, but it is also very adaptable and could be a platform process," says Alois Jungbauer, one of the authors of the study, and also a member of both the PREP 2015 and ISPPP 2015 Scientific Advisory Committees. When Jungbauer and his team first started investigating continuous processing, they hit a dead end. "The time was not ready for continuous manufacturing and all of my colleagues from the pharma industry told me that this technology is not relevant. But now things are changing."

Jungbauer believes that the team's continuous principles can compete with conventional protein A capture steps in terms of yield and speed, and they've now done studies with several antibodies using feedstock from pharmaceutical companies. "Protein A is still the workhorse in the biopharma industry for antibody manufacturing," says Jungbauer. "It works very well and it is a complete, mature technology. It is the benchmark that any new technology has to beat."

The big question is: can you use Protein A to bring antibody production down to less than $10 per gram? One thing is certain: lower manufacturing costs = increased access to medicine.

Jungbauer, alongside Giorgio Carta and Alan Hunter, will be giving a workshop at PREP 2015 on preparative chromatography for biomolecule purification. And Mark Schure (featured on page 19) will tackle multidimensional LC for bioseparations at ISPPP.

PREP 2015 (the 28th International Symposium on Preparative and Process Chromatography) will take place July 26–29, and ISPPP 2015 (the 35th Symposium and Exhibit on the Separation and Characterization of Biologically Important Molecules) will take place July 29-31, both at Loews Hotel, Philadelphia, PA, USA.

For more information, visit:
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In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

Rewarding Industry

The Uwe D. Neue award recognizes the impact that breakthrough work by industrial scientists has on analytical science. 2015's recipient is Mark Schure – and here's why.

By Martin Gilar, Principal Investigator, Waters Corporation, Milford, Massachusetts, USA.

Established in 2013 by Waters Corporation, the annual Uwe D. Neue Award in Separation Science at the HPLC Symposium, honors a distinguished industrial scientist who has made a significant contribution to the field of separation science for at least 15 years post graduation. Importantly, awardees should be instrumental in the embodiment of technology in commercial products.

There are two main reasons for establishing the award in separation science: we want to honor the legacy of Uwe Neue, late scientist and Waters Corporate Fellow, and we want to recognize and promote outstanding researchers working in industry – people whose scientific work generally goes unnoticed. These people tend to be encouraged to patent rather than publish; they may design instruments used by other researchers rather than make discoveries themselves. In short, the Uwe D. Neue Award recognizes industrial researchers who are helping to shape the landscape of separation science.

Uwe Neue was a unique individual. With his big mustache, German accent, and wide smile, he was a person you noticed when you met him in the hallway. A visit to his office made an equal impression; piles of papers, manuscripts and books covered his desk, chairs, shelves, and floor. Although not known for his filing skills, he always knew the location of any document hidden in his office.

Uwe's impact on Waters HPLC columns and instruments is undeniable. He found time to write papers and he published "HPLC columns: Theory, Technology and Practice", a very popular book. And he was always ready to discuss a research problem with junior colleagues, whether they came from academia or the industry. In the latter part of his career, he attained the highest scientific rank at Waters (a category created specifically for him), which gave him the opportunity to publish more freely than before.

Following his untimely death, I met with my Waters Corporation colleagues to discuss whether we could establish an award that would fulfill the above-mentioned goals. I reached out to members of the HPLC symposium permanent committee – Barry Karger (North Eastern University), Attila Felinger (University of Pécs), and Peter Schoenmakers (University of Amsterdam). I also sought advice from Gerard Rozing (Rozing.com Consulting), who managed a poster award sponsored by Agilent Technologies at the HPLC symposium. From their feedback, the idea of the Uwe D. Neue award took shape. With the support of the Waters chief technology officer, Dan McCormick, I proposed the award to the top-level leadership at Waters Corporation. It hit the right note with them, they offered their sponsorship, and the award was born.

J. Jack Kirkland (Advanced Materials Technology, Wilmington, Delaware) was the recipient of the first award presented at HPLC 2013.It recognized his life-long contribution to separation science and the development of superficially porous particles, now one of the most efficient

sorbents used for HPLC separations. In 2014, the award went to Gerard Rozing for his active role in Agilent LC research, including the field of microfluidic based liquid chromatography.

This year, at the HPLC 2015 symposium in Geneva, I will present the award to Mark Schure, also featured here. His contribution to LC theory, including the theory of sampling in two-dimensional LC (2D-LC), quantitative definition of 2D-LC orthogonality, and modeling of chromatographic processes, is well known from his papers, tutorials, presentations, and as a previous chair of the HPLC symposium (Philadelphia 2004). Researchers in the field of biopolymer separation will also recognize him as a co-chair of the International Symposium on the Separation of Proteins, Peptides and Polynucleotides. Schure's career has parallels to Neue: they both used mathematics to solve separation problems.

The Uwe D. Neue award has a growing reputation and I hope that it will continue to help with providing the recognition that many other industrial scientists deserve. Although I have my own views on the awardees, the rest of the selection committee – Monika Dittman (Agilent Technologies), Guowang Xu (Dalian Institute of Chemical Physics), Wolfgang Lindner (University of Vienna) and Barry Karger (who replaced the late Georges Guiochon in 2015), may offer their own reasons why they voted for the awardees.

Candidates for the Uwe D. Neue award are nominated by their peers. The nomination letter should include the individual's impact on separation science – a strong list of patents, publications, and presentations undoubtedly helps. The nomination package is valid for three years but can be updated, if necessary. Please send nominations to: martin_gilar@waters.com.

A Rewarding Life

This year, I feel proud and humbled to have received two special accolades – the Dal Nogare Award and the Uwe D. Neue Award. After many years in both industry and academia, it's extremely gratifying to be recognized. Here's how I got where I am today.

By Mark Schure, Adjunct Professor of Chemical Engineering at the University of Delaware, and Chief Technology Officer at Kroungold Analytical, Blue Bell, Pennsylvania, USA.

At Pittcon 2015, I received the Dal Nogare Award from the Chromatography Forum of Delaware Valley. I was thrilled because, as I explained to the audience, all four of my chromatography mentors – J. Calvin Giddings, my post-doc adviser, Peter Carr who is also a dear friend, Jack Kirkland who has mentored me for 30 years and is a wonderful person, and the late Georges Guichon, to whom I was very close – were recipients of the Dal Nogare Award. Mary Ellen McNally read one of the seconding letters; a long list of my accomplishments written by Georges made the occasion very special.

I guess my "big-ticket item" is that I have more than 30 years of simulation science experience to bring to bear on chromatography and other separation problems. It's proven to be powerful for solving problems that are far too difficult and complex for mathematical investigation and for which experimental investigation

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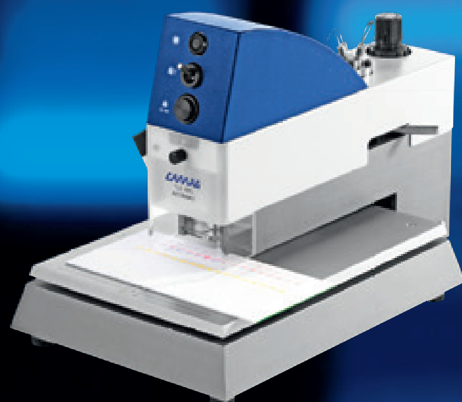

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is not definitive. Simulation is the third paradigm of science and I was probably the first to bring these types of techniques, which require advanced computer power. But it's not all about introducing new techniques, it's about using them to unveil the mechanism of separations.

Let me elaborate. I've worked with two types of particle simulation. One is a molecular simulation technique called "Configurational Bias Monte Carlo in the Gibbs Ensemble". This works very well for phase equilibria problems, which is essentially what chromatography is about. In chromatography, no one seems to be able to agree on the separation mechanism because it requires "further study" or it is very difficult, if not impossible, to elucidate experimentally. Ilja Siepmann from the University of Minnesota Department of Chemistry and I have been working on this problem for over 15 years.

Ilja had developed Configurational Bias Monte Carlo, which we believed to be the best option ever for obtaining equilibrium results. Other simulation techniques that looked at liquid chromatography (LC) used molecular dynamics. Having worked in that field for years, I knew you could not rely on molecular dynamics to provide good equilibrium results. However, the Configurational Bias technique coupled with the Gibbs Ensemble (developed by Athanassios Panagiotopoulas at Princeton University, Princeton, New Jersey, USA) worked well for phase equilibria. So, we used this approach to solve problems such as how does reversed-phase LC work. We knew we'd got it right because we compared our energetics – free energy calculations – to experimental results and they were nearly an exact match. This matching with experimental results is typically how you validate a simulation, and, in our case, the result was striking.

The other type of simulation I've worked on is Monte Carlo Transport; it is also known as Brownian dynamics and molecular dynamics, but it is a particle-based technique – not molecular-based. It's used to solve transport problems. One example of this is simulating the flow through a packed bed incorporating retention, convection and diffusion – all the elements of packed-bed LC. And we'd already solved the dispersion calculations you need for LC by running tracers through model packed beds under non-retention conditions. This is a very powerful simulation technique, but let's be clear, it's not molecular, it's transport based. You begin with the flow field, send your tracers through the porous medium and watch their arrival times as they leave the column. It's just like watching a physical solute leaving a chromatography column. This approach has been applied to packed-bed LC, pore diffusion in ion exchange chromatography, capillary electrophoresis and a host of other techniques used in separation science.

It also has applications for field-flow fractionation (FFF) and its various versions; in fact, we figured out a long-standing mystery of what Coriolis forces do in sedimentation FFF. From our simulations, we discovered that when people did sedimentation FFF, they were rotating the channel in the wrong direction. It made me realize how powerful these techniques were because you can figure out from the simulation what the experimentalist may not know. So, simulation is a closer match to the actual experiment than it is to theory-based investigation.

Keeping it practical

One thing I've also known is that it is important to keep practical applications in the headlights! Indeed, if you are doing anything – and this includes deep theoretical and deep simulation work – it needs a practical outlet. Let's take reversed-phase LC: if you know how it actually works, you can play with the parameters, which gives you a better feel for what the experimentalist wants to do with the technique. It also gives you a test bed for further discovery and optimization.

That point really hit home with elucidation. Where do the solutes you want to separate go into the phase – do they embed in the bonded chains or do they sit on top? And, of course, you can do this without actually having to run a series of experiments. This is clearly something that interests industry and I'm all for sharing academic work with industry, having spent many happy years working in industrial settings. Indeed, Uwe Neue also had good academic connections and he knew the power of doing basic experiments.

For most of my industrial career, I've been a modeler and have had to deliver practical results. I could use any approach I thought would shed light on the problem and that generally entailed using a lot of computing power. For example, there was an ion exchange division at Rohm and Haas and I would often be asked by my colleagues to explain how things worked at the molecular level. I've also done many biomolecule calculations to investigate binding sites – the business recognized that asking me was the most practical way to get an answer, especially because computing power had increased a lot, relieving us of inexact simulations or the need to solve differential equations by mathematical methods that often gave broad-brush but not definitive results.

As computing became cheaper and more powerful, and people began building their own computer clusters, investigations became increasingly more practical. It encouraged them to predict that in 10 years the majority of their work would be simulation-based with people running powerful computer programs. The reality of the 10 year estimation is proving elusive and today we are still writing our own software for about half the work we do, even though there is more commercially-available problem-solving software on the market.

Industry meets academia

Uwe Neue understood the need to link

industry with academia and he reached out to universities to augment his own knowledge and studies. For my own part, I've been an adjunct professor in the chemical engineering department of the University of Delaware for 20 years. The department has many bright people – faculty and students – and I'm fortunate to work with them. Additionally, I consult for Advanced Materials Technology in Wilmington, Delaware, USA, and the company willingly funds work-study students from the chemical engineering department. I currently have four people working with me – all honors students and exceptionally gifted.

I also have good access to other professors whenever I need their insights. It's great to have such high caliber people to work with to augment my own knowledge and experience, which is important because separation science is interdisciplinary and you need input from academics who understand other issues that may not relate immediately to injecting a sample and seeing a chromatogram.

I've been lucky to have good co-workers at the University of Minnesota as well – that's where Peter Carr is. Pete introduced me to Ilja Siepmann and continues to provide input to our studies, which have been funded by the National Science Foundation for the last 14 years.

The team approach

Academia is clearly different to the industrial world. In industry, you concentrate on product development and effective utilization of resources to execute a development process. This isn't really a focus of university work. Nevertheless, universities like Delaware are becoming more team-oriented even though academia generally focuses on basic research. That said, I sometimes had to do basic research in industry as part of the product development cycle – in reality, there is not a very clear separation between the two. Indeed, the most successful people in industry are those who can work on both sides.

I said earlier that separation science is interdisciplinary – especially in theoretical separation science. I need to be a computer guy, a chemical engineer, a mathematician, and even an electrical engineer, which is one of my lesser-known specialties. And when you run out of ideas you have to find someone with a different knowledge base who will become part of your team – and that's the case in both industry and academia.

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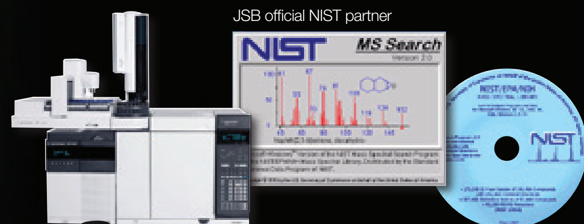

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Zeroing in on Zero Gases

How can a metrology project to measure zero help in the goal of cleaner air?

By Stephen Harrison, Global Head of Specialty Gases & Specialty Equipment and Peter Adam, Customer Applications & Engineering, Special Products & Chemicals, Linde, Munich, Germany.

Back in March, we witnessed the UK government issuing health warnings as a dangerous cloud of smog from the continent blew across Britain. In France, the situation was considerably worse, with such dense smog that a 14 mph speed limit was imposed on Paris motorways. And though we know there are many triggers for respiratory ailments, it's generally recognized that certain VOCs can have severe, adverse influences on human health. Unfortunately, it's a disquieting fact of life that we're routinely exposed to VOCs throughout our daily activities, from driving our cars to working in our offices or even just sitting at home.

With the ever-increasing awareness of the potential for negative health effects from the air we breathe, it's not surprising that requirements for low-level traceable calibration standards are becoming of greater importance. We recently saw the Euro VI automotive emissions legislation enacted – the most stringent environmental law to date in the European Union. It not only requires automotive manufacturers to use multiple new calibration gas mixtures and high purity gases for monitoring and analysis, but also prescribes detailed specifications to calibrate and zero instruments. But the use and importance of so-called 'zero gases' goes way beyond the application in automotive emissions testing to more general ambient air quality monitoring.

As many readers will know, the calibration of high precision analytical instruments used for environmental testing, such as gas chromatography and Fourier transform infrared spectroscopy often requires two or three set points to create a calibration curve and a zero point setting to establish the baseline reading. To create this zero point setting, a zero grade gas is needed. The increasingly lower concentrations of pollutants permitted by new legislation mean that an accurate zero set point has now become extremely important.

We are also seeing a trend towards national and international specifications being driven by atmospheric monitoring stations and metrological institutes who recognize the value of having accurate reference points when measuring impurities anywhere in the world. Today, we need a measurement approach for the single and simultaneous assessment of the impurities of zero gases and a protocol for their certification.

National metrology institutes, industrial gases manufacturers and instrumentation companies have recently come together to do just that by playing a collaborative role in a project called MACPoll (Metrology for Chemical Pollutants in Air). MACPoll is part of a European metrology research program focusing on zero gases R&D for air quality applications. Special focus is being placed on reactive impurities, such as nitrogen oxides, sulphur dioxide, ammonia and hydrogen sulphide, with a view to minimizing these components to quite challenging concentration levels. The impurity levels used in the MACPoll project defined very ambitious specifications for zero gases, including 0.5 ppb – or even 500 parts per trillion nitrous oxide, 2 ppb ammonia and 1 ppb sulphur dioxide. Considering that as recently as 10 years ago, a typical impurity of nitrous oxide would have been in the order of in 100 ppb, technological development appears to be moving at a very rapid rate.

Many technological challenges have been successfully tackled through the MACPoll project and its achievements are highly regarded, providing good preparation for the high quality calibration gas mixtures of the future. Its work on accreditation of zero gas began about five years ago and whilst the first phase of the project is now complete, we believe that full commercialization of its outcomes is about three years away.

> *"With higher accuracy comes a greater degree of confidence in the analytical data being reported."*

Undoubtedly, higher accuracies in calibration mixtures enable better accuracy, standardization and precision of measurement internationally, bringing measurement in different countries onto a more level playing field. With higher accuracy comes a greater degree of confidence in the analytical data being reported. And because the mix is traceable back to a national or international standard, all gas producers, end users and environmental agencies can have a higher degree of assurance that the analytical data being reported is accurate and uniform across the world.

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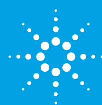

Agilent Technologies

Pulling the Trigger on Complex Problems

As associate scientific director of Nutritional Chemistry and Food Safety at Covance Laboratories, Katerina Mastovska must tackle the most challenging analytical questions. And she loves every second.

Born into a family rich in chemistry heritage (both of her parents are chemists/food technologists), Katerina (Kate) Mastovska has been working in laboratories since the age of 14, surrounded by chemistry and talk of food safety. No surprise then that, despite her mother and father's protestations, Kate was keen to follow in their footsteps when it came to choosing a route through academia. In particular, food analysis was top of the list. "You would earn more money doing economics or law," her parents insisted. But Kate's mind was made up. Keen on mathematics and with a fondness for puzzles (just like Mike Thurman and Imma Ferrer: tas.txp.to/0515/mikeandimma), chemistry – and its practical application in food analysis – was a logical move in many ways.

When did your passion for contaminant analysis begin?

Back in the early 1990s, I joined Jana Hajslová's lab at the Institute of Chemical Technology (ICT) in Prague, working on pesticides amongst other things. And that's really where my passion for contaminant analysis began. In this field, our overall goal is to make food safer – and our work has a real impact. On the analytical side, my passion stems from the challenge – food samples are highly complex; measuring over 100 compounds at very low levels in a complex matrix is about as tough as it gets. And every compound has a story – I really enjoy designing methods to meet those challenges.

After my PhD, I worked as a research chemist at the US Department of Agriculture (USDA) for about seven years. I joined Covance in 2009. In essence, my role at Covance is all about solving problems. Some people dislike problems, but I actively seek them.

So, you've been in academia, government labs and now industry – has your work changed?

Definitely – the environments are very different. And I have to say I enjoy my current role the most, predominantly because it is so fast-paced. I also like the client relationship aspect – I can really see the immediate impact. Whatever I do really needs to be very practical and – in all honesty – commercially valuable. It also offers freedom for research – an excellent combination.

There is certainly more pressure working in a client-facing environment, but I actually enjoy it; as I said, I love complex challenges. We work a lot with the food supplement industry – botanical extracts and other highly complex matrices – and that adds yet another layer of complexity. But of course, it's more about the satisfaction in finding a solution than the problem itself. A problem without a solution is no fun at all.

How do you typically approach problems?

Of course, different problems require different approaches and tools, but in general, my first step is always to gather as much information about the issue as possible. In other words, we must first analyze the problem itself. Sometimes,

I already have a solution in mind, if not, I employ a logical and stepwise approach to find the root of the problem – and then I isolate it. Researching the literature and reaching out to peers is essential – someone may have been down the same path before. The rest is down to the right tools and experience.

So, what's in your analytical toolbox?

One big issue, especially for contaminants in complex matrices, is the need for assured compound identification; finding compounds that should not be in the sample has a huge impact for our clients or their suppliers, so correct identification is essential. How do we approach this? Well, we ensure that we have as much information available as possible. When it comes to pesticides, for example, we routinely use both GC-MS/MS and LC-MS/MS, which gives us orthogonal overlap in terms of compound coverage. In many cases, we go above and beyond international guidelines on minimum requirements for identification. More specifically, in LC-MS/MS, we are now routinely using triggered multiple reaction monitoring (MRM), which allows us to acquire more information from the compound by looking at more than two MS/MS transitions. The extra information that triggered MRM provides is a huge benefit when it comes to compound identification because it increases our confidence. We cannot make avoidable mistakes.

Our clients may come back to us and ask, "Are you really sure this compound is in our sample?" And though we may not be able to be 100 percent confident, it's great to know that you have the additional information you need to support your findings.

Part of my job is to let clients know what the information actually means. The fact that a compound is found in a sample may not be a problem from a regulatory point of view, but it may kick start an investigation into the source of the contamination. After all, many of our clients also need to go above and beyond the minimum requirements to protect their brand and reduce potential risks. And certainly, when sourcing new suppliers, the more information I can give our clients, the better.

And presumably "above and beyond" is a moving target?

Absolutely. Global trade is far more intensive and complex than it used to be – and the rate at which we exchange information has also increased. Both of those factors account for an increased vigilance from a contaminant analysis point of view.

But let's not forget that there have also been huge increases in analytical technology and software over the last 20 years – and that too has had a big impact. We can measure much lower concentrations and more compounds than ever before. And it's also much easier, so we can run more samples. No doubt, that has made life difficult for decision makers in the food safety arena – especially on the industry side – where the question is always "how low do we need to go?"

For Covance, increased globalization also means that we must use the same methods, reporting systems and quality systems at all locations around the world. We've also harmonized our analytical instrumentation to ensure that we have the same capability across the globe.

Could you share any methods you have recently introduced?

Since we got the new Agilent 6490 system we have developed several methods to include triggered MRM. In particular, we implemented an LC-MS/MS method for low-level analysis of regulated mycotoxins in infant formula. It's a very high-stake analysis area, and so the additional information afforded by triggered MRM on the potential presence of contaminants is hugely beneficial.

> *"One big issue, especially for contaminants in complex matrices, is the need for assured compound identification".*

The biggest change – which is a project I've been working with Agilent on – is a new method for the analysis of over 500 pesticides. On the LC-MS side, that's over 450, once again using triggered MRM. We're currently in the process of finalizing that method ahead of validation and global roll-out. The new method has doubled the number of pesticides (for LC-MS) and added about 200 pesticides overall. We've improved the LC separation and, of course, the triggered MRM allows us to gather that additional information for compound identification.

Where do you think food analysis is heading?

Well, we're still learning and still moving forward. We are a long way from being able to use a "tricorder" style device to identify every contaminant in food. Cast your mind back to the melamine incident... that took us all by surprise. We weren't looking for melamine in food products. Now, we test melamine routinely. I think as a field, we all want to prevent the next melamine-type tragedy.

Embracing technology that allows us to gain as much information about our samples as possible is our route forward – until we get our hands on a tricorder!

Pioneering Proteomics

My love of biology, mass spectrometry and computer programming has led me down an uncommonly rewarding path of discovery and innovation. Here, I share some of my story and look towards the horizon of my exciting and ever-changing field.

By John Yates III

1983... Somewhere in the United States of America...
"I really should combine my interests in biology, chemistry and mass spectrometry. What's out there? OK. MS-based protein sequencing looks like the future. And that means there are really only two serious options: the laboratory of Don Hunt at the University of Virginia or Klaus Biemann's lab at MIT..."

The wonder years

I grew up in a military family, which meant moving often, changing schools and making new friends. One advantage to the nomadic lifestyle was crisscrossing the US several times and visiting spectacular sights like the Grand Canyon, the Painted Desert, redwood forests, and meteor craters – you can't look at the Grand Canyon and not wonder how and why! It was a really natural introduction to wondering about 'life and nature'.

I was first excited by science and medicine when I was a freshman at high school. It wasn't very far into the American football season (the first game) when I broke my leg in a tackle. I was hospital bound in a military facility, and for the first time, I began to consider the science behind what was going on around me. Certainly, the experience made me take science – particularly biology and chemistry – more seriously at school. And when my chemistry teacher presented a demonstration in class making nylon, I was captivated; the fact that you could mix two chemicals together and create something new simply blew me away.

After high school I went to the University of Maine to study zoology, and during this time I took a course in organic chemistry. I loved it and was tempted to change my major, but I was already a third year student, so changing my major would delay my graduation. Instead, I stayed in zoology, applied to medical school in my forth year and didn't get in, so I had to think about a Plan B. I figured I'd do chemistry for a year, and then re-apply to medical school at a later stage. However, it turned out that the chemistry department had just invested in a mass spectrometer. It was amazing – in part because it was attached to a computer, which was about as close as you could get to a PC at the time (1980). It was a Hewlett Packard GC-MS with a HP computer and it was so cool; you could do library searching of spectra! This serendipitous experience completely changed my plans, and I never once reconsidered medical school.

I was immediately hooked on chemistry and made plans to move onto a PhD. I decided I wanted to study MS-based protein sequencing, as it combined my interests in chemistry and biology. Don Hunt had just published a paper on fast atom bombardment (FAB – invented by Michael Barber in 1981) for soft-ionization of peptides (1). It was at the cutting edge – and I wanted in on it. Don Hunt is a great innovator and his lab was an exciting place to be in 1983; joining his group turned out to be one of the best decisions of my life.

A source of change

Advances in mass spectrometry are very often driven by changes in ionization techniques. New methods come along that allow you to do new things – then instrument manufacturers modify their instruments to take full advantage, like boosting mass range in the case of FAB. And so throughout the 1980s, researchers were all racing ahead with FAB, and in Don's group that meant understanding what it could do for peptide analysis. While our main competition focused on using tandem double-focusing magnetic sector instruments (behemoths that required a million bucks and a room of their own), Don was really pushing the idea of using triple quadrupoles for the sequencing of peptides. He believed in the power of triple quads and by 1986 we made it work to sequence peptides (2).

Of course, when electrospray ionization (ESI) came along in the late 80s, it totally changed everything. ESI worked well with triple quads, and as a result, sector instruments all but disappeared since it was difficult to do HPLC with their slow scan speed. At about the same time, I completed my PhD and joined Lee Hood's lab at Caltech for a post doc. Lee's group had developed the gas-phase protein sequencer that was a 1000 times more sensitive than Edman's spinning cup protein sequencer and was pushing to use it with 2D gels. Lee's lab (with Reudi Aebersold – also a post doc in the Hood lab) was championing the use of two-dimensional gel electrophoresis to do entire proteomes – by pulling spots off the gels and running them through the gas phase sequencer. Once again, I was immersed in a lab that was leading advances in the field, and surrounded by colleagues who went on to make significant contributions in science.

The human genome distraction

Hood's new sequencing technology helped ignite the biotechnology boom in the US. That breakthrough meant that people were sequencing proteins that they hadn't been able to before, and coupled with recombinant technologies, many of these new proteins could be used as therapeutics.

It was really fortuitous that I joined Lee's lab, but it didn't seem like it at first. Lee had about 70 people working with him and had just put together the crucial components that made up the microchemical facility – the ability to sequence and synthesize both DNA and proteins. Naturally, many in the lab were turning their attention to sequencing the genome and there were big discussions on how best to do it. I was in the protein sequencing section of his lab and remember thinking to myself,

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"what's the future of protein chemistry?!" Despite concerns about the relevance of protein sequencing, I started working on how I could interface mass spectrometry with information that would come out of the genome projects. I also considered the possibility of using mass spectrometry to sequence DNA – after all, the search was on for faster and more accurate sequencing technology. Where did I fit in the grand scheme?

I didn't get far on the latter idea, but I had a bunch of different software tools and a small database of protein sequences; it occurred to me that we might be able to use mass spectrometry information with the database to identify proteins in a faster timeframe, which led me to a peptide mass fingerprinting strategy in 1993 (3). A number of other groups came up with the same idea – there were about five papers on the subject that year. But ultimately, I noticed that it was pretty easy to get fooled with mass fingerprinting in terms of false positives, and that triggered my thoughts on how to use tandem mass spectrometry data.

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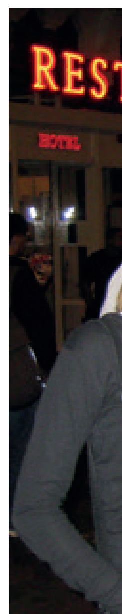
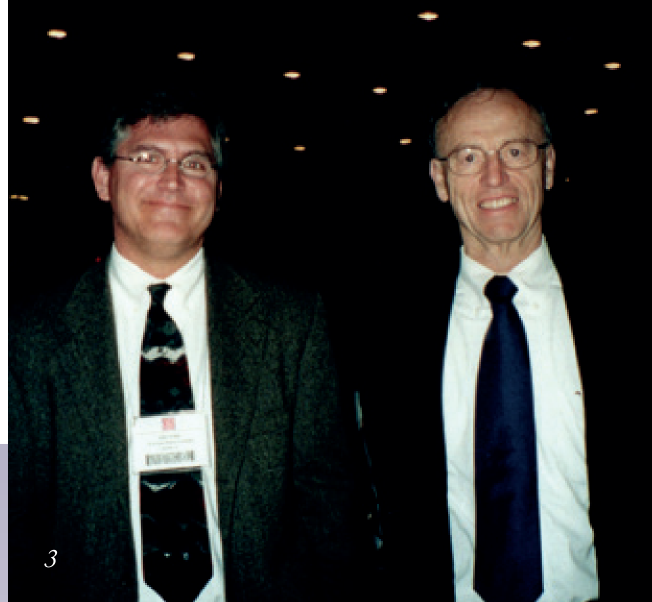


Code master

While we were manually sequencing some MHC Class 2 proteins, and waiting for some partial sequencing results to come back from the BLAST server at NIH, I started to wonder why we couldn't just send the entire spectrum off for a database search so we didn't waste time sequencing things that were already known. I took a day off to figure out how to match spectra to sequences and wrote some trial computer code. I convinced myself it would work and hired a programmer – Jimmy Eng – to work on the project full time. He was an electrical engineer but his masters project involved neural networks for language processing – and that intrigued me. Could we use the same strategy to interpret mass spectra? Jimmy didn't know what a protein or mass spectrometer was, but I figured I could teach him that. He had some programming experience, but not as much as I thought might be needed. But Jimmy was exceptionally



1) Yates Laboratory summer party ~2003. 2) At Pittcon with Steve Gygi (left) and Mike Washburn (right) – both have been leaders in proteomics. 3) At ASMS with Don Hunt at ASMS. 4) At ASMS in the early 1990s with Don Hunt and Klaus Biemann (right side). 5) Celebrating promotion to full professor. 6) Me as a graduate student interpreting tandem mass spectra with treasured PC. 7) At ASMS with Ian Jardine. 8) Out on the town with Thermo scientists in Munich at HUPO 2004. 9) Me as an assistant professor at the University of Washington ~1994. 10) Me as a graduate student at the University of Virginia with Patrick Griffin (left) and Jeff Shabanowitz (center). Jeff was an instrumental wizard and built many important instruments in Don Hunt's laboratory. Pat was a fellow sequencer of proteins by mass spectrometry.

bright and hardworking, and hiring him turned out to be a lucky turn of fate and the beginning of a great partnership. There were a couple of technical problems to solve at first, like getting information out of proprietary MS files, but somewhat surprisingly, the program worked pretty well from the get go. And so, in 1993 SEQUEST was born, but it took several rejections of the paper before it was published in 1994 (4).

Disruption often seems to come from people new to a field rather than those who have been around for a long time. And I was well prepared to take the leap of faith – I knew how to code, I knew how to manually sequence peptides from mass spectra, I'd gotten interested in databases through working with a program called PC/GENE, and I had smart, enthusiastic people in my group willing to take risks with me. Everything just came together at the right moment.

I guess what separated me from the crowd at the time was my love of coding. Going back a couple of years, a lot of my classmates at Maine used to moan about how hard computer programming was and I felt quite intimidated by it. Eventually, I forced myself to take a class to see for myself. I loved it, but it was not easy to do on a regular basis, as it required having time on a mainframe computer. But when I came to Virginia, PCs were becoming more widely available and I picked the programming back up. I even convinced my wife to let me buy a PC (it cost about $1500 – quite an investment for a pair of grad students!) and I started writing programs in Turbo Pascal, back when programs were limited to 64kb or something (hard to imagine for some of the younger readers I'm sure...)

I have a philosophy that anything you do is not necessarily wasted effort as it may be useful in the future. In that spirit I wrote a lot of programs at Virginia that were not necessarily practical or useful. One of the programs I wrote predicted all aspects of a protein sequencing workflow. I could take a protein sequence, do a virtual tryptic digest, predict the HPLC retention time and plot out the chromatograms for the resulting peptides and predict and plot the fragmentation patterns for all the peptides. Therefore, for a given protein sequence I could generate quite a bit of theoretical information. Working in that way really got me in the right frame of mind for what was to come a few years down the road.

> "Disruption often seems to come from people new to a field rather than those who have been around for a long time."

Must scan faster

Tandem MS is a fantastic mixture analysis tool and armed with a way to quickly interpret the data I started thinking about a way to circumvent the use of gel electrophoresis. What became clear from database searching strategies was that the tandem mass spectrum for a peptide was more or less a zip code for a protein. By extension, you could digest a mixture of proteins and run them through the mass spectrometer and match the individual peptide MSMS back to the protein. While I was at Virginia, Don Hunt used to say that you could sequence two proteins simultaneously from a mixture with tandem MS methodology. While true, the data interpretation was slow and data collection needed to be comprehensive. The creation of sequence databases would make this concept both possible and practical.

LC was getting to the point where we could run increasingly complex mixtures and we really started pushing the technology to identify proteins in mixtures. We moved from complexes, to organelles to cells and finally to tissues. Of course, each step required more and more sophisticated separation technology, more advanced mass spectrometers and better software tools.

Ian Jardine has already told his story in The Analytical Scientist (tas.txp.to/0515/Jardine) and he was indeed heavily involved in a lot of the work I was doing. I took SEQUEST to Ian, and he immediately understood the potential and capability in mixture analysis without gel electrophoresis. Basically, he recognized that it was a game changer and wanted to exclusively license it from the University of Washington where I was working at the time. Part of Ian's genius was being able to cut through all the academic noise to figure out what direction the field was heading. Ian wanted to push mass spectrometry into the biochemistry field, and considered SEQUEST to be one of the main stepping stones towards that goal.

I remember visiting Thermo (Finnigan back then) to see the beta version of the new LCQ Ion Trap – Ian's first big project – and because mixture analysis was at the forefront of my mind, my mantra was "must scan faster, must scan faster." The guys were justifiably proud of the instrument and I was so impressed

and excited when they showed it to me, but to their dismay I kept asking, "Can't you make it scan any faster?" I think they were dumbstruck, but I explained why – and sure enough, every model that followed was able scan faster. Indeed, faster scanning speed is still at the heart of discussions today.

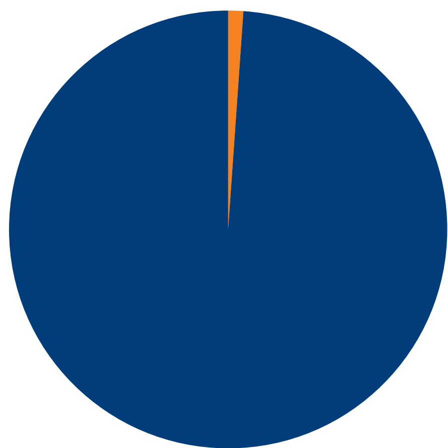
As part of the licensing agreement for SEQUEST, I got an LCQ Ion Trap and that set us on a long road of collaboration on direct protein mixture analysis using LC-MS. Essentially, I wanted to use technology to understand diseases. As the mass spectrometers got better, we were able to collect more data, so we needed to develop more advanced software tools. It was a very positive cycle of invention and re-invention.

Even back when I was at Caltech, Ian put a mass spectrometer in my lab, a move that was absolutely key at the start of my career but also an important catalyst for moving mass spectrometry into the field of biochemistry as Lee's laboratory was one of the epicenters of protein biochemistry. I remember giving talks on mass spectrometry and proteins in the early days and there would always be someone from conventional protein sequencing grilling me. It was with great satisfaction that about five years later, they were all moving to do mass spectrometry. Needless to say, traditional sequencing was being obviated pretty quickly by the rapid changes in the mass spectrometry world.

Most of the equipment in my lab today is from Thermo Fisher Scientific – partly because of my long-term collaboration with them, but also because it just works. I bought a QTOF once – and while the instrument looked spectacular on paper in terms of mass accuracy, it just didn't seem up to the task in terms of robustness. On the other hand, my ion traps were working 24/7 and they never broke down – and even though I wasn't getting the same mass accuracy or resolution, it has to be said that it's better to have data than no data. I eventually got to the point where I would only buy Thermo equipment. Later, when the Orbitrap was introduced, it was a complete game changer for the field because it produced robust high resolution, high mass accuracy data – now everyone has to have one.

The Yates Lab today

Unsurprisingly, I'm still very interested in proteins from a very disease-centric perspective. We're trying to increase



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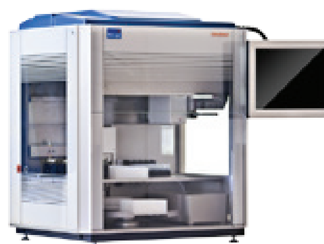

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our understanding of how protein networks, protein-protein interactions and protein modifications change as a function of disease. Today, the tools and developments we've been working on for the last 20 years have led us to the point where we are asking very specific questions about disease.

Unfortunately, in terms of funding, proteomics frequently seems to take a backseat to genomics. Funding for genomics is quite good and funding agencies don't hesitate to spend a billion dollars on certain strategies even if the results aren't that great. Proteomics simply doesn't get that kind of attention. And I think that's because genomics has a very significant track record for finding disease genes, with the hope that gene discovery will lead to cures.

A good example is cystic fibrosis. The gene was found in 1989 with genomic technologies, but it's taken 26 years of traditional biochemical and proteomics studies to better understand the biochemistry of the problem. Clearly, discovering disease genes is a good thing (and can lead to spectacular careers in science – the current head of the NIH was on the team discovering the CF gene), but equal resources need to be devoted to the technologies that allow genomic discoveries to both be understood and to be turned into cures.

To that end, we've been working on cystic fibrosis for over a decade and we've developed some interesting approaches to help understand the mechanisms of the disease. Now, we are looking at six very clear drug targets. One candidate can rescue the disease to the same extent (in cell cultures) as a drug that is about to come on the market. There are several other disease projects in my group at various stages, but the CF project is the most advanced.

I guess I have come full circle; I started out wanting to go into medicine, and in a sense I have. As a physician you can have a positive impact on the lives of thousands of people, but as a scientist, you can make a difference to millions by making discoveries that change the way disease is diagnosed or treated. I remain grateful that what seemed like a setback early in my life turned out to be the auspicious opportunity that led me to a fulfilling career in science.

“I have a philosophy that anything you do is not necessarily wasted effort as it may be useful in the future.”

Protecting our proteomic future

The big difference between genomics and proteomics (apart from the complexity) is funding. There was a very deliberate and well-crafted push by US funding agencies to develop next-gen sequencing technology, with the aim of getting down to the $1000 genome. One of my former post docs now works at NIH and was involved in developing a focused program to create disruptive, next-gen tools for protein analysis. Unfortunately, the NIH decided not to pursue it. This is disappointing not only from the loss of funding to the field, but also because a focused and deliberate strategy could have yielded big results.

Mass spectrometry and proteomic methods are having a very broad impact on biological science. I feel strongly that the breadth of this impact is not yet well understood or appreciated. Almost every study published that involves proteins will have used mass spectrometry in some form or other. But very often that aspect of the work gets buried in the supplemental methods – if it's reported at all. It's almost become commodity science (Rich Whitworth focused on this problem in his editorial last month based on our discussions: tas.txp.to/0515/commodity). When science is treated as a commodity, people stop citing papers. Consider electrospray ionization – no one cites the work of Yamashita and Fenn (5) when they use it anymore, but they do cite BLAST if they use it. I really don't understand why it's acceptable for some areas of science and not others. I worry that with increasing commoditization of proteomics, funding agencies don't appreciate its impact either.

Without recognition, it's difficult to develop new technology that allows you to ask new questions. To quote the theoretical physicist Freeman Dyson, “[Technology] is the mother of civilizations, of arts and of sciences.” Indeed, new technology allows us to do things that we couldn't do before. And while I think people appreciate this fact if they stop and think about it, they quickly forget. Almost all technology began life in a laboratory somewhere – likely funded by a fundamental science grant. It can be frustrating to see my own field of science being commoditized before its time.

Regardless, mass spectrometry instruments are rapidly

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evolving and continually pushing the frontiers of bioanalysis, particularly in proteomics. But has the technology reached incremental status or is there a potential disruptive innovation that could emerge and completely alter the landscape?

We have to ask: how can proteomics gain access to the kind of massively parallel next-generation sequencing strategies that genomics have adopted? Mass spectrometry is inherently serial; we need to spend time and effort on speeding up proteomics platforms. At this point, it is hard to imagine a new and disruptive technology emerging to replace mass spectrometry. But then again – I guess that is the nature of a disruptive technology: you don't see it coming!

I am keenly aware of the need for disruptive innovation in proteomics, but once you have a large established group, moving in a new direction can feel like turning the Titanic. I'd love to have a 'Skunk Works' section in my group, working on exotic, high-risk projects, but NIH would probably need to be better funded for that to happen…

Maintaining our "momentome"

From a technical standpoint, we're on the verge of being able to do whole proteomes. Admittedly, the term 'whole proteome' is still somewhat up for debate, as expression and modifications change over time, unlike the genome. My definition is our ability to identify all of the proteins present in a particular mixture in a routine and robust way – and I think that's coming in the next few years.

Once we've achieved that goal, we can increase the amount of sequence coverage (from a bottom-up perspective) so that we can start asking questions about modification states. While we're doing that, we need to focus on improving top-down approaches, where there are still a lot of technical challenges. The latest generation of mass spectrometers will somewhat democratize the world of top-down proteomics because we won't all need 15-Tesla magnets – and that should move the field along much faster. Over the next 10 years, I expect to see increasingly improved ways of fragmenting larger and larger proteins, which will also have a huge impact on top-down approaches.

As I indicated, finding ways to parallelize analyses must also be high on the agenda to drive technological advances. We've been working on ways to look at entire networks of proteins in a single experiment so that we can investigate the dynamics of pathways. And we are also developing new software approaches to meet the needs of new methods of analysis. These advances don't parallelize mass spectrometry per se – we're still acquiring data in a serial fashion – rather they parallelize the questions being asked.

Software is still key, but I think the standard proteomics tools are now pretty robust –you know, we probably don't need a 40th version of SEQUEST. Instead, the community must focus on new tools that allow us to ask new and different questions.

"I'd love to have a 'Skunk Works' section in my group, working on exotic, high-risk projects"

Seeking new technologies that can help solve new problems always stimulates me. However, it can also be frustrating when we gain access to those technologies; how do you choose between 10 different great applications of that technology when resources are limited? The answer is "with difficulty," but I'm immensely proud of the work that my group has done, is doing and will continue to do.

Fundamentally, what motivates and excites me the most is solving problems and finding clarity. And that is what I will continue to do.

John Yates is Ernest W. Hahn Professor of Chemical Physiology and Molecular and Cellular Neurobiology at The Scripps Research Institute, LaJolla, California, USA.

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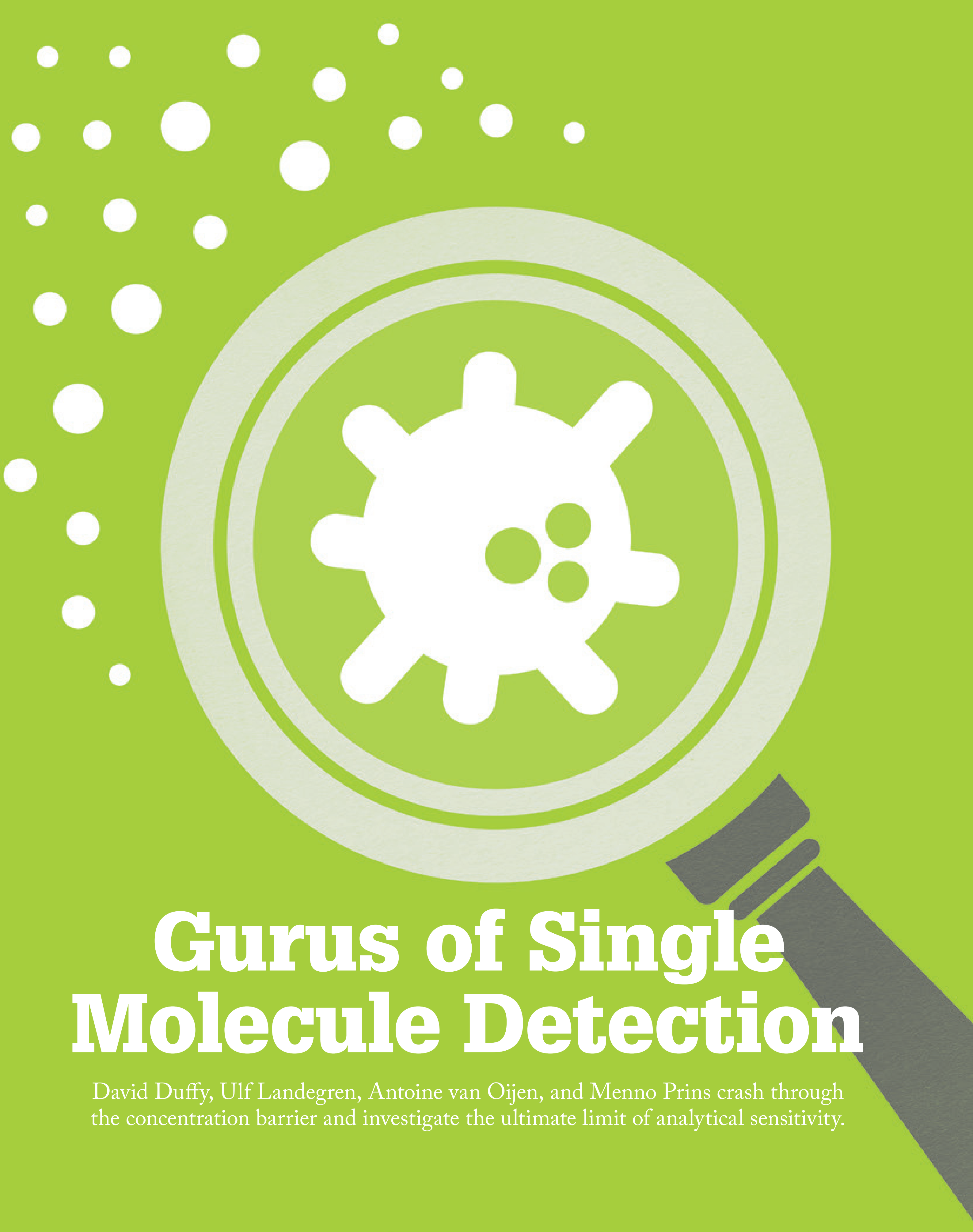


Gurus of Single Molecule Detection

David Duffy, Ulf Landegren, Antoine van Oijen, and Menno Prins crash through the concentration barrier and investigate the ultimate limit of analytical sensitivity.

How are you involved in single molecule detection (SMD)?

Antoine van Oijen: My academic research group develops single molecule biophysical tools to study complex, multiprotein systems. We are interested in understanding the basic molecular mechanisms that underlie processes such as DNA replication, viral fusion, and membrane transport. As a professor and group leader, I focus on defining scientific projects, applying for funding, recruiting personnel (PhD students, postdoctoral fellows) and advising and mentoring them throughout their research.

Ulf Landegren: I am professor of molecular medicine at Uppsala University and I'm on the board of Olink Bioscience, a spinout company commercializing protein assays; I am also involved in a few other companies. Both in academic and industrial contexts, we are developing tools for life sciences; some of these are for single molecule level analyses for diagnostic applications.

David Duffy: We develop instruments for single molecule detection aimed at revolutionizing the measurement of proteins to improve healthcare. My role is developing and refining our fundamental single molecule array (Simoa) technology and associated instrumentation and I collaborate with clinicians and researchers to develop new applications.

Menno Prins: I am a professor at Eindhoven University of Technology and lead a research group that investigates molecular biosensing technologies, based on single particles and single molecules, for fundamental research and for near-patient testing applications.

Can you offer some historical perspective?

AvO: Up until a few decades ago, the ability to observe individual molecules seemed like science fiction. As a PhD student, I was fortunate to be involved in the early years of single-molecule detection, at a time when not many people worldwide were attempting to visualize single molecules in highly idealized model systems. Over the years, these tools have been applied increasingly to the understanding of how proteins work and have revolutionized how we think about molecular mechanisms and protein dynamics.

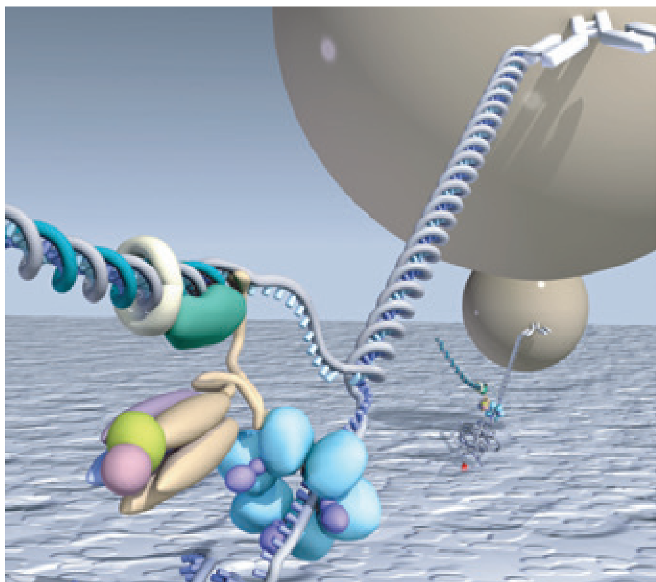
UL: Kary Mullis, inventor of PCR, saw how ridiculous it was to talk about 1.66 yoctomole instead of simply saying one molecule. Now, with digital PCR, we can count individual DNA molecules, and there are also routine detection techniques for single proteins, although not every single molecule is detected in these protein assays – it's more like one out of every hundred or so.

SMD in life science applications requires great specificity as well as detection sensitivity to identify individual molecules in the vast complexity of real biological or clinical specimens. For example, our padlock probes can detect unique gene sequences or (reverse transcribed) transcripts. Once specifically reacted, the probes are amplified locally using rolling circle amplification (RCA) for convenient SMD by microscopy or in flow imaging.

Our proximity ligation assays (PLA) allow detection of individual proteins or protein complexes by pairs of antibodies modified with DNA strands (1). Upon proximal binding by the antibodies, the attached DNA strands guide the formation of DNA circles, which are replicated locally via RCA, just like padlock probes (2). This is now a standard single protein detection technique, combining specificity and sensitivity, which has more than 1000 users worldwide, and is applicable for analyzing clinical samples prepared for microscopy.

MP: The development of instrumentation for biochemical patient testing always starts with mechanical automation for increased throughput, and thereafter proceeds to a phase of miniaturization and integration to allow decentralized and more flexible testing. A sizable market has developed for decentralized point-of-care testing, with blood glucose testing being the most mature application. Point-of-care testing technologies continue to be developed for biomarkers such as small molecules, proteins, and nucleic acids. Beyond point-of-care testing, the next step in miniaturization and integration will be the development of devices for real-time patient monitoring, with biochemical sensors integrated into medical devices that remain for some time in contact with patients. This latter field is still in a very early phase. I believe that SMD technologies will progressively penetrate into all three segments: high throughput, point-of-care, and patient monitoring. In the single-molecule regime, every molecule counts and biology becomes digital, which can be advantageous for extracting maximum information out of a biological process.

DD: We developed a method for measuring proteins called "Digital ELISA", a single molecule version of the analog enzyme-linked immunosorbent assay (ELISA) used for measuring proteins since the early 1970s. Our objective was to use single molecule sensitivity to drive huge increases in sensitivity over existing immunoassays. The technique has its roots in digital PCR, where single molecules are confined within very small volumes (picoliter to nanoliter) and quantification is achieved by counting them. To get digital ELISA to work for counting single proteins, you must measure single enzyme labels in wells that are much smaller (about 50 femtoliters [fL]) than those used for digital PCR. In 2005, David Walt at Tufts University and David



Artist's impression of a single-molecule experiment that allows the visualization of the DNA-replication process at the single-molecule level. A long DNA molecule is coupled between a bead and glass surface with a laminar flow mechanically stretching the tether. Tracking the positions of the individual beads reveals kinetic information on how fast the DNA gets unwound and duplicated by the protein machinery that supports replication.

Rissin, his graduate student, managed to do this with trapped solutions of beta-galactosidase in 50-fL well arrays etched into glass. The wells were sealed in the presence of the enzyme substrate with a silicone gasket. A single enzyme generates about 3,000 fluorphors in 30 seconds, and when sealed within 50-fL it can be detected using a standard laboratory fluorescence microscope. By counting enzymes in this way, we assumed that we could obtain huge increases in sensitivity to immune complexes labeled with beta-galactosidase. At Quanterix, we used this idea to develop digital ELISA by first capturing proteins on microscopic beads, labeling them with the enzyme, then sealing and counting them in femtoliter well arrays. We published this approach in Nature Biotechnology in 2010.

What is your view on current developments?

AvO: We are now at an exciting time when single-molecule biophysical approaches that unravelled basic molecular mechanisms are being used in biotechnological applications. The use of SMD in next-generation sequencing (Pacific BioSciences and Oxford Nanopores) and in super-resolution imaging (Nobel Prize Chemistry 2014) are the best examples. I think that many exciting developments will emerge in the future that will use single-molecule tools in diagnostic applications.

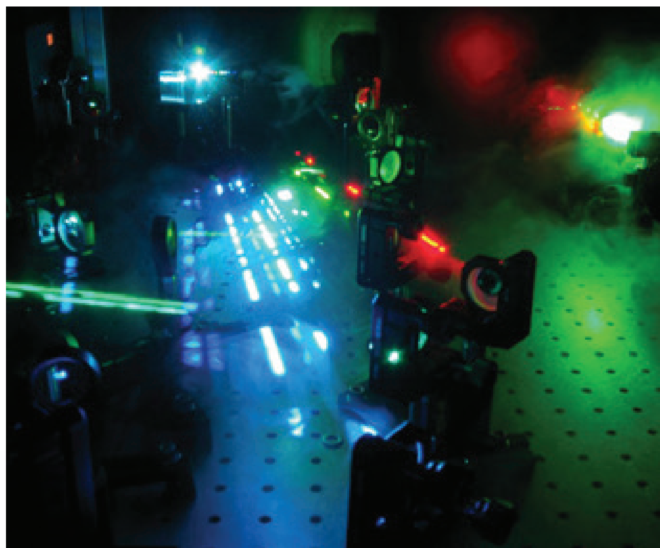
DD: SMD technologies are now entering the mainstream and are influencing the analytical measurements that scientists make. Digital PCR is now available in multiple platforms, and it is in wide use by researchers to study rare biological events. The improved quantitation of digital PCR is also helping adoption of this technology, and its implementation in diagnosis for humans is on the horizon. For our own protein detection technology, we are seeing widespread adoption from basic researchers in universities, to pharmaceutical companies developing drugs, to companies developing laboratory developed tests. By developing the technology for in vitro diagnostics, the dream of tests based on measuring single protein molecules will soon be a reality.

MP: Glucose testing represents the best example to date of near-patient medical biosensing. Rapid finger-prick blood tests are available, as well as devices for continuous glucose monitoring in skin. We should aim to measure much more than a patient's glucose level, such as small molecules and proteins; however, these have much lower concentrations than glucose and it becomes very challenging to develop the necessary real-time monitoring technologies. In monitoring applications, sample preparation procedures should be minimized and direct detection is preferred. Current research focuses on miniaturized detection methods and novel molecular and cellular systems. The challenges are large, so new technological concepts are needed in this domain, not only for future applications in humans, but also for research on artificial tissues and other live biological systems.

UL: Single molecule analysis is increasingly in focus, including in the context of single cell analyses. There is a great demand for analyzing heterogeneity among cells and for precise and sensitive protein measurements. The protein assays we develop depend on simultaneous binding by sets of two, three or more antibodies for enhancing detection specificity and for single molecule sensitivity. The new capability of highly sensitive protein detection now provides opportunities – as well as a strong desire – to identify new generations of diagnostic protein biomarkers that depend on this increased sensitivity.

For you, what are the most important milestones?

UL: There has been much progress in miniaturizing SMD of proteins, which confines reaction products to volumes where nonspecific background species are negligible. By contrast, we have solved SMD problems at both nucleic acid and protein levels by establishing new probe designs that depend on recognition of two or more affinity reactions before amplified detection. The design of the assays means that only the correct combination of reagents can give rise to detectable signals, thus enabling



Fluorescence imaging tools have been developed that allow researchers to visualize the emission of individual, fluorescently tagged proteins. Lasers and optics are key components in these experimental approaches.

multiplexing without associated background problems - another important aim of current molecular assays.

DD: First, the demonstration of a thousand-fold improvement over standard ELISA that we published in Nature Biotechnology (3). Second, the low cost consumable that we developed with Sony DADC, which showed that we could provide cost effective single molecule analysis, making it accessible by the wider biomedical community. Third, the commercial launch of our fully automated instrument that gave researchers access to the sensitivity improvements provided by Simoa. In terms of clinical milestones, we demonstrated a test that detected cancer recurrence months rather than years after surgery; we showed that protein biomarkers of neurological disorders could be measured directly in blood; and we showed that Simoa could detect viruses and bacteria at the same levels as nucleic acid testing.

AvO: I think one of the biggest milestones was psychological: the technology for detecting the fluorescence of a single molecule has been around since the 1970s (lasers, sensitive photodetectors), but it had been considered impossible to detect the weak fluorescence of a single molecule amidst background contributions. Throughout the 1990s, and with the first demonstrations of SMD, it became clear that the technical challenge was not necessarily in the physical detection equipment, but rather in the selective and specific labeling of biomolecules needed to allow single-molecule biophysical studies without perturbing function.

MP: In the coming years, point-of-care biosensors will come to market for small-molecule and protein testing with an analytical performance level that meets laboratory equipment. In the long term, I think an important leap will be the development of technologies for continuous biochemical monitoring of patients, integrated into medical devices and disposables.

How does SMD fit into the broad picture of analytical science?

AvO: Strictly speaking, single-molecule studies represent the ultimate analytical science in that the focus is on the development of tools to analyze biological processes at the ultimate sensitivity level.

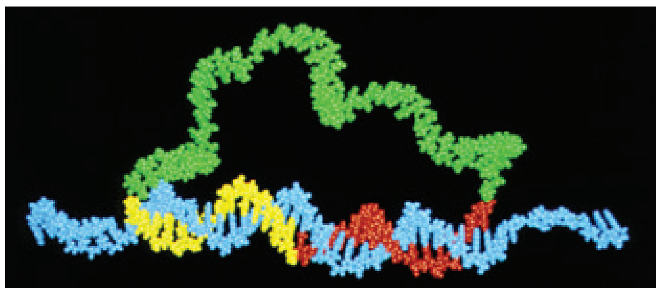
DD: Our founder and early employees were analytical chemists, and we are dedicated to developing analytical methods based on SMD. Measuring very low concentrations is also in our genes. It is one thing to detect if a molecule or organism is present in a sample, but having the ability to precisely and accurately measure the concentration of molecules across a wide dynamic range is a large technological challenge, albeit a somewhat unglamorous one. It's something we strive to do well even when making multiplex measurements a thousand times lower in concentration than normal!

UL: Obviously, there is a desire to measure rapidly increasing numbers of biomolecular properties in the human body, both for healthcare and in wellness research. There is also a rapid price reduction per datapoint, balanced by vast increases in amount of data collected. One particular instance of single or rare molecule detection that will increase in importance with the advent of improved tools is measuring exceedingly rare mutant versions of genes present in blood plasma from tumor patients for monitoring minimal residual disease.

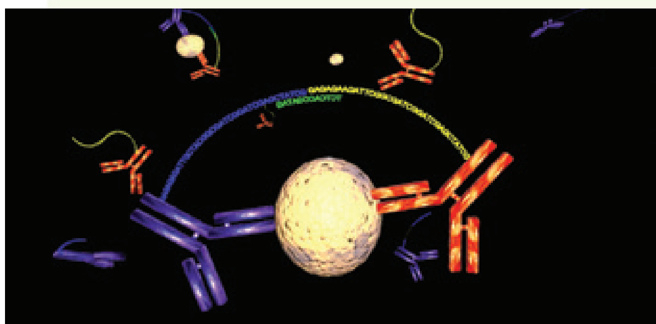
MP: Our research includes the development of detailed mechanistic understanding of interactions between molecular probes, particles, surfaces, and complex biological matrices. Specific and non-specific interactions, multivalency, protein coronas, heterogeneities in space and time, for example. Research methodologies based on single-molecule resolution will generate breakthrough insights into these complex questions that are very important for analytical science.

What is your mission for society, health and science?

DD: We wish to inspire a revolution in personalized healthcare by exploiting the power of the protein. Most of the incredible progress in molecular diagnostics and personalized medicine



Padlock probe for single molecule DNA detection. Single molecule detection in complex biological samples requires excellent specificity of recognition, and detection signals that exceed any background. Padlock probes achieve single molecule detection by depending on target recognition by the two ends of the probe (yellow and red). This allows the ends of the probes to be joined by DNA ligation, giving rise to a circular DNA strand, which can be locally amplified by a process of rolling circle amplification for easy visibility.



Proximity ligation for single protein molecule detection. Pairs of antibodies specific for a protein of interest are conjugated to DNA strands. When both antibodies bind a target protein the DNA strands are brought in proximity and can be joined by DNA ligation. Ligated DNA strands can then be amplified for easy detection using molecular genetic techniques such as PCR or rolling circle amplification. Illustration by Jonas Jarvius.

in the last 10 years or so has been driven by detection of nucleic acids. This revolution is due to two major analytical developments: nucleic amplification technologies (like PCR) and next-generation sequencing. We believe, however, that protein is a much richer, biologically-relevant molecule for detecting disease, so has a greater potential for understanding biology and improving diagnosis. Simoa is a major development that I believe will spark a revolution in protein-based molecular diagnostics in the next few years. Other technologies will also contribute to this coming revolution, including next-generation mass spectrometry techniques that can drive the discovery of new protein markers, and new approaches to protein-binding reagents that allow these new markers to become validated diagnostics.

UL: Our aim is to improve human health, both in the developed and developing countries around the world, primarily by permitting earlier diagnoses in the course of disease, in preference to late and chronic therapy. Although we work in academia, successful technologies are transferred to industry to make the methods more broadly available.

AvO: The mission of our academic group focuses on understanding molecular mechanisms. Our main goal is to develop single-molecule technology that allows us to understand how DNA is replicated. While this goal is driven by basic science, the technological spin-offs have an impact in diagnostic tools, and the results of using these tools contribute to greater understanding of disease mechanisms and antibiotic resistance.

MP: My mission is to train and inspire young scientists and engineers by involving them in top-level biosensing research. We investigate and develop novel biosensing principles based on single particles and single molecules, for fundamental research as well as for applications in healthcare. An important aim in healthcare is to enable people to live a high-quality life in their own environment. Novel biosensing technologies will help to achieve this aim, by recording the biochemical health status of people in need of care.

What are the major hurdles?

MP: Science involves new ideas, consistent working – and of course some luck. For biosensing research, good collaborations are needed between experts in physical sciences and molecular engineering. And finally, special skills are required for the translation of scientific results to medical applications, which is the topic of YouTube video "Translate to Innovate" that I have recently published (4).

UL: It takes a long time to develop a molecular tool and a successful technique, so that they can be widely available for parallel, highly sensitive molecular analysis. It can't be rushed; and, in our case this process regularly extends over more than 10 years. Most of the delay is in finding the right student or students for the job, but we have been blessed with many talented students.

DD: The first hurdle was time. As a small company with a big idea, we needed the time and money to bring the idea to fruition. Fortunately, we had understanding investors who funded the early development of the technology. We were also fortunate to receive support from government agencies and collaborators. The second hurdle was scalability. A big idea can only become really big if it can meet the financial and implementation expectations of customers who have high

demands for both – again a challenge for a small company. And that's why we partnered with some larger companies to achieve our vision.

AvO: While our research group receives the funding we need to pursue our scientific goals, I am worried about the reduction in funding for basic sciences and the shift of focus to translational efforts. In the long term, success as a society in research and development needs both types of research. Shifting the balance to applied work may result in economic benefits in the short turn, but we shall lose momentum in the end.

Tell us about some of your successes.

DD: Publishing in Nature Biotechnology demonstrated to the world that we had a scientifically-sound approach to SMD. It created a lot of credibility and interest for the technology. Launching a fully automated single molecule instrument just two years after development began was also a significant achievement, and we now have dozens of customers using the instrument around the world. It's just the start for Simoa, but it is exciting to see all the amazing uses of SMD.

UL: It is a great pleasure to see our methods in widespread use, even if not always correctly attributed. Some eleven large biotech or diagnostic companies have licensed techniques from our lab, and we are now spinning out our sixth company.

AvO: I feel fortunate in being able to work with a fantastic group of researchers who are all driven by a desire to understand the world around us and to leverage that understanding for the betterment of society.

MP: While working at Philips Research, I initiated and then developed with a team a point-of-care biosensing platform based on magnetic nanoparticles (5, 6). In this technology, all assay steps are controlled by electromagnetic forces, and for low biomarker concentrations the nanoparticles are bound to the sensing surface by single molecular bonds. I became a fulltime professor at Eindhoven University of Technology last year, and my aim is to once again develop innovative biosensing technologies, starting from novel basic concepts.

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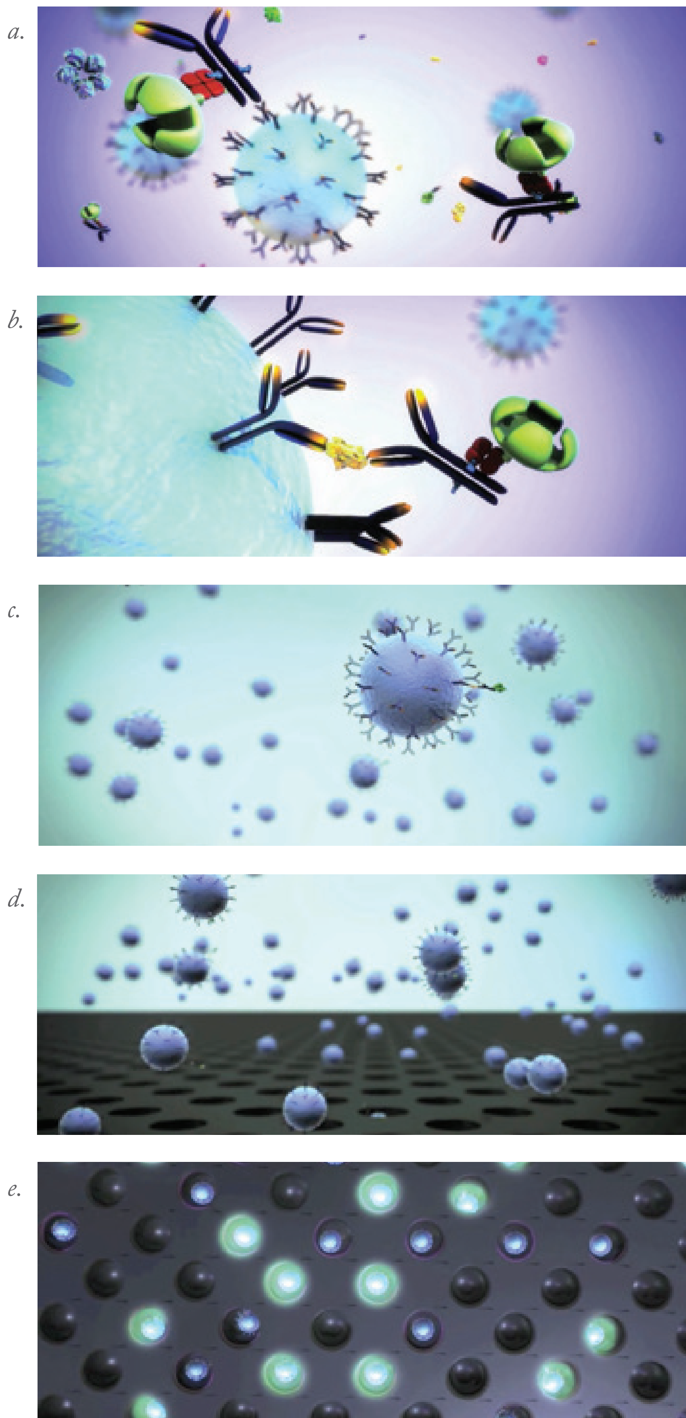

Counting of single protein molecules in blood using Simoa.
(a, b) Single protein molecules are captured and labeled on antibody-coated beads. (c, d) Single beads are isolated in arrays of 216,000 50-femtoliter wells. (e) Single molecules are subjected to fluorescence imaging.

How do you hope your work impact society?

AvO: We want to understand in molecular detail how the bacterial DNA-replication machinery works. By combining our single-molecule biophysics efforts with modern structural biology, biochemistry, and computational modeling, I feel we should be able to make great progress over the next three to six years. Such knowledge will help us with understanding antibiotic resistance and potentially identifying new approaches that will help us with this problem.

UL: In the following order: I am hoping that we will have identified new analytical challenges, proven that those we are currently working on are robust, and brought more of the earlier ones to clinical applications. Simple!

MP: My goal is to address the question of how single-particle and single-molecule methodologies can improve biosensing. And, in the long term I hope to contribute to a new sensing technology that is suited for integration into medical tools and for real-time biochemical monitoring of patients.

DD: We hope to be well on our way to achieving our mission of changing healthcare through measuring proteins. In three to six years, the Simoa technology should be widely adopted in the research market and being used for in vitro diagnostics. I am confident that many new insights and applications for measuring single protein molecules will have emerged in that time. We also hope to have expanded the use of the technology into new markets, like point-of care, and to have augmented Simoa with other enabling protein technologies. Hopefully, a protein molecular diagnostics revolution will be well underway!

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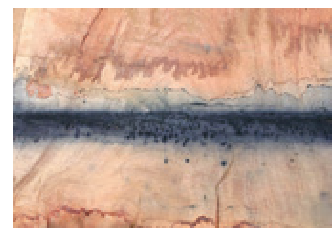
Top Three Trends in Lipidomics

State-of-the-art lipidomic analysis is based on two mass spectrometric approaches used for quantitation: shotgun lipidomics (using electrospray ionization MS/MS) or LC-MS. The shotgun approach provides fast quantitation, while LC-MS can provide more detailed information on isomers. Michal will discuss the potential of various chromatographic modes, including SFC, and also cover MS imaging – the third analytical platform used to determine spatial distribution of lipids and other biomolecules. Finally, Michal will introduce useful multivariate data analysis methods.

Webinar date: 4 June 2015, 14:00 UTC (Coordinated Universal Time)
Register Free at: http://tas.txp.to/0515/thermo-3trends-reg

TLC-MS: What are you missing?



Coupling thin layer chromatography to mass spectrometry – a combination of two powerful analytical methods. The surge in interest of coupling thin layer chromatography (TLC) with mass spectrometry (MS) is due to the availability of commercial instruments that make the routine use of this technique possible. Coupling the advantages of TLC, such as the ability to separate many samples in parallel and the high matrix tolerance, with MS creates a powerful tool for the modern analytical environment. Learn how you can ensure reliable results with the TLC-MS as Michael Schulz walks you through application examples.

Webinar date: 11 June 2015, 14:00 UTC (Universally Coordinate Time)
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Polymer Analysis in 2D

How to perform advanced polymer analysis with two-dimensional chromatography. Many macromolecules are products with multiple distributions, such as molar mass, chemical composition, end groups or others. Two-dimensional analysis allows the simultaneous measurement of two distributions with enhanced peak capacity. This webinar will describe how 2D analysis works and share the best techniques for comprehensive characterization of (co)polymers.

Webinar date: 18 June 2015, 17:00 CEST (Central European Summer Time)
Register Free at: http://tas.txp.to/0515/pss-webreg

Cutting-Edge Metabolomics

As new technology platforms push us to the limits of what's possible, the metabolomics community is closing in on the future of the field: routine and rapid quantitative analysis.

By Karl Burgess, Head of Metabolomics, Glasgow Polyomics, University of Glasgow, Scotland.

Believe it or not, I started out as an undergraduate in computer science and cybernetics. Unfortunately, the world of robotics involved a lot more mathematics than I expected. And so after a year of computer programming, I switched to pathobiology. But I never lost my interest in computers and programming, and that has been invaluable as I've progressed through my career; during my postgrad days I moved into bioinformatics and molecular modeling, which brought my two halves together. I soon realized that I wanted more time in the lab, which led me to do a research-based masters degree in biological and biomedical science.

I ended up in the proteomics lab at the University of Glasgow in a world where robotics, wet-lab work, biology and computer happily co-existed. I'd found my calling – at least for a while. Using mass spectrometry coupled to computational techniques that make sense out of the biological data is where my broad interests now lie. In many cases, it's not about creating new algorithms, it's about processing data and presenting them in a useable format that biologists can understand.

Orbitrap temptation

So, why the shift from proteomics to metabolomics? One of the reasons was to get my hands on an Orbitrap instrument to be honest... I actually started out uncomfortable with the idea of metabolomics – it's a completely different ball game. In proteomics, we could use Mascot to provide a probabilistic score for a given protein based on the mass spectra. You can use a cut-off system and, much like a court of law, you end up with an innocent or guilty verdict on the identity. In metabolomics, we were working entirely on mass and retention time – it's a very binary way of working and felt quite limiting; it was a "yes" or "no" answer to identification without knowing how certain you were in either case. Now, we're building fragmentation libraries and the requirements for supporting metadata in studies are increasing all the time.

Indeed, metabolomics is now very rigorous – and it's been a big learning curve for me in terms of quality control. Excellent reproducibility is key; dozens of replicates may be necessary to get the statistical quality for quantitation. And that's the point where clinicians start to become very interested – robust, quantitative data on biomarker-style molecular relationships they are used to working with.

I did most of my PhD work on a relatively fast-scanning but pretty low-resolution ion trap instrument. When I first got an Orbitrap instrument (an XL), I was showing my boss the data at 100,000 resolution, and he actually thought it was centroided – I had to zoom in about 20 times before I could demonstrate the reality of the peak widths. It was a really great moment! I've also done some work on high-resolution QTOFs, but stability of mass accuracy was a problem. The Orbitrap has always been rock solid in that regard. In fact, when we bought our ex-demo XL, it had been boxed up in the demo lab, left in a crate for three months, unboxed outside the building and bumped up a rough slope into the lab. After pumping the instrument down we found that it was still within 3ppm...

Metabolomics today

Heading up metabolomics at Glasgow Polyomics means that I get to work on some really diverse projects – all sorts of crazy samples. Indeed, the whole facility is geared up to apply state-of-the-art technologies to investigate biological systems by combining multi-level, multi-omics datasets.

As an example, we've had a lot of success partnering with Matt Dalby's group on the analysis of stem cell differentiation and interaction with surfaces. With Matt, we've got some fantastic collaborations (Nikolaj Gadegaard and others, who make nanopatterned materials) where we explore how different nanostructures promote different kinds of differentiation. Obviously, if differentiation occurs, there are lots of complex modifications to the metabolome. Tracking these changes over the course of differentiation on different surfaces is enormously powerful.

I'm now trying to tie up my interests in infectious disease with the surface attachment work in the area of bacterial biofilms. Infection of medical implants is a

really significant problem, especially with antimicrobial resistance increasing. We're looking into novel antimicrobials that modify biofilm formation with endogenous metabolites and repurposed drugs. I've got a great collaborator: Gordon Ramage, who works in the Dental School, and has been analyzing multispecies biofilms for many years. With his expert clinical microbiology knowledge, and the three PhD students we've got on the project, we're now starting to get some interesting results.

On the software side, we're working on probabilistic annotation of metabolites from data using a Bayesian clustering approach. This is part of the drive towards providing a meaningful probabilistic analysis of identification. In many ways, it's a first step towards creating a framework in which we can slot multiple measures of physicochemical properties to determine the likelihood of a particular ID.

GC Orbitrap joins the party

We've already put GC-Orbitrap technology to the test in a really cool project called 'the way of all flesh' with Richard Burchmore, which is essentially analyzing the decomposition process of of dead bodies. Time of death is really tricky to work out once liver temperature has dropped to ambient. And so, the search is on for biomarkers of death, using metabolomics and proteomics. First, we let a big piece of steak decompose over a 12-day period, taking MS datasets as time went by. We got some very interesting leads in terms of amino acid biomarkers.

Whilst at Thermo Fisher Scientific in Runcorn, UK, we were able to move onto rat models. First of all, the data reproduced the work we'd done on LC-MS previously, but the added resolution and the presence of the NIST libraries allowed us to distinguish things like sugar isomers that we have difficulty with on our untargeted LC-MS method. In fact, the software on the GC-Orbitrap system allows us to automate metabolite identification using enhanced spectral deconvolution, NIST library candidate searching and accurate mass filtering. Sensitivity was phenomenal; with a 1 µL injection we were overloading the system, so we had to move to split injections.

In the final stage of the project, we managed to acquire samples over various time periods from a body farm (or more correctly, a forensic anthropology research facility) in Texas. We are gearing up to run the human work on the freshly installed GC-Orbitrap system in our lab right now – exciting stuff. We're hoping that GC-Orbitrap technology can deliver better coverage of the biomarkers we've discovered, as well as the opportunity to perform good quantitative measurements.

We'll be presenting all of our findings at the 11th International Conference of the Metabolomics Society in San Francisco bay area towards the end of June.

In the near future, I'm also looking forward to doing a lot of biofilm work on the instrument. I actually started this research area as it provided a platform for pushing metabolomics innovation, but once you've got your own bit of biology to investigate, it all gets quite exciting. High-resolution separations and mass accuracy are really key to analysis of biofilms.

Moreover, the GC Orbitrap enables untargeted metabolomics because it provides accurate mass full scan data rather than targeted transitions, as you would get on something like a triple quad. The array of quorum sensing molecules that bacteria use to communicate with each other, triggering, for example biofilm adherence and dispersal, are very diverse, and not yet well characterized. An untargeted approach gives us the potential to identify new compounds; accurate mass EI fragments allow us to characterize them. Additionally, high GC resolution allows us to separate isomeric compounds and, with some extra chemistry, even chiral compounds, which are extremely important in bacterial signaling and peptidoglycan synthesis.

In metabolomics, we're essentially looking for everything. Therefore, access to NIST libraries is enormously powerful as it allows us to make unexpected discoveries in a non-targeted fashion. Targeted metabolomics by definition narrows the field.

Metabolomics of 2025

In my view, GC-HRMS is fast approaching the point of being the ultimate metabolomics platform. And LC-MS is catching up rapidly. In 10 years, I predict that metabolomics will be easy (!) You'll buy an instrument and a set method, and advanced software will do the work for you. In an ideal world, we'll have contributed heavily to the development of that software. We've got quite a few publications in software and algorithm development for MS, and they're beginning to coalesce into one single web-based platform. Once again, it's about providing people with useful, interesting data. I would say software is the biggest challenge right now; the hardware tools we need are here.

As far as GC-Orbitrap technology goes, I'm deliberately trying to keep my acquisition a bit of a secret (this article won't help). The people I have told are extremely excited about the prospect of running samples and, candidly, I don't want a never-ending backlog just yet.

Even if I'm 10 percent more confident in the data, it's really important – and in reality, it's a lot more than that because I can provide compound matches to fragment patterns in percentage terms and then use accurate mass to really drill down into specific fragments. To put it simply, GC-Orbitrap technology gives us extra confidence. And confidence is an extremely important asset in our field.

Video interview with Karl Burgess: tas.txp.to/0515/KarlBurgess
To find out more: thermoscientific.com/HRAMGCMS

To Teach... Perchance to Learn

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Some people are born teachers. Some "achieve teaching" during their lifetimes. Others have teaching thrust upon them. Here, I compare and contrast life in business and academia, and share my experiences in the latter for those considering the move.

By Lee DesRosiers

I find myself attempting a graceful "glissando" into greying semi-retirement. And what better way (other than Volvo-driving) to add gravitas and prestige than by teaching? A large part of the scientific community has been involved with teaching and might fall into all three categories. In most life science fields, many researchers start out with some teaching responsibilities – a course "load" as it's called. That is, many start off teaching and gradually extricate themselves from the tentacles of correcting, corralling and guiding students – students with mixed intentions and varying levels of ambivalence.

Listen to many, speak to a few

In the case of scientific researchers, bogged down in ever-ramifying pursuits, teaching can be seen as an impediment. Who wants to pontificate at length on introductory subjects to smartphone-clutching young people? Are current students – our distraction-era pioneers – really as oblivious as they seem? Well, only if you teach with disinterest and don't listen.

Some become teachers as a conscious effort to apply a practical career choice on a non-applicable field, such as politics, history or others too numerous to insult. Subjects that, while they can be studied, cannot be learned as a career. Teaching is the only real option for a huge swathe of academic pursuits. And by continuing to teach these subjects, more (similar) teachers are created, thereby sustaining a peculiar human loop of academia. That is not to say these subjects are not worth teaching – there is more to life than getting a job, although living without one is challenging.

Others come to teach later in life. They may be retired, passing the torch to the dynamic, openly driven younger crowd that will survive them. Or they have been marginalized, dismissed, "smart-sized" or made redundant; rejected from their professions in a disorienting haze. No longer motivated exclusively by money, title or position, they attempt to be useful, they seek to enlighten. In the tired words we all know, to share and give something back; to make a difference. Such ossified words persist of course, because they are true.

And this is where I find myself. After decades of high intensity upper management lifestyle, I am now non-corporate. In fact, I've been through the five stages of management:

1. Who is Lee DesRosiers?
2. Get me Lee DesRosiers
3. Get me the Lee DesRosiers type
4. Get me a young Lee DesRosiers
5. Who is Lee DesRosiers?

Typically, business people come late to teaching, if at all. I say "if at all" because, oddly, teaching business, that most pragmatic of pursuits, is a highly theoretical process carried out largely by academics, who are a world unto themselves. A brief glance at authors' bios from a typical management course lists an astonishing array of individuals who run management institutes, with such rarified qualifications as PhDs in Management. No sane sustainable business organization would ask for a higher degree for a non-technical position – but our universities do.

Management textbooks are written almost exclusively by academics, and are taught by similarly described individuals. Certainly, these people have something to offer, but so do experienced business people. Good decisions come from experience, and experience comes from bad decisions. Nonetheless, the value of business experience is dismissed; the academic world believes that experience, while quaint and amusing, is not really relevant. Academics refuse to value experience in the business world because they see themselves above it and, most importantly, they don't have any.

Alas, poor Yorick! I knew him, Horatio

The French word for company is "société". And we speak of social guests as "company". There is a distinct sense of exclusion (or possibly excision) for me now, having left business; exclusion from society.

The fact that I came late to teaching has given me an idiosyncratic acuity apparently lacking in my colleagues. In many ways, teaching is analogous to business. Certainly, teaching is one characteristic of great managers – the best managers teach but the best teachers don't really manage anything, other than their considerable administrative burden.

There are distinct benefits to teaching as compared to business.

In business, they say you should only attend the meetings that cannot happen without you; as a teacher, every class is like that. The class is analogous to a business meeting and cannot start without you. You cannot be absent or late – it is very much "your" meeting.

As the "leader", it is not difficult to infuse a culture of mutual respect in a classroom; the students are not really competing with each other. You teach respect by example, by practice, by encouragement. Respect is a constant uphill battle in commerce, continuously undermined (most insidiously from above).

Students, unlike normal meeting participants, don't want your job. No student has any interest in seeing you fail, your success truly is their success. In business, meeting participants may well be secretly hoping for your failure and often do want your job.

I have no "boss" as a professor, only my students. They are, for three and a half months, more like family than colleagues (if you can imagine a transitory, temporary family). I am responsible for them and the challenge is all mine. I decide what they must learn, from where and how it will be evaluated. The course becomes a self-contained, breathing organism, adapting to both students and teacher as it evolves over its short life.

Most delightful of all is the "HR" situation. Disruptive person keeping you up at night? Hang tough and they will automatically be gone in a few weeks. In fact, you will decide their fate. The students move through your class like a whirlpool – the form remains while the content shifts.

My fellow professors are companionable enough, but we are ships sailing in different waters. We don't spew jargon at each other and we don't speak of "delighting our stakeholders". We are disturbingly empirical. What is the best way for our students to learn?

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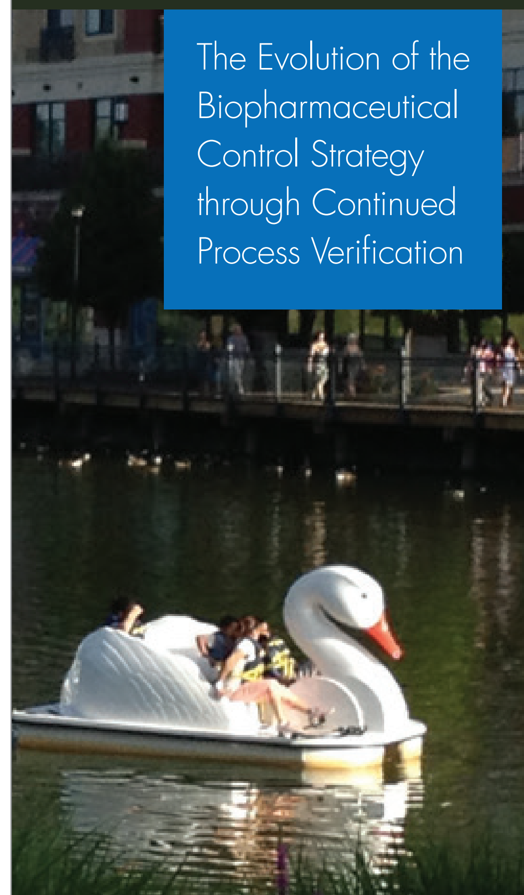

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As far as rewards go, in business roles, you get up early to be with people you don't like to do things you don't want to do in a place that you don't want to go to. Are you happy? You might say so, but only after you've been given a car and many thousands of dollars. You wouldn't do it for free. Teaching for free is actually not inconceivable.

All the world's a stage

Teaching is the highlight of my week. It's an intense social interaction like some wild complex party. All too soon it is over and my long silent week begins. I love that silence too, and I rejoice when class ends, high as a kite when I imagine or believe that I really had an impact. Only rarely in business, perhaps after a particularly successful presentation, have I ever had that rewarding sensation. Now I can't live without it.

More pragmatically, you cannot drift aimlessly through your day in a twilight level of consciousness, as most of us do at work some or all of the time; you are always center stage. You must be as alert as a cat in front of your class. There are some similarities to being a stand-up comedian – including stage fright. Every class is an important performance and you can certainly "bomb".

A fool thinks himself to be wise, but a wise man knows himself to be a fool.

When people ask what I do, I don't say "I'm a teacher" but rather "I teach". The corporate equivalent, "I manage," doesn't work. No longer do I offer vague titles like "Global Senior Business Development Team Leader" or "Commercial Director, German-speaking countries". Functionally, there is no doubt about the usefulness of teaching; occupationally, it is unassailable.

I like to think of myself as a glowing talent with a certain distinction and a hint of decay. In fact, much of what I know about business still applies. Nonetheless, many of my business "skills" are obsolete. I remember when an advertising mock-up was delivered as a "blue" (a strange intermediate print), and how to bind catalogues – all those seemingly ancient activities that dominated marketing 30 years ago.

Students today don't "own" music, movies or even books anymore. Soon they will see no reason to own knowledge. Why not just look it up? The best software wins.

And yet in my profession, things are often still paper only. The hand-written, in-class case studies are priceless – as irreplaceable and unrepeatable as a Charlie Parker jam session.

I absolutely cannot lose hand-written final exam papers. My university will not mail them to me as there is a miniscule chance they will be lost. Of course, this is another process that will disappear as exams go completely on line. But for now it is a wonderfully refreshing glimpse into the arcane world of valuable, diligently prepared documents.

Aye, there's the rub

Some teach all their lives. They are compelled to explain, to guide, to enlighten… or at least try to. Among them are the great, charismatic, kindly individual landmarks who have shaped many of our lives and will guide our children. It is an aspiration I reach for every day.

A teacher is only evaluated by their students, which is much like your customers deciding your next promotion in a business setting (and maybe they should).

You don't usually fail as a teacher – you give up.

Lee DesRosiers is a lecturer at McGill University School of Continuing Studies in Montreal, Canada.

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Bright Spark

Sitting Down With... Richard Pollard, President of Ocean Optics, Dunedin, Florida, USA.

You have an unusual background...

I guess I'm different to many people in the analytical space because I got here via an engineering path. My father was a bank manager and a real "people person". His job meant that I grew up in various parts of the north of England before heading down to Brunel University in London to do a degree in manufacturing engineering. Engineering was a good fit for me; I focused on mathematics, physics and chemistry at school. In essence, I recognized that I liked building stuff and solving problems. The great thing about Brunel was the mix of work and studying, which is how I ended up on several placements at Ford.

An emphasis at Ford back then was on low-weight engine materials and reduced environmental impact – so I got to work on some fun and thought-provoking projects. It was particularly interesting to see how much science was being applied to engineering problems – there was a lot of innovation. And I was surprised by the pace and ambition.

And you stayed with Ford?

That's right. I stayed on at Ford working on engineering problems in several business units for seven years. I also headed up several production facilities and quickly found that I had an aptitude for working with people – even though I was thrown in at the deep end as a graduate manager. I had every trick in the book thrown at me. But honesty and understanding helped me earn respect.

I learnt early on that it's not just about being a boss – it's about understanding the goals of teams and individual employees, and learning how to align those goals with business objectives. Now, I very much believe that you must find the right people first and then put your plan together around them. Certainly, you need a vision – that's what gets people on board, but the A to Z route of how you get there needs to flex with the talent of the people around you.

So, what exactly is the route from Ford to Ocean Optics?!

After Ford, I joined an electronics firm who were working at the cutting edge of printed circuit board technology. There was a lot of chemistry involved, but it was also my introduction to optics. I was put in charge of imaging processes. After a while, I decided I wanted to work for a smaller organization; I joined the board of a great company called Keeler, which makes ophthalmic instruments. Keeler exists within the same UK-based umbrella group – Halma – as Ocean Optics, along with about 45 other companies.

What did you bring to Keeler?

We were trying to refresh products and inject some innovation. Innovation is hard – but I could apply lessons learnt from previous experiences. You have to listen, to be open to ideas from inside – and outside – the company, and you have to fully understand the roadblocks and priorities. One project stands out for me – it seems obvious now, and at the time it was groundbreaking – like all the best innovations! A key product at Keeler was a head-mounted indirect ophthalmoscope that depended upon a very bright light. Bright lights required a lot of energy back then, so the device was connected to an electrical outlet. We went in search of a suitable power pack and ended up with a totally integrated solution. There was a big concern about the weight of the battery, but actually it balanced out the optics on the front, something we realized on the first prototype. Innovation can also be about usability or extending applications.

Ocean Optics is a fast-growing company – an exciting next step?

Absolutely. I joined in 2006, running operations, and it was incredible to see the scope of applications that Ocean Optics were involved in – and we've expanded significantly since then. The diversity now is simply astonishing. And importantly for me, in many cases we're helping other companies improve life.

You're now president – how are you shaping the company?

Much of it is about vision – crystalizing our company mission. And it's about sharing passion for innovation. I also recognized the true value of the business, which is in applications rather than products.

Research (and working with other researchers) is tremendously important to us. We want people to think about Ocean Optics first and we want them to know that they'll be working with a team that will be completely focused on solving their problem. I hear that from customers now – and more than anything else, I want us to stay in that place as we grow. Needless to say, we have some very exciting research collaborations.

Spectroscopy applications are soaring right now – where is the field heading?

The next wave of spectroscopy is likely to enter a space that is much closer to the consumer market. We all have phones in our pockets that have cameras and processing power – and there are some missing pieces that could transform them into very meaningful devices. I foresee a lot of medical/health and food monitoring – all outside of the lab of course. We all want more data and knowledge about how the world around us is affecting our health. Our part in all of this? Well, we're taking a key-partner approach – all covered by non-disclosure agreements – but you don't need to research too long before hazarding a guess about who some of those partners might be. There's a pace and investment behind some of these projects that is refreshing. Within 18–24 months you may see some of that work come to fruition. These are very exciting times.

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