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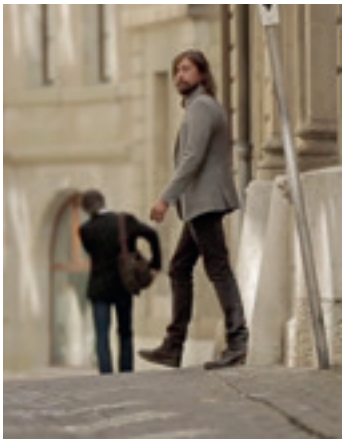




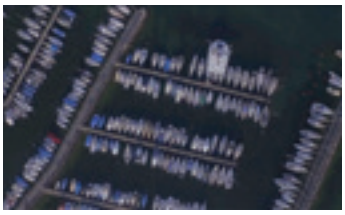

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Contents

On The Cover

Multidisciplinary collaboration in the clinic is about to transform healthcare.

Upfront

In My View

34

Features

The Toolbox

Translated

In Perspective

Reports

Sitting Down With

The Translational Scientist

ISSUE 1 - JANUARY 2016

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Collaborate or Stagnate

Can pharma get over its IP anxiety and embrace meaningful partnerships?

Collaboration within companies is clearly vital, and these days big pharma working with biotechs is a given. Pharma companies have a long history of partnering with academia too – on page 10, University of California professor Greg Weiss reports that he has already had a number of calls from companies wanting to explore the exciting technology he has developed.

Unsurprisingly, collaboration between rival companies is much less common. However, in "Meet The Green Team" in the last issue, green chemistry consultant Andy Wells drew attention to the "remarkable" increase in pre-competitive industry collaboration over the past 10 years. Why? To find leaner, greener manufacturing strategies. As well as joining forces on sustainability initiatives, companies have been collaborating on large-scale R&D projects (1) and working together to help develop new licensing pathways (2).

Collaboration – or at least meaningful collaboration – is by no means easy. Historically, pharma has had a propriety culture, not well suited to sharing knowledge. Many big pharma companies still struggle to be team players. A recent survey of biotechs shows the perceived 'partnering skills' of top drug companies lagging well behind their technical capabilities (3). If the biotech–big pharma relationship, with its clear benefits for both sides, can be difficult to manage, partnerships between rivals are harder still. Like a marriage, a good partnership requires hard work and compromise from all parties.

Ultimately, increased collaboration may be a necessity rather than a choice. The days of big pharma profits generated solely by in-house R&D are long gone, and perhaps that's no bad thing. Done right, collaboration allows us to become more than the sum of our parts. It broadens our horizons, helping us look beyond the obvious to see new and creative solutions. In fact, what if we stepped outside the limits of the pharma industry altogether? GlaxoSmithKline did just that when forming a partnership with Formula 1 team McLaren. An odd combination? Not really. McLaren can replace all four tires on a racecar in four seconds – imagine the savings if pharma manufacturing could harness that efficiency.

There is no doubt that pharma companies can collaborate successfully. During World War Two, US and UK drug makers formed government-backed coalitions to scale up manufacturing of penicillin, saving countless lives on both sides of the Atlantic (4). Today, as well as conflict and disease, we're facing new challenges caused by our reckless use of resources. If we can harness the collective brainpower, skill and commitment of this industry towards solving the world's problems, we will all benefit.

Charlotte Barker
Editor

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Claire Thompson

When Claire was five years old, her father taught her how to play football. He said, "The difference between an average footballer and an exceptional footballer is their ability to look up. They know where the ball is - they look up to see where the opposition is and pick out the next pass." Claire has applied this advice throughout not only her football career (where she played at international level), but also in business. She now fast tracks nanotechnologies into products and profits, advising investors on how to pick the winning assets; innovators on looking up from the bench and getting to clinic or market; and large corporations on strategic acquisitions and entering new markets. Claire has a degree in Biochemistry from the University of St. Andrews and a PhD from the School of Pharmacy, University of Nottingham.

Claire asks if nanotechnology can pick up where personalized medicine leaves off on page 17.

Abbe Steel

Abbe Steel is Founder and CEO of HealthiVibe, a company that enables patients to contribute to clinical trial design. Previously she was the Vice President of Patient & Physician Services at UBC-Express Scripts, worked at Sanofi on global marketing programs, and was Senior Director, Patient Programs at PAREXEL. When Abbe isn't busy tweeting (@AbbeSteel) about patient centricity, you can usually find her walking a funny-looking black dog named Harper.

Abbe calls for more patient involvement in clinical trial design on page 21.

Peter Seeberger & Andreas Seidel-Morgenstern

Peter Seeberger's research covers a broad range of topics from engineering to immunology. He is a director at the Max-Planck Institute for Colloids and Surfaces in Potsdam and Professor at the Free University of Berlin. Through his work in the area of neglected diseases, Peter has also become involved in philanthropic causes – he is a co-founder of the Tesfa-Ilg 'Hope for Africa' Foundation that aims to improve healthcare in Ethiopia.

After receiving his PhD from the Academy of Sciences in Berlin and working as a postdoctoral fellow at the University of Tennessee, Andreas Seidel-Morgenstern defended a Habilitation at the Technical University, Berlin before working for Schering AG. In 2002 he joined the Max Planck Institutes, where he is head of the Physical and Chemical Foundations of Process Engineering group. Andreas is interested in heterogeneous catalysis, the development of new reactor concepts, crystallization, adsorption and preparative chromatography. The results of his work are published in almost 400 research papers.

The story behind Peter and Andreas' award-winning production method for antimalarial drugs is revealed on page 46.

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Upfront

Reporting on the research, personalities, policies and partnerships that are shaping translational medicine.

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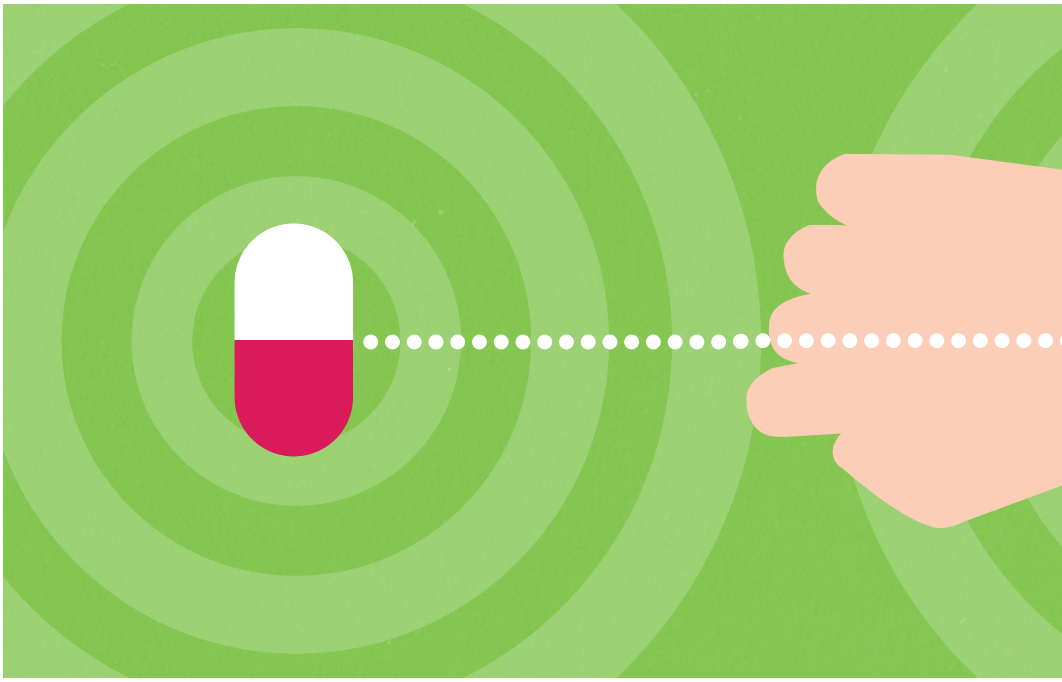

Google's Nanoparticular Ambition

Tech firm's latest project aims to detect disease using a nanoparticle pill and a wrist-worn device

The Google[x] life sciences division is innovating again. This time with nanoparticles. No less ambitious than other projects preceding it (such as its plan to build the largest and most detailed human genome database: http://bit.ly/1GxMmeq), the tech giant's latest undertaking aims to create nanoparticles capable of reporting signs of cancer and other diseases using a wearable, magnetic device.

Speaking at the Wall Street Journal Global Technology Conference (1), project leader, Andrew Conrad, said "Essentially, the idea is simple: You swallow a pill with nanoparticles, and they're decorated with antibodies, or molecules that detect other molecules. They course through your body and because the core of these particles are magnetic, you can call them somewhere. These little particles go and mingle with the people, we call them back to one place, and we ask them, 'Hey, what did you see?'" This is certainly an oversimplified way of explaining it, and although magnetic nanoparticle diagnostics are not a new concept, making it work is far from simple.

According to Conrad, a wearable sensor containing a magnet would be used to attract and analyze the particles. As well as detecting cancer, it's hoped the system could be used to spot other disease signs, such as fatty plaque in blood vessels and high potassium levels – potentially creating opportunities for clinicians and pathologists to monitor patients and spot disease before symptoms develop.

That's certainly ambitious; with many technological and regulatory challenges to overcome, industry experts give the project at least five years before they expect any meaningful results. There is a social factor to consider too: Google's detractors already point to many of their data collection practices as an invasion of privacy, and there could also be concern over how Google might collect and use private medical data.

Despite possible privacy concerns, a system that continually monitors for signs of disease could provide a wealth of information for healthcare providers. According to Conrad, the system is part of a paradigm shift that would see medicine become much more proactive. "Every test you ever go to the doctor for will be done through this system," he said, "that is our dream." *RM*

Reference

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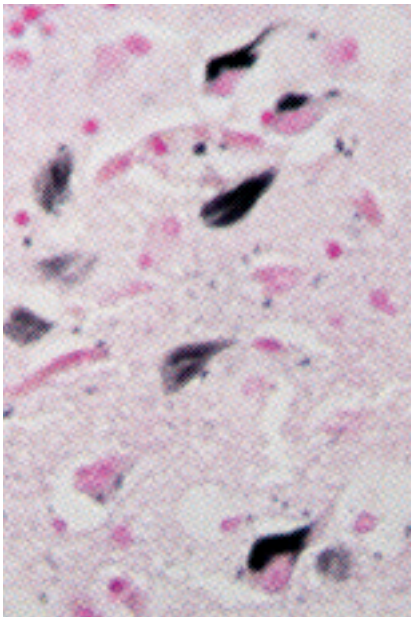
Alzheimer's Analytics

Predictive biomarkers in saliva may yield a simple, noninvasive diagnostic test

The baby boomer population is aging and the incidence of Alzheimer's disease on the rise, but a definitive diagnostic test for the condition has been elusive. Currently, Alzheimer's is diagnosed by a series of cognitive tests, with biological confirmation possible only by postmortem examination of the brain. But cognitive tests aren't always reliable, and postmortem examinations aren't much help to patients.

New research from the University of Alberta, Canada, suggests that the answer may lie in saliva. At the recent Alzheimer's Association International Conference, neuroscience student Shraddha Sapkota reported that metabolomic analysis of salivary samples by liquid chromatography-mass spectrometry allows clear discrimination between patients with Alzheimer's, those with mild cognitive impairment, and those with normal cognitive aging (1). The researchers were also able to identify the top metabolites for distinguishing between conditions. Most importantly, the study results revealed directional associations between certain metabolites and cognition states – that is, biomarkers upregulated in patients with known cognitive impairments were predictive of episodic memory problems and slow neurocognition when elevated in those with normal aging. Detecting such biomarkers in saliva tests may eventually allow doctors to identify Alzheimer's patients before they become symptomatic. Not only that, but gaining this knowledge



before cognitive issues become evident allows patients to take part in decision-making processes while still able, and allows researchers to identify potential clinical trial participants for Alzheimer's disease therapeutics or preventatives.

The benefits of the research extend beyond identifying predictive biomarkers. The saliva test itself is a new approach to Alzheimer's diagnosis and has advantages of its own. Saliva testing is easy, painless and noninvasive, and the fluid itself is easy to transport – ideal given the likelihood of repeated testing over a long time span. The work is still in its early stages, but salivary biomarkers appear to show clear diagnostic promise. *MS*

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I Can See a 'Brainbow'

New neuroscience reveals that the synaptic connections between the retinal ganglion cells and the visual cortex are not as simple as first thought

It's well-known that the developing brain starts off with many more connections than it has in adult life, and that some of those connections are strengthened while others are eliminated in a processed called synaptic pruning. In terms of the brain's visual function, this means that we start out with synaptic connections from many retinal ganglion cells (RGCs) converging on the cells of the lateral geniculate nucleus (LGN) in the thalamus (whereupon signals are relayed to the visual cortex). As we age, the number of connections decreases. Or so we thought.

Now, thanks to Michael Fox and his team at the Virginia Tech Carilion Research Institute, we know that retina-to-brain connections don't work quite the way neuroscientists previously thought. The researchers used a technique called "brainbow" (Figure 1), in which each individual neuron is tagged with a different fluorescent color, to trace what they assumed would be a single RGC – the source of several axon terminals forming synaptic connections. But when Aboozar Monavarfeshani, the graduate student who did the tagging, looked at the samples, he saw a variety of colors indicating terminals from more than just one or two RGCs. "The samples showed a true 'brainbow' – I could see, right in front of me, something very different than the concept I learned from my textbooks." The researchers verified their results by electron microscopy and reached the same conclusion: that

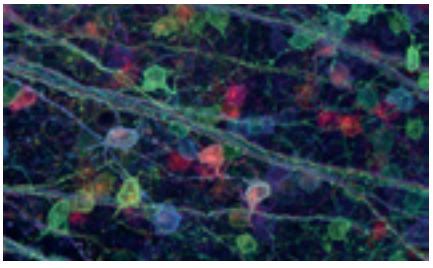

Figure 1. A "brainbow"-tagged image of a retinal whole mount, showing multiple distinct colors.

the axon terminals from numerous RGCs, rather than the few previously thought, make synaptic connections to the LGN (1).

What does this mean for vision research? "These results are not what we expected, and they will force us to reevaluate our understanding of the architecture and flow of visual information through neural pathways," said Fox. With such new and unanticipated information, scientists who seek to understand how the brain contributes to sight may have to reconsider theories that might once have been taken as read. But though these experiments raise more questions than they answer, it's simply a sign that we have a long way to go to fully understand the complex visual machinery of the brain. *MS*

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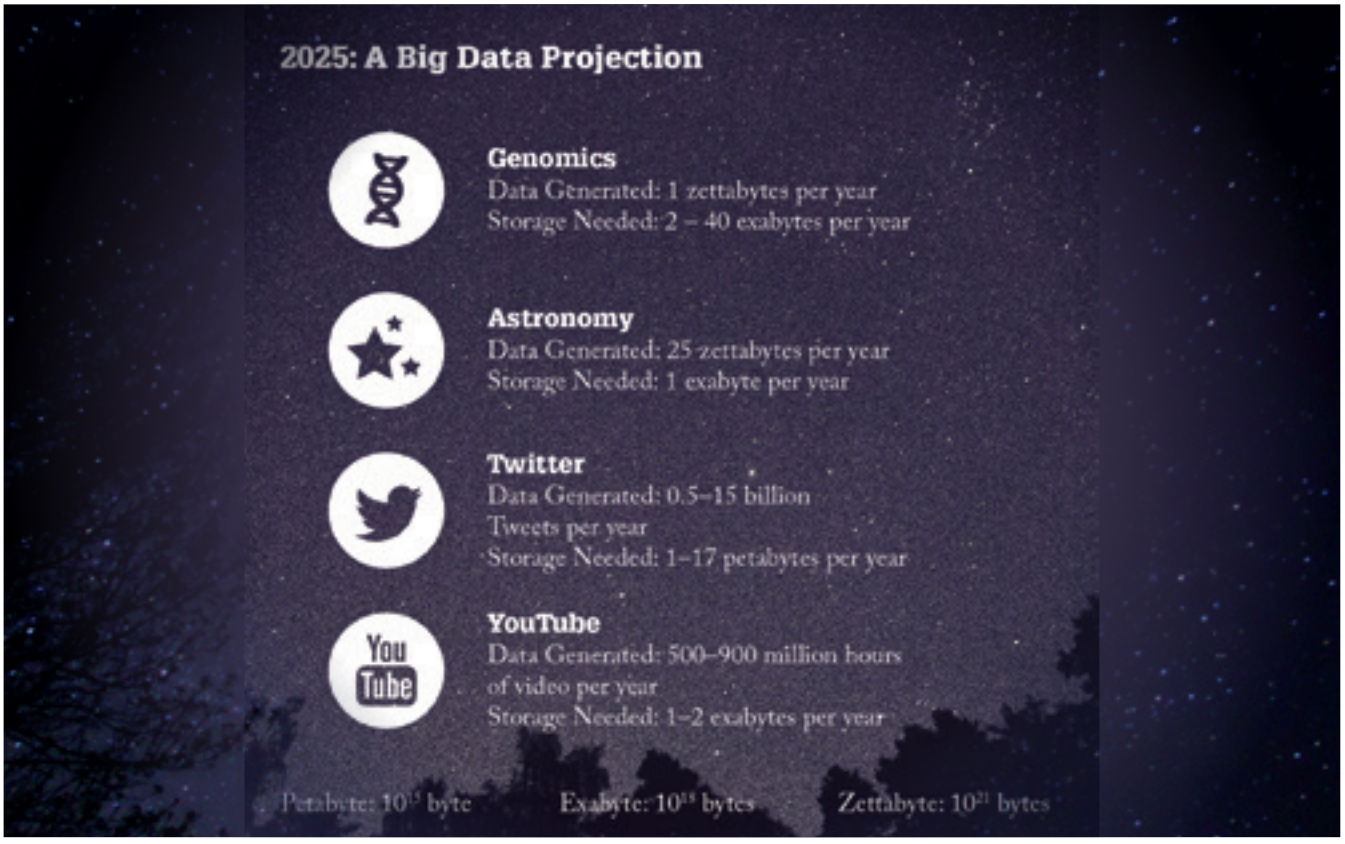

Forget Astronomical, Try Genomical

Genomics could soon outpace other scientific disciplines as the king of big data, but there could be problems ahead…

The genomics revolution is uncovering more insights into human biology than ever before, but some researchers foresee a problem on the horizon: just what will we do with all that information? A recent study by a team of US biologists and data scientists has concluded that the speed of genomic data generation has now outstripped that of YouTube (1), and at the current rate, the amount of genomic data produced every day is doubling every seven months.

Right now the storage and analysis of genomic information is manageable, but as sequencing becomes cheaper and more common, issues are likely to arise. It's predicted that by 2025, up to a billion people may have had their genomes sequenced, creating the need for a huge amount of storage, and producing vast amounts of data on par with social media platforms, and disciplines such as astronomy (see Figure).

Genomics is a "four-headed beast", explain the researchers, with four key areas: acquisition, storage, distribution and analysis; all posing their own particular challenges. This means that no one solution will solve the impending problem – improved sequencing technologies, data storage and sharing solutions, and optimized computing infrastructures and data libraries will all need to play a part as genomics grows at lightning speed. "For a very long time, people have used the adjective 'astronomical' to talk about things that are really, truly huge," says Michael Schatz, co-author of the associated paper, "but in pointing out the incredible pace of growth of data-generation in the biological sciences, my colleagues and I are suggesting we may need to start calling truly immense things 'genomical' in the years just ahead." *RM*

Reference

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Amine Alchemy

Transforming common chemicals into complex amines is the pharmaceutical equivalent of turning lead to gold

Researchers from The Scripps Research Institute claim to have developed a new method for synthesizing amines, which they describe as being akin to "taking dirt, and then adding a bit of rust, putting it all in a blender and ending up with gold" (1).

In fact, complex amines are often more expensive than gold because they can be challenging (or even impossible) to make using traditional methods. This includes many with particularly desirable properties for drug developers, such as resistance to breakdown by enzymes in the body.

"Amines are very polar and therefore difficult to purify and manipulate without protecting groups," says Phil Baran, the Darlene Shiley Chair in Chemistry at the Scripps Research Institute, and leader of the study. "I think that one of the most exciting aspects with our method is that it might one day be used widely by industrial drug and agrochemical makers for the betterment of humanity. Many companies have already told me they are using the new method, including Bristol-Myers Squibb (BMS), who are actively using it in current medicinal chemistry programs."

BMS and Scripps have been working together for some time as part of a multilaboratory partnership. In one of their latest projects, scientists from Scripps and BMS found that mixing two abundant (and cheap) feedstock compounds – nitro(hetero)arenes and olefins – with an iron catalyst could generate a variety of complex amine-containing compounds, under very mild conditions. According to Baran, most scientists have nitroarenes and olefins on a shelf in their lab, but it is only when they are merged in a precise way that they are able to produce such a wide range of amines. He adds that the method is very simple and practical to carry out. His team has successfully used the reaction to synthesize over 100 different amines, including drug compounds, in fewer steps than would be used in traditional methods. Indeed, some of the amines produced contained sensitive functional groups that couldn't survive conventional amine synthesis reactions.

Baran says, "Our lab has always been driven by pragmatic considerations and cost. We feel that if you're going to invent a reaction it should probably be accessible to as many people as possible and not be over-engineered," says Baran. "As for how our technique will fare in the future, time will be the ultimate judge of any synthetic method so let's check back in five years. At the moment though, we are working on improvements such as rendering alkyl amines in higher yields." *SS*

Reference

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Biomimetic Protection of Biomolecules

Porous shells – inspired by sea urchins – help defend biomacromolecules from the outside world

Vaccines and other biomedicines can easily be denatured or decomposed by unforgiving conditions. But does nature already have the answer for protecting at-risk biological material? Well, many organisms collect and process minerals to fabricate tissues that serve structural or functional purposes, and Australian scientists believe that the process of biominerialization can be used to protect biomolecules. Starting out with some preliminary experiments, researchers from Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) added proteins to an aqueous solution containing the chemicals needed to produce synthetic porous shells (1).

"Firstly, we investigated the chemical reaction to determine the best 'biomineralization' conditions for the production of the porous shells. It was surprising to see that biomacromolecules were acting like micro-reactors, bringing ligands and cations (the precursors of the porous shell) together. The conditions were enough to trigger the formation of a tri-dimensional porous hybrid network, also called a metal-organic framework. Next, we investigated the generality of the method, and discovered that the vast majority of biomolecules were triggering the formation of shells within a few minutes," says Paolo Falcaro, a researcher in material engineering at CSIRO.

Subsequently, the team studied the properties of the protected system by exposing the encapsulated biomolecules to extreme conditions such as boiling organic solvents and high temperatures. The result? The shells prevented deformation and decomposition that would almost certainly result in loss of bioactivity. In addition to the potential ability to preserve and deliver proteins, antibodies, vaccines, DNA and enzymes, Falcaro believes that the method could open up a new world of applications for biomolecules in chemical processing and biostorage.

"It was fascinating learning that useful natural biomacromolecules were acting like a living entity, bringing together chemicals and generating a self-protective crystalline porous shell," says Falcaro. "Interestingly, the shell is porous and allows small molecules to diffuse through it. The shell can be dissolved on demand by adding a weakly acid solution."

How is the shell made? Easy, says Falcaro – akin to preparing instant coffee. The addition of two chemicals (Zn2+ ions and 2-methylimidazole) to an aqueous solution that contains the biomolecules is enough to promote the formation of the protective porous shells.

At the moment, the shells can be prepared only with certain organic ligands and metal ions. "From the chemistry perspective, we are currently investigating other ligands in order to tailor the pore size and topology. From the biochemistry perspective, we are trying to predict the 'biomineralization' efficiency of proteins with different hydrophobic/hydrophilic domains and surface charge," says Falcaro. "We are also investigating the efficacy of the shell for different biological systems." *SS*

Reference

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In My View

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

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

Contact the editors at edit@texerepublishing.com

A Blast From Potions Past

Are ancient texts hiding the answer to antibiotic resistance?

By Christina Lee, School of English, University of Nottingham, UK.

Some view science as a relatively modern invention, but scientific knowledge and ingenuity go back thousands of years, even if the language used to describe phenomena sound ridiculous to modern sensibilities. As an example, in medieval times diseases were thought to be transmitted by dragons. It sounds outrageous, but it means that there was at least an understanding that some diseases are airborne.

I want to share a fascinating research project that stems from studies in arts and humanities rather than science. Subjects that fall under the umbrella of arts and humanities are sometimes perceived to have less practical value, but there is potential for these subjects to make a difference in many ways. They should not be dismissed or forgotten. Let us consider the problem of antibiotic resistance. Pharmaceutical companies are urgently seeking an answer, but have they thought to look in the past?

I recently became involved in a pilot study where I am working alongside microbiologists to test Anglo-Saxon recipes for antibacterial effectiveness. My colleagues and I didn't really set out with any intention of making an important medical discovery; we simply found it fascinating.

I study history, particularly Anglo-Saxon England and the Viking world. One day, a colleague asked me about Anglo-Saxon antibiotics; after all, infections have been around forever. Because we had a wider network of people around us who were interested in infectious disease we decided to test an old remedy to see if it had any antibiotic effects. We turned to Bald's Leechbook – an old English medical text that contains various recipes (some of which sound insane) passed on from various sources, including the Romans – and decided to test a remedy for styes – eye infections caused by Staphylococcus aureus.

And so off we went to recreate the recipe as authentically as possible, with ingredients such as garlic, wine and bile from a cow's gall bladder. The recipe had very precise steps; to start, we had to pound it and strain it through a clean cloth (not pleasant!). Not just any cloth, either – Bald's Leechbook is often quite specific about what textiles to use. It sounds crazy until you consider that wool, for example, has compounds such as lanolin, which affect different chemical reactions. We also had to use a brass vessel, leave the mixture to stand for nine days, and so on… There were many steps and we followed as best as we could. The finishing touch? Applying the ointment with a feather. It was an entertaining exercise, but when we applied the ointment to cultures of MRSA bacteria, I didn't think it would work.

But I wouldn't be writing this following a failure. Sure enough, it killed the bacteria. We were astonished and did a few other experiments; none of the ingredients worked separately, but together the outcome was always the same, resulting in up to 99 percent of the bacteria being killed. So we decided to try it on mice, with the help of a bacteria expert in Texas. The recipe still worked. Our collaborator was stunned!

Our university press officer thought it was intriguing too so she thought we should put out a press release. Soon after that, it seemed that the entire world was interested. The story went viral – we were receiving hundreds of emails every day from newspapers, as well as patients demanding that we hand the recipe over. Nothing can prepare you for that kind of attention!

We've written a research paper on the

work, which is due out soon. And now we're looking further into the research to see if there is more potential there. Of course, this is extremely early work and we have no idea if it can be transferred to humans.

It is amazing to think that somewhere in Europe all those years ago someone wrote down this knowledge. Which begs the question, where will the spark of inspiration for the next antibiotic or blockbuster medicine come from? Future scientists? Scientists of the past? Or maybe from academics studying arts and humanities? We're certainly looking forward to testing more recipes from Bald's Leechbook…

A Personal Question

Can nanotechnology pick up where personalized medicine leaves off?

By Claire Thompson, Director, Nanoscientium, London, UK.

Personalized, precision or stratified medicine – we can't agree on what to call it but we all agree that we desperately need it. With even the most promising modern medicines showing therapeutic activity in only around 30 percent of the population (1), we need to get better at predicting, detecting and targeting diseases across populations.

There is no shortage of international initiatives and funding to fuel innovation in this area. For example, in January 2015, US President Barack Obama launched a Precision Medicine Initiative, while in the UK the 100,000 Genomes Project is already well underway. These programs will lead to a much greater understanding of the diversity of our genomic make up and a plethora of new targets for drug development or diagnostics.

But this is where the current remit of personalized, precision and stratified medicine tails off, and we need to go further. For medicine to be truly personalized, we cannot simply become better at predicting disease. We must be able to routinely and reliably detect, monitor and target disease, and this is where nanotechnology comes to the fore.

Nanoparticles or nanocomplexes are already routinely used in diagnostics. From pregnancy testing to malaria diagnosis, they enable precise, accurate and reproducible data. Rather than medical professionals taking blood samples and sending them for analysis off-site, we are starting to see more point of care diagnostics, where nanosensors allow rapid readout from small samples. Nanotechnology is also used in diagnostic imaging. For example, EndoMag has developed a handheld magnetic probe (SentiMag) and magnetic tracer (Sienna+) to localize lymph nodes for cancer detection and staging. The products will be launched in the US in 2015.

The Qualcomm Tricorder X Prize is catalyzing the convergence of PoC diagnostics with digital or e-health. The prize professes to be "turning science fiction into reality" and who would argue with them? It is a global competition to make a portable device that can diagnose 20 different medical conditions and readout continuously to "put healthcare in the palm of your hand". The top 10 teams, including teams from the US, Canada, India, Taiwan, Slovenia and the UK, all use nanosensors.

The biggest issue with delivering precision medicine is exactly that – delivery. Targeting drug molecules to the right organ, tissue or cell is an ever-present problem in pharma and biotech. Nanotechnology from BIND Therapeutics is helping to overcome this challenge. Founded by Professor Robert Langer from Massachusetts Institute of Technology, BIND's flagship technology, Accurins, deliver targeted and programmable therapeutics. Accurins are functionalized nanocomplexes that target disease-specific cells or tissues and deliver their therapeutic payload directly to the site of disease. Such targeting enhances efficacy and minimizes adverse effects on healthy tissues. BIND has already demonstrated positive Phase II results for non-small cell lung cancer and has inked deals with Amgen, Pfizer, AstraZeneca, Roche and Merck.

The field of theranostics, which combines diagnostic and therapeutic capabilities into a single agent, is another emerging technology, with the potential to diagnose and deliver the right dose to the right tissue at the right time.

Personalized medicine doesn't just relate to genomic subsets of a population – targeting drug delivery to specific age groups can also make a big difference to patients. For instance, VaccineTab has developed a liposome-encapsulated nanotechnology for vaccine delivery. It is a needle- and pain-free delivery system, so is has particular benefits for children's vaccinations. In addition, the VaccineTab technology is thermally stable, thus reducing the need for cold chain product supply. In the developing world, where the cost of vaccines and lack of cold chain logistics prevent effective vaccination programs, VaccineTab could have a huge impact.

With its health market predicted to reach US$1 trillion by 2021, it is abundantly clear that nanotechnology will pick up where genomics ends, driving disease detection and the targeted delivery of personalized medicine. They do say that good things come in small packages...

Reference

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Diagnosing Malaria Sooner

Our third effort at developing a simple analytical diagnostic for malaria looks promising. It could help prevent the spread of the most devastating disease on the planet.

By Bayden Wood, associate professor, Chemistry Department, Monash University, Victoria, Australia.

Over three billion people are at risk of malaria. In 2012, according to World Health Organization estimates, 207 million people were diagnosed with malaria and 600,000 people died from the disease; the main casualties were children under five and pregnant women.

There is an urgent need for diagnostics to detect the early stages of the parasite; they must be highly sensitive, cost effective, simple to use, and rugged enough to be transported to remote areas in tropical jungle communities. Current diagnostic tools include optical microscopy, which has a sensitivity of around 40 parasites/µl but which requires an experienced microscopist; monoclonal antibody-based rapid diagnostic tests (RDTs), which are easy to perform but do not quantify parasitemia (parasite load) and take about 20 minutes per test; and polymerase chain reaction (PCR) assays – the current gold standard – which have excellent sensitivity (one parasite/µl) but require expensive technology and reagents – and results take up to two hours to generate.

For a diagnostic technique to be effective it must be able to detect both the immature asexual (ring) stage of the parasite (the only stage that is present in the peripheral circulation in new infections) and the mature sexual stage, which appears later and is the only stage capable of transmission to mosquitoes.

We initially investigated the potential of Raman microspectroscopy in combination with multivariate data analysis (1). The technology showed potential for detecting hemozoin, a by-product of the catabolization of hemoglobin and also known as malaria pigment, but it took several hours to produce results, which is not acceptable.

To accelerate the analysis, we next detected hemozoin in a whole drop of blood. Using an ultrasonic acoustic levitation device we could probe the droplet with a Raman microscope with a right angle lens. An acoustic levitation device consists of a piezo electric transducer and a reflective sound plate, which together generate a standing wave with very stable ultrasonic nodes. A droplet of blood can be placed in one of the central nodes and levitated in air, which has the advantage of concentrating the droplet though evaporation and reducing the attenuation of Raman laser light, as there is no container. This enabled us to record high quality spectra and detect later stage ring-form parasites. However, it was not conducive to routine analysis as the droplets can become unstable after time and explode, and it did not detect the early stage rings found in peripheral blood. But it did demonstrate the ability to investigate a large population of cells with a spectroscopic modality.

Building on this, we identified a unique fatty acid signature for each stage of the parasite's life-cycle at the single-cell level using the FTIR microscope on the infrared beamline at the Australian Synchrotron (2). Since a synchrotron clearly cannot be used as a routine clinical tool, we turned to total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. This detected the earliest ring forms of the parasite and the gametocytes by analyzing specific fatty acids associated with the parasite membranes rather than relying on hemozoin. ATR-FTIR in combination with partial least squares (PLS) regression analysis enables parastemia detection down to 0.00001 percent in laboratory-spiked red blood cell samples – an improvement upon the PCR assay. Moreover, the technique is portable and rugged, so it can be placed in a bag and transported to remote jungle communities. It quantifies parastemia and does not require highly trained technicians. Sample preparation involves blood centrifugation, removal of the plasma and white blood cells, and the addition of methanol. A 20 µl aliquot of packed red blood cells is placed onto the ATR-FTIR window and the spectrum recorded in about 20 seconds. The spectrum is run through the PLS algorithm and the diagnosis, including degree of parastemia is determined in seconds (3).

The ability to detect very low levels of parastemia is crucial. People with low levels of malaria parasites often show none of the classic fever symptoms but infect more vulnerable members of their communities via mosquito bites. We will soon conduct a pilot study in Thailand to test the efficacy of the ATR-FTIR approach with clinical patients in remote communities.

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Black-Box Data Analysis for Spatial Metabolomics

Automated and reliable tools for spatially annotating metabolites from imaging mass spectrometry data are essential.

By Andrew Palmer and Theodore Alexandrov, European Molecular Biology Laboratory, Heidelberg, Germany.

In recent years, metabolomics has been recognized as a field of major importance that promises to advance our understanding of cell biology, physiology, and medicine. Metabolites are the 'small cogs' in the cellular machinery and consist of small molecules that are ingested, altered, and catalyzed within the cellular machinery, including not only those molecules synthesized within cells but also those gained from the environment, such as vitamins and nutrients. Such molecules are indicative of cellular processes both from the underlying genetics, cell differentiation and the immediate environmental pressures – and they provide a real-time read out of the state of individual cells and cell populations. Cellular activity can be highly spatially localized and so being able to image markers of metabolic activity may provide researchers with new perspectives on biological problems. Traditional methods often treat samples as homogeneous bulk materials, but this risks missing important biological information; for example, the degree of penetration of an anti-cancer drug into a tumor or a secretion of an antibiotic in proximity to invading bacteria.

Imaging mass spectrometry (MS) is essentially a chemical camera that can map the distribution of chemicals across a sample with micrometer precision using highly accurate measurements of the molecules' masses. Its unique feature is that effectively millions of images are recorded showing the distribution of potentially thousands of molecules. Unfortunately, this makes the datasets very large; a single image can be over 100 GB of data and processing the data is currently the main bottleneck in gaining further biochemical and biological knowledge.

What we need to exploit the full potential of such an advanced technique are algorithms for high-throughput molecular annotation of our 'big data' data. Successful algorithms must incorporate existing molecular knowledge databases, efficiently exploit both the mass spectral and spatial information inherently present in imaging mass spec data, but also, importantly, control annotation confidence.

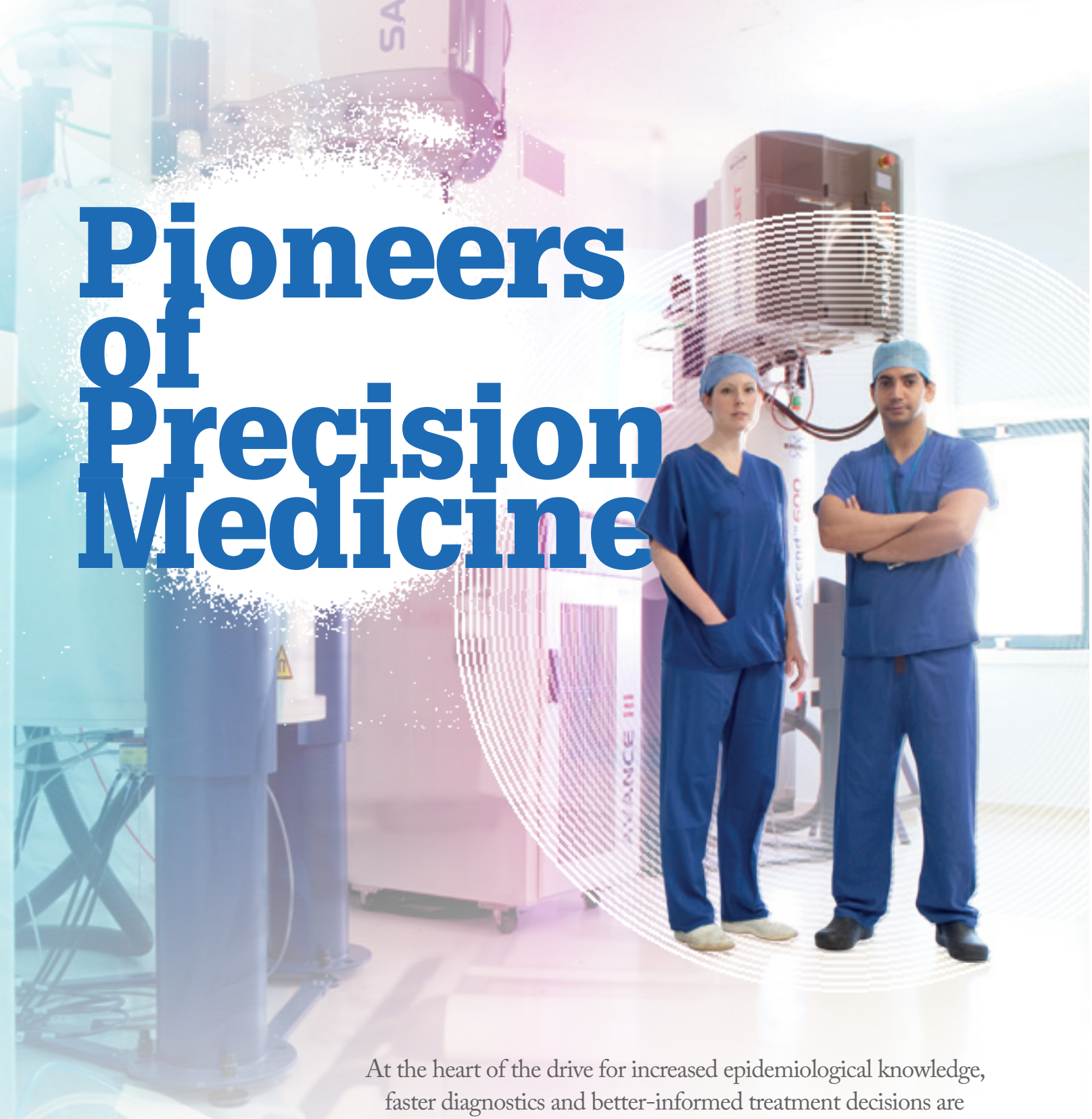
A large body of knowledge on metabolites and metabolic pathways has been accumulated for specific biological systems and recorded in curated databases (for example, HMDB, KEGG, LIPIDMAPS, ChEMBL). We are developing novel spectral and image analysis tools to assess whether these molecules are present in imaging MS data – and where. In fact, this approach is quite different to the usual methods for analyzing mass spectrometry data, which typically focus simply on individual spectra. Our tools will be wrapped up as an online 'black box' search engine to which researchers can directly submit their data. Users will receive molecular images corresponding to detected metabolites as an output, which shifts the perspective away from MS peak analysis of individual spectra to high-level analysis of metabolic images linked to molecular knowledge bases.

"Our tools will be wrapped up as an online 'black box' search engine to which researchers can directly submit their data."

Over the past few years, we have developed the algorithms that form the cornerstone for such a black box system and evaluated them within the biological analysis pipelines of several collaborators. The next step will be to provide it to the community as an open source engine so that everyone can use it online or offline to turn the chemical pictures produced by imaging MS into functional maps of metabolic activity. This is the core aim of the European Horizon2020 project METASPACE we have just launched that unites eight partners from five countries.

For more information, visit: www.embl.de/research/units/scb/alexandrov. And on page 36, enjoy an artistic representation of the future of 3D chemical mapping from Alexandrov.

Pioneers of Precision Medicine



At the heart of the drive for increased epidemiological knowledge, faster diagnostics and better-informed treatment decisions are collaborations between medical doctors and analytical specialists. Here, Jeremy Nicholson shares the vision behind the UK's MRC-NIHR National Phenome Centre, Ron Heeren showcases the new M4I institute and Steven Olde Damink offers the surgeon's perspective as a new age of healthcare dawns.

Our Phenome Future

By Jeremy Nicholson, Chair in Biological Chemistry and Head of the Department of Surgery and Cancer at Imperial College London.

I have quite an unusual background in chemical sciences and pathology, which culminated in me becoming the Chair in Biological Chemistry at Imperial College London in 1998. I'm still in that role, but I also became the Head of the Department of Surgery and Cancer at Imperial about five years ago. I'm probably the only non-clinical professor in charge of a clinical department anywhere in the world.

How did that happen? Well, contrary to popular belief, managing clinicians is not necessarily a clinical job – it's actually about research vision and coordination of scientific activity, especially in such a large department. Notably, the department is more than just surgery and cancer; I also have reproductive medicine, anesthetics, pain medicine, critical care, obstetrics and gynecology, hepatology and gastroenterology all reporting to me in terms of academic structure. And there is a big division in the department called Computational and Systems Medicine, which has about 180 non-clinicians. The MRC-NIHR National Phenome Centre is part of this latter division and took on the legacy of the state-of-the-art drug testing analytical laboratory from the 2012 Olympics games. In July 2013, we repurposed the analytical tools therein for epidemiology-scale population phenotyping.

We also have a clinical string to our phenome bow. At the Imperial Clinical Phenotyping Centre, I look after the stratified medicine research team. The focus there is on personalized healthcare – trying to identify new targets, looking at unmet disease needs, and understanding the areas where personalization does and does not work. A very important part of this is molecular phenotyping,particularly metabolic phenotyping. We're also interested in the overall patient journey and how we can optimize it by understanding metabolic changes. We can use metabolic information from NMR or mass spec at the beginning of the journey as a diagnostic tool, but also in the interventional stage, when we are interested in monitoring patient progress over time. Beyond that, prognostic modelling based on previous patient journeys can be used to predict the outcome of a particular therapy, which brings us back to personalization. By using metabolic phenotyping, we can stratify patients not only into disease subclasses but also into treatment regimes. It's an extremely advanced stratified medicine program that integrates genomics, metabolism and meta-genomics.

Going back to the MRC-NIHR National Phenome Centre, we use a range of similar technologies – NMR and mass spec – with a high degree of analytical overlap with the Clinical Phenotyping Centre. Clearly, the aim is quite different – epidemiology informs future healthcare policy –so we are interested in linking metabolic phenotypes with disease risk.

What's unusual – perhaps unique – about our department (apart from having a non-clinician running it!) is this alpha–omega approach to metabolism. We go all the way from disease risk and general population phenotyping, through to monitoring patient journeys and into instantaneous diagnostic tools like the iKnife, the iEndoscope and associated technologies that could potentially affect second-to-second decision making by surgeons.

The iKnife story

I recognized quite quickly that Zoltan Takats – inventor of the iKnife – matched our profile for advanced technology in the department perfectly. I also realized that he would probably be able to build on that technology faster in a world-leading surgical setting. The iKnife – and the rapid evaporative ionization mass spectrometry (REIMS) technique at its heart – gave us another angle on metabolic diagnostics. It also fit in well with the long-standing strategic relationship we have with Waters Corporation, who actually acquired the REIMS technology in July 2014.

Funnily enough, a year or more before I heard about Zoltan's research, I'd been talking with Waters about an idea for an electrosurgical knife adapted for mass spectrometry. It seemed obvious to me that the smoke could be a rich source of information. In fact, Lord Darzi (ex-Minister of Health and a surgeon in my department) and I received a grant from our National Institute of Health Research (NIHR) Biomedical Research Centre to work on the development of a prototype iKnife technology. We'd only had the grant about three days when I saw Zoltan's first paper in Angewandte Chemie. But if you can't beat them – join them. Or get them to join you.

We hired Zoltan and his team shortly after, and it turned out to be one of the best moves I've ever made. I have to give credit to Zoltan as the inventor of the iKnife – but it could have been me! Actually, Zoltan is a world-class mass spectroscopist and I couldn't really hope to match his efforts in that technology area.

It seems that everyone wants an iKnife – if you could get a truckload you could sell the things in a hospital car park... But seriously, we have to be careful about who we partner with because it needs to go through clinical trials, as it is extremely patient facing. There is still a lot of work to do, but we move into clincial trails this year.

Taming a Clinical Department

Scientists are clearly trained in very different ways to doctors. What we do in medical education (which in my opinion is completely out of date) is to completely silo people. By the time someone becomes a proficient expert – a nephrologist, hepatologist, or neurologist – they end up knowing very little about anything else. Because the Department of Surgery and Cancer is so broad, it contains the whole gamut of doctor phenotypes – from surgeons to baby specialists – that don't mix well scientifically. When I inherited the department it was a shambles in terms of strategy, with no unifying features.

As a non-siloed scientist I was able to step back and look at the big picture, and start connecting people in different ways. In fact, I decided to unify the department by focusing on a personalized healthcare theme based on systems biology and computational/analytical technologies. It enabled an entirely different approach to interlinking multidisciplinary teams and projects. It also proved that having a research plan and sticking to it does actually generate money...

Of course, it wasn't easy. Part of the success stems from understanding what everyone does. Paraphrasing Chinese military general Sun Tzu: if you know your troops and know your enemy, you can fight a hundred battles without fear of loss.

And so, when I became head, I visited every research group to find out what they were doing. It took me 18 months – there are nearly a thousand people in the department (over six campuses). I then had to map out the activity and figure out what groups could work well together. I actually used systems biology modeling methods to survey the department, using self-organizing mapping and advanced multivariate statistics to optimally bridge groups.

Introducing such a large amount of core science into a clinical department has turned out to be an extremely successful strategic gamble. Many major medical institutions support basic connections with physical science, engineering, mathematics, computing, and so on. But adding groups, such as systems biology, directly into the clinical department is a much more efficient way of working. It means that the scientists, engineers and mathematicians gain a better understanding of the clinicians, the challenges and the big picture. In essence, lowering the communications barrier to facilitate collaborative research is key.

Likewise, desorption electrospray ionization (DESI) imaging – another of Zoltan's inventions – was transformed when it came to Imperial. Previously it was being used in a univariate-imaging mode, but we've really pushed the capabilities into multivariate imaging, which is vastly more informative and makes DESI an exciting new, orthogonal molecular pathology tool. The chemistry that's generated in DESI imaging has a great deal of overlap with REIMS – so you can use one database to populate the other. If you link histopathology with mass spectrometric imaging, a pathologist can identify a particular carcinoma and the associated chemical signature, which can link back to the iKnife. It's beautiful because you can slip data from one to the other as it builds a bridge between real-time diagnostics and pathology. And by using statistical total correlation spectroscopy (something we invented for NMR-based biomarker structure elucidation) with DESI-MS , we've built a bridge between biology and network biochemistry. I find it very satisfying analytically that these aspects connect together in such a way.

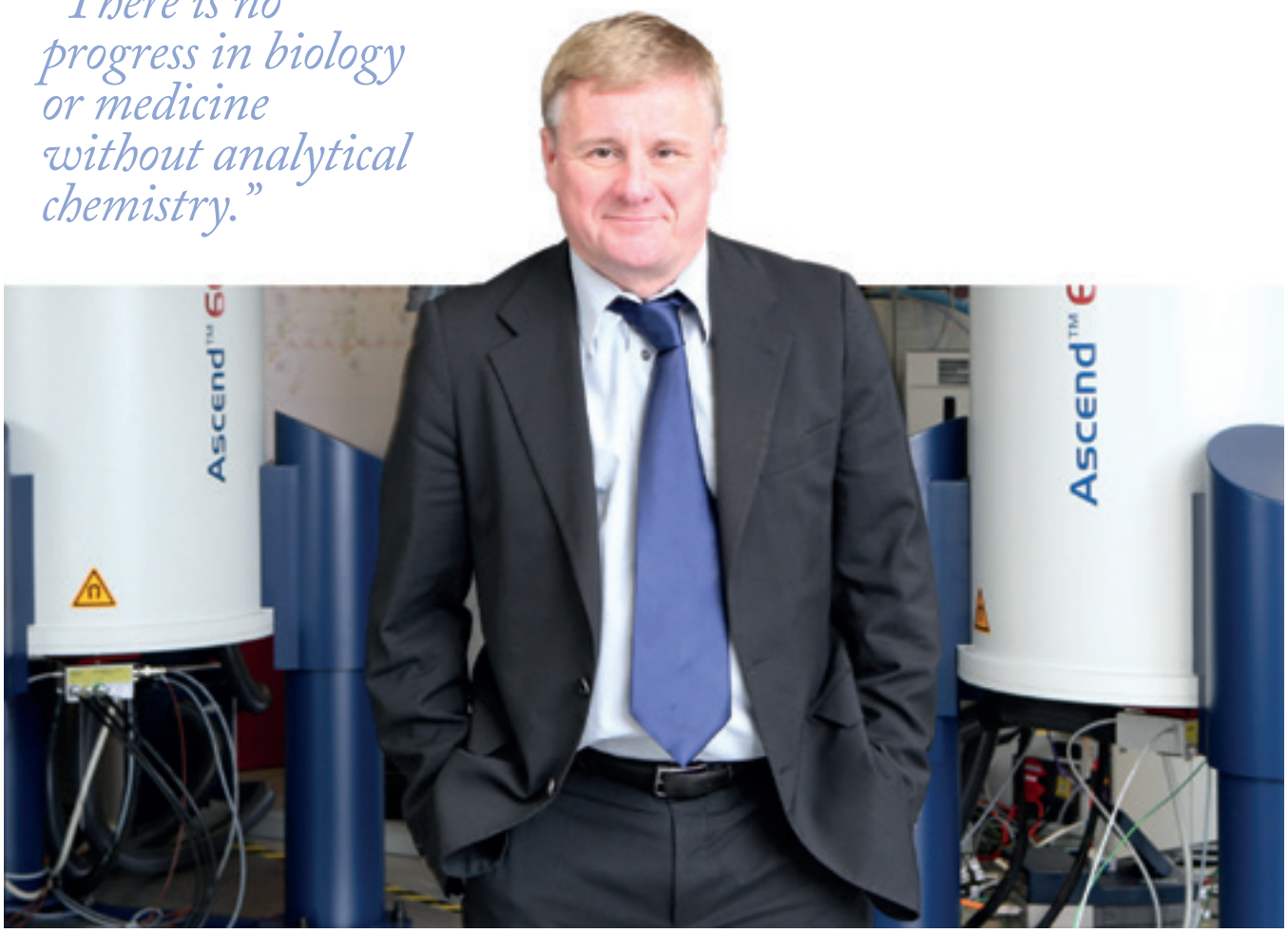
Alignment of the planets

Strategy is clearly important (see "Taming a Clinical Department"). But it's not just about strategic vision, it's also about good timing and bit of luck – like most things in science. I happened to be the right person to pull this together at the right time. And we have the funds to make it work. Working in a clinical department gives you access to a great deal of funding that you could never hope to attain as a basic scientist. I can honestly say that the last five years have been the most challenging, interesting and enabling of my entire career as a scientist.

It's also fair to say that if it weren't for the 2012 Olympic games, we would have found it difficult to pull together the instrumentation needed to make epidemiological phenotyping a reality. Acquisition at such scale is unprecedented, but given industry funded front end payments for Olympics drug testing and a grant from the Medical Research Council (MRC) and NIHR, we acquired a suite of instruments that includes about 20 mass spectrometers and three NMR systems, which gives us the bandwidth needed to deal with potentially up to 100,000 samples each year, with anything up to 10 assays per sample.

How does it work? Well, we might take 10,000 samples from a population study on blood pressure, and attempt to link diastolic blood pressure to metabolic variables to understand the roots of high blood pressure. We have National Phenome Centre projects running on cardiovascular and respiratory diseases, Alzheimer's disease, ovarian cancer – and the list is growing. Many of the studies also have genomic data, which allows us to perform a statistical data fusion with our

"There is no progress in biology or medicine without analytical chemistry."



Courtesy of Imperial College London Department of Surgery and Cancer

phenotypic data to produce system level models that can inform us about risk factors and the pathogenesis of diseases. We've got lipodomics platforms, reversed-phase UPLC-MS platforms, hydrophilic interaction chromatography for very polar molecules, and NMR-based assays for metabolome exploration. But we also have UPLC-triple quad MS for multiple targeted assays.

In essence, the National Phenome Centre is a combination of analytical methodology and huge bioinformatics capability (to the tune of about £30 million, thanks to the MRC, NIHR, EU, Waters Corporation and Bruker Biospin) and the strong basis for a successful template, from sample handling to analytical chemistry to statistics, which can be recreated anywhere in the world. And the same can be said for the clinical phenotyping center.

Indeed, now that the strategy is working, others are looking at the model and hoping to replicate it. In fact, we are setting up a network of phenome centers that use harmonized technology and methodology. If you do a study in our lab or at Nanyang Technological University in Singapore, you should be able to get the same answer. Historically, that hasn't been true in metabolic science because people have their own pet methods and instruments. Clearly, if you want to implement technology in a clinical setting – whether it's the iKnife or urinary metabolic profiling – it must be standardized, validated and widely accepted.

Analytical chemistry is at the heart of our efforts. There is no progress in biology or medicine without analytical chemistry, which is something that people can lose sight of. Imagine the Human Genome Project without DNA sequencing...

The next generation of medicine is going to be enabled by analytical chemistry. In a way, it's my job to educate medics that they need more analytical chemists around. Creating a research strategy that entirely revolves around an area of science where we are considered to be world class has certainly helped get everyone on board. But in the modern world you have to run to stand still, and race to stay ahead. There is no time for complacency; achieving the translational goals that we have set is a great and ever shifting challenge that will take many years of hard work. Per ardua ad astra!

Photo by Harry Heuts

MultiModal Molecular Imaging

By Ron M.A. Heeren, co-director of the Maastricht MultiModal Molecular Imaging Institute (M4I).

Molecular imaging based on mass spectrometry provides a broad scope of analytical, molecular and local information that can be employed for patient phenotyping. Within M4I – the Maastricht MultiModal Molecular Imaging institute – we develop innovative technologies to generate and discover more detailed knowledge that enables surgeons to perform tissue typing 'on-the-fly'.

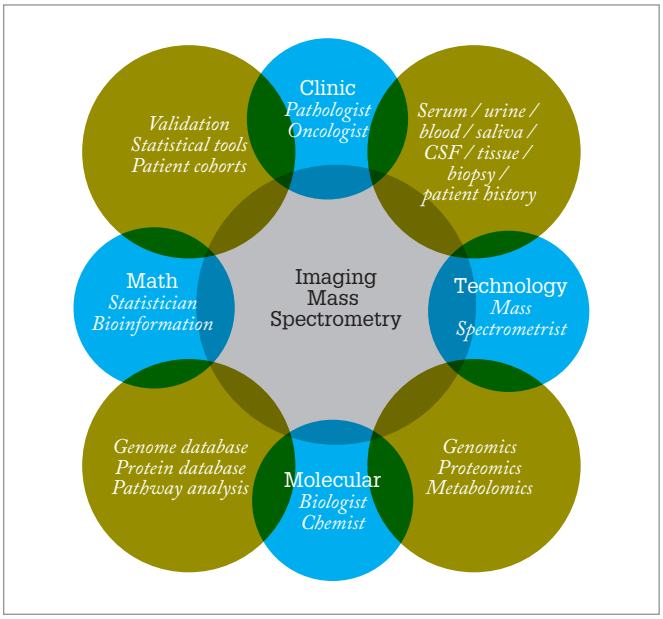
Coming from a technological environment (FOM-AMOLF), I felt that we needed to translate our new imaging technologies into the clinic – becoming one of two directors at M4I enabled me to do that. The possibility to collaborate closely with Steven Olde-Damink (a surgeon), Peter Peters (a nanobiologist) and Clemens van Blitterswijk (regenerative medicine) was an enormous motivator for me and it also essentially allows me to take my research to a higher level – in the pursuit of personalized medicine.

Personalized medicine requires different molecular datasets to be generated in a concise manner. Imaging mass spectrometry is a unique discovery method and I would say the main enabling high-throughput technology for this purpose. Personalized diagnosis relies on the quick and complete characterization of, for example, patient biopsies. Once we gain sufficient information, it can be directly employed to 'train' smart surgical devices, such as the iKnife.

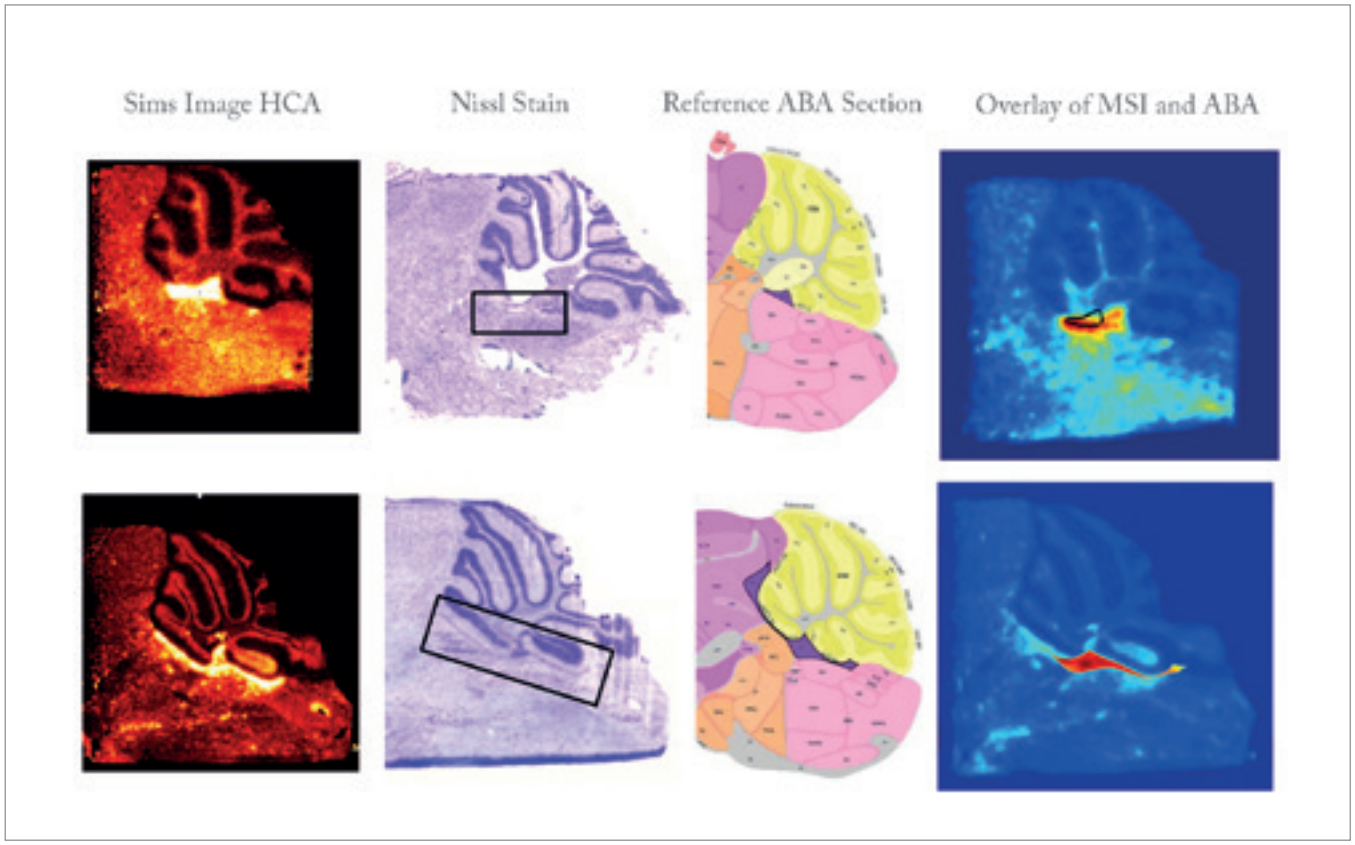
Maastricht University and the regional government have invested to strengthen the knowledge infrastructure for life sciences and health so that we can lead the European molecular imaging scene. Indeed, M4I offers a unique combination of enabling technologies for personalized medicine at the molecular scale, the cellular scale, the tissue scale and, most importantly, at the systems level scale.

In particular, we are developing new technologies that push the boundaries of MS imaging. We strive to enhance throughput, speed, spatial resolution, sensitivity and molecular resolution with a combination of fundamental, instrumental and applied research. We cover the whole story from nanoscopy to patient diagnosis within one institute, and everything must be embedded within clinical practice. And that isn't easy; the biggest hurdle was a logistical one – how could we house dozens of researchers and instruments in an existing infrastructure and bring everything up to the state of the art?

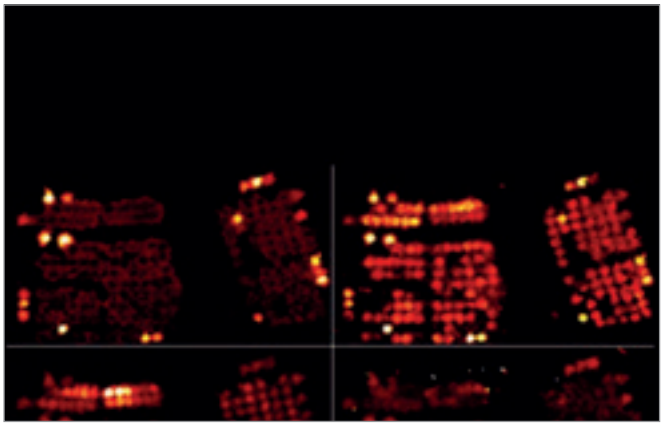
The answer was getting the right people. The new molecular imaging teams within M4I have the right expertise and capabilities to conduct truly translational research. The team



H&E stained tissue micro-array section of different human breast tumor models.



Automatic correlation of clustered SIMS data with Nissl stained histology is used to co-register regions in which a specific molecular signal is found with annotated regions in the Allan Brain Atlas (data by K. Škrášková and A. Khmelinskii of the Heeren group).



Hierarchical cluster analysis of mass spectrometric image data employed to determine tumor therapy response based on molecular classifiers. This method can be employed to rapidly build databases filled with molecular information. This in turn is one of the information sources for personalized medicine (data by N. Mascini and G.B. Eijkel of the Heeren group).

is made up of a broad range of analytically-driven scientists, including (but not limited to) physicists, (bio-)chemists, material scientists, bioinformaticians, pathologists and clinicians.

As a young university, Maastricht offers a stimulating environment. Facilitating and embracing multidisciplinary teams is a prerequisite for success in a day and age where the problems we tackle have become too complex for a single discipline. I think the unique element in our new endeavor is that different scientific cultures are able to open up and collaborate without hesitation. Finding surgeons who are willing to ask researchers how they can best optimize surgical protocols to improve personal molecular diagnosis is a real eye-opener. And it is indicative of the innovative attitude found at the Maastrict University Medical Center (MUMC).

Success will come from a shared goal. The surgeons and the rest of the team at M4I know that better molecular diagnosis will result in more targeted treatment, which will in turn improve the patient's prognosis and reduce the use of less effective therapies. For example, precise surgical margin determination during surgery reduces the chance of cancer recurrence, which has a clear impact on the quality of life for patients in the clinic.

One thing is clear. The institute's output in three years must feed directly into the clinic and contribute to improve health care. That is how we will make personalized medicine a reality.

The Surgeon's Perspective

By Steven Olde Damink, Consultant Surgeon Hepato-Pancreato-Biliary Surgery at Maastricht University Medical Center, Associate Professor of Surgery, and Director Research Laboratories Director, Department of Surgery, Maastricht University.

Two years ago, I built up the courage to travel to Amsterdam to convince Ron Heeren that he and his team needed to move to Maastricht, build a world-class MS imaging (MSI) institute, and directly embed it in a clinical environment. Such an institute, I believed, could bridge the gap that traditionally exists between the development of new technology and its clinical application. Indeed, the project's big ambition is to fully exploit the integration of new technological imaging developments within the clinical environment.

In the process of developing the idea for the Maastricht MultiModal Molecular Imaging Institute (M4I), Frans Ramaekers (scientific director of the MUMC research school GROW) was also able to attract Peter Peters (now co-director of M4I). Although Peter had a great offer on the table to move from NKI to Delft, the integrative nature of M4I convinced him to settle in Maastricht. Today, my role in the project is as initiator and clinical bridge/liaison.

The primary value of M4I really lies in the investment in human capital (rather than simply MSI hardware). Ron's team is at the

"Clustering objective patient information provides more insight in diagnosis and therapeutic success (response)."

forefront of the development of MSI equipment (fundamentals, technology) and application (desorption, detection). But we also needed buy in from specialists in instrument development, physicists, chemists, medical specialists, pathologists and translational scientists. Bringing MSI techniques into clinical practice requires rapid standardized measurements. Up until this point, technical development typically focused on discovery, so we needed a different slant. The multimodal approach appears to easily attract the talent we need from various research fields, but the key to our success will be based on the willingness of all team members to have an open mind to each other's field, specific scientific language and modus operandi.

To optimize collaboration, we decided to mix the research teams across all research offices and to have common research meetings and seminars. That said, the clinical research culture is very different from the research culture of chemists and physicists. According to the science philosopher Thomas Kuhn, the two sides may have difficulty learning each other's language and adapting to it. But hopefully this cultural border will create just enough research tension to result in Kuhn's predicted "scientific revolution."

Why mass spectrometry imaging?

Mass spectrometry imaging provides insight into the molecular basis of a clinical problem. Consequently, the nature of a problem can be defined more accurately – and the treatment adjusted accordingly. It becomes even more powerful when it is merged with other omics data, such as genomics and transcriptomics. Clustering objective patient information provides more insight in diagnosis and therapeutic success (response) – and it is independent of personal interpretation or insight (the various 'schools' of teaching). It can also help ensure that patients receive optimal treatment or, perhaps even more importantly, prevent the use of unsuccessful or harmful treatments; for example, chemotherapy being given to 'non-responders'.

As a surgeon, it is of the utmost importance to know the nature of the tumor you are about to operate upon. However, it is sometimes still difficult to obtain the correct diagnosis using standard pathological screening. Mass spectrometry imaging may give us the opportunity to gain a more specific and objective diagnosis ahead of surgery.

Joining forces

The research group of the Department of Surgery actually has a strong history of translational (metabolic) research. Specifically, we are strong in developing of specific human (patient) models to answer research questions, which allows us to move away from experimental animal models and therefore avoid the (patho-)physiological differences between species.

It is my belief that most real changes are driven by technological (and that is to say analytical) advances. The close collaboration between hardcore scientists all focusing on the development of new tools that help guide treatment and predict (and evaluate) treatment success makes this initiative fascinating. There are also exciting developments in real-time diagnostics; we hope to implement MSI into the operating room were it could provide on-the-spot information on the tissue the surgeon resects. Such techniques could potentially avoid incomplete tumor resection by giving detailed molecular information about the cut-section, allowing surgeons to continue operating if necessary. And working with surgeon scientists allows Ron and Peter's team to obtain optimally prepared human samples for imaging research. I think the biggest challenges we face are the standardization of analytical techniques and the building of metabolic profile libraries of diseases. In this latter endeavor, access to to the right patient samples is essential.

No doubt, the introduction of new techniques into clinical practice requires both an acceptance of its potential and a change to the current workflow. I believe that we can only succeed by collaborating closely with our colleagues in the field. Our objectives will take some time to realize, but the first Horizon 2020 grant proposal has already been submitted.

Shark Attack

Could the unique properties of the shark immune system help us create biologics with more bite?

By Charlotte Barker

When scientist Caroline Barelle tells people that her research involves sharks they tend to jump to conclusions. "People often imagine I work next to a tank full of great whites – and that anyone who disagrees with our science becomes lunch!" she jokes. In fact, the sharks Barelle and her team work with are not man-eaters but spiny dogfish (Squalus acanthias – shown above), a common species off the coast of Scotland, where their lab is based. Though the diminutive dogfish may not be auditioning for a part in Jaws any time soon, the technology that Barelle and her colleagues are developing has dramatic potential.

Sharks have been attracting attention as a potential source of therapeutic proteins since the 1990s, when scientists found that, despite evolving some 450 million years ago, they have an adaptive immune system that is surprisingly similar to a mammalian one. The researchers identified an antibody-like molecule – immunoglobulin new antigen receptor (IgNAR) – that forms part of this adaptive response (1,2). While standard mammalian antibodies are made up of heavy and light chains, IgNARs have only heavy chains.

Barelle first came across shark proteins in 2006 when she joined the antibody drug discovery biotech Haptogen. The company spotted the potential of these small antibody-like molecules for targeted therapies. The first thing to catch their eye was that the molecules are very small, less than a tenth of the size of human antibodies, and yet bind with great affinity to their target. They also have much greater stability than mammalian antibodies, thought to be a result of an interesting quirk of shark biology – their blood has a very high concentration of urea. The resulting high osmolarity prevents saltwater from dehydrating the animal, but also creates a harsh environment for proteins. The secret of how shark antibodies retain their stability in these conditions is being unraveled by researchers in Germany, who hope they can apply the knowledge to improve monoclonal antibody therapies (3).

Spin-out soloMERs

Convinced of their therapeutic value, Barelle has been working on shark proteins ever since, first at Haptogen, then at Wyeth and Pfizer, before joining the University of Aberdeen to head up their shark protein research program. But it wasn't until now that she had the opportunity to bring together the whole IP portfolio into one company, focused solely on the technology.

The resulting company – Elasmogen Ltd, soon to be spun-out of the University of Aberdeen – will use humanized versions of the IgNAR variable domain (VNAR). The VNAR domain is produced either by immunizing the sharks and taking a blood sample or, to spare the sharks (and perhaps researchers' fingers), assembled from a synthetic library of billions of shark VNARs created in the laboratory. The team then isolates VNAR domains against the target, selects the most effective, and humanizes it to produce a product suitable to be further developed for clinical use – a soloMER™.

"Antibodies are incredibly successful therapies that generate a lot of revenue", says Barelle. "However, mammalian antibodies have a number of limitations. They are very large, complex molecules that cost a lot to make, so antibody therapies are typically expensive. The size also limits what tissues the therapy can penetrate and the type of targets they can go after."

VNARs are single-chain domains, which makes them easier and cheaper to manufacture. Indeed, their small size and an unusual shape means they can reach targets that

Perfect fit

It was these special qualities of soloMERs that led Almac Discovery to approach Barelle's team. "We knew about the work at Aberdeen and the Elasmogen team," says Iain James, Vice President of Preclinical and Clinical Development at Almac. "We have a site-directed coupling technology for proteins that we are interested in using for antibody–drug conjugate (ADC)-like

approaches, and this seemed like a great match," he adds. Almac Discovery is focused on anticancer therapies, and the group was hunting for technologies that would allow better penetration of protein conjugates into solid tumors.

The collaboration was equally attractive to Elasmogen, who are looking for partners with complimentary technology to bring soloMERs into the clinic. Almac's proprietary coupling technology joins small-molecule drugs (in this case cytotoxic anticancer therapies) exclusively to the C terminal end of the protein, rather than the random coupling more commonly seen in antibody–drug conjugates. "We will know exactly where the cytotoxic is coupled, and how many cytotoxics are coupled to each protein. That way we can be in a lot more control of the manufacturing process and we have a much better defined product and possibly a better side-effect profile," says James.

The initial stages – production of the protein and screening for the most promising VNAR domains – will be carried out in Elasmogen's lab in Aberdeen. They will send the candidate VNAR domains to Almac Discovery, based in Northern Ireland, who will further develop the proteins and be responsible for pre-clinical and clinical development.

But the potential for soloMERs goes beyond cancer therapies, according to Barelle. Their extreme stability means that they could survive the harsh environment of the gastrointestinal tract (even after boiling, the team found the soloMERs would still bind to their target). An oral formulation for biologics is somewhat of a holy grail right now, and the team are keen to investigate this further. Another avenue to be explored is delivery to the eye. "Current ocular antibody therapies may involve regular injections into the eye – we hope that the small size of soloMERs could make them suitable for topical or site-specific application," says Barelle.

To find out what soloMERs are capable of, the team at Aberdeen are on the lookout for further collaborations with pharmas or biotechs developing compatible technologies.

Camels and convergence

Sharks are not the only animals whose immune molecules are inspiring interest. Camelids (a group that includes camels, llamas and alpacas) also produce single-domain heavy-chain antibodies,

From the Deep…

Shark VNAR domains were discovered by chance and scientists believe that many more potential therapies are hiding in the animal kingdom. Given that 95 percent of the earth's oceans have yet to be explored, who knows what life-saving compounds could be lurking in their depths. Here's just a few secrets the sea has given up so far…

Ocean oncology

Lissoclinum patella, commonly known as the sea squirt, could provide a new class of drugs in the fight against cancer. It's not the most glamorous of marine creatures, but the symbiotic microbes hosted by the sea squirt have been found to contain compounds, known as patellamides, active against a number of cancers. Another species of sea squirt was the original source for the chemotherapy drug trabectedin, now being used to treat soft tissue sarcomas.

Ouch!

A cone snail can cause a painful sting to unlucky humans who cross its path. But it seems that the venom also contains a number of analgesic molecules, which could have unique benefits for patients suffering chronic pain. Biochemist David Craik told a meeting of the American Chemical Society that a compound based on the venom was up to 100 times more potent than morphine in a rodent model, with no evidence of addiction.

Caring coral

Coral reefs have been a rich source of medicines for decades, with azidothymidine for HIV and anticancer agent cytarabine just two of the drugs that have their origins in these fragile ecosystems. A type of soft coral, gorgonion, produces several compounds with promising anti-inflammatory properties, with applications both in medicine and cosmetic skin creams.

in addition to the usual mammalian immunoglobulins. "I find it fascinating as a scientist that such diverse animals have evolved such similar molecules as part of their adaptive immune response," says Barelle. "It's a great example of convergent evolution."

What do sharks and camels have in common that means they independently evolved stable, single-domain antibodies? We can't know for sure, but Barelle speculates that an extreme environment may be a factor – after all, many camelids face an inhospitable desert environment, while sharks must overcome the dehydrating effect of salt water.

Camelid antibodies are being commercialized for the clinic by Belgian company Ablynx in collaboration with a host of big pharma companies, with several programs already at clinical stage, a success that Barelle's team finds encouraging. Although there are some similarities with the single-domain antibodies of camelids, VNARs are quite distinct – they have more binding loops, are smaller and, importantly, are phylogenetically distinct from antibodies, which the Aberdeen team hope will make them even more clinically effective and commercially attractive.

"It's been a very exciting time for me over the last few years," says Barelle. "I have moved from being a director of science to building a business and setting up new partnerships, like the one with Almac. To be even a small part of developing a drug that gets all the way into the clinic is immensely rewarding and I look forward to starting Elasmogen as a fully independent company in the coming months."

"The diminutive dogfish may not be auditioning for a part in Jaws any time soon, but the technology has dramatic potential."

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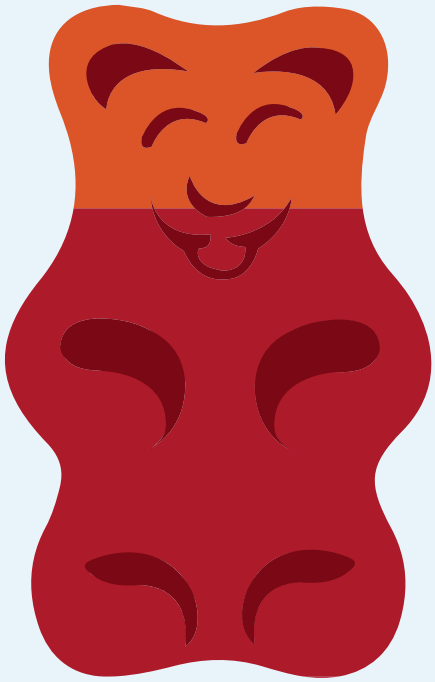
A Sweet Revolution

Glycan analysis poses major challenges for the biopharma industry; how can new technology lighten the load?

About 70 percent of preclinical and clinical candidate biopharmaceuticals are glycoproteins, with carbohydrate structures attached to amino acids in the protein. These glycan groups can have a huge impact on safety and efficacy, so accurate and efficient analysis of glycans is crucial.

In this two-part series, we'll be talking to the scientists who are applying cutting-edge analytical science to unravel the complex role of glycans in biotherapeutics.



Glycoproteins make up 70% of candidate biopharmaceuticals

Pick 'n' Mix Glycobiology

Jonathan Bones, Principal Investigator at Ireland's National Institute for Bioprocessing Research and Training (NIBRT), uses the latest technology to help biopharma overcome challenges in glycan analysis. Here, he shares his work and divulges how using several complementary techniques is the key to (sweet) success.

What's the mission of NIBRT?

At NIBRT, we work in close collaboration with industry partners to solve some of the key problems they face. The mix of fundamental science and real-world problems makes this a very stimulating environment; we're applying the latest analytical science to as many aspects of bioprocessing as we can. My lab has a major focus on glycan analysis.

Why is glycan analysis so important for the biopharma industry?

It's a regulatory requirement to provide detailed characterization of the glycan structures attached to therapeutic proteins; glycans can modify both the efficacy and safety of the molecule. For example, glycans present in the Fc region of a monoclonal antibody can modulate the interactions with Fc receptors in the immune system, which affects efficacy. In terms of safety, when proteins are expressed in non-human systems, such as CHO cells, you run the risk of non-human epitopes on glycan groups, which could elicit an immune reaction in the patient.

New technology allows us to make informed choices throughout the bioprocess, from selecting a cell line, to process testing, to the final product.

How are glycans analyzed?

A typical approach would be to detach the sugars from the protein and attach a fluorescent tag, then use high-performance liquid chromatography (HPLC)/ultra-HPLC or capillary electrophoresis (CE) to separate the fragments by size and polarity. Once you've separated the glycans, you usually want to characterize their structure. One method is exoglycosidase digestion, which uses enzymes to break down the sugars in a very specific and sequential manner. By looking at what you have removed and what remains, you can fit the puzzle pieces together to work out the structure. The other key technology is mass spectrometry (MS), typically used in combination with LC or CE to give you the full picture.

You make it sound relatively straightforward…

Not exactly! One of the biggest challenges is the complexity of glycans. The sequence of a protein is linear – you can visualize it as a string of beads – and we can use enzymes to break apart the beads for analysis in a predictable manner. Glycans, far from being linear, are complex branched molecules with multiple points of connection – more like LEGO® blocks than beads. That adds huge complexity because we not only have to identify the sequence of monosaccharides that makes up the glycan, but also their position and linkage orientation.

Glycan analysis for monoclonal antibodies is hard enough, but when you start looking at the larger therapeutic proteins like interferons, recombinant hormones or erythropoietins, it's a whole new ball game, with large, complex branching glycans and modifications with inorganic substituents or sialic acids. To unravel this complexity, you need not just one analytical technique but a range of complementary, orthogonal techniques to confirm that what you found with the first technique is what's truly there.

How are you helping to overcome these challenges?

Right now, we're doing exciting work on new technology for quantitative and full structural characterization of glycans in biotherapeutics. We're starting to adopt advanced technologies from proteomics – we're robbing the proteomics toolbox and making it our own!

Over the past five years there has been a lot of progress in quantitative analysis of glycans, with new tandem mass tags and isotope labels being developed. Currently, most analyses rely on relative results – so it's sometimes hard to be sure whether seeing the same-sized peak on a chromatogram indicates exactly the same glycan profile. We are looking at new, stable isotope differential labeling methods, in which two independent samples are labeled separately, then run together (multiplexed) through the same LC-MS analysis. Such an approach allows us to minimize the technical variation and identify genuine changes in the molecules.

What technological advances have had big impacts on your work?

In recent years, new analytical technology has made it easier to generate the information we need. High-resolution accurate MS has really helped us to nail down structures and characterize modifications with confidence. We also do a lot of MS/MS work, using negative-ion MS to both sequence the glycan and provide additional structural and positional information. Ion mobility MS is another technology we are exploring as an add-on to LC separations – it provides another level of selectivity.

What makes glycan analysis such an exciting area?

The complexity in glycobiology gives you a lot of scope as a researcher, plus new technology and methods become available all the time. My background was in small molecules, but when an opportunity came up to work with Professor Pauline Rudd here at NIBRT, and subsequently Barry Karger at the Barnett Institute, Northeastern University in Boston, I couldn't resist taking on a new analytical challenge.

The most satisfying aspect is seeing the science we do coming to fruition – working with the team here to translate research concepts into actual solutions that benefit the industry, and ultimately the patients.

Instrumental Sugar Rush

With Ken Cook

In the past, it's been difficult to measure and analyze glycans – carbohydrates in general have weak polarity, do not easily stick to common column types and are difficult to detect after separation. But times have changed. More effective columns, advanced mass spectrometers, and new fluorescent reagents have made glycan analysis faster and easier. This, in turn, has generated ever-increasing interest in this field, and new advances for biopharmaceutical companies.

Safety is the top concern for all drug manufacturers. It's crucial that anti-self glycans are not inadvertently included in glycoprotein therapies, or they could potentially kill rather than cure. Glycans also play an important role in efficacy and can act as highly effective biomarkers. For example, changes in the complex glycan structure of serum glycoproteins can be used to detect heavy drinking – something that patients often lie about.

So how can new technology help harness this potential? First, the ease of analysis has improved greatly. The first really effective columns for glycans were all amide hydrophilic interaction liquid chromatography (HILIC) columns, which can only separate by size and heterogeneity. Now, we've brought out two new columns that can also separate different charge states. These are particularly suitable for complex glycans, such as those found in serum proteins, which can have up to six charge states.

Monoclonal antibodies typically have much simpler glycan groups, and with the high-resolution mass spectrometers that have come out in the last couple of years, such as Thermo Scientific™ Orbitrap™-based instruments, we are now able to analyze the whole antibody at once, including any glycan groups. A single analysis obviously offers a big time-saving compared with the traditional method of deglycosylating the protein, separating out the carbohydrate, adding fluorescent labels and then carrying out liquid chromatography, often coupled with mass spectrometry (LC-MS).

Of course, getting good data from chromatography or mass spectrometry is only helpful if you can interpret it. In recent years, there has been a lot of work done on bioinformatics, both by universities and vendors, and there are now several software packages (including SimGlycan® from PREMIER Biosoft) available that can accurately identify the glycan structure from the results of an analysis.

In combination, these new techniques and technologies are allowing biopharma companies to characterize glycans with more accuracy and in more detail than ever before.

Ken Cook is EU Bio-Separations Manager at Thermo Fisher Scientific.

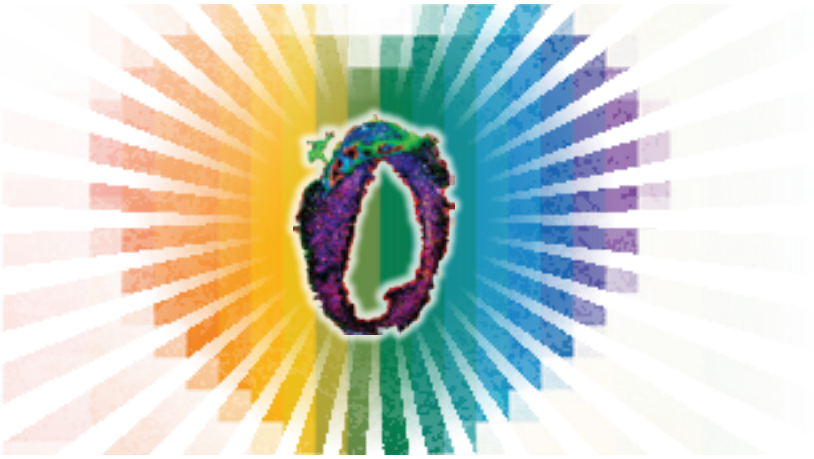
Toolbox

Key tools
New technology
Emerging techniques

MS Imaging Targets the Clinic

The field of mass spectrometry imaging is enjoying constant – and exciting – progress. Here, Ron Heeren and Axel Walch highlight current research applications and anticipate the impact that the technology will have on clinical practice.

By Rich Whitworth



Give us a snapshot of mass spectrometry (MS) imaging

Axel Walch: From a clinical perspective, MS imaging – unlike most imaging methods – removes the need for target-specific labeling whilst measuring the required broad spectrum of endogenous and exogenous analytes in tissues within the histological context. The highly multiplexed analyses offered by MS imaging enables not only diagnostic (targeted) assays but also the potential for biomedical discovery applications. Multi-class analysis capability (from proteins and peptides to drugs, lipids and beyond) is a further strength of MS imaging. The practical simplicity of MS imaging and its ability to gain reliable information, even from the smallest tissue samples, means that it has the potential to complement traditional histopathologic evaluation for assisting in diagnosis, risk assessment, or response prediction to therapy.

Ron Heeren: Right now, there is a strong push for higher information content imaging to unravel the molecular complexity of biological surfaces. This includes a drive for higher resolution techniques – both higher spatial resolution and higher molecular resolution. The latter is being pushed by high-resolution mass spectrometry (MS) techniques (Fourier transform (FT)-MS, TOF systems and so on), as well as the incorporation of ion mobility separation to enrich the wealth of molecular information obtained from complex organic surfaces.

Another important development is the move towards rapid quantitative MS imaging strategies that use selective reaction monitoring (SRM) approaches and dedicated internal standards. In particular, this is a requirement for pharmaceutical applications, such as pharmacokinetic and pharmacodynamic (PK/PD) studies.

Ambient imaging technologies are opening up a whole new area of MS imaging. Essentially, samples that are not vacuum-compatible can now be imaged at the molecular level. In some cases, such as with laser ablation electrospray ionization (LAESI), multiply-charged ions can be produced, and that offers various analytical advantages. This step change now allows true top-down imaging to be developed.

What trends do you see in the application of MS imaging?

RH: MS imaging is developing into an analytical tool for the fundamental discovery of signaling pathways of disease, leading to validated molecular disease profiles in the area of clinical molecular imaging. These disease profiles can be used for staging a disease, that is, for diagnostic and prognostic purposes. Even more importantly, the breadth of molecular information that MS imaging offers is

a key input parameter for personalized medicine. In reality, it is rare to find a single molecule or biomarker that can be used to determine the course of a disease. Rather, it is an understanding of the interplay between proteins, lipids and other small molecules that offers the true solution, and that is exactly what MS imaging offers. It is this that will define its role in the future.

The heterogeneity of tumors can be fully disclosed using MS imaging. Lipids, primary metabolites and peptide/protein images reveal the tumor margins and provide insights into molecular signaling pathways. This makes MS a key tool for various aspects of oncology, such as identfying surgical margins, developing personalized therapies and tumor staging.

Other areas of medicine will also benefit from the fundamental understanding of the spatial molecular composition. One example is cardiovascular research: atherosclerotic plaques and the interaction of stents with arterial walls can be much more fully explored. Another is neurodegenerative diseases: altered molecular signals that lead to tissue loss or the formation of protein plaques in the brain can be dissected. The power to examine all molecules simultaneously will provide true mechanistic understanding of disease.

In the more distant future, I predict that there will be discovery-based approaches, drug distribution and effect monitoring… possibly even MS imaging within the GP's office.

AW: To answer that, let me offer a brief description of the current status of pathology in personalized medicine. The task of the pathologist is to assist physicians in the correct diagnosis of diseases at the earliest possible stage; this allows the optimal treatment strategy to be developed for each patient. Surgical pathology (the traditional tissue diagnosis of biopsies or surgical resection specimens) is simply a tool based on histology. "Molecular morphology" aims to combine novel molecular and genetic information with the well-established histomorphologic features that continue to define cancer and many other types of diseases mentioned by Ron. Certain molecular and genomic technologies for tissue analysis are already useful in diagnosis of disease, taking us towards treatment tailored to individual patients. Personalized medicine – the future of patient health care – demands personalized pathology. This would integrate the flood of new molecular data with traditional surgical pathology, digital histopathology, and clinical data in electronic medical records. MS imaging of tissues has a central role in combining traditional tissue diagnosis by histopathology with highly multiplex molecular analysis. MS imaging data sets are enormously complex, comprising thousands of unique ion images. These images can be registered to high-resolution digitized histology images, correlating molecular information and adding a new dimension of understanding to the histopathology and thus disease.

The future for personalized pathology is that molecular signatures of proteins, peptides, lipids, and molecules of cell metabolism obtained by MS imaging directly from patient tissues, will improve diagnosis and therapy response prediction for improved patient stratification. In PK/PD, the application of MS imaging to determine the tissue distribution of drugs and their metabolites will have a dramatic impact on both drug discovery and development.

What have been the biggest MS imaging breakthroughs of the last decade?

RH: The birth of atmospheric desorption and ionization techniques and their combination with mass spectrometry have opened up a new field of imaging and analysis. This has allowed analysis to be performed in situ, for instance during surgery and has also enabled researchers to study non-vacuum-compatible samples. Another huge advance was the recent development of top-down imaging MS in which large proteins are localized and, importantly, identified in the same high resolution MS experiment. The recent application of active pixel detectors coming from high-energy physics has increase throughput and sensitivity. In the secondary ion mass spectrometry (SIMS) community, new particle beams (C60, Argon and H2O clusters) have enabled detailed depth profiling and 3D imaging studies with larger intact molecular species. All very exciting breakthroughs.

AW: From a preclinical and clinical research point of view, there has been a plethora of methodological publications about MS imaging, but only a limited number of studies clearly demonstrated an impact on MS imaging in biomedical and clinical research applications – my main area of interest. The following impressive highlights show the potential for biomedical discovery:

- Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues (1)
- Mammalian heart renewal by pre-existing cardiomyocytes (2)
- Ambient mass spectrometry for intraoperative molecular diagnosis of brain tumors (3)
- Imaging mass spectrometry reveals modified forms of histone H4 as new biomarkers of microvascular invasion in heptocellular carcinomas (4)
- Chemo-informatic strategy for imaging mass spectrometry-based hyperspectral profiling of lipid signatures in colorectal cancer (5).

What are the greatest challenges facing MS imaging?

AW: MS imaging technology has huge potential, but do we want to see improved spatial resolution, the detection of higher molecular weight compounds, greater

sensitivity, better quantitation, and increased molecular coverage? Of course we do! And, as with other analytical techniques that have made the leap, increased system stability, reproducibility, and user-friendliness will be essential, if we are considering MS imaging for routine use in a clinical setting. This is where vendors can help; to ensure a successful transition, fully integrated systems are an absolute must. Some are moving in the right direction, but a complete solutions platform – from sample prep to MS analysis to bioinformatics – is not yet available. I would love to know what's in the pipeline…

RH: While the technology is evolving rapidly and resolution and speed are improving annually, challenges still exist. Research is striving for ever more detailed insights but we are falling orders of magnitude shy of the challenges in bioinformatics and clinical validation of results. Standardization and validation are two issues that must be addressed for clinical maturation. More and more data is being generated using protocols that are fit-for-purpose rather than being standardized, making it difficult to generate large-scale, validated e-biobanks with assured quality. There is a clear need for standardization of protocols and tissue standards to evaluate the quality of the data being entered into the databases. The Human Tissue Atlas is a perfect example of how immunohistochemistry (IHC) images are consolidated around different tissues and diseases. Something similar for MS tissue imaging would be an incredible clinical resource. The projection – or fusion – of MS images onto existing tissue atlases is another challenge that needs to be (and is being!) tackled by the MS imaging community. One example is the Allen Brain Atlas (www.brain-map.org), currently used by researchers involved in brain tissue imaging with MS. European network initiatives, such as the Cooperation in Science and Technology (COST) action on MS imaging, have the potential to address these challenges.

Clearly, you both envisage a strong role for MS imaging in a clinical setting…

RH: The vast amount of molecular information generated by multimodal tissue imaging experiments is ideal for generating biobanks for different diseases. MS delivers a lot of immediate information on many different levels which can be used to make, for instance, well-informed clinical decisions during surgery or selecting personalized therapies. However, as stated earlier, this is only possible if the clinician can assess the information in context or immediately compare it to molecular disease profiles obtained from existing knowledge stored in an appropriate biobank or database. Once this infrastructure is established, the clinical diagnostic and prognostic applications of MS imaging will bloom. Biobanks will quickly be generated by analyzing large amounts of tissues and profiling approach (a concise set of quick local analyses) will be used to personalize therapy or adapt surgical procedures.

AW: MS imaging does have huge clinical potential, especially in surgical pathology but also in other areas. I agree with Ron's remarks on the need for development, standardization, and validation. Take the example of sample preparation, something we all hope to spend less time and effort on. While certain solutions have been developed, for example, pre-coated slides, spotting robots and spray coaters, much less has been done to standardize usage across laboratories or platforms. In order for diagnostic assays to become routine:

- Validated methodologies (for a statistically significant sample set) must be developed
- Methods for analyte identification must be improved
- Assay specificity must be confirmed (identification of the analyte in situ provides final confirmation that the assay sufficiently specific).

Can you comment on quantitative aspects of MS?

AW: Improvements are essential. While qualitative assessment can distinguish disease states based on the presence or absence of certain molecules, increasing numbers of applications require the ability to discriminate between subtle changes in analyte abundance. In these cases, we need absolute quantitation to make a valid clinical decision. This will require improvements to sample preparation, such as the addition of standards to the tissue, alongside increased instrumental range and more robust data analysis to advance MALDI imaging quantitation.

For early drug discovery, quantitative MS imaging could be deemed to be adequate. However, making accurate quantitative judgments (on a pixel-by-pixel basis) is some way off and must be addressed.

From a data analysis point of view, a number of software packages are attempting to address the time-consuming nature of both analysis and calibration. This will have a positive impact on the potential of quantitative analysis. Validation will be key to unlocking the true applicability of quantitative MS imaging – robustness is of paramount importance.

RH: The quantitative aspects of many MS-based surface analytical techniques are understudied. We still do not have sufficient insight into why certain molecules ionize well in one tissue environment but not at all in another. This mechanism is referred to as tissue suppression or, more colloquially, "the matrix effect." That's a term well known by those using other MS analyses, employed here to indicate that we have few clues as to what determines which molecules we can and cannot image with MS based microscopes. The difficulty can, in part, be ameliorated by the use of clever internal standards or multiple reaction monitoring (MRM)-based approaches.

Forensic applications are beginning to benefit from this. However, there are still simply too many unknowns to make MS imaging quantitative and other targeted approaches currently offer a better solution when the compound of interest is known. In this light, MS imaging is a discovery tool for looking for the "unknown players" or the specific combination of molecules that make something happen in a living system.

What is the most tantalizing analytical application?

RH: From the many possible responses, I will offer three. One, in conservation science, MS imaging can be applied to the study of paint cross-sections. Two, investigations of bacterial colonies under environmentally-challenged conditions can be employed to investigate bioremediation of polluted environments. These studies can also offer insight into efficiently producing bio-fuels.

And three, study of the interfaces between new biomaterials and living systems will enable better biocompatible materials and drug delivery systems to be designed.

AW: The most tantalizing analytical applications of MS imaging are those that otherwise could not be performed directly with intact tissue in the natural histological context. Examples include the multiplex analysis of drugs, drug combinations and their associated metabolites; and imaging of cell metabolism molecules in situ, such as energy metabolism or the citric acid cycle. The simultaneous or sequential analysis of these different analytes on the same tissue section is possible, further increasing the uses of the technology. Such analyses at the level of individual cell populations within tissues could be not performed before the advent of MS imaging.

What specific choices need to be made to push progress?

RH: We stand at a transition point. The technology has matured enough to be applied in the clinic, but small-scale efforts will lead to disappointments; a large-scale infrastructure is needed to take full advantage of the benefits. Investment is essential, as is the concerted effort of all involved. Large-scale molecular imaging centers, with participation of national science and national health councils will result in more efficient translational research. The imaging centers in Harvard and Vanderbilt universities are key examples that I hope Europe will follow, developing large-scale multimodal imaging approaches complemented by solid bioinformatics and e-biobanking infrastructure. All of this requires integration at the national and international level (EU/Horizon 2020 and WHO).

Lastly, talk us through the next decade

AW: We've discussed challenges and limitations here. Despite these, MS imaging has seen exponential growth in both technological development and potential applications. I can only imagine it continuing to do so, joining a host of other established imaging techniques to form an ever more powerful toolbox combining broad coverage and exquisite specificity for the molecular analysis of tissues. MS imaging has already demonstrated its usefulness for biological discovery, and applications that display real clinical value are starting to surface more frequently.

Given continued progress in the challenging areas mentioned above, MS imaging will increase its foothold in biomedical research and, as we move towards personalized medicine, begin to adopt its greatest role in personalized pathology.

RH: New high throughput detectors, such as the IonPix detector from Omics2Image, and new high resolution imaging modes will be introduced. Parallel detection combined with imaging of increasingly large molecules or complexes will become possible and will take off with these new detectors, directly impacting the speed of analysis. In addition to new technologies, novel and efficient bioinformatics approaches are needed and, therefore, inevitable. All large-scale analytical data collected will be embedded in e-biobanks and made available to the larger medical community. This in turn will enable the introduction of small-scale MS-based analytical devices into health care systems. From here it is not hard to imagine a future where GPs have more informative tools at hand to quickly screen patients or determine drug levels and adjust therapy. Ultimately, this should reduce the cost of health care and improve quality of life for patients and their immediate environment.

Ron Heeren is group leader of Biomolecular Imaging Mass Spectrometry (BIMS) at Foundation for Fundamental Research on Matter (FOM) Institute AMOLF, Amsterdam, The Netherlands. Axel Walch is head of the Analytical Pathology research group, Helmholtz Zentrum München, Germany.

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Translated

Translation in action
Celebrating success
Bench-to-bedside

Nocturnal Light Saves Sight

An illuminated sleep mask that exploits the Troxler effect might transform the treatment of diabetic retinopathy

By Ian Grierson and Richard Kirk

The retina uses more oxygen per unit mass than any other tissue in the body, due to the fact that photoreceptors have a phenomenally high metabolic rate. That oxygen demand becomes even greater at night, rising by around 40 percent, as rod photoreceptors dark-adapt. Under normal physiologic circumstances, this isn't a problem; the additional demand for oxygen is met by increased blood flow through the retinal vasculature. However, problems start to occur when that vasculature becomes damaged.

A growing body of research has found that diseases such as diabetic retinopathy (DR) and diabetic macular edema (DME) are driven, at least in part, by retinal hypoxia. People with diabetes commonly have microvascular damage, which can start to compromise retinal blood circulation. Once circulation is sufficiently compromised, the result is retinal hypoxia. This leads to upregulation of vascular endothelial growth factor – VEGF – with the consequence being retinal neovascularization. As those new vessels are leaky, the result is DME (Figure 1).

The current standard of care for DME treatment, depending on the degree of edema, is either laser photocoagulation or intravitreal injection of anti-VEGF drugs, typically bevacizumab, ranibizumab or aflibercept. The anti-VEGF agents are highly effective in the majority of cases, but even with pro re nata or treat-and-extend regimens, they still require patients to make regular hospital visits to receive their injections. For a limited population of patients, there's also the option of steroid implants. Two are currently available: Allergan's Ozurdex and Alimera's Iluvien, both of which provide longer-lasting therapeutic action (of 6 and 36 months, respectively) than anti-VEGF agents, but are reserved for patients who, according to their summaries of product characteristics, are "insufficiently responsive" or "unsuitable for non-corticosteroid therapy." That's partly because intravitreal steroid use carries the risk of raised intraocular pressure, and the development of cataract in the phakic eye – so careful consideration is required before their use. The other factor is cost; these agents don't come cheap.

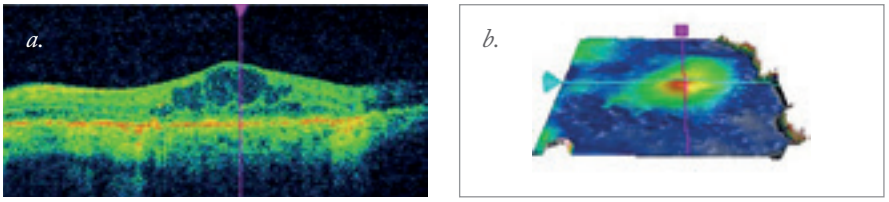

Figure 1. OCT-derived images of macular edema – cross-sectional (a) and topographical (b).

Diabetes is a huge economic burden on healthcare systems around the world. In the UK, where we're based, diabetes costs our National Health Service (NHS) more than £10 billion each year – 10 percent of the total healthcare budget – and a large proportion of that cost is drugs to treat the ocular complications of diabetes. It's only going to get worse as diabetes prevalence rises, potentially by as much as 50 percent by 2030 in the UK, with obvious pharmaco- and socioeconomic implications. Anything that could reduce the number of hospital visits, and particularly, anti-VEGF injections required, could make a huge difference to patients' lives and healthcare costs.

Better than sleeping with the lights on

If at least part of the genesis of DR or DME is retinal hypoxia, and this can be caused by dark adaptation, then might preventing dark adaptation from occurring reduce the metabolic demands on the retina and alleviate the disease processes? Since humans only dark-adapt at night during sleep, sleeping in an illuminated environment should prevent or reverse the condition.

Figure 2. Noctura 400 fabric mask and light-emitting pod. The mask shines light through a patient's eyelids during sleep.

The problem with sleeping with the lights on is that patients might sleep on one side, on their face, move under the blankets… and this doesn't lend itself to rigorous scientific assessment or consistent clinical effects. The way around that is to create sleep masks that emit light to uniformly illuminate the eyelids during sleep. It's important that the masks are comfortable to wear and do not disturb sleep; constant illumination should not be disruptive, as the eye adapts quickly due to the Troxler effect.

Ultimately, the result of this thought process was the development of the Noctura 400 (Figure 2), developed by biophotonic research and development company PolyPhotonix. It contains a removable lighting pod that emits a mellow green light into closed eyes throughout the night while a patient sleeps. The light source is an organic light-emitting diode (OLED), which is tailored to emit precise wavelengths to suit the application and powered by an onboard coin cell battery that has a three-month life span.

You may have heard of pill bottles that can track patients' drug regimen compliance; Noctura 400 can do something similar. It has a compliance monitoring system that senses and records how long the light mask is worn, and provides nightly data on the amount of therapy being administered (Figure 3), meaning that clinicians can examine treatment adherence and link that to the condition of patients' retinas.

Figure 3. Noctura 400 compliance graph. Green blocks show when the mask has been worn by the patient.

"Clinicians can examine treatment adherence and link that to the condition of patients' retinas."

Clinical validation

Developing the sleep mask involved a number of collaborations across the UK. The Northern Design Centre of Northumbria University helped determine the mask's shape and appearance, and the School of Medicine, Pharmacy and Health at Durham University, provided valuable insight into patient issues. A close relationship between PolyPhotonix and the Eye and Vision Department at Liverpool University established the safest wavelengths and light intensity to use, and now a clinical trial has been completed demonstrating the safety and acceptability of the treatment. In addition, the Noctura mask has been evaluated by a Czech patient group in a six-month clinical trial that involved patients with advanced diabetic eye disease, with favorable results. It was the data from these trials that allowed the Noctura 400 to be awarded a CE mark.

A large three-year randomized controlled clinical trial is underway, based at 15 hospital eye departments throughout the UK. The trial was designed to clarify whether mask wearing can effectively combat diabetic eye disease, and to identify which patients derive the most benefit. Three hundred patients were randomized to light masks or control masks, in order to determine whether the therapy can prevent development and progression of DME, or even lead to regression of the disease.

The implications

Today, the Noctura 400 sleep mask is currently being clinically evaluated in more than 35 NHS hospital clinics and has already recorded over 150,000 hours of use. The UK's National Institute for Health and Care Excellence (NICE) has recognized the clinical potential of Noctura 400 for the treatment of patients with diabetic eye disease, and the mask is on course to be fast-tracked through the NICE evaluation process. NHS funding has also been made available to carry out evaluations in the community alongside the clinical trials, and a number of commercial pilots are underway; one with a number of optometrists in the North East of England and one national pilot with a group called The Outside Clinic, that visits patients' houses and provides an optician service.

Of course, the technology that underpins Noctura 400 may also benefit patients with other retinal degenerative diseases. PolyPhotonix is currently in the early production stages of the Noctura 500, a version designed for the treatment of wet age-related macular degeneration (AMD). This latest incarnation has been made available for its first pilot trial, to be conducted in centers in both Cardiff and Bristol.

Today's treatment of DME and wet AMD is invasive, costly, inconvenient, and hospital-based. The sleep mask is noninvasive, dramatically less expensive than chronic anti-VEGF treatment regimens, and will help allow patients to take charge of their own treatment at home, under the care of their physicians. If it lives up to the promise already shown in clinical trials, Noctura could profoundly change the future management of diabetic eye disease and wet AMD.

Ian Grierson is Emeritus Professor of Ophthalmology, Liverpool University, Visiting Professor University of Cardiff, UK, and Special Trustee Moorfields Eye Hospital and Scientific and Medical Adviser to PolyPhotonix.

Richard Kirk is Chief Executive Officer of PolyPhotonix.

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Welcome to the Molecular Diagnostic Revolution

There's a whole new world of molecular diagnostics out there. Why has innovation been restricted? What are the upcoming challenges for manufacturers? Is it time to take a tip from Mother Nature? Biocartis' Rudi Pauwels has some of the answers.

How far has the field of molecular diagnostics come?

PCR technology essentially established the molecular diagnostic field as we define it today. PCR has been – and still is – a phenomenal research technique, but if you consider how the technology evolved in the laboratory medicine space, much of the innovation focused on automation of the PCR component. Unfortunately, the risk of contamination, among other issues, means that every lab needs dedicated PCR infrastructure, trained technicians, specialized equipment, and so on. The result? PCR technology has not penetrated the space as much as other clinical diagnostic systems, such as biochemistry, hematology, and protein detection.

But every challenge is also an opportunity, which is why a number of companies realized that the next step in pushing this great technology into clinical practice was to take a fresh, holistic approach and taking the entire sample-to-result workflow into consideration. Another key hurdle that needed to be addressed was the need to measure a growing number of biomarkers in a single clinical sample and develop so-called 'multiplexed assay' solutions.

Multiplexed analyses are the way forward?

Mother Nature shows us that it's feasible to simultaneously carry out multiple reactions in a single reaction environment – as long as the system and its components are well designed. Although these multiplexed technologies still have some way to go, the field has in fact made a lot of progress both in terms of system designs and molecular techniques. Advances in next generation sequencing, microarray analysis and multiplex PCR have all contributed to achieving performances incomparable to those of a decade ago. And we're getting better all the time.

But techniques such as multiplexed PCR are just the analytical components of the total solution. As diagnostic technology developers, we must always keep focusing on true diagnostic needs of the physician and patient. The prime diagnostic objective is to provide accurate, reproducible results in close space and time proximity to where patients and physicians interact and first-time-right therapeutic decisions need to be made. This requires new, flexible systems that can process even complex clinical samples directly with minimal manual interventions and without requiring any specific laboratory infrastructure.

Do you feel that innovation has been restricted?

Yes. Molecular diagnosis has traditionally been a technique requiring specialized infrastructure and operators and, as a result, the service has been thus far mostly centralized in high-volume reference labs. In line with that trend, manufacturers built automated solutions for higher volume testing. The flexibility to step away from the traditional batch-based workflows is only a recent development. The challenge of fully automating sample preparation and creating an uninterrupted workflow to analysis has long been underestimated. Sample preparation was traditionally done as a separate, mostly manual operation necessarily carried out in a laboratory environment. It is well known that manual procedures can be the source of variation and errors in the final results, even with excellent analytical equipment.

How has the lack of innovation stunted growth in personalized medicine?

In most oncology practices, molecular diagnostic results from tumor tissues may take two to three weeks to be returned. These delays, also requiring access to specialized laboratories, not only prolong the anxiety period for patients, but also are an impediment for wider and global adoption of personalized medicine. The true objective of the diagnostic industry should be to develop solutions that can be scaled up on a global level. We need compact, high-performing, high-precision, technical solutions that do not require specialized infrastructure or trained people. Is that enough? Probably not. I think technology is a key enabler of this revolution but there are other factors. For instance, I am often surprised to hear the debate about the price of diagnostics. Though the high value is – and should be – reflected in the prices of personalized drugs, payers should take a holistic view and also recognize the intrinsic value of the diagnostic solution when considering pricing. *In vitro* diagnostics (IVD) manufacturers and labs should be sufficiently incentivized and rewarded.

It's important to recognize that laboratories, especially the CLIA (clinical laboratory improvement

amendments) reference laboratories, play an important role because they can rapidly develop new biomarkers and make them available. I think we should applaud collaborations between labs that can rapidly develop biomarkers and IVD companies that develop the diagnostic solutions, and recognize the value they bring to personalized medicine.

How can new molecular technologies be made attractive to small volume labs?

It all comes down to the current and future needs of the patient, which are complex and often don't involve a single set of symptoms. In this light, the importance of multiplexing capabilities in the platform become more obvious.

Clearly, we also need to develop solutions that are flexible for use in both big laboratories and smaller, point-of-care settings.

How challenging is this approach?

It's a big undertaking. For example, when working on biomarkers that will help stratify your patients for a specific drug, the development of an IVD is expensive and time-consuming. The ideal solution – and the challenge that we have as manufacturers – is to develop fully flexible platforms that use the same basic technology and are amenable to both point-of-care and bigger lab settings. It would lead to a single IVD development of a new assay that can be run in multiple assay volume settings.

Finally, the technology must also be efficient, cater to varying throughputs and skillsets, and be easy to use. No easy task.

Communication and connectivity

are also extremely important. When using molecular diagnostic techniques, a lot of very valuable information is collected but is not always fully utilized. As with many other aspects of our life, when things become interconnected it is useful to capture as much information as possible – and in the best way.

I've been very much inspired by Apple and other consumer technologies in terms of developing a flexible molecular diagnostic platform that is designed for a potentially wide range of applications. I therefore want to challenge the older dogma that diagnostic systems need to be designed for specific purposes. If we want high-precision medicine, we will need high-precision diagnostics yielding clinical actionable results irrespective of where or how or by whom the test is performed. From a patient and physician perspective, these technologies are essential.

Can the dream be turned into reality?

It's our *raison d'être* at Biocartis. And it's absolutely why I created the company in 2007. We have invested heavily in developing a new molecular diagnostic system that goes from sample to results.

However, I think the role of diagnostics is still underestimated. After my years of research in HIV diagnostics and next-generation therapeutics, I moved into industry because I really wanted to make an impact. As an industry, we have high ambition – and I believe we are well on our way.

Rudi Pauwels founded Biocartis in 2007, where he is CEO. He (co)founded several biotech companies, including Tibotec, Virco and Galapagos Genomics. For more than two decades, he focused on the search and development of anti-HIV drugs – a number of which have been approved and introduced on the market – and the development of diagnostic tools to allow personalized HIV treatment.

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Finding Humanity in Science

What drives someone to throw aside selfish pursuits to focus on projects with true philanthropic impact? Here, the winners of the 2015 Humanity in Science Award provide their answers.

By Rich Whitworth

The 2015 Humanity in Science Award (www.humanityinscienceaward.com) was presented jointly to Peter Seeberger and Andreas Seidel-Morgenstern, directors at two collaborating Max Planck institutes in Germany, by our sister publication The Analytical Scientist. Their groundbreaking work in drug synthesis also won both scientists a spot on our 2015 Power List. By coupling flow chemistry with advanced chromatography methods, Seeberger and Seidel-Morgenstern were able to manufacture artemisinin-based therapies – the most effective drugs to treat malaria – from plant waste material, air and light. The science is innovative and exciting, and the potential impact of their project – and the concepts born from it – could really shake things up in the pharmaceutical industry.

The key active pharmaceutical ingredients (APIs) of all artemisinin combination therapies are produced in one or two chemical steps from artemisinin (see Figure 1). The majority of artemisinin (~200 tons per year) is extracted from the Sweet Wormwood plant (Artemisia annua) cultivated for the purpose, and prices fluctuate with harvest yields, driving up prices. With demand outstripping supply, up to 50 percent of anti-malaria drugs sold in Africa and Asia are counterfeit – useless and sometimes toxic.

Seeberger came up with a process for photochemical continuous synthesis of artemisinin from a waste product of the plant, DHAA. Yields were low at first – 40 percent yield at 200g per day – but careful optimization resulted in a greatly simplified process and a significantly improved yield. To demonstrate the power of the fully continuous synthesis/purification regime, Seeberger and Seidel-Morgenstern developed a continuous three-stage, multi-column chromatographic/crystallographic purification method for artemisinin-

derivative α-artesunate (see Figure 2).

The combined process produces artemisinin of greater than 99.9 percent purity and is now being implemented in a pilot plant in Vietnam. Not only does the new process enable production of less expensive anti-malaria medications, it also increases participation of developing nations in the value chain of drug production.

Now, let's focus on the personal stories of the duo that led the project, to discover what seeds humanity in science.

You can read the full submission to the Humanity in Science Award online: www.humanityinscienceaward.com

Using Entrepreneurship and Chemistry for Good

Peter Seeberger, Professor and Director of the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.

How did you get into chemistry?

I grew up in Nuremberg in Bavaria and was the first member of my family to go to university. I guess I was a good high school student, because I qualified for the highest possible scholarship for Bavaria, something that is awarded to just a select few. I could have studied anything, but I chose chemistry.

I then had to do my mandatory national service in the German army, which further motivated my pursuit of chemistry – the armed forces were definitely not for me. I studied both chemistry and business to begin with, but I eventually focused on chemistry because it gave me the chance to stand out from the crowd. I studied chemistry for three years at University Erlangen-Nuremberg with a full scholarship. The program normally took five to six years to complete, but after three years I had finished and was nominated for a full graduate scholarship to go to the US for a year. I applied to both Berkeley and Colorado universities and ended up going to Colorado, which was great as I like skiing...

I finished my PhD in Colorado working with Marvin Caruthers – a member of the US National Academy who famously automated DNA synthesis and set up many companies, including Amgen. Working with him made me realize that doing very good chemistry could also help you to do very good biology. The idea of starting up companies was also interesting.

So you moved again?

Right. Bruce Merrifield won a Nobel Prize in Chemistry in 1984 for chemical peptide synthesis on a solid matrix and I thought I could do something similar for carbohydrates. To prepare myself, I applied to work with the best-known carbohydrate chemist of the time – Sam Danishefsky, professor at Memorial Sloan Kettering Cancer Center and Columbia University. He accepted me into his lab in New York, where I worked extremely hard – 18-hour days, seven days a week – for two years. I focused on developing methods for carbohydrate synthesis.

I'd already lined up a job in Germany as an assistant professor, but before I could accept it Danishefsky called me to his office at 1am on December 23 and asked me what I'd be doing after leaving his lab. He encouraged me to apply to Massachusetts Institute of Technology (MIT) – and I did. They invited me to give a talk and then I had a day-long interview. The following day I received an offer to be an assistant professor at MIT.

I accepted.

Why was Danishefsky so keen to push you to MIT?

Danishefsky encouraged many of his people to apply to leading institutions. I'd published 12 papers with him and he seemed to think I would be a good match for MIT.

Moving to MIT was the best career decision I ever made, so I'm thankful to Danishefsky for pointing me in the right direction. Often, your choices in life are due to the influence of your mentors and role models. Without their influence, I would never have considered applying to top universities. When you come from Bavaria, places like Harvard and MIT are pretty far away – and not just in distance.

I did not know what to expect at MIT, but it worked out OK. I remember that in the first three days of starting my job, one of my colleagues asked me to go to the faculty lunch room and the provost said to me, "Young man, what do you think of your chances of getting tenure?" I replied that I had no idea, but I guess I was lucky because after about four years I was promoted to tenured professor at MIT at the age of 35.

Sometime later, ETH (the Swiss Federal Institute of Technology) in Zurich made me an offer I could not refuse. I'd been in the USA for 13 years and thought I would probably stay there my whole life. If I turned down ETH, I thought it would

be difficult to return to Europe.

Initially, I didn't find life at ETH easy because I'd been Americanized both in the way I spoke English and in my etiquette. It was a learning process for both sides I think. I was there for six years, met my partner (who was a professor in Berlin) and had a daughter. The commute between Zurich and Berlin needed fixing.

The Max Planck society offered me a job to take over a directorship at the Institute for Medicine in Heidelberg. It was a good offer, but it would not improve my family situation (travelling between Heidelberg and Berlin is actually worse than travelling from Zurich to Berlin). However, the people at Max Planck were persistent and suggested that I join an institute in Potsdam where they would erect a new building for us. I have to say I was anxious about the move. When I left MIT it was one of the most difficult days of my life because I was not sure whether I made the right decision. I was in a similar situation and knew there would be a lot of things I would miss about Switzerland. That said, I have been lucky in life and felt it would turn out well.

What brought you such success?

First of all, you have to pick a good area to work in. Glycosciences is a fantastic area with seven Nobel Prize winners up until the early 1970s – and glycans are everywhere. The advances in molecular biology of the mid-1970s and the new-found ability to manipulate DNA for proteins meant carbohydrates took a back seat and the technologies for enabling glycomics and glycobiology were lacking. I had expertise in DNA and peptide and carbohydrate chemistry that no one else had at the time. Many said that my idea for automated synthesis of carbohydrates wouldn't work, but it was a smart choice given my background.

I also work really hard – I'm very driven. I don't think I'm more intelligent than the next guy, but perhaps I am able to see interesting areas that enable long-term programs rather than just solving little puzzles.

And that approach fits in with your work at Max Planck where you are building platforms?

Yes – and that's how I got interested in flow chemistry, which is part of the work we received the Humanity in Science Award for. It's something I've been involved in since my days at MIT, where I remember hearing a talk by a physicist who had begun working on flow chemistry while he was in Germany. He talked about how you do chemistry in pipes instead of buckets, and that really appealed to me.

I started building systems and platforms. And we slowly began to get involved in medicine – after all, though my students are very well trained in carbohydrate chemistry, they need experience with drug molecules to improve their employment prospects! In fact, most of the people we train go into industry; more than 200 of them have left to get really good jobs. But I've also seen 47 professors come out of my lab.

It's fantastic to have the opportunity to convince talented young chemists to work with me. I give directions to make sure we get to a certain point, but the important work is done and implemented by the young scientists we train. If our students weren't as diligent, things could have gone in a very different direction.

Preparing to Change the World

Andreas Seidel-Morgenstern, Director of the Department of Physical and Chemical Foundation of Process Engineering, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany.

Take us back to the early days...

I grew up and worked in what was East Germany – right up until I got my PhD, which I did in 1987 in the former Academy of Sciences' Institute of Physical Chemistry in East Berlin. It was an interesting time, but also very political. In fact, my scientific career was more or less over after I completed my PhD because I refused to become a member of the East German communist party. Later in life, I read my Stasi [The Ministry for State Security] file, which noted that I wasn't loyal and should not benefit from promotion. At the time, I did not really appreciate how strict the system was.

Then in 1989, the wall came down. It was a new world for me. I had my PhD and I wondered what I should do next. Because the "iron curtain" no longer trapped me, I contacted the Technical University in West Berlin where a colleague (who later became my boss) helped me become a more active member of the German chemical engineering community. Later, I thought I should move to an English-speaking country to see more of the world.

So you moved to the USA?

Right. My wife is a chemical engineer as well – we met in the old East Germany and we already had a family, but we were not married. One day she came home and said the company she was working for was collapsing, but there was an opportunity in Tennessee, USA. That evening I was thinking about a few papers I'd read from a guy in Tennessee – I could not recall his name so I searched my files and found him: Georges Guiochon, University of Tennessee, Knoxville.

The next day I wrote him a letter to ask if he had any positions open. About three weeks later the answer came back: "yes." I included a research project idea in the letter – a smart move – Georges liked it. He offered me a post-doc position and my wife and I married to make it easier to move to the USA with the children as a family.

Why do you think Georges was so keen to work with you?

Georges' mathematical oriented views on chemistry and in particular chromatography meant he always had very good foreign experts. I guess he liked to work with anyone – foreigners included – that fit in with his strategy. I brought a new idea that was related to my PhD (how to calculate competitive adsorption isotherms) and I think he saw this field as a chance to enrich his own scope.

I could have stayed longer in the USA, but I applied for and received a grant from the German Science Foundation to support three-years at the Technical University of Berlin to do my habilitation. At the same time, Georges suggested that I should stay in the USA – this was a tough decision. However, we returned to Germany. We had young children and were still very poor; it was financially risky to remain in the USA – and my widowed mother was alone back home in Germany.

So, in 1992, we returned to Germany and I did a relatively rapid habilitation. I had many results so it was easy. Unfortunately, I did it too quickly because the grant rule agreement essentially said, "when you finish, we stop paying you". To make ends meet, I got a job with Schering in Berlin, but I wasn't there for very long; my boss encouraged me to apply for academic positions, noting an opportunity in Magdeburg, which I took.

And that was the connection to the Max Planck Society?

Yes. Three years later, there was an unexpected situation in Magdeburg. Germany was united and the Max Planck Society (supported by taxes) started to invest in the former East Germany to ensure an even distribution of funding throughout the German states. Up until then, Magdeburg and the federal state Saxony-Anhalt had not received much support. The society decided to form a new institute on Dynamics of Complex Technical Systems, which now houses more than 200 people.

I became director of the institute in 2002, and we now have many projects in various engineering areas – chromatography is just one of them. By training, I am a reaction engineer and so we also do a lot of analyzing and quantifying reaction processes, which broadens our separation science based scope.

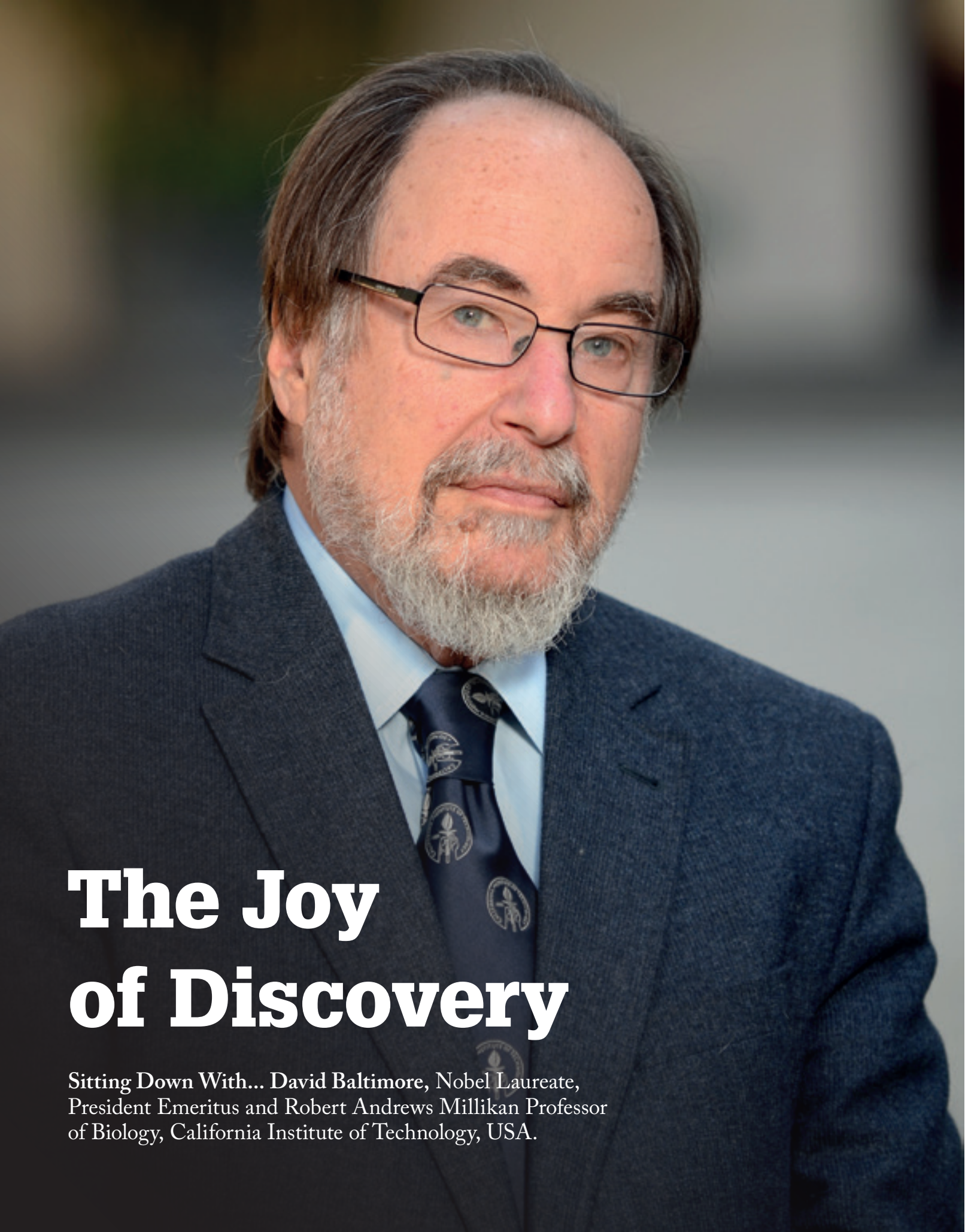
The Max Planck Society meets once or twice a year at annual meetings, and that's how I met Peter Seeberger. I quickly realized we were well matched; his group was strong in chemistry whereas we had expertise in designing continuous separation processes. We connected and now have quite a few stories to tell.

How do you work with Peter?

Peter's group focuses on certain target molecules and constantly comes up with new and fascinating chemistries. Very often, his reaction pathways are challenging (and very clever), but connecting them directly to our separation processes can be difficult. We started studying a simple model reaction. Then we worked intensively on artemisinin and artesunate. Currently, we are looking together for new target molecules to further contribute to this area.

My overarching goal is to develop universal methods that can be applied generically. Devising strong, widely applicable technologies is what interests us the most. Nevertheless, we are not naïve; we know that every separation problem brings its surprises. We need sufficient flexibility to fine-tune what we do.

The malaria story is wonderful. Our job was to establish a separation concept to isolate a valuable component from a complex mixture. And we have many other examples of similar problems in biotechnology. Typically, a single product will exist alongside many conflicting by-products. How can we structure the separation problem in a more generic way? If you think of chromatography, you could have a sample containing 100 components, but component 17 is your target. How do you get it? We consider this a pseudo ternary separation problem. One to 16 forms a big fraction before the target – the first fraction. The second fraction is your target – component 17 in a very narrow window, followed by another big third fraction (18–100). If you find a process that looks at the problem in the same way – for example, ternary simulated moving bed (SMB) chromatography – you can tune various pump flow rates representing the crucial process parameters to enable you to isolate any target from any mixture. Of course, in practice you have to connect several process steps together and you need to recycle streams if you want to be efficient and not lose valuable materials. That's characteristic of our way of looking at problems.



The Joy of Discovery

Sitting Down With... David Baltimore, Nobel Laureate, President Emeritus and Robert Andrews Millikan Professor of Biology, California Institute of Technology, USA.

Going back to the beginning, why experimental science?

Growing up, you often question yourself about what you're good at. And it became clear to me as I progressed through high school that I was good at science and mathematics. In 1955, I had the opportunity to spend a summer at The Jackson Laboratory (www.jax.org) and it introduced me to the huge potential of experimental science. It was very inspirational and essentially determined the rest of my life.

When I went to graduate school (Massachusetts Institute of Technology) back in 1960, I looked at what people were doing in experimental science; the most interesting work was being done with viruses, particularly ones that grow in bacteria. But I thought that the field was very limiting. I wanted to see if viruses could be used as a probe for the behavior of animal and human cells. I left MIT to go to Rockefeller University because there was a professor there – Richard Franklin – whose work was very closely aligned with my aspirations.

Later on, I met with Renato Dulbecco – one of the people bringing animal virology into the late 20th century – and he invited me to join him at the Salk Institute in La Jolla. I moved there in 1965, where I spent two and a half years before moving back to MIT.

You achieved a great deal at MIT. What are your highlights?

The discovery of reverse transcriptase in 1970 was the biggest; nothing beats that. But we did move into cancer research where we discovered that the Abelson virus made an oncoprotein that phosphorylated tyrosine. It stays with me as a great moment because we had found a new kind of protein modification that was linked to cancer. It eventually led to the "miracle drug" Gleevec.

Next, I decided to move the laboratory into immunology. A highlight there was our discovery of RAG genes, which encode the proteins that recombine DNA and give the immune system its variability – and ability – to react. And we also unearthed the NF-kappa B transcription factor – plus a whole host of other transcription factors that control immune function. It was a period of incredible and important protein discovery on the part of the post-doctoral group I was working with. And there is no higher high than a discovery.

But you won a joint Nobel Prize in 1975 for "discoveries concerning the interaction between tumor viruses and the genetic material of the cell" – that must have been a high point...

The main highlight there was that the call came from my wife! She was at a scientific meeting in Europe and heard before the official announcement, and she called me – woke me up, in fact. Going to Sweden for the ceremony was like entering fairyland. I was treated like nobility and given the Prize by the King of Sweden.

Did your research focus change after receiving the prize?

The prize was coincident with my movement into immunology, but it wasn't the reason for the move. I had already made the decision based on the rise of recombinant DNA methods. Indeed, the new ability to use recombinant DNA methods to understand mammalian cell biology was very real in 1975. And at that point I decided to enjoy myself and take advantage of the new methodology to work on the adaptive immune system.

What are you focusing on right now?

After stepping down as president of the California Institute of Technology in 2005, I decided to try something a little different. I wanted to see if we could translate some of our findings from the laboratory into humans – either as therapeutics or prevention. In particular, I focused on gene therapy methods. We've been doing that now for the last 10 years and we have several projects in clinical development; cancer and HIV are major focuses.

As gene technologies advance, what are the major concerns?

One concern of mine dates back to my early experience with recombinant DNA methods. Indeed, when they were developed, I was part of the group that produced the Asilomar Meeting in 1975 to address the potential dangers that might arise from this new technology. Now that genome-editing technologies have appeared, the concern is a reality rather than a theoretical concern. I was also part of a group that called on the National Academy to take some action to limit the use of these technologies until we could at least come to a consensus about what's right, what's appropriate and what's inappropriate. That of course is always a judgement in the context of technology. The National Academy is now actively evaluating the technology and the larger societal concerns that surround gene editing.

What advice would you give to today's scientists?

Science today is such a different world than it was when I started out. But in retrospect, I think I made some pretty good decisions back in the 1960s; I found the right places to work, and the right people to work with. The most important consideration should be your scientific environment and the people around you.

Today, I think that science has been so 'professionalized' that some of the joy of discovery is lost. Anything we can do to give young scientists an opportunity to be independent and to express their own particular notions about science and creativity is positive for the forward movement of science.

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