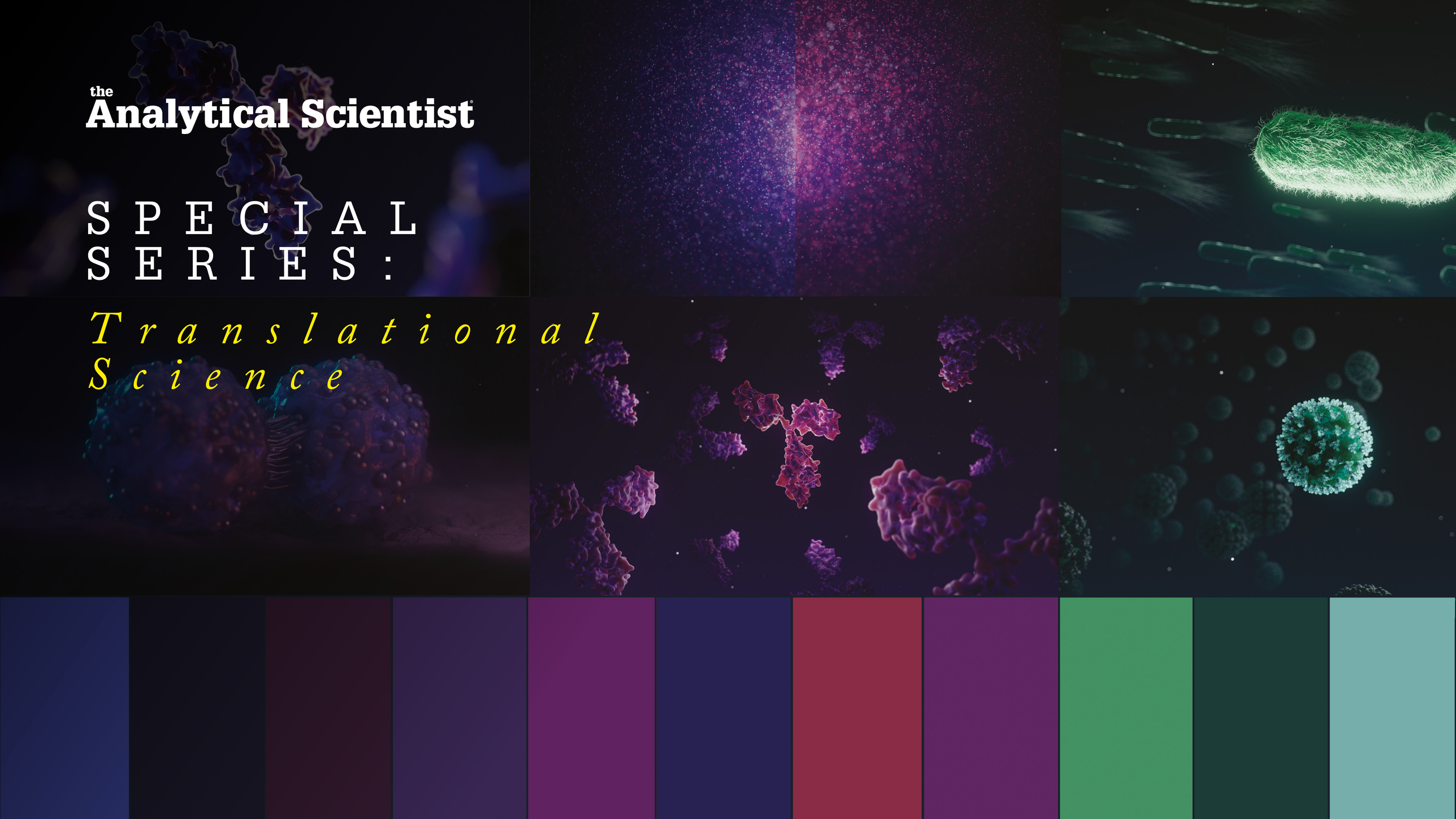


the Analytical Scientist

SPECIAL SERIES:

Translational Science

UPFRONT

A Picture Paints a Thousand… New Therapies?

How AI-enabled tools can democratize cell painting to speed up drug discovery and reduce failure rates

"Cell painting is a high-content profiling technology that uses up to six fluorescent dyes to visualize specific cellular components at the single-cell level. The assay essentially "paints" images of various components of the cell, including the nucleus, mitochondria, endoplasmic reticulum, cytoskeleton, and more. After microscopic images are captured, image analysis software converts them to data by extracting various measures of cellular morphology, called features. The results allow researchers to understand the effects of perturbagens, such as chemical compounds or genes, on the behavior of cells, feeding drug discovery and the characterization of bioactive molecules.

Here, we speak with Angeline Lim, Senior Scientist at Molecular Devices, and David Egan, co-founder and CEO at Core Life Analytics, about a collaboration focused on using AI to make cell painting faster and more efficient.

What are the current limitations of cell painting?

Cell painting workflows often prove time- and labor-intensive – a full screen may take several days to complete and generate massive amounts of data. Next, researchers face the challenge of extracting meaningful information from the resulting data, which can be overwhelming – either demanding the expertise of a data scientist or – in a worst-case scenario – wasting valuable data simply because of the lack of tools.

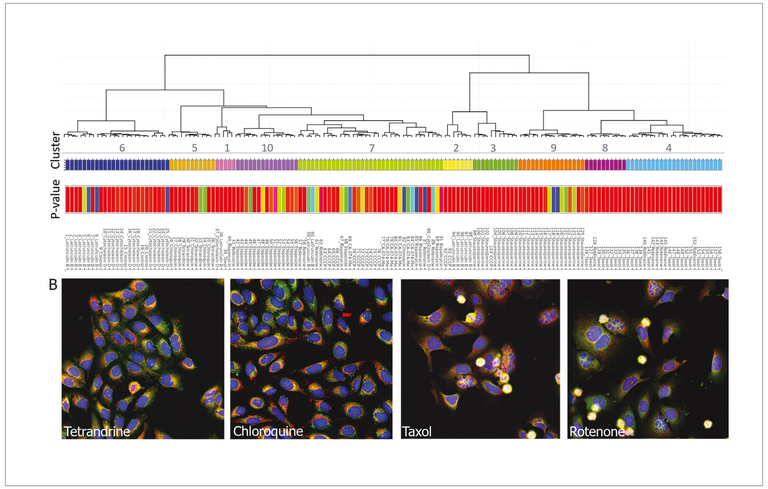
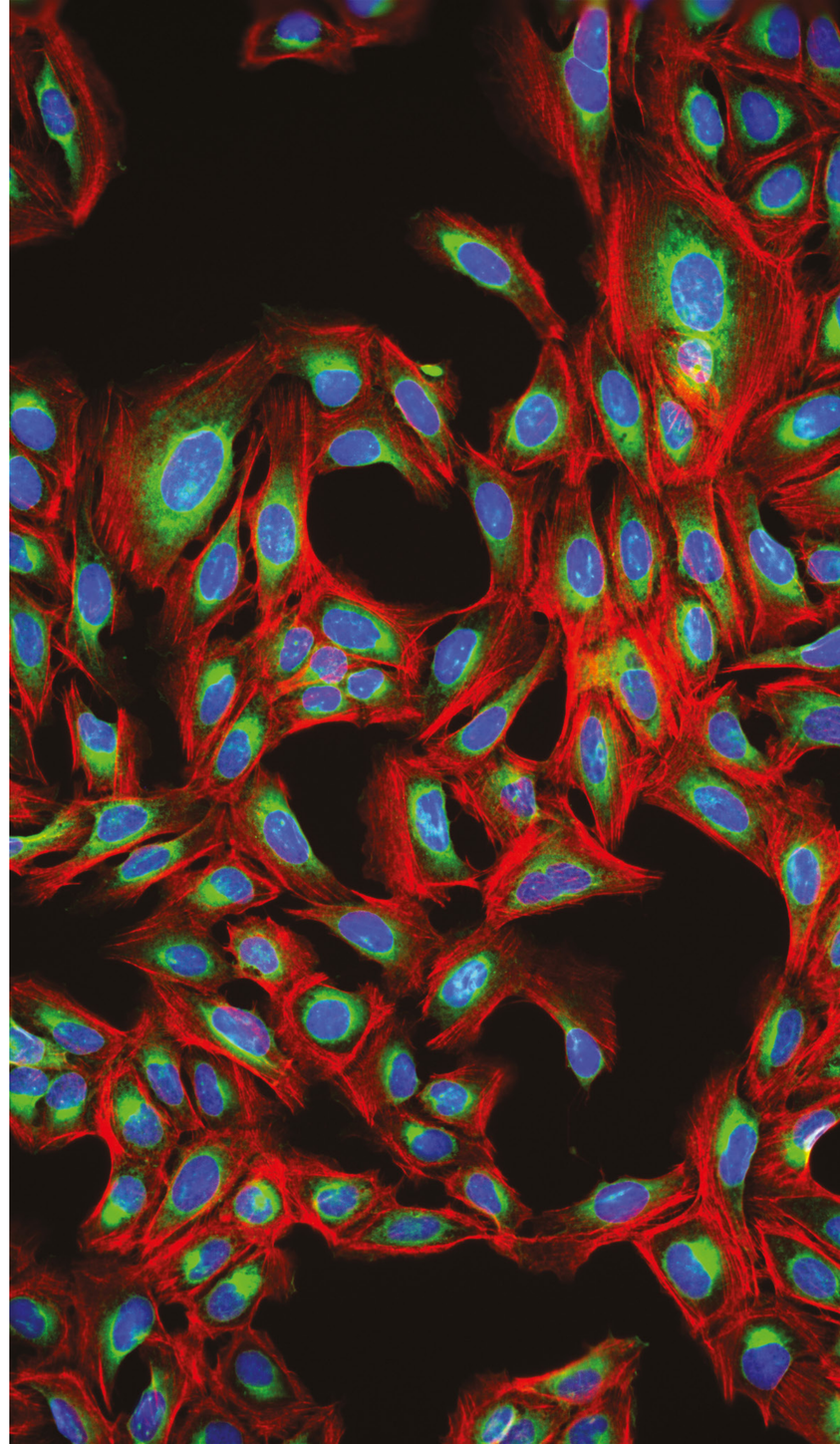


Figure 1. Cluster analysis. A) A dendrogram that represents hierarchical relationships is shown. Wells belonging to the same cluster (numbered) are represented by colored bars. P-values based on the distance score are shown for each well. Note that cluster 9 consists of only staurosporine treated cells, whereas cluster 10 consists of only etoposide treated cells. B) Examples of compound-treated cells belonging to some of the clusters are shown. Cluster 5 consists of tetrandrine- and chloroquine-treated cells. Note the increased number of ER punctae in both wells. Cluster 4 consists of rotenone- and paclitaxel-treated cells. Note the presence of blebbing in some of the cells belonging in these wells, suggesting cytotoxic effects.



How can AI-enabled technologies help?

Overcoming the aforementioned hurdles requires additional computational tools. In the past, only a team of statisticians, data scientists, and software developers was capable of analyzing cell painting data with any reasonable and timely success. Now, AI can quickly facilitate the analysis of large image datasets. In addition to machine learning-enabled software that handles data extraction from images, AI-enabled tools such as StratoMineR – a data analytics software from Core Life Analytics – can also help scientists iteratively mine data for information without the help of a data scientist.

Could you share more details about your collaboration?

At Molecular Devices, our customers are largely biologists in the life sciences field – not data scientists – who seek us out for guidance around analyzing large datasets coming from their cell painting assays performed with our high-content cellular imaging systems. The cloud-powered data analytics solution from Core Life Analytics, called StratoMineR, offers users a self-guided workflow that enables researchers to easily derive meaning from their high-content data.

Our collaboration provides more biologists with the AI-enabled tools that deliver advanced data mining and analysis – reducing the dependency on data science expertise.

And how might this benefit drug discovery?

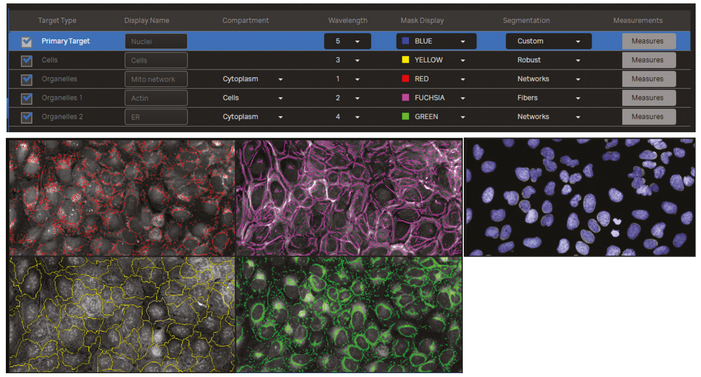
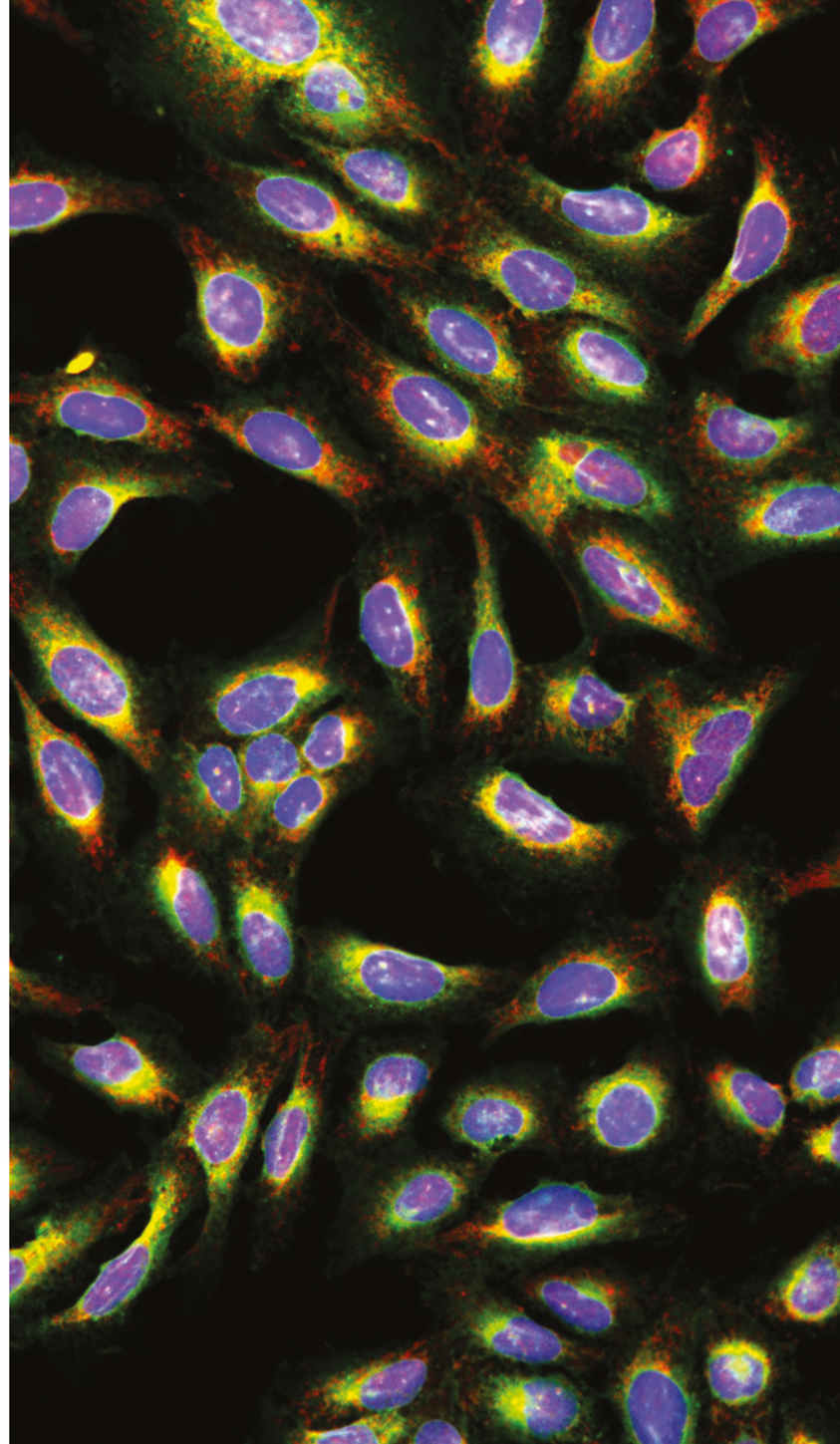


Figure 2. Feature extraction in IN Carta software. A) An analysis protocol was created in the IN Carta software to segment the various cellular structures. Here, we used the built-in nuclei model to achieve robust segmentation of nuclei across all treatments. Other cellular features such as cytoplasm compartment, actin filament network, ER, and mitochondria were also segmented.

The democratization of data science with AI-enabled analytic software enables data-driven decision making. It has enormous potential for speeding up the drug discovery process and reducing failure rates.

Angeline Lim supports scientific, technical, and applications initiatives for Molecular Devices' portfolio of ImageXpress high-content imaging systems and David Egan co-developed Core Life Analytics' StratoMineR platform with his co-founder Wienand Omta to help biologists independently



IN MY VIEW

Cell Therapy's Live Analytical Challenges

Analytical science can take cell therapy manufacturing to the next level: in-line measurement of critical quality attributes

Cell therapies are a new, exciting, and complex branch of medicine that uses living cells as drugs. And with that complexity comes multiple analytical challenges – depending on the initial cell source and the nature of the manufacturing process.

One main analytical challenge is establishing invitro potency assays that represent the therapy's mechanism of action in vivo, which is either undefined or far more complex than one would encounter with small molecules or monoclonal antibodies. Plus, cellular products cannot be terminally sterilized. The regulatory agencies understand this challenge and have issued specific guidance (1) to address the raw material (donor cells, master, and working cell banks) and in-process and release testing parameters.

At release, the product should be tested for sterility (ruling out the presence of microbiological and adventitious agents), identity (product characterization), purity (testing for acceptable limits of contaminants), and potency. Autologous therapy products are manufactured as one batch per patient and are infused back to the patient as soon as possible – a 14-day sterility test for release testing isn't ideal here. Aseptic techniques are maintained throughout product manufacturing, and sterility may be tested 48 to 72-hour prior to final cell harvest/formulation or after the last re-feeding of the cell culture. The product may be released at risk with a STAT Gram stain on the final formulated product, with a 14-day culture in progress.

Developing rapid, reliable, and validated analytical assays for microbiological agents is another major challenge for cell therapy. And it's equally important to control critical quality attributes (CQAs) – defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution (2). As the industry matures, analytical sciences will play an increasingly significant role – providing the tools to enable cell and gene therapy developers to define the ranges and limits and test it at various steps of the manufacturing process.

The primary analytical techniques used for cellular product characterization are cell counting, cell size, and viability analysis, using dyes and image-based analysis. Other CQAs, such as chimeric antigen receptor (CAR) expression, are assessed by phenotypic characterization using flow cytometry. Enzyme-linked immunosorbent assays (ELISA) are used to quantify the residual cytokines, with various cell activation agents used in the process. If the activation is bead-based, the residual bead amount in the final drug should be quantified – microscopy-based methods quantify the bead agents. Finally, PCR-based methods are used to quantify the vector copy number in gene-modified cell therapies.

Analytical science has a plethora of tools that can potentially be employed to the cell and gene therapy space. For example, Fourier-transform infrared spectroscopy (FT-IR) could potentially enable in-line measurement of metabolic data, such as glucose and lactate values, which are currently measured using off-line or at-line enzyme-based sensors.

To develop these kinds of in-line process control tools, industry players are collaborating. For example, the UK's Cell and Gene Therapy (CGT) Catapult has formed a consortium of over 20 organizations to assess the application and combination of multiple technologies for process analytics within the cell and gene therapy industry (3). I also think we'll see a move towards AI-based process control tools in the next 5–10 years. In-line monitoring of manufacturing processes, off-line measurements of cell and gene products, and in vivo data on cell product potency and persistence should allow AI tools to make better decisions in manufacturing processes.

Dalip Sethi, Director of Scientific Affairs, Terumo BCT

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IN MY VIEW

We Need to Talk About Clinical Representation

Alzheimer's research should benefit all, regardless of ethnicity or race

As of today, there is no cure for Alzheimer's disease (AD). Historically, Black people and other ethnic minorities have been underrepresented in clinical research. We have a duty to ensure that our greatest advances in scientific research are for the benefit of all. These statements, at least in my view, are simple facts. Therefore, excluding minority groups from Alzheimer's research is not only a disservice to them, but to the entire population, because it means we do not have a complete understanding of the disease. If we want to get serious about finding a cure for AD, we need to ensure we're including representative samples in our clinical studies.

Of course, this holds true across many different research areas, but our group's focus – and my expertise – is in AD. In recent months, we've focused a lot of effort on understanding the proteomic and lipidomic influences of AD across underrepresented groups – and it has become apparent that we have many challenges still to overcome in ensuring that clinical research is truly representative.

First, we need to ensure that everyone values true representation in their study populations. This may seem obvious to some, but the lack of diversity still seen in many cohorts would suggest that not all research communities agree. To start with, we should look at cohorts' diversity and ask whether they are inclusive. Some progress has been made with certain funding agencies, such as NIH, recognizing the importance of this work and trying to move toward equity in research – but there is still much more to be done to understand how systemic racism impacts our ability to perform outstanding clinical research.

I believe it is imperative that diversity, inclusion, and equity are built into the peer review process. We might have trainees from diverse backgrounds, but we need to ensure that work that is creating inclusive studies is valued and supported. We also need to recognize that there is some way to go to rectify issues of the past (for instance, current discrepancies with the level of funding and support available to researchers interested in doing diversity and inclusion work). To support this effort, I believe there should be more accountability within these processes; we should be asking people to justify the lack of diversity in their cohorts and to contribute to making their studies more representative.

Second, even if you do want to have diverse cohorts, you need to identify enough biospecimens from minority groups. I'm involved in encouraging research participation among different population groups – African Americans in particular. We need to improve educational awareness and share with our communities the importance of research overall, but especially with respect to disparities faced by particular communities.

One way we are trying to do this is through sharing positive messaging around research participation within the African American community. Until now, we've focused mostly on negative messaging and the barriers to research and not on facilitating that research. To counteract this, we try to capture authentic (and positive) messages from people who have participated in studies, and highlight why they did so. We then include these stories in resources that are handed out at various centers recruiting participants for clinical research. We are

also running a social media campaign and have created videos that can be used in outreach events, community settings, doctors' offices, and more. The idea behind all of this is to see whether this particular approach to storytelling is more effective than traditional (passive) approaches to recruiting people.

Clearly, much work still remains to ensure that the research community is undertaking truly inclusive studies and to fully understand the disparities present in AD. It's simply not possible to see the complete picture of this disease if we don't understand how it works – in everyone.

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FEATURE

Of Mice and Monkeys

Animal models remain an unfortunate necessity in some fields of research and development, but analytical advances and new technologies are paving the way to reduce – maybe even one day eliminate – our reliance on living creatures

It's not "antiscience" to highlight problems with animal models. Our (sometimes) furry friends have made many sacrifices on our behalf. In fact, estimates suggest that 110 million mice and rats are killed in US labs each year. And then there are the frogs… And dogs… And monkeys… And please spare a thought for the ubiquitous zebrafish…

But the use of animals in the pursuit of (scientific) knowledge is not only a modern-day practice. In ancient Greece, the dissection of human bodies was considered taboo and anatomical exploration thus relied on live animals. It was through conducting studies of this kind that Herophilus ("the father of modern anatomy") started his own studies; the likes of Aristotle and Diocles also partook.

Today's animal studies represent a significant departure from these barbaric practices. Subject suffering is limited wherever possible – and backed by legislation – and animals can even be housed with friends. But the drawbacks with animal models don't start and end with ethical dilemmas – there are also concerns regarding their applicability to human disease and the logistics surrounding their maintenance.

Luckily, advances in analytical science are helping us to move forward in new directions. Advances in instrumentation and methodology are allowing us to use smaller samples (and thus fewer animals!), and new models that don't rely on animals at all are emerging as alternatives.

"In proving old models redundant or unfavorable, we not only spare animals the bother of ineffective studies, but we also underscore the need for alternatives"

Assuming that animal lovers outnumber the animal ambivalent, we decided an exploration of this exciting analytical frontier was long overdue.

Reduce, replace, refine

The three Rs initiative (replacement, reduction and refinement) for animal research began many years ago, providing widely accepted principles that guide the way we conduct animal studies. They ensure that we approach this work in the most efficient way possible when it is needed. An example: if these studies must be used, use the minimum number of animals possible. Another: extract the maximum amount of information from those animals. Cutting-edge analytical chemistry is crucial in achieving such feats.

"When I started in the pharma industry some 40 years ago (not in the 19th century, as some of my students may have thought), you needed over 40 rats to gather pharmacokinetic (PK) data, and a further six rats per dose route for the drug metabolism studies," says Ian Wilson, a Visiting Professor at Imperial College, London. "This is because you needed 1 ml of plasma per time point for the LC-UV methods used in the PK studies, and this means you needed 2 ml of blood per sample. Six rats per time point were sacrificed to provide data of the required statistical quality. And that equates to an awful lot of rats."

"Today, we can do the same study in three rats or mice," he continues. "With modern analytical chemistry, we can assess PK and drug metabolism, and even conduct omics analyses using blood samples as small as fifty microliters in runs as short as five minutes." In such cases, the combination of microsampling with chromatography and high-resolution MS can dramatically reduce animal usage while increasing data quality and hitting two of the three Rs: reduce and refine. Ian's own work on gefitinib in mice, which used rapid UPLC methods followed by MS detection (1), is testament to these capabilities.

Organoids from outer space

In proving old models redundant or unfavorable, we not only spare animals the bother of ineffective studies, but we also underscore the need for alternatives. These alternatives can come in many shapes and sizes, but in vitro systems are one of the leading choices. But, as Ian says: "There's no such thing as an in vitro rat just yet." Luckily, we have organoids to take their place.

Organoids are three-dimensional organ models derived from stem cells that are able to mimic some of the complexities of tissues in the human body in terms of their spatial organization and cellular distribution. They represent a welcome departure from classical cell line and animal systems, which are limited by their culture-altered biochemical processes and physiological separation from humans, respectively.

Today, organoid models have been reported for almost every major human organ (including complex systems like the blood-brain barrier and fallopian tubes) and many of our most troubling diseases. Cancer

is perhaps the most successful example. Oncological research has benefited from an organoid invasion due to their ability to replicate the pathophysiological features of naturally occurring tumor processes, such as growth and metastasis.

The application of high-throughput and omics profiling technologies, as well as MALDI imaging, allows us to evaluate drug effects in these in vitro tumors. And, in the case that organoids are patient-derived, these approaches can even help us to personalize treatment and improve outcomes for patients - both in cancer and beyond. The close interaction between organoids and analytical technology thus helps us to protect humans and animals alike, while also opening doors to innovative research approaches for the future.

On the topic of three-dimensional cultures, so-called "tissue papers" are also making a name for themselves. These systems use plant tissue material loaded onto paper-based scaffolds to mimic human tissues, allowing them to act as models for processes like cellular tissue invasion (common in cancer) (4). Tissue papers can also be implanted into animals with relative ease when the occasion calls for it as the plant-derived materials do not tend to elicit inflammatory responses!

Do you want chips with that?

"The advent of organ-on-a-chip technologies show great promise," explains Steven Ray Wilson, a Professor from the Department of Chemistry at the University of Oslo. "These systems can be very representative of human functionality and they can be automated with high throughput and precision. They certainly represent a very attractive alternative to animals, but I don't think that we can completely replace them just yet."

The key tricky question that we must consider here is: can organ-on-a-chip technologies represent humans more accurately than animals? It's a tricky question, but analytical science will likely lead the way to an answer.

"We must explore how these systems metabolize drugs," Steven says. "And we must also map the protein makeup of these tiny and complex organ models if we are to compare them to the real counterparts in humans and animals. Some spectacular new breakthroughs suggest that they are very accurate indeed, as is the case with the embryo-like gastruloids. The field is exploding right now. Who knows where we will be in a couple of years!"

Of course, one core consideration in mimicking the human body is connectivity between organs; after all, when in practice does a human organ act totally alone? By connecting multiple organs on a single chip, researchers can study the interactions between the pancreas and liver in diabetes patients or the lung and brain in cancer patients. Analytical chemistry plays a central role here, too – often by the coupling of these systems to high-throughput MS systems for rapid readout.

As you might imagine, these inter-organ interactions are somewhat complicated and can differ greatly between species, so chip-based devices are a welcome addition to our research arsenal. We can even go one step further with "human-on-a-chip" technologies, which comprise many organs to replicate – to some degree – the whole human body. But if using animals for research is ethically questionable, how do we feel about using a "human-on-a-chip?"

Beyond the science

Such a question might seem odd or even silly at first. And my initial reaction to ethical considerations surrounding organs- and humans-on-chips was completely dismissive, but the field does raise unique and interesting concerns. A couple of quick questions from Ian highlight an area of potential concern: "At what point does a collection of glial cells constitute a human brain? How many interacting cells do we need to label something living?"

The next big question: if we consider these systems as living, how should we treat them? "It's an interesting question," admits Steven. "Thinking about it quickly leads us towards debates about the nature of life itself, and that provides an important opportunity for us to have interplay with the humanities. I imagine we will see more of this."

Steven suggests that we should expect to see organ-on-a-chip technologies find a routine role in drug testing and developmental biology. In this latter application, ethical concerns become even more complex. For example, it is possible to mimic embryonic development by loading a chip with a spatially controlled patterning of pluripotent stem cells (4), but does that mean that we should?

Zebrafish are used widely for developmental studies because their eggs are fertilized and develop outside of the mother's body. Observation is simple, and the fish are also cheap to house on a large scale. But how do we make the most ethical choice between experimenting with zebrafish versus embryo-like structures on chips?

From a research standpoint, it's likely that both approaches will be employed to provide the most holistic view possible. And that is very much the case with animal models now; alternatives are here, but are often used in parallel with the minimum possible number of animal studies. Though this is likely to remain the case for some time, it's exciting to imagine the doors that analytical technologies may open for us (and our animal friends) in the years to come.

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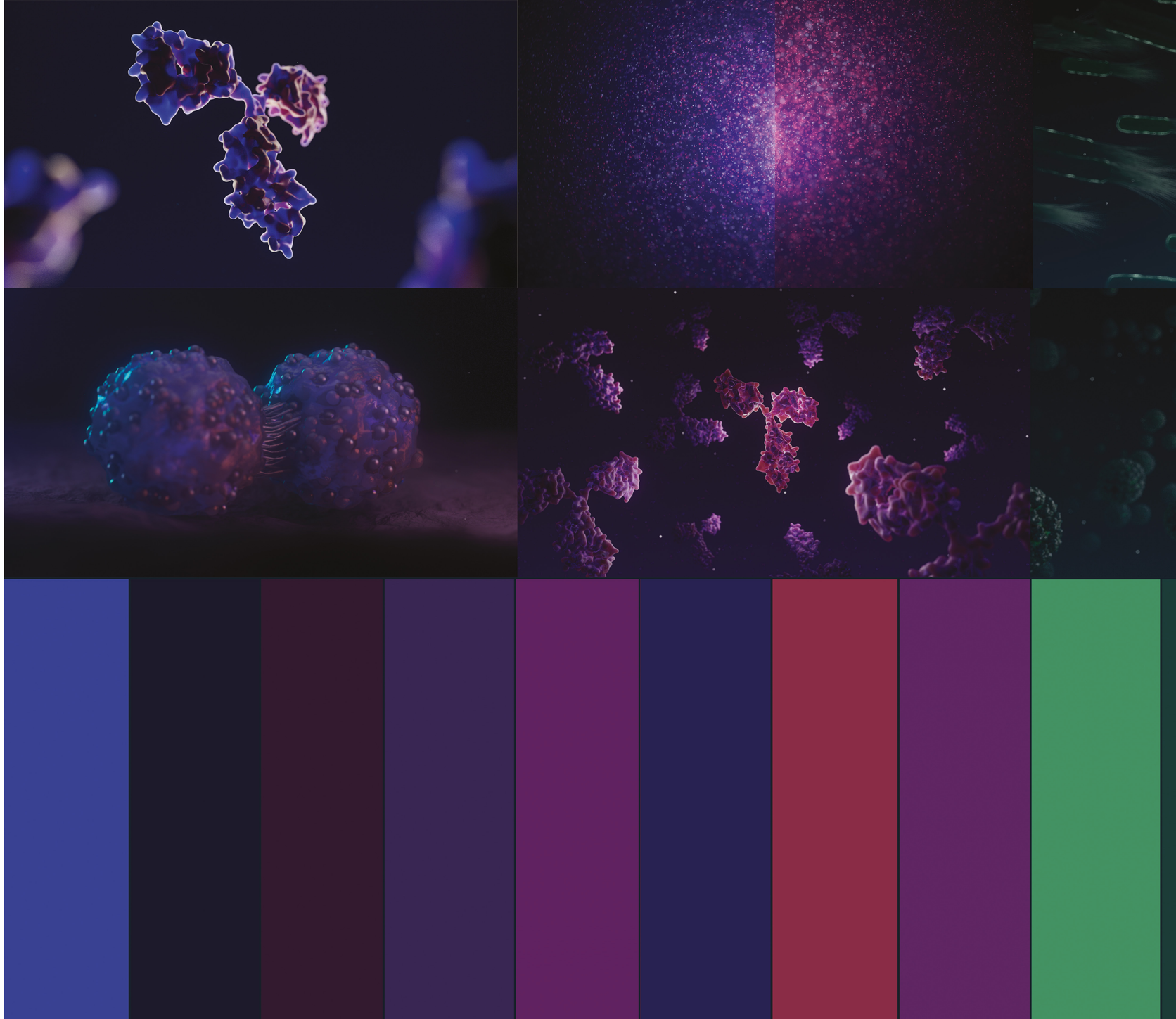
FEATURE

Talkin' 'Bout a (Protein) Revolution

How native MS may help rewrite the textbooks on immunology – and life itself



For researchers in the life sciences, the "protein revolution" happened quite some time ago. But for the general public, proteins are still somewhat of an alien concept – unless you're talking about counting your "macros" as part of the latest dieting trend. Though most people are well aware that DNA carries genetic information and that this has an effect on our lives – from the color of our hair to our predisposition to certain diseases – most do not fully understand the vital role proteins play in every living process.

One of the few positive things to come out of the COVID-19 pandemic is that people have (in general) gained extra understanding around concepts like viruses, antibodies, and vaccines – and, in turn, the action and function of proteins. As scientists, we should welcome the public's newfound (non-literal) appetite for proteins with open arms – not only is it a sign of how far we've come, but of how much there is still left to explore and understand.

As we learn more and more about our proteins, scientists around the world are making fascinating discoveries. My own team recently uncovered evidence that we are even more unique at the proteome level than was ever previously thought – and that this can explain a lot about how and why we all react so differently to diseases, drugs and vaccines. But more on that later…

First, I hope to bestow upon you all the same passion I have for native mass spectrometry – an exciting technique that could help us write the next chapter in the protein revolution.

Going native

So what is native mass spectrometry? Native MS is a term that we initially coined here at Utrecht University. (There's a similar well-known technique in biochemistry called native gel electrophoresis, where non-denaturing gels are used to analyze proteins and protein complexes in their folded state – and that's partly where the name came from…)

In contrast to traditional MS, with native MS we try to keep the structures of the proteins and protein complexes that we analyze as close to what they were in their native environment of the cell. To do this, we use special solvents that not only maintain the integrity of the protein interactions, but are also compatible with the electrospray ionization process that transfers molecules from the liquid to gas phase. By using this unique set-up, it means even non-covalent complexes can remain intact for analysis. In the words of the Nobel Laureate John Fenn, through electrospray under these pseudo-physiological conditions, we can make elephants fly (1).

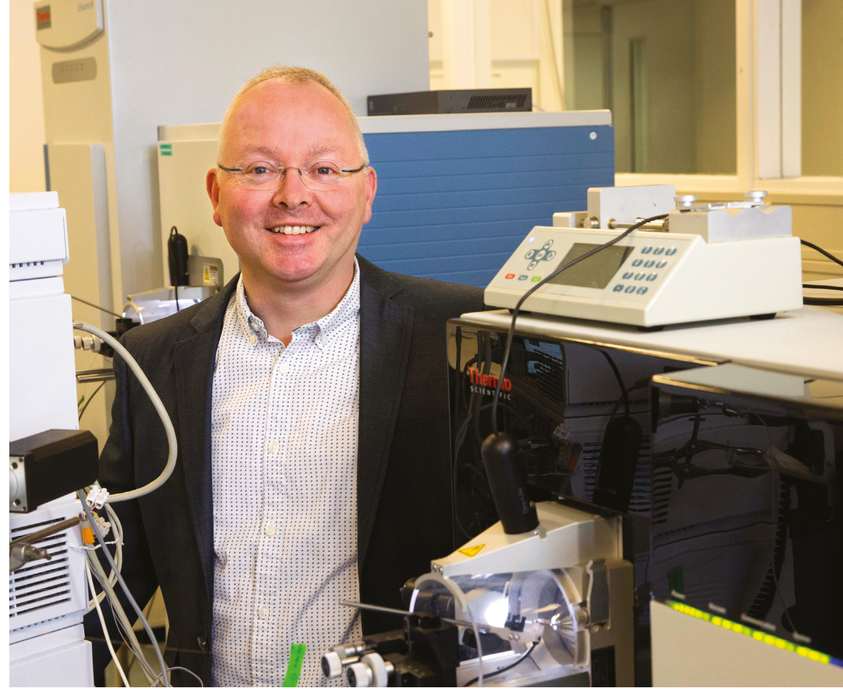
By keeping proteins and protein complexes intact in the mass spectrometer, we're able to accurately measure the masses of interesting biomolecular machineries, such as intact antibodies, viruses or ribosomes. It also allows us to confirm the composition of these systems; for instance, do we have a monomer or tetramer (the tetramer will have a mass that is four times as great as the monomer). Even when a protein complex consists of about 20 different proteins and RNA; as for instance in an intact ribosome, with a mass close to two million Daltons, we are able to accurately measure its mass in a native state and observe changes in its composition.

Accuracy is the real key to native MS. After all, we are only measuring the masses of the complexes, but we do it so accurately that we are able to learn about their composition and whether this changes over time – like when they exchange or add new subunits. And that means we're also able to learn a lot more about the function of these macromolecular complexes. For example, a change like a phosphorylation or glycosylation, which also induces changes in mass, may either activate a protein or inhibit a protein's function. By discovering whether a protein is modified or not, you can learn how to activate or deactivate a protein. In turn, this means you could aim to regulate entire biological processes. And that's just one reason why measuring these masses so accurately is important – there are also many other incredible applications (see for instance our work on a bacterial biological clock).

Gene therapy

When my team and I first started using native MS, we looked at virus-like particles (VLPs). VLPs resemble viruses; they form beautiful, very rigid spheres made up of self-assembling proteins – this is known as the capsid. However, these VLPs lack the genetic information needed to infect a host cell, making them safer to analyze. On the other hand, intact or "native" viruses contain not only the capsid, but also all the genetic information that enables the virus to reproduce within the host. One well known example of a native virus – and one that is not harmful to humans – is the adeno-associated virus (AAV). In recent years, biopharmaceutical companies have used AAV as a vector for delivering certain genes into human cells by replacing part of the viral genome – the basis for gene therapy. A notable example of this is Zolgensma; a prescription approved AAV vector-based gene therapy for the treatment of children less than 2 years old with spinal muscular atrophy introduced by Novartis.

Even more recently, adeno-virus (ADV) vectors have garnered attention because of their use in the AstraZeneca, Janssen and



Sputnik vaccines for COVID-19. Without a doubt, these gene-loaded viral particles are an exciting class of emerging biopharmaceuticals. However, they are also extremely complicated to produce and structurally very heterogeneous and therefore raise huge and specific novel analytical challenges.

We work with several pharmaceutical companies to help them overcome some of these challenges by analyzing virus-based gene delivery vectors using native MS. Why do they need our help? First of all, it's about sensitivity. It's very hard and laborious to produce these particles, so you never have many of them available to analyze – unlike recombinant antibodies where you can produce grams. There are also further complications because these particles are huge – we are talking 4–5 million Dalton. And they are extremely heterogeneous, containing several different proteins and gene products, which makes them difficult to analyze with a single technique.

We pioneered the use of a particular type of native MS – charge detection MS – for this exact purpose (2). This technique's big advantage is its ability to count, while accurately measuring the mass

of, single viral particles. And it's really opened up a whole new avenue of analysis, allowing us to look closely at gene vectors and discern whether or not the genome of choice has been successfully loaded. Understanding whether the gene has been incorporated is vitally important for the biopharmaceutical companies producing these particles. As such, I foresee that native MS will play a crucial role in the quality control of gene delivery factors for this exciting new field of medicine.

Rewriting the textbook on immunology

More recently we also started to focus on antibodies, as they are naturally present in huge quantities in our blood. According to the textbooks, antibodies are made by certain cells in our body called B cells. The number of different B cells in our body is enormous – we're talking 1015 , which to put it in perspective is several times more than the number of people on the planet. I had been wondering myself already for some time: how many (and which) antibodies does the human body really produce that end up actually circulating in our blood to counteract invading pathogens? The commonly accepted answer within immunology was that this could be an almost infinite (or at least, immeasurable) number. When we suggested measuring them all, many within the field thought it would be impossible. We've now published research that proves the task was not as insurmountable as people thought (3). To our surprise, we found that just a couple 100 different antibodies dominate the repertoire in each person's blood – a number so low in comparison to what was previously believed, that we knew these findings were already very exciting. But then we also discovered that each person had their own personal repertoire of antibodies – equally exciting.

You might be thinking that such a finding is somewhat obvious; "Of course we would make different antibodies against every virus or pathogen we encounter," you say. "I'd expect there to be a unique signature to align with this fact." However, we observed that, even if people are exposed to exactly the same pathogen – or vaccine in the case of COVID-19 – they still make their own personal repertoire of antibodies against it. Fascinating. This clearly implies that every person reacts to incoming pathogens differently. We can see this working on a grand scale when we look at how everyone has reacted to the COVID-19 vaccines; some people get ill, others don't, and the vaccine works better at protecting some people from the virus than others. We now know that these differences are at least partially due to the specific antibodies each person makes. (For more information, see the sidebar: What's the deal with antibodies?)

In the long run, I hope that we will be able to measure and identify the antibodies that each person makes against a certain pathogen using MS. This would open up the possibility of taking antibodies from someone who does mount a good response, and using them to help those who don't. In this case, we'd be using recombinant molecular biology to produce the best antibodies against the disease and treat someone who has a sub-par response. Such treatment could be used against all COVID-19 variants, but I'm equally eager to see how this could be applied to any other viral or bacterial infection – or even other diseases, such as rheumatoid arthritis and cancer. Basically, new understanding in this arena could be applied to any disease to which our bodies respond with antibodies – and that's quite a few! Ultimately, it could open a new way of producing personalized biotherapeutics.

Our finding was extremely exciting and totally unexpected – and one that is bound to find its way into the textbooks in the coming years. It's not often you get to make such discoveries, so I'm extremely proud that our team of about ten researchers, who worked on this for a couple of years, was able to achieve this.

The horizons of MS

MS has come a long way since its inception more than 100 years ago, but some challenges seem to always remain the same. Can we get faster? Can we get more sensitive? Can we get higher mass resolution? Can we get better mass discrimination? Though many great advances have been made, the answer to all these questions is yes, we could and should. It's incredible to think that we now only need tiny amounts of material to run our analyses, which means we can uncover the entire proteome of a single cell. This is an enormous sensitivity jump from where we were. However, the cell is still made up of billions of proteins. The area I'm most excited about for the future – and an area we've also contributed to ourselves – is in increasing the sensitivity of MS to measure single molecules.

My ultimate dream is to take a single cell, take out every molecule one by one and measure its mass. And though it might sound like a pipe dream, it's already theoretically feasible. Practically, I'm not sure when we'll manage it – but, because I can imagine how it might happen, I'm sure someone will figure it out soon enough, and of course we hope to contribute to that.

For me, progress is all about understanding, fundamentally, how life works. In turn, we increase our knowledge about our wellness, health, and the planet. The horizons of MS are already beautiful. But beyond that horizon, there are undoubtedly new and wonderful stories – applications that many of us haven't even dreamed of yet.

Albert J.R. Heck is a Professor at the Science Faculty, Utrecht University, and Scientific Director of the Netherlands Proteomics Centre, The Netherlands

REFERENCES AVAILIABLE ONLINE

SITTING DOWN WITH

The Analytical Neuroscientist

Sitting Down With… Jonathan Sweedler, James R. Eiszner Family Endowed Chair in Chemistry, Director of the School of Chemical Sciences & Professor of Neuroscience and Molecular & Integrative Physiology at the Beckmann Institute, University of Illinois, USA

How did you get into analytical science?

I was always into science – be it using a ham radio or making model rockets. In fact, in California, when I grew up, they had a ban on model rockets, so I was busy writing to state legislators to try and change the law – at about 12 years old. Soon after I became honorary first president of the local rocket club (which was mostly adults). I had around seven years' worth of science courses in high school having changed my schedule around to accommodate the extra classes. I was always going to study science in college, and I guess I liked exothermic reactions, so chemistry was the one I went for. Though I did also study classical Greek as a minor…

The hard part was figuring out what I actually wanted to do as a career. I was interested in applying chemistry to biology and the brain, but I ended up getting a fellowship to work at the Lawrence Livermore National Lab as an undergraduate. Livermore is one of the two main weapons labs in the US – though I was working on analytical projects, which is what made me decide to stay in analytical chemistry. But I remained interested in neuroscience and, once I finished my analytical chemistry PhD, that's what I decided to focus on during my postdoc at Stanford.

The Livermore Lab – is that where you met Tomas Hirschfeld?

Yes. He was my first mentor and, really, the reason I am an analytical chemist. He was the only person I've ever met who genuinely had a photographic memory. You could ask him a question and he'd tell you to look up a paper – he'd know the journal, year of publication, the page number and even where on the page the relevant paragraph was. It was remarkable. And yet he'd sometimes forget to meet you after lunch! More importantly, he was a truly creative thinker with a broad knowledge base who always had a unique perspective on any given problem – and he'd encourage his students to explore new and crazy ideas. (It helped that the budget at Livermore was limitless, as far as I was concerned; I was working with FTIR and MS instruments back in the early 1980s, which just wasn't possible anywhere else.) Creative problem solving is something I've valued throughout my career – and often just asking myself "What would Tomas have done?" does the trick!

Has your career ever taken a serendipitous turn?

In planning my postdoc, I was trying to figure out how I could study the brain from the point of view of a chemist – a daunting prospect. One way would be to simplify the problem by working on a simpler organism. So I started looking into researchers working on things like sea slugs, which have around 10,000 neurons, a number I can comprehend. I also needed to find a chemist to support this idea, something I was able to do at Stanford with Richard Zare and Richard Scheller. At UIUC, I continued this research area. I was discussing these research ideas with a physiology professor who suggested I go to Friday Harbor Marine Lab, San Juan Island, Washington, to learn about some of these organisms. I even ended up doing a sabbatical at Hopkins Marine Station in California learning fundamental neuroscience of marine organisms. I got paid to learn, be on the beach, and to scuba dive – it was great! A lot of people said I was crazy trying to learn new skills at the stage in my career when you're supposed to be your most productive, but it was invaluable. I've used the practical skills I learned throughout my career and it also redefined me as a researcher.

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