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Analytical Scientist

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IN MY VIEW

What's in Your Capsids?

Are your capsids full, half full, or empty? We need better analytical techniques to tell us the answers to these crucial questions in the development of gene therapies.

It's just over 30 years since the first approved gene therapy procedure was performed – and I'm sure we're all amazed at how the field has progressed since then. The FDA expects approvals for cell and gene therapy products to reach around 20 per year by 2025 (1). A key component enabling the growth of the field of gene therapy is recombinant adeno-associated virus (AAV) vectors. There have been three recombinant AAV-based gene therapies approved for commercial use so far (2), and there are hundreds of active clinical trials worldwide for a variety of diseases.

As development of gene therapies increases, there is a growing demand for accurate and efficient techniques for characterizing AAV vectors. Many existing methods for analyzing AAV vectors, particularly for determining the full/empty capsid ratio, are labor-intensive and time consuming. Analytical methods using anion exchange (AEX) chromatography, however, are supporting the analysis of AAV capsids and could be a key technology for further advancing gene therapies.

Viral vector characterization is essential for assuring product quality. Critical quality attributes (CQAs) include viral potency, identity, quantity, process residuals (i.e., Triton and deoxyribonuclease), aggregation, empty capsids, capsid protein content, and product safety. To meet purity requirements the proportion of empty, partial, and full AAV capsids must be determined. Unsurprisingly, full capsids are required for therapeutic efficacy; empty capsids, which do not contain genetic material, or partial capsids, which contain only a fragment of the genetic material, are simply by-products of AAV production and can negatively impact product efficacy and safety – potentially producing adverse reactions, such as an immunogenic response in the patient.

There are various analytical methods for characterizing capsid levels in the laboratory, including transmission electron microscopy (TEM), analytical ultracentrifugation (AUC), charge detection mass spectrometry, and UV spectrophotometry. The two main methods



“Today, chromatographers have learned they need to search across multiple column chemistries to find the solution that provides the best resolution for empty/full capsid separation.”

are TEM and AUC. TEM involves a sample vitrified by rapid freezing to preserve the structure of a biological specimen. When imaged, there is a clear difference in structure between full and empty particles. The AUC method distinguishes and quantifies the different AAV capsids either by density (sedimentation equilibrium) or mass (sedimentation velocity).

As AAVs are relative newcomers in gene therapy, the industry has not yet decided upon the most effective method of analysis. Certainly, existing methods for determining the full/empty ratio of AAV capsids have limitations; for example, TEM provides direct visualization and counting of the empty and full particles, but quantification heavily relies on image quality and field selection.

And although AUC has excellent resolution and is highly accurate, it often requires a dedicated facility and specially trained analysts, who must spend hours on data interpretation. Moreover, AUC also consumes hundreds of microliters of valuable samples.

In short, TEM and AUC are both low throughput – and neither is readily scalable.

In my view, AEX chromatography is a useful technique for the analytical toolbox. When genetic material is encapsulated in the capsid, the surface charge on the particle changes. This physiochemical difference between full and empty capsids makes them ideal for analysis with AEX chromatography methods. AAVs are also small (20–25 nm) and suitable for both traditional and monolithic columns.

The AEX chromatography method requires only several microliters of material – clearly a real benefit when gene therapy samples are so precious. Furthermore, no sample preparation is needed, which simplifies the analysis and increases throughput.

Historically, use of the AEX method for separation of empty, full, and partial capsid was less than optimal due to analysts narrowly exploring anion exchange column chemistries. For example, a user may begin method development with a column that would be recommended for nucleotide analysis because this would work well for characterizing genetic material inside the AAV. However, this same column chemistry may not be best suited to separate empty, partial, and full capsids. Today, chromatographers have learned they need to search across multiple column chemistries to find the solution that provides the best resolution for empty/full capsid separation.

Right now, AUC provides the better resolution, but I believe that ongoing developments with AEX column chemistries and chromatography systems make it a technology to watch for the future. Chromatography systems are already up and running in the QC environment for other processes and specialist expertise is not needed. Also, chromatography lends itself well to automation opportunities, opening the door for high-throughput capsid analysis, ultimately providing a more cost-effective way of speeding up product development and reducing time to market.

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References

1. FDA, “Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies,” (2019). Available at <https://bit.ly/3ryl9mp>
2. FDA, “Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs),” (2020). Available at <https://bit.ly/3t728b6>

IN MY VIEW

ICP-MS: Taking the “Gold Standard” to the Next Level

Today, ICP-MS allows us to understand how cells interact with their environment – but we have a long way to go before we can measure these dynamic processes as they occur. What comes next?

Cells are the basic unit of all living organisms – “the building blocks of life” – so it isn’t surprising that analyzing cells and their constituents is central to life science research. One challenge for researchers is that cells vary considerably – even cells of the same culture behave differently at a chemical level because of their different life cycles (or cell status). ICP-MS is the only method that provides quantitative metal data in single cells with multi-element coverage and high sensitivity, which has led to its rise as the gold-standard tool for measuring how cells interact with their environment.

Elemental single-cell analysis comprises both the assessment of biomarkers through metal tagging of antibodies (denoted as mass cytometry or imaging mass cytometry), as well as the determination of endogenous/toxic metal accumulation and the uptake of nanoparticles from cell suspensions. Isolated cells and cell agglomerates in tissue samples have been investigated with laser ablation (LA)-ICP-MS in an imaging mode, which has focused on detecting metals – either directly or with metal tagged antibodies.

Though ICP-MS has been on the market since 1983, mass cytometry is a relatively young technology (commercially available since 2009, launched by DVS – Dmitry, Vladimir and Scott – under the brand name CyTOF Mass Cytometer). The basic idea is similar to fluorescence-based flow cytometry. Instead of using fluorescence to tag antibodies, single-enriched isotopes of rare earth elements (REE) are used. This approach overcomes the problem of “bleaching” or high-background interference associated with autofluorescence, while also allowing the use of a greater number of isotopes in a single assay. Then, to bind metal isotopes to antibody polymers, tags are applied, which mostly bind to surface receptors of single cells or, in the case of phenotyping, the antibodies bind to intracellular proteins after cells are permeabilized (the approach for cell functional assays). Because each polymer can bind up to 100 atoms, it is possible to amplify a single protein/receptor by the same factor. In fact, a cell can have up to 1,000 surface receptors of the same kind, which can really increase the amplification for extremely high sensitivity!



“ICP-MS is the only method that provides quantitative metal data in single cells with multi-element coverage and high sensitivity, which has led to its rise as the gold-standard tool for measuring how cells interact with their environment.”

After incubation of the target cells with different antibodies, which are tagged individually by enriched nuclides from lanthanide elements, the cell suspension is transported to a pneumatic nebulizer. Stochastically spaced cells in the laminar flow are then delivered concentrically to the plasma core, where the cells get ionized. Since each cell generates ion signals of a few hundred μs peak width, a very fast mass spectrometer (a time-of-flight instrument) is required to measure each cell time resolved.

There have been a number of improvements in ICP-MS over the years. For example, higher sensitivity, higher time resolution, and dedicated sample introduction systems. For laser ablation systems, lasers have been developed with higher repetition rates (up to 200 Hz), the ability to wash out aerosols more quickly to achieve better lateral resolution at the sub-micrometer level.

With novel LA-ICP-MS devices, sub-micrometer resolution has been achieved, which is needed to compete with light microscopy! Early

results have demonstrated multiparametric analysis of cancer tissues with up to 40 parameters measured in the imaging mode by laser ablation. Indeed, such analysis has revealed the role of the neighboring community of cells in cancers – going some way to explaining why patients respond differently to cancer treatments.

There have been thousands of papers published on the use of ICP-MS, covering nano-particle interactions, metallo-drug research, various types of cancer research, and many more areas. However, some big challenges remain. For example, at the cellular level, we don't have reference materials, which holds true for imaging and mass cytometry. And that means we must use our own standards to calibrate devices – not an ideal situation. Validation reference materials and inter-lab comparisons are urgently needed. And concerning mass cytometry, we still wait for it to be accepted as a routine diagnostic tool for detection of cancer, inflammation or other diseases.

In the future, through improvements in reagents, it may be possible

to detect single viruses or even single molecules. Currently, we can measure the end-point of a disease, such as a cancer cell, or the toxification of a cell leading to cell death, but we cannot measure these dynamic processes as they occur (time resolved analysis). We would need multimodal spectroscopies to measure – in a time resolved manner – the complex chemistry underpinning living processes.

In fact, we know very little about the complex machinery of life, which is why we need more scientists – especially younger scientists – applying analytical chemistry to the complex reactions taking place within cells at the nano-meter level and at time scales of μs - or milli-seconds. Single cell analysis – including ICP-MS – can take us a long way, but we need to measure living processes in real time to truly understand what's going on. Who will step up to the plate?!

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Use the (OptoFluidic) Force

With a wide array of particle analysis methods available, what does OF2i bring to the table?

With Christian Hill, CEO of BRAVE Analytics

How does OF2i differ from methods already in use?

DLS, NTA and laser diffraction measure particle size by evaluating Brownian motion. With OF2i, we take a different approach – focusing a laser through the liquid sample to exert a force on the particles that pushes them forward and turns them around the beam's center. This causes the nanoparticles to move along a spiral trajectory, which minimizes interparticle collisions that would disrupt measurement. A microscope records the velocity change due to these optical forces – this correlates with particle size.

OF2i was actually intended as a PAT method for continuous monitoring of pharmaceuticals. Observing and adjusting the size of particles in nanoemulsions and nanosuspensions directly during production prevents product being bottled that later fails the batch test – so online control saves money.

This new method is available in a laboratory device, how did that come about?

To develop the new OF2i method for process control, we took part in the NanoPAT project funded by the EU. We've been working with our partners in industry to fine-tune the method for their applications, which involves small-scale installations to test the performance. As a spin-off of the Medical University of Graz, we are also in contact with researchers in various biomedical fields. We began to get requests for measurements on our "beta device" and realized the potential for a laboratory nanoparticle analyzer based on OF2i.

Where is OF2i already in use?

One of our earliest customers has used OF2i to monitor the molecular processes of early-stage condensate formation in order to optimize drug delivery for treating Alzheimer's and other age-related diseases. OF2i delivers a second-by-second rolling results curve that shows what is going on in the sample as it occurs. In this case, it's possible to observe the formation and size distribution of the proteins in question as they change over time. Another researcher was interested in the dissociation dynamics of coated nanoparticles under different pH conditions. OF2i measures the particle concentration over time and the results tracked the particle dissociation in the acidic buffer as it happened.

In another recent study, OF2i was used to investigate the leaching of nanoplastic particles out of PPE bottles into water over minutes and hours. The method is well-suited to detecting ultra-low numbers of particles in a medium.

And only last month we got a request for a test installation coupling our device to an ICP-MS. The researcher wants to capture, sort by size and concentrate particles, and then monitor particle size and particle concentration alongside other ICP-MS measurements.

Where do you see OF2i in the future?

We certainly know that for particle analysis there is no "one-size-fits-all" answer. Dynamic light scattering may be the most suitable method for one application, whereas TEM or SAXS may work better for others. We see that OF2i's capability for time-resolved measurements brings benefits whenever sample kinetics need to be observed and understood, and its capacity for measuring and capturing ultra-low particle concentration open up new fields. We are already working on two add-on Raman modules to allow the identification of substances via Raman spectroscopy and particle analysis on trapped particle populations.

And the long-awaited online sensor will bring OF2i to production facilities by the end of 2024.

FOR MORE ABOUT OF2I SEE:
WWW.BRAVEANALYTICS.EU



FEATURE

An Elemental Regeneration

Emerging applications in life sciences and environmental analysis are driving renewed interest in the unique properties of ICP-TOFMS – in particular, its ability to detect complete elemental mass spectra from short transient events, such as single nanoparticles.

Prior to moving to Switzerland, I did my PhD at Indiana University in Gary Hieftje's group, where I studied a new form of velocity-based mass spectrometry – distance-of-flight mass spectrometry (DOFMS). My research into DOFMS was formative, and taught me about mass spectrometer design, construction, and operation. I've carried the interest and expertise I gained in velocity-based mass spectrometry to my current work at ETH Zurich, developing analytical methods for the high-throughput analysis of diverse inorganic nanoparticles from environmental samples by ICP-TOFMS.

As I began searching for a postdoc position, I was aware of renewed interest in full-spectrum atomic mass spectrometry, largely because of key applications in biomolecule detection and laser-ablation imaging. The potential encouraged me to join the Günther group, who were central to the revival of inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS).

ICP-TOFMS rediscovered

ICP-TOFMS is not a new technique; it was pioneered in the early 1990s by the Hieftje group and then commercialized by two instrument companies. However, ICP-TOFMS had limited commercial success because the sensitivity and dynamic range lagged too far behind other scanning-based ICP mass spectrometers. Notably, the initial limitations of ICP-TOFMS were largely the result of the inadequacies of high-



“Another promising application of ICP-TOFMS is single-particle ICP-MS analysis, in which particle number concentrations of nanoparticles and elemental compositions of single-nanoparticles are measured.”

speed digitization electronics at the time. Today, high-speed detection electronics have improved (and continue to do so), so ICP-TOFMS has matured into an approach that can compete with other ICP-MS instrument designs in terms of sensitivity, limits of detection, and linear dynamic range. Moreover, it offers unique measurement possibilities through high-speed, full-spectrum ICP-MS analysis.

In my time at ETH Zurich, I've worked predominantly with two ICP-TOFMS instruments: a prototype instrument that was developed through a collaboration between TOFWERK (Thun, Switzerland) and the Günther group, as well as a commercial ICP-TOFMS instrument (icpTOF, TOFWERK). The collaboration between the Günther group and TOFWERK is a great example of how a university-based research group can work in symbiosis with a company in developing a new tool; the academic group provides vision and expertise in compelling and significant applications, while the company provides technical know-how and the environment to develop a user-ready instrument.

I work mainly on two applications: elemental imaging with laser ablation, and single-nanoparticle detection and quantification. In both of these research areas, ICP-TOFMS provides measurement characteristics that are not available and often fundamentally not possible on other elemental mass spectrometers. The key benefit of

ICP-TOFMS is that it provides full spectral coverage and very rapid data acquisition speeds. For laser ablation and single-particle analysis this is a major advantage because it means that we can measure complete atomic mass spectra for discrete transient events, such as single laser ablation events or single nanoparticles. From a practical standpoint, full mass spectrum detection is also beneficial because it simplifies mass spectrometer experimental setup and means that we never miss any unexpected – but pertinent – isotopic signals.

New approach, new measurements

In laser-ablation ICP-MS, a pulsed laser is used to ablate a small quantity of a solid material and the resultant aerosol is transported to the ICP-MS, where the elemental and isotopic composition of the ablated aerosol is measured. Laser ablation is a clean way to sample solid materials and, by controlling the position of the laser, it can be used to measure sample material with lateral and depth resolution. Combined with ICP-MS, laser ablation can provide element and isotope composition at high lateral resolution. Laser ablation is fundamentally a pulsed technique: a laser impinges on a material surface, which causes ablation, and a cloud of sample material is generated – all in a timescale of nanoseconds to hundreds of microseconds. In conventional LA-ICP-MS, sample transport systems are used to “smear” laser-ablated aerosol

particles from many laser-ablation events so that timescale of the mass spectrometry analysis matches that of the laser-ablation sampling. Spreading the time profile of LA aerosols leads to more accurate quantification for analysis with scanning-based ICP-MS instruments; however, it also means that information about each LA shot is lost and that lateral and depth resolution is limited by the overlap of signals from temporally (and physically) adjacent laser shots. The high-resolution LA-ICP-TOFMS system we've developed in the Günther lab is fundamentally different to this conventional LA-ICP-MS approach.

In our lab, we use a “pulse-resolved” LA-ICP-TOFMS imaging strategy – the complete transient signal of each LA event is measured without overlap from adjacent LA event signals. This imaging strategy prevents the image blur that arises when ablated aerosols from different locations are mixed in the aerosol transport system. To perform high-speed pulse-resolved LA-based imaging, research in the Günther lab has focused on development of a low-dispersion LA cell and aerosol transport system that can deliver ablated aerosol from individual laser-sampling events within 10 ms. Several other groups are also pursuing low-dispersion LA aerosol transport; however, to provide meaningful and quantitative information about the material sampled at each LA position, we need an ICP-MS instrument that has the speed to match the laser sampling – this is where ICP-TOFMS comes in. By combining low-dispersion LA sampling with ICP-

TOFMS, we are able to generate multi-elemental high-dynamic-range quantitative elemental images at lateral resolutions down to five μm and at high speed (100 pixels/sec). The figures of merit of LA-ICP-TOFMS imaging are simply not available with other ICP-MS instrument designs.

More importantly, the elemental images that can be generated with this approach are compelling and offer access to otherwise unavailable information about trace-, minor-, and major-element spatial distributions across a specimen. In my research, I have focused on the application of LA-ICP-TOFMS imaging for analysis of geological samples, where high-resolution elemental imaging combines with observations about specimen textures to provide insight into rock formation and history. LA-ICP-TOFMS has also been shown to be very promising for elemental bioimaging. By staining tissues with isotope-labeled antibodies and performing LA-ICP-TOFMS imaging experiments, highly multiplexed distributions of biomolecules can be measured at subcellular lateral resolution. This approach is called imaging mass cytometry (commercialized by Fluidigm, USA) and was partly developed in collaboration with our lab. Apart from antibody-labeled elemental imaging, LA-ICP-TOFMS will also have substantial impact in the burgeoning field of elemental bioimaging, where LA-ICP-TOFMS approaches will help researchers hone their understanding of the roles and diagnostic potential of native metals in biological systems.

Addressing a (nano)particular problem

Another promising application of ICP-TOFMS is single-particle ICP-MS analysis, in which particle number concentrations of nanoparticles and elemental compositions of single-nanoparticles are measured. The expanding use of inorganic nanoparticles in a variety of consumer goods (everything from lotions to fabrics to food packaging) and industrial processes, makes measurement of individual nanoparticles, especially in complex natural media, an emerging analytical challenge. Nanoparticles are hard to detect – they are small (hundreds to millions of atoms), dilute in terms of total mass concentration, and often present in matrices that contain naturally occurring colloids with similar element compositions.

We have a good understanding of how nanoparticles behave within the products or processes for which they are intended; however, their eventual fate is harder to predict. Nanomaterials make their way into the environment through many routes, whether through disposal into water or solid-waste handling systems that are not designed to deal with nanomaterials, or through incidental release, such as from automotive exhaust. Predicting the impact of nanomaterials and nanoparticles being released into the environment requires the modeling of particle fate and transport, understanding routes of exposure, and performing toxicology experiments. All of these studies need to be supported by analytical measurements. It's important that we better understand the amount and chemical species of inorganic nanoparticles in natural systems, and develop systems for monitoring them.

Multi-Element Single-Particle ICP-TOFMS Signal

Currently, there is no single measurement (or even a defined group of measurements) that is used to characterize a broad range of nanoparticles in natural systems. We are working to develop single-particle ICP-TOFMS as a comprehensive measurement system for non-targeted analysis of diverse inorganic nanoparticles. In single-particle ICP-MS, liquid samples with dispersed inorganic nanoparticles at environmentally relevant concentrations ($\sim 10^5$ particles/mL) are introduced into the ICP-MS instrument. When a nanoparticle passes through the ICP, it is vaporized, atomized, and ionized so that a dense cloud of ions is produced and detected as a signal burst by the mass spectrometer. If this signal burst is more intense than dissolved background signal levels, we are able to count the particles. In fact, single particle-ICP-MS can even detect nanoparticles in a matrix that has a higher mass concentration of analyte in the dissolved fraction than the nanoparticulate fraction. Because the ICP is a robust atomization and ionization source, we can also quantify the amount of element(s) in each nanoparticle. With conventional ICP-MS instruments, only a single isotope can be monitored across the short timescale of a single-particle event; TOFMS allows for all elements to be measured within each single-particle event. By using ICP-TOFMS, we can measure both

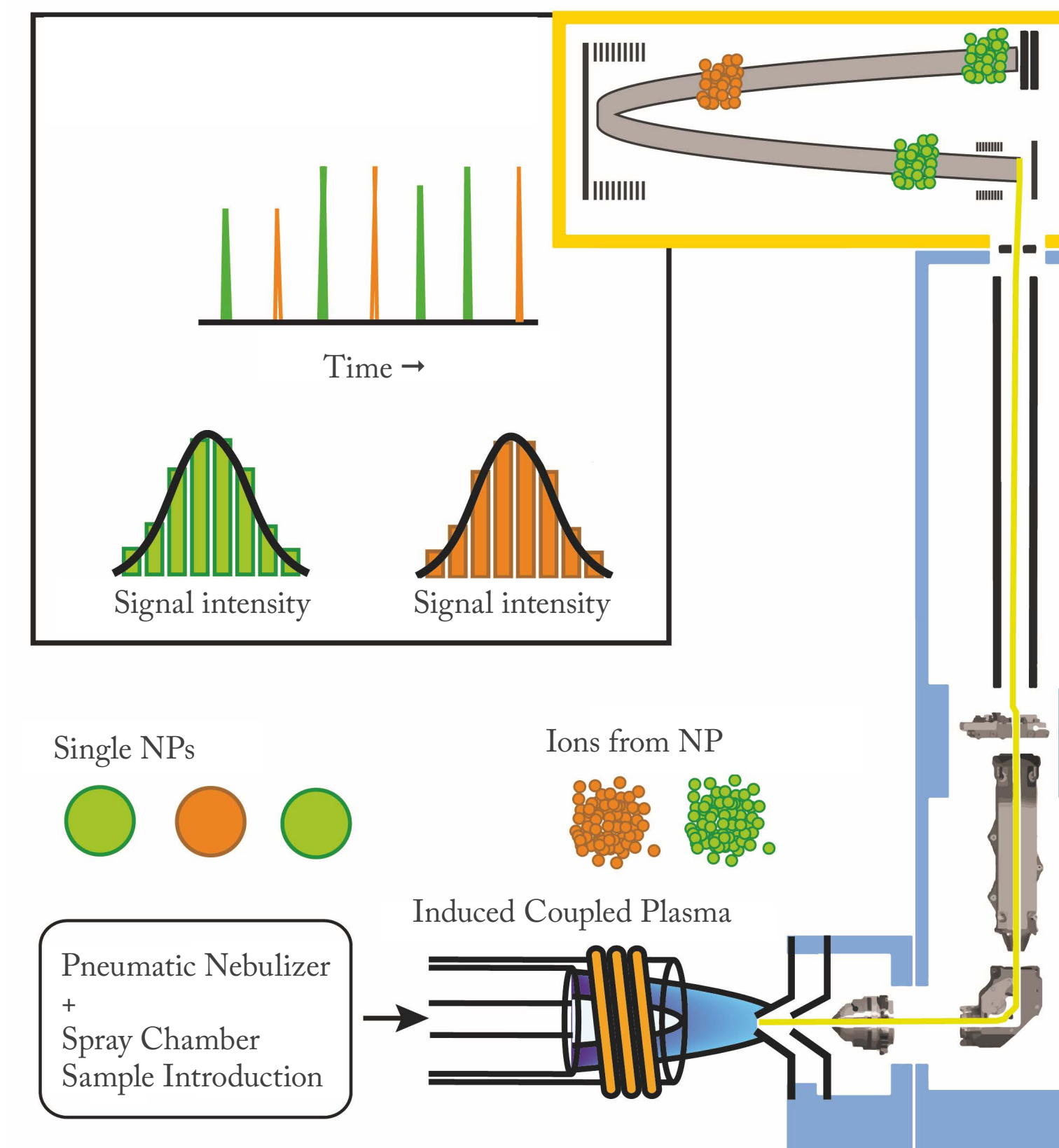


Figure 1. In single-particle ICP-TOFMS (schematic of icpTOF shown here), dispersed NPs are introduced into the ICP, where they are vaporized, atomized, and ionized. Each NP produces a dense cloud of ions that is recorded as a short burst (300–500 μs) of signal by the mass spectrometer. The magnitude of the single-particle signal correlates to particle mass and the frequency of bursts to particle number concentration. With ICP-TOFMS, ions of all m/z are measured simultaneously, so a diverse range of NPs (including multielemental NPs) can be analyzed in a single run.

“Because the ICP is a robust atomization and ionization source, we can also quantify the amount of measured element(s) in each nanoparticle.”

multi-elemental signatures of individual particles and a diverse range of nanoparticle types – in a single analysis.

To illustrate the advantages of ICP-TOFMS for multi-element detection of single nanoparticles, consider our proof-of-principle study, measuring engineered nanoparticles of cerium dioxide (CeO₂) in a soil matrix with cerium-containing natural nanoparticles. In this study (1), we were able to distinguish the two types of cerium particles (engineered and natural), based on conserved multi-element fingerprints from each nanoparticle type. With a machine-learning classification algorithm, we were able to quantitatively count engineered nanoparticles in a matrix with over 100× more natural than engineered cerium nanoparticles. Importantly, without TOFMS, we would have only been able to measure the signal of ¹⁴⁰Ce⁺ for each nanoparticle, and could not have differentiated the Ce-containing natural nanoparticles from the CeO₂ engineered nanoparticles – single-particle ICP-TOFMS has enabled a real leap forward in measuring inorganic nanoparticles in complex matrices.

ICP-TOFMS impact

I have described just a few of the many applications where fast, multi-element analysis is critical. Other applications primed for

ICP-TOFMS include multiplexed isotope tags for biomolecule quantification, and combination with fast separations. I believe that this next generation of commercial ICP-TOFMS instruments will become a mainstay in atomic mass spectrometry. Such instruments provide sufficient sensitivity for most trace-element analysis laboratories and offer measurement capabilities – high-speed full-spectrum detection – to meet the challenges of contemporary elemental analysis. Even more exciting is the potential for more powerful ICP-TOFMS instrumentation in future; higher sensitivity and dynamic range is certainly achievable. Emerging applications – especially in biological sciences and environmental analysis – that simply cannot be tackled with alternative elemental mass spectrometry approaches will no doubt drive further development of ICP-TOFMS. All in all, it is a fun time to be working with ICP-TOFMS – and a thrill to be a part of this renaissance of elemental mass spectrometry.

Alexander Gundlach-Graham is a Research Scientist at ETH Zurich, Switzerland.

Reference

1. A Praetorius et al, “Single-particle multi-element fingerprinting (spMEF) using inductively-coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) to identify engineered nanoparticles against the elevated natural background in soils”, *Environ Sci: Nano* 4, 307–314 (2017).



FEATURE

Where Are They Now? With Russ Algar

In 2018, Russ Algar, Associate Professor at the University of British Columbia, told us his ultimate goal was “continued growth” and solving problems that seem “daunting or unimagined today” – working in partnership with other research groups and clinicians. Has Algar been working towards these objectives since we last spoke? We explore.

Broadly, how has your career progressed over the past four years?

Things took a challenging turn for my team over the last two years – as they did for most people. Research was progressing well before the pandemic and, after a hiatus and some setbacks, is now thankfully accelerating again. One example of an evolution of our research over the past four years has been a shift toward cellular analysis. Looking back, ideas that were rough sketches in 2018 have now come to fruition. It is also the case that more people have noticed our work, and some of that attention definitely came from my inclusion in The Power List.

I have also been grateful to have received some new honors since 2018. One of these was the McDowell Medal from my institution for excellence in pure or applied scientific research by a young faculty

member – it was very exciting for analytical science to be so highly regarded in a pool of outstanding research from across science and engineering. Another honor was the McBryde Medal from the Canadian Society for Chemistry. This award has a 35-year history, so it was very cool to see my name added to a list of great analytical scientists that I’ve admired since I was a student.

What has been your main lesson learned?

My most recent recognition is a Killam Accelerator Research Fellowship. It provides some lab funding but is most valuable in its protection of time for research. Between the pandemic and normal advances in life and career, I have definitely learned lessons about protecting time. Things like family, personal health, and research seem to exponentially benefit from more time spent on them.



“There are links between the two streams of research, but contrast in the technical requirements of the research keeps things interesting. Ultimately, the high-tech answers to fundamental questions will inform some very practical advancements.”

There is definitely still room for improvement, but I’m now more cognizant of the “busy work” that generally has the same outcome regardless of time spent. I’m thus getting better at maximizing the time I spend with my research team, so there’s also more time for the family – fatherhood is still the best, although I have notably less hair now than four years ago...

Are you working on anything particularly exciting at the moment?

I’m excited by everything we’re working on – my team and our research are truly my biggest professional motivators. That being said, one interesting thing about our current work is that our research directions are, in a sense, diametrically opposed. On one hand, we are continuing to develop our capability for single-molecule and single-particle fluorescence measurements, and have a long list of fundamental questions that we plan to answer with these measurements. The instruments are large, expensive, and high-tech. On the other hand, we are continuing to develop portable, low-cost, smartphone-based devices for molecular diagnostics and cellular analysis. For example, we most recently combined a 3D-printed

device with supra-nanoparticle assemblies to enable flow cytometry on a smartphone. We hope that assay methods and devices that come from this research will improve the equity and quality of healthcare in rural or remote communities that have fewer resources than urban centers.

There are links between the two streams of research, but contrast in the technical requirements of the research keeps things interesting. Ultimately, the high-tech answers to fundamental questions will inform some very practical advancements.

Do you have any major career goals for the future?

More collaborative and translational research is definitely a goal for the future. To some degree, I consider my group to be toolmakers, so I want our R&D to answer fundamental research questions in biology and health, and to develop technologies that are used in the clinic. My team has started down this path with some great collaborators and discussions with potential end-users but really we’re just getting started.

What is the single-most exciting development in your field today?

The most exciting ongoing development in my particular area of research is single-molecule detection. There is no better detection limit than a single molecule, and nothing is hidden when measuring molecules one at a time. With technological advancements, single-molecule fluorescence imaging has gone from exotic to almost routine. That’s exciting in itself, but I think I’m more excited by the number of different approaches to single-molecule detection that now exist. Of course, there are challenges to address, but single-molecule detection and digital assays have tremendous potential. I’m definitely enamored with the idea of low-cost, portable devices capable of single-molecule detection.

Any advice for young people entering analytical science?

Good advice for young analytical scientists is to learn broadly and to take on projects that push them in new directions. If you already know how to complete a project before you start it, then the project might not have been worth doing in the first place. I think I’ve been better served by knowing a little about many things than by knowing a lot about a few things.

FEATURE

Advanced Medicine's Analytical Wish List

Which analytical techniques could revolutionize cell and gene therapy development? Rakel Lopez de Maturana, Qualified Person & Quality Control Director at VIVEbiotech, reveals her wish list

Broadly, what are the main analytical challenges in cell and gene therapy?

Advanced therapy medicinal products (ATMPs), or lentiviral vectors in VIVEbiotech's case, are so inherently complex that they pose a huge analytical challenge. Of all hurdles, I would underline two.

First, the availability of sample volumes for testing is very limited. Lentiviral vector batches manufactured at early pharmaceutical development stage have a rather small size so it is critical to optimize any use of this very costly and scarce product. As the demand for the number of release tests and assays linked to stability studies increases, this is clearly a limiting factor for thorough product characterization.

Second, there is a lack of rapid analytical methods for in-process control (IPC) testing. Lentiviral vector manufacturing is a continuous, multi-step process. To monitor productivity, total viral particles can be quantified in hours by certain techniques, such as ELISA or interferometric light microscopy (ILM), but measuring infectious particles – the “real” active product – takes days, so it cannot be used as an IPC. Similarly, some safety assays – for instance, sterility – also

require incubation. In other words, the manufacturing process is performed from beginning to end with little knowledge on yield or product quality; on-line analytical testing would greatly help to adjust production parameters to increase productivity.

As the cell and gene therapy industry matures, is analytical science becoming more important for the field?

In addition to the two obstacles mentioned above, alternative testing approaches using small sample sizes and rapid analytics for IPC testing, there are increasing regulatory requirements to characterize ATMPs, with an emphasis on a more extensive list of release assays and the use of fully validated, accurate, and robust techniques.

Moreover, there is a trend towards customized, project-specific assays; for example, we are noticing that in addition to lentiviral vector titration, product characterization requires measuring the expression of the particular transgene by a relevant bioassay. Analytical scientists play a key role in developing assays and methods with good performance that are aligned with their phase-specific regulatory demands.



“I believe we are moving towards a more quantitative and more automated future. Automation will bring rapid, accurate, and more reproducible characterization with lower-volume samples.”

What are the main techniques used in cell and/or gene therapy analysis?

Most batch release assays tend to be compendial methods or well-recognized techniques that are relatively easy to validate, so they are favorably seen by regulatory bodies. At VIVEbiotech, we mainly use cell-based assays for viral titration or for the detection of viral contaminants; nucleic acid testing (NAT) as the key quantitative tool in many tests (for example, infectious titer, residual DNA, mycoplasma); ELISAs for the quantification of viral proteins or process contaminants; and physicochemical assays for the measurement of parameters such as pH or osmolality.

What techniques used by analytical scientists in other fields could help cell and gene therapy developers?

There are a number of techniques that could help overcome analytical challenges in ATMP characterization; for example, mass photometry, interferometric light microscopy (ILM), micro-flow imaging (MFI) microscopy, dynamic light scattering (DLS), and multi-angle light scattering (MALS) coupled to separation technologies (size exclusion

chromatography [SEC]-MALS, field-flow fractionation [FFF]-MALS) can be used to assess physical titer, full/empty ratios, size, and aggregation or subvisible particle impurities.

Other methodologies that are routinely used in other fields but with a low profile in viral vector characterization include next generation sequencing (NGS) (for example, RNA-Seq for lentiviruses) to detect all DNA/RNA variants in a sample and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze vector identity at the protein level.

What other challenges do you face with viral vector characterization?

Besides the analytical challenges already pointed out, I think the main difficulty in lentiviral vector characterization is testing biological activity (or potency). This is a cell-based assay with, as expected, high variability. Great efforts are continuously being made to optimize and improve intermediate precision of this method.

On top of this, there is no harmonization of protocols and no reference standard for potency testing, making inter-laboratory

comparability very difficult. Thanks to the Lentivirus Vector Reference Material Initiative from the ISBioTech (International Society for Bioprocess Technology), such reference standards may be available in the not-too-distant future and managed by an unbiased repository at minimal cost. In the meantime, we have developed our own internal controls at VIVEbiotech to overcome the problem in the near term.

What will the future of cell and gene therapy analysis look like?

I believe we are moving towards a more quantitative and more automated future. Automation will bring rapid, accurate, and more reproducible characterization with lower-volume samples. Molecular biology will also continue advancing. On the one hand, there will be a wide switch from qPCR to digital PCR, improving sensitivity and precision of data. On the other hand, Sanger sequencing for assessing identity will be replaced by NGS. Finally, some of today's state-of-the-art techniques may become more mainstream, proving effective/feasible for ATMP characterization to substitute (or at least complement) traditional assays.

SITTING DOWN WITH

Confronting Challenges – Big and Small

Sitting Down With... Christy Haynes, Elmore H. Northey Professor, Associate Department Head, Department of Chemistry, University of Minnesota, Minneapolis, USA.

We hear you're currently in Valencia – what are you working on?

I received a Guggenheim Fellowship to support a research collaboration between the Polytechnic University of Valencia (the MPV) and the Institute Tecnológico. The lab here is focused on biomedical applications of nanoparticles, and the team has developed some really nice mass spectrometry methods for characterization of protein absorption. It felt like a good time for a sabbatical...

How so?

I've been on faculty for 13 years, and I wanted to take a step back and re-evaluate. Sometimes in academia, people have a very singular focus – and become famous by working on their own. But I don't fit into the

traditional chemist box – I'm interested in the messy spaces between fields and in truly collaborative work. This sabbatical allowed me to come in with a "beginner's mind."

How did you find yourself in analytical science?

I don't have a very traditional trajectory. There are no scientists in my family, and mine was the first generation to go to college. All I knew was that I wanted to have an impact on the planet. For me, chemistry is really pragmatic; I had to get through college faster than most – I couldn't afford a fourth year of college and so I did it in three – so part of me wanted to stick with what I knew.

My PhD focused on physical chemistry but I was orientated towards the analytical, not least because it helped me cross



“There are no scientists in my family, and mine was the first generation to go to college. All I knew was that I wanted to have an impact on the planet.”

disciplines. As a grad student I did single-cell chemistry, which was a great gift; ultimately, I ended up with unique skillset that really helped when applying for jobs. I tell my grad students that it’s important to have many strings to your bow, so that you can set yourself apart.

What are the pressing issues in nanoscience?

Nanoparticle toxicology as a field emerged about 15 years ago and, for a decade or more, it has been largely focused on the exposure of organisms to high concentrations of nanoparticles for short periods of time. That is important, of course – but even more important is to understand the impact of long-term low-dose exposures. A great example are lithium-ion batteries, which contain nanoparticles such as nanoscale nickel manganese cobalt oxide (NMC). Electric vehicles are using these batteries, which is great, but there’s something like 40 kg of this NMC in each one – how will that impact on the environment in the long term? I think

we have responsibility to be proactive and consider issues of safety and sustainability in the lifespan of those batteries. My focus is on bacterial interactions with nanoparticles – the bottom of the food web.

What challenges do you encounter in nanoparticle analysis?

Our biggest problem is that we need to find methods that can track nanoparticles in real-time in complex matrices and, at the same time, help us to understand how the particle is transforming chemical signatures for dissolution or adsorption. Right now, that is impossible, so we pull together complimentary methods to try to paint the whole picture – such as electron microscopy, dark field scattering, hyperspectral imaging and EDF. Unfortunately, most of those methods can’t be employed in situ, in real time. One of the really complicated aspects of this work is identifying thoughtful and appropriate controls, so that you don’t misinterpret data.

In a more general sense, when you’re straddling fields, it’s sometimes hard to reach the audience that needs to hear about our results. With nanoparticle sustainability, for example, we would like to be able to talk directly to policymakers, to help create smart regulations.

You’re a champion for diversity in science – why tackle these thorny issues?

It’s still important to acknowledge the challenges that women – and other minorities – face in science. However, I must admit that it’s sometimes hard always being the person who points out gender discrepancies or discrimination. In a way, I’d just like to get my science done without bothering about social politics – but I recognize that comes from my own privilege. Plus, I believe that if you have any sort of power, influence or visibility you have a responsibility to talk about these issues. Being an underrepresented person has definitely influenced me – and it has driven me to try to improve things for those who follow. It’s also important to remember that diversity contributes to the vibrancy of any field.

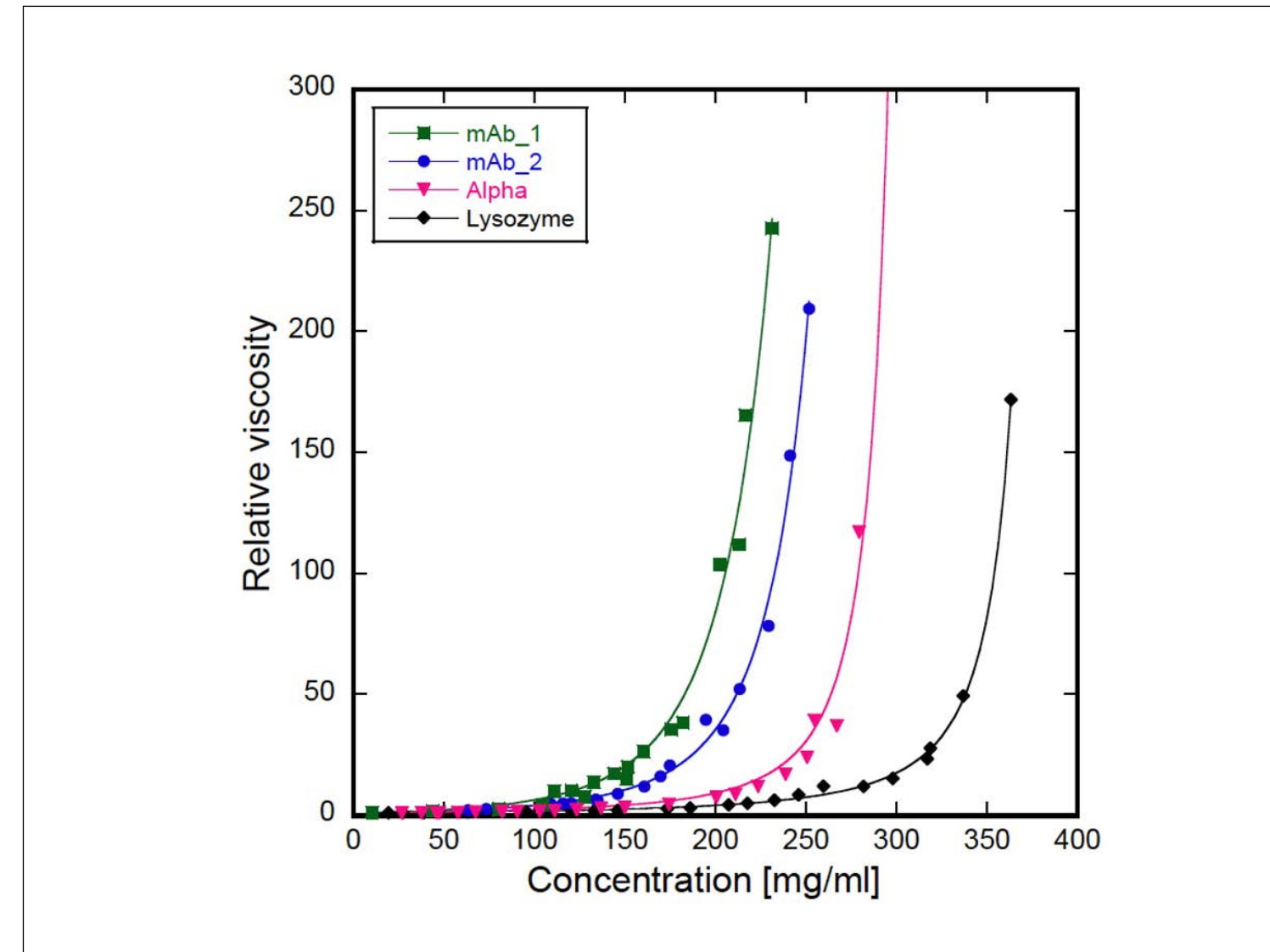
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By Danila Gaudino and Ricardo Armenta

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SPOTLIGHT ON...

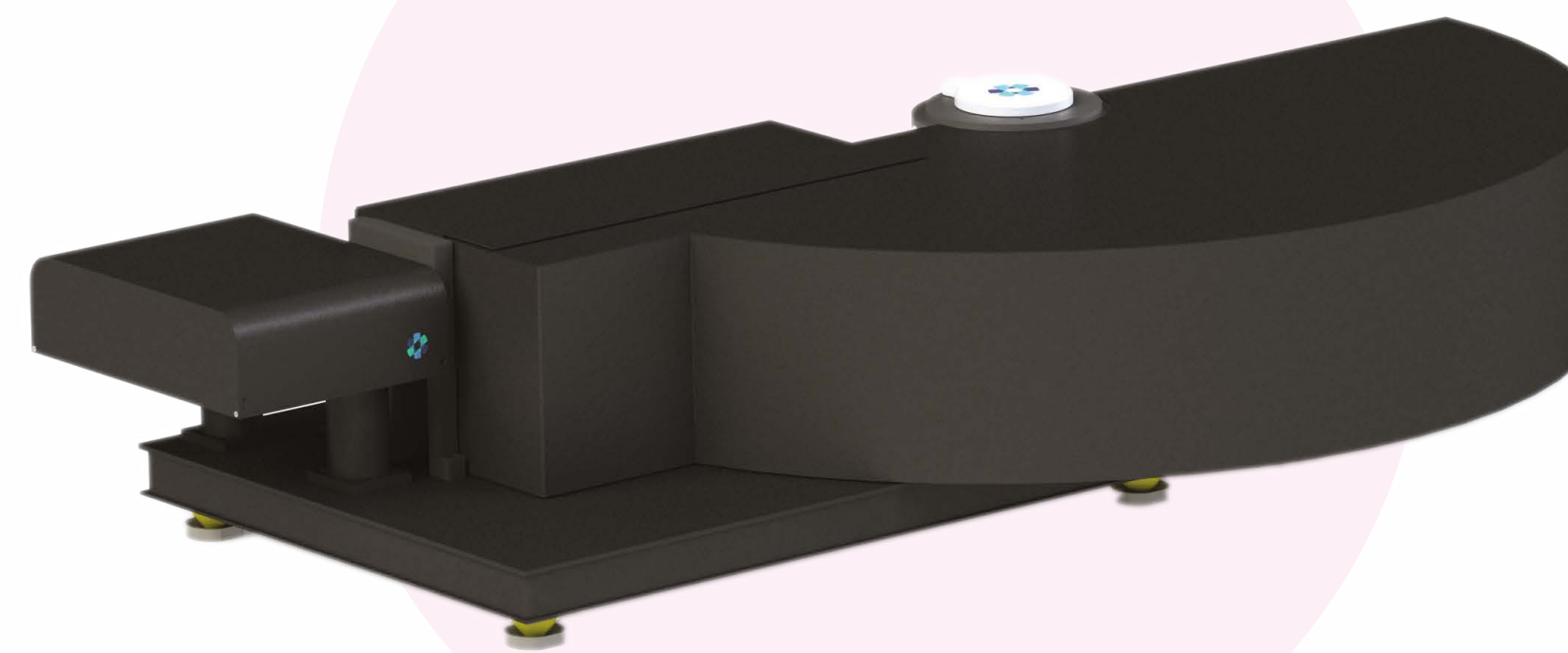
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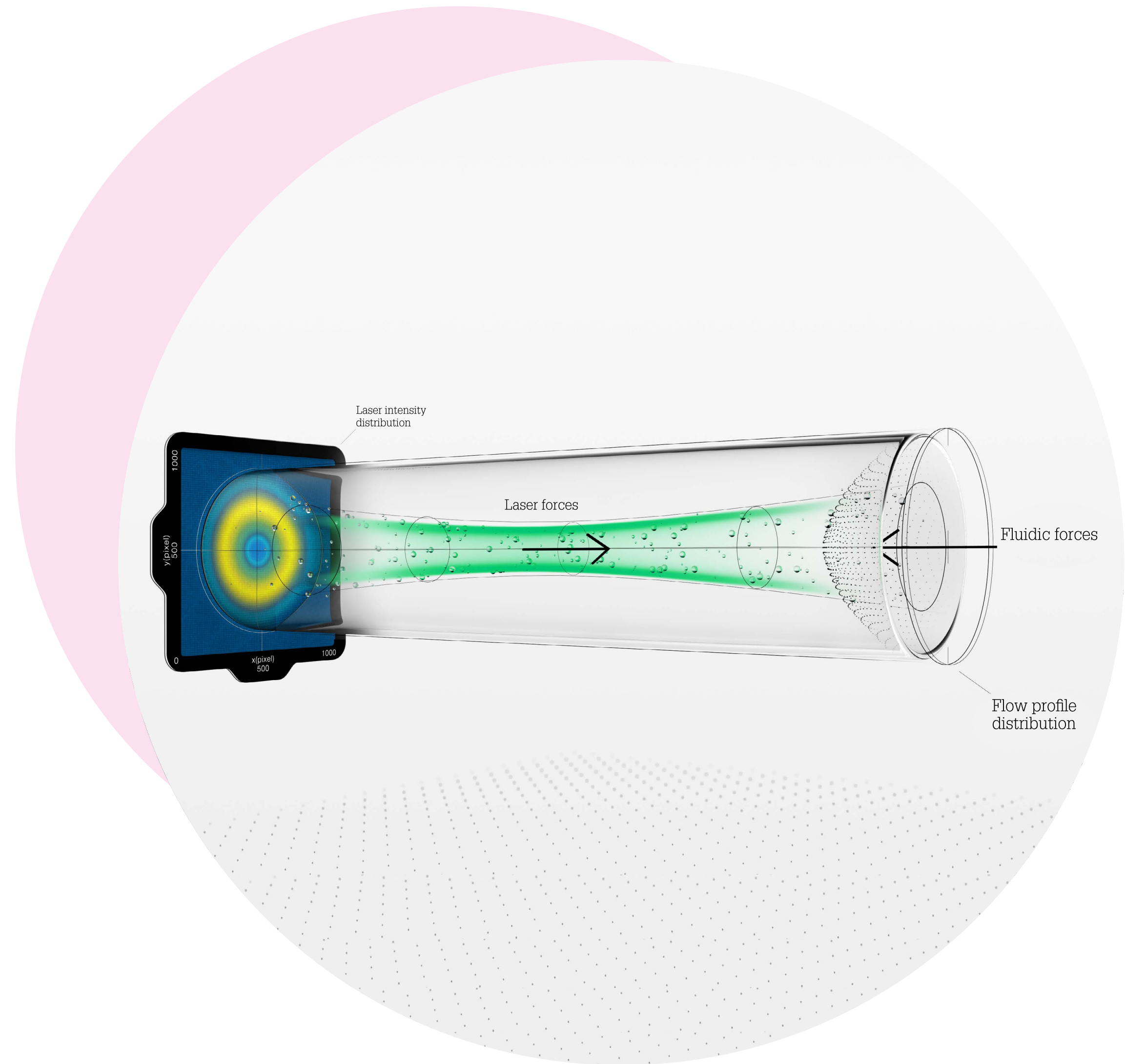
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