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June 2023 | issue 1

PREMIER LEARNING FOR ANALYTICAL SCIENTISTS

ASMS 2023

Annual Conference: Find out what to expect

BREAKING BOUNDARIES

The Orbitrap Story

Uncover the past, present, and future

THOUGHT LEADERSHIP

Advances in Single Cell Proteomics

The Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer provides a major boost

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Leading Lights in Mass Spectrometry

Discover the technologies shaping this field

THE FUTURE

of Mass Spectrometry and Allied Technologies


thermoscientific
Orbitrap Astral

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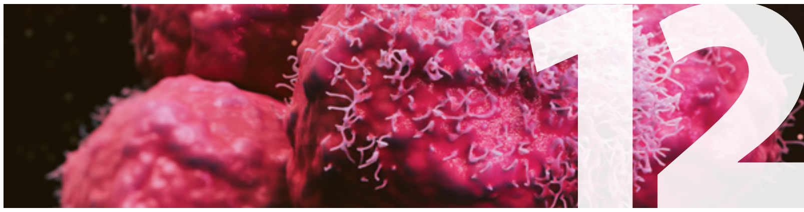
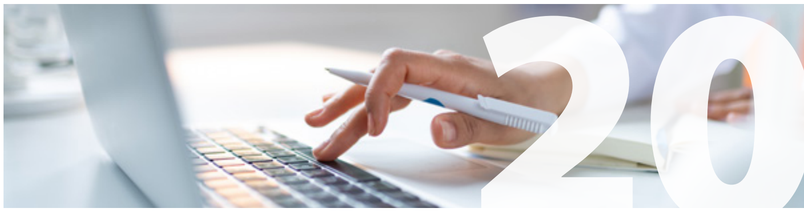




EDITOR'S NOTE

Welcome to Separation Science's special issue, "The Future of Mass Spectrometry and Allied Technologies," released to coincide with the 2023 ASMS Annual Conference. While mass spectrometry has a rich history in the analytical world, we continue to see innovations and breakthroughs within this powerful technique and its allied technologies. This issue delves into the most exciting developments, trends, and stories in the ever-evolving mass spectrometry landscape.

We begin with a sneak peek into the ASMS 2023 conference. Joseph Loo, Vice President of the American Society for Mass Spectrometry (ASMS), reveals the organization's history and explores what we can expect from some of the latest advancements in mass spectrometry. We then embark on a captivating journey as we hear Alexander Makarov discuss the past, present, and future of the groundbreaking Orbitrap™ analyzer. "Breaking Boundaries: The Orbitrap Story" highlights the history, development, and impact of this impressive technology, which has become a mainstay in modern analytical laboratories. Our theme of mapping history to the future continues as we trace the roots and milestones of mass spectrometry in our timeline infographic. Discover the breakthroughs and key discoveries that have shaped mass spectrometry into the indispensable tool it is today.

A key theme of modern mass spectrometry and coupled techniques is the vast amount of data produced. Processing and deciphering this data is a common challenge that is only set to expand as technologies advance. This theme is highlighted on page 14—we talked to Erin Baker about navigating the complexities of IMS-MS data processing. It's also a notable theme in our "Leading Lights of Mass Spectrometry" article, where we hear from our experts as they weigh in on which technologies will be pivotal in the future of the field.

Of course, there would be no advancements if we didn't foster knowledge-sharing and collaboration. On page 16, Tom Jupille of Chromatography Forum explains how this vibrant hub has evolved over the years and continues to attract both novice and experienced members of the analytical chemistry community. Finally, we recognize the importance of continued learning and professional development by highlighting online training solutions that ensure analytical scientists excel in this field.

The Separation Science team is dedicated to delivering premier learning for analytical scientists. We welcome your questions and feedback about our editorial coverage—please reach out to me at acichocki@sepscience.com.

***Aimee Cichocki**, Senior Editor*

Aimee Cichocki

business operations leader
Dean Graimes
dean.graimes@sepscience.com

scientific director
David Hills
david.hills@sepscience.com

senior editor
Aimee Cichocki
acichocki@sepscience.com

editor
Janet Kelsey
janet.kelsey@sepscience.com

science writer
Adam Dickie
adickie@sepscience.com

sales manager
Melanie Dunlop
melanied@sepscience.com
1.888.781.0328 x231

commercial director
Jeroen Reiniers
jeroen.reiniers@sepscience.com
31.20.893.2850

group art director
Danielle Gibbons
danielleg@labx.com

senior graphic designer
Janette Lee-Latour
jlatour@labx.com

graphic designer
Julie Davie
jdavie@labx.com

Published by
LabX Media Group

president
Bob Kafato
bobk@labx.com

managing partner
Mario Di Ubaldi
mariod@labx.com

executive vice president
Ken Piech
kenp@labx.com

creative services director
Trevor Henderson
thenderson@labx.com

production manager
Greg Brewer
gregb@labx.com

Separation Science
PREMIER LEARNING FOR ANALYTICAL SCIENTISTS

1000 N West Street, Suite 1200
Wilmington, Delaware, 19801
888.781.0328

ASMS 2023: A Preview of the Latest Trends in Mass Spectrometry

Find out what to expect from the world's largest mass spectrometry conference

by Adam Dickie

With the ASMS 2023 annual conference just around the corner, we spoke with Vice President of Programs, Joseph Loo, to find out more about the valuable work of the organization and what to expect from this year's event.

"The American Society for Mass Spectrometry (ASMS), formed in 1969, promotes and disseminates knowledge of mass spectrometry science and related topics," explains Loo. "The society has a membership of over 7,000 people who hail from academic, industrial, and governmental institutions. ASMS is a non-profit corporation governed by an elected Board of Directors. The Board works in close partnership with Jennifer Watson, ASMS Executive Director."

ASMS' annual conference is the world's largest conference focused on mass spectrometry, with an average of over 6,500 attendees. "The annual conference is a smorgasbord of activities for the mass spectrometry enthusiast," says Loo. He explains that newer members can learn the fundamentals of an extensive range of topics by enrolling in short courses. Meanwhile, undergraduate students attending the conference are encouraged to participate in a special poster session and competition. There are also tutorial lectures, morning and afternoon oral sessions, evening workshops, and more.

"Our corporate sponsors hold breakfast seminars and evening hospitality suites," adds Loo, "while vendors host exhibit booths in the convention center where information on their newest products is displayed." And the annual conference is not just for learning—it has traditionally been a helpful venue for those seeking employment in the mass spectrometry area.

So what are some of the key themes we can look out for at the 2023 conference? "We're all looking forward to seeing what's the latest and greatest in all areas of mass spectrometry," acknowledges Loo. "Increasing analysis throughput and sample capacity has always been emphasized at the conference, and by all indications, this will continue at this year's event. Central to this theme is on-line separation strategies, both solution phase, for example, chromatographic and electrophoretic, and gas-phase, for example, ion mobility, coupled to mass spectrometry measurements."

ASMS Annual Conference:

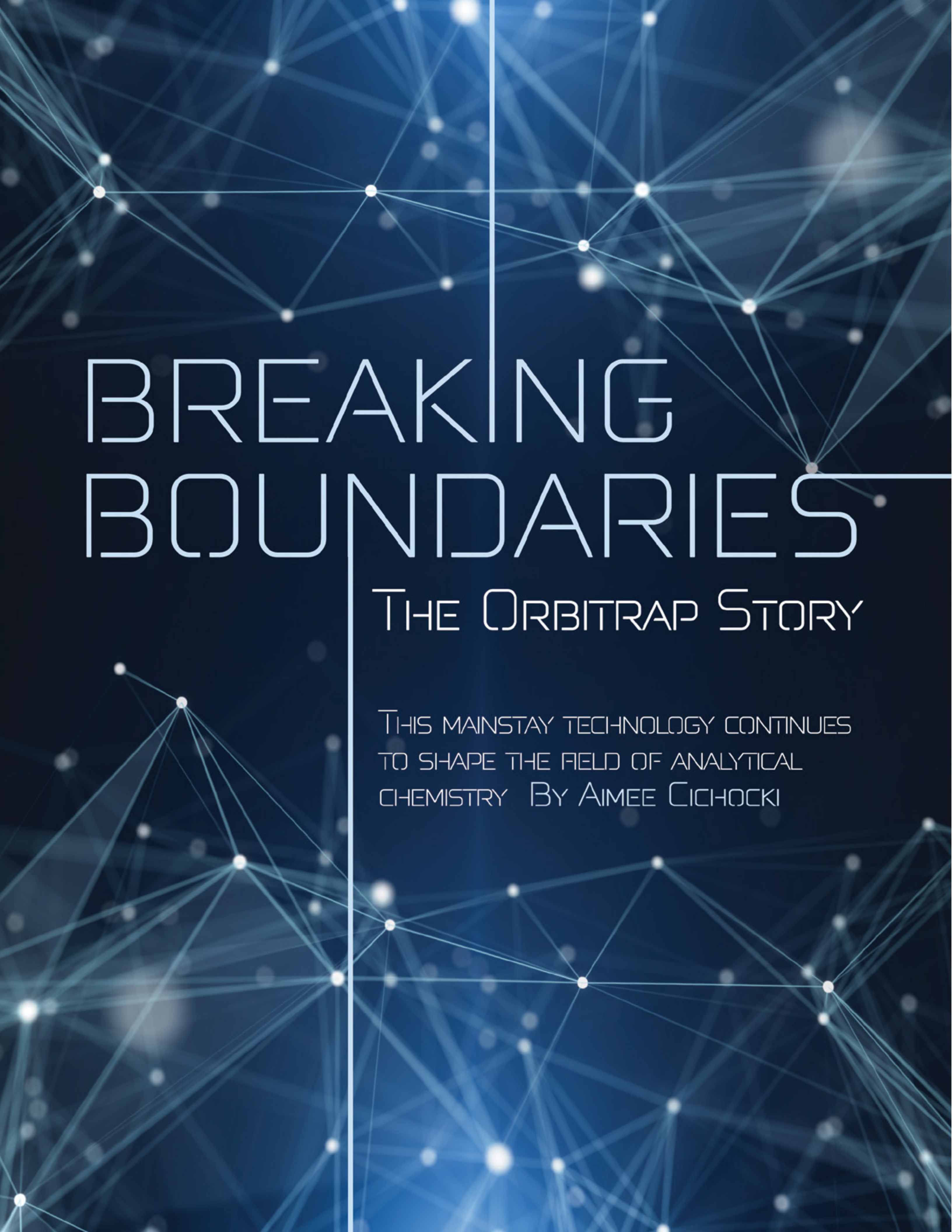
6,500 attendees
3,500 presentations
64 oral presentations
50 evening workshops
16 hours of poster viewing

Loo expects attendees will learn about new mass spectrometers with increased resolution, dynamic range, and sensitivity. He notes that single ion charge detection is a relatively new technology that offers special advantages for large molecule measurements. "And single cell mass spectrometry (metabolomics, lipidomics, proteomics, and imaging) appears to be growing exponentially. The recent ASMS Asilomar Conference that featured this topic was very popular, and I expect this momentum to continue at the 2023 annual conference in Houston." Loo adds that coupled with the improvements in technologies, there will be new applications covering the vast spaces in the biomedical, pharmaceutical, environmental, and clinical arenas.

Looking beyond the conference, what does the future of mass spectrometry hold? "The area that immediately comes to mind is the intersection of computational sciences and mass spectrometry, and all of analytical chemistry for that matter," says Loo. He explains that mass spectrometry can generate massive amounts of data, and computational tools are needed to process this data and convert it to knowledge. "Relatedly, artificial intelligence/machine learning (AI/ML) is increasingly found in aspects related to mass spectrometry data, similar to today's headlines about AI's impact in society."

Find out more about these and an array of other topics at the 71st ASMS Conference in Houston, Texas, June 2–4.

Adam Dickie *is a science writer for Separation Science. He can be reached at adickie@sepscience.com.*



BREAKING BOUNDARIES

The Orbitrap Story

This mainstay technology continues to shape the field of analytical chemistry

By Aimee Cichocki

Mass spectrometry has played a pivotal role in the evolution of analytical chemistry, enabling researchers to identify and quantify molecules with extreme precision and sensitivity. Among the various types of mass spectrometers, Orbitrap^{TM} technology has emerged as one of the most powerful and versatile platforms for chemical analysis.

The basic principle of the Orbitrap analyzer is to trap ions in a hyper-conical electrode assembly and measure their mass-to-charge ratio by detecting their oscillation frequency in an electrostatic field. The high mass resolution and accuracy of Orbitrap technology have revolutionized analysis in various areas, including proteomics, clinical research, forensic technology, and environmental analysis.

THE ORIGIN AND EVOLUTION OF ORBITRAP TECHNOLOGY

The development of the first iteration of the Orbitrap mass analyzer began in 1996, driven by a team inspired and led by Alexander Makarov, who is now Director of Global Research LSMS at Thermo Fisher Scientific. Makarov had just left his postdoc position at Warwick University. “I started my first and only job in a small startup, HD Technologies,” he recounts. “I moved with my family to Manchester, and we started our journey.”

Makarov was joining a small group of scientists who had accepted voluntary redundancy from the company that is now Shimadzu. The promise of their technology was evident from the beginning, with the team beating more than 180 competitors to secure an initial funding grant of £50,000 from the Department of Trade and Industry. “We won because, essentially, I was able to prepare the very first picture and the very first promise of what was later called Orbitrap based on my previous post-graduate work,” explains Makarov. “Of course, it was a completely different design than what eventually reached the market.”

Makarov admits that when starting out, he barely knew the difference between peptides and proteins. “But we desperately wanted to do something great—in a small company, you either run at maximum speed just to keep afloat, or you do something unusual.” Ultimately, it was the company’s time of flight (TOF) technology that led to its acquisition by Thermo Electron (now Thermo Fisher Scientific) in 2000, but the Orbitrap project sweetened the deal. “Our company was mainly doing time of flight for different sources—electron input source, electrospray source, MALDI, and so on—but the Orbitrap analyzer was the icing on the cake.” While nobody really knew if the technology would take off, it was garnering a lot of interest.

The period between project inception and market launch was not all smooth-sailing. Although exciting, this was also a worrying time for the development team as there were questions around scalability. “If we had too many orders of LTQ Orbitrap instruments, there were concerns we couldn’t ship LTQ FT anymore. But how do you ship so many instruments if you’ve never produced them before?” There were also challenges with the various unprecedented aspects of the Orbitrap analyzer. “For example, we had an unusual combination of voltages, and everything was compressed over a length of eight centimeters. A pressure drop of eight orders of magnitude over eight centimeters was something that was never realized before. And then there were technologies for thermal stabilization which were never tried.”

Some of these components only started working months before the launch. Makarov remarks that the team got lucky but admits there was a lot of work behind the scenes to ensure the instruments were shipped and that customers were happy with the results. “This combination of high resolution and mass accuracy with very high sensitivity was essentially unparalleled,” reveals Makarov. “And it was orders of magnitude more sensitive for this type of resolution than any previous instrument. This drove Orbitrap applications, and even now, this high transmission from the front-end optics to the C-trap, then from the C-trap into the Orbitrap analyzer to the detected signal is really what determines the acceptance of Orbitrap mass spectrometry in so many different fields.”

One aspect of the development process that helped overcome challenges was Makarov’s special effort to over-engineer the initial Orbitrap backend of the LTQ Orbitrap instrument. “Whenever I had doubts, for example, whether or not the wall was too thin, I would rather go for more,” he explains. “If I didn’t know what amplitude of voltage I needed, I went for reserves just in case.” He adds that from there, the instruments were optimized, with feedback from the field providing valuable guidance.

Makarov notes that another key driver of success was the team itself. “The team was very small, with a core of five people,” says Makarov. “Most of us were completely new to the company, so we had to prove ourselves. It wasn’t just a job for money—it was a matter of our reputation and our entire standing future in the company—so everybody was highly engaged.” He notes that although they didn’t have time to talk much, discussions during the odd team lunch often revealed something that needed to be urgently addressed. “Eduard (Denisov) would mention that something wasn’t quite working properly. What seemed like a small problem on the surface would often turn out to be something completely unexpected. And then we had to change course and repair things on the fly."

Eventually, by 2005, the instrument was brought to market. Makarov describes the classic imposter syndrome he felt during the 2005 ASMS conference in San Antonio, the setting in which the concept of the new technology was introduced. "We had just one poster, which was repurposed because when we submitted it in January, we didn't know whether we would be able to launch or not," says Makarov. "So it was a poster on LTQ FT, but I strategically titled it something like "FTMS" without speaking about LTQ FT specifically." He adds that the poster viewing became quite an event. "The crowd was 15 people deep in every direction, and then we had another huge crowd in the hospitality suite."

Many questions were being asked about this new and exciting technology, including what the instrument itself looked like and what it was capable of. Although Makarov admits the launch may have been a little premature, the team was in a hurry to show its innovations to the world. "Since then, a five-digit number of Orbitrap instruments have been produced and distributed to laboratories across the globe," he adds.

KEY APPLICATIONS FOR ORBITRAP TECHNOLOGY

While Orbitrap mass spectrometry is now considered a mature technology, new iterations continue to be developed. Makarov describes how this development takes two major directions. "On one side is commoditization—we try to make it as boring and primitive as possible. You will see this in instruments such as Exploris MX, which just has an MS detector with no fragmentation, used for quality control in biopharma," he explains. "But on the other side, we try to raise performance and push it in all directions. You will see this on Orbitrap Ascend or Q Exactive UHMR MS, where we try to expand the mass range, range of fragmentation methods, new detection methods like direct mass technology, and other interesting advancements."

Makarov notes that while Orbitrap MS is known as a workhorse in proteomics, it can cover a vast range of applications from structural biology to pesticide analysis. Indeed, Orbitrap technology remains at the center of many mainstay and pioneering applications. One example is CosmOrbitrap, an exciting technology touted to assist in exploratory planetary missions—it could even be instrumental in answering questions about the origin of life.

Although the development of CosmOrbitrap technology has transpired more slowly than Makarov anticipated, progress is being made. A key player here is the CosmOrbitrap consortium, a group of laboratories combining efforts to propel advancements. "As a commercial company, we have to concentrate on our core markets," explains Makarov. "I would love to work more on CosmOrbitrap, but we've never had sufficient bandwidth, so the CosmOrbitrap consortium continues the work." And while these efforts have thus far progressed at what Makarov describes as glacial speed, that is set to change. "Now there are two projects in NASA that fund these developments, so things are moving much faster."

Makarov also describes some other exciting uses for Orbitrap technology. "One interesting use involves performing cancer diagnostic tests during surgery, as developed by Professor Livia Eberlin and her team." The surgeon can open the tissue, immediately detect healthy versus cancerous tissue, and avoid unnecessarily cutting away healthy tissue. And there are other pioneering uses in the medical field. "I like the newest applications where Orbitrap MS is used for direct analysis of intact viruses, mRNA vaccines, or very advanced protein therapeutics," shares Makarov.

He also sees great promise in leveraging the technology for screening applications in the food and pharmaceutical industries. And he acknowledges its impact as a leading technique in anti-doping. "This technology is behind many of the scandals of the last decade," he remarks. "Suddenly, you're able to see not just what you're looking for but also what you didn't want to look for. And while this results in some tricky situations, we need to utilize this technique to make sports clean and fair."

"Ultimately, it opens quite a lot of different opportunities and questions," says Makarov. "This is all very challenging but, at the same time, seems to be within reach for mass spectrometry."

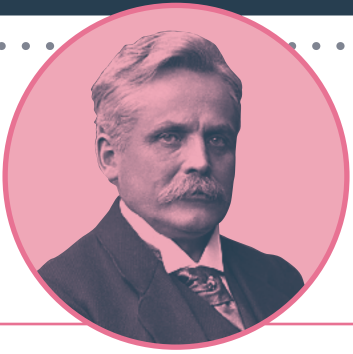
Orbitrap mass spectrometry has established itself as a versatile and powerful platform for chemical analysis, revolutionizing a wide variety of fields. The technology's high mass resolution, accuracy, and versatility in identifying and quantifying molecules have made it unparalleled in the realm of analytical chemistry. Despite Makarov and his team facing challenges during its development, the Orbitrap success story serves as a testament to the impact of innovative thinking and determination.

As the technology further develops and its capabilities expand, Orbitrap mass spectrometry will undoubtedly play an increasingly significant role in advancing scientific understanding and addressing global challenges.

***Aimee Cichocki** is the senior editor for Separation Science. She can be reached at acichocki@sepscience.com.*

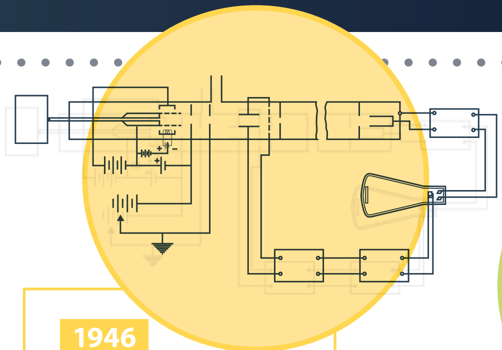
The Evolution of Mass Spectrometry

A journey through time and technology



1898
Wilhelm Wien discovers that beams of charged particles are deflected by a magnetic field.

1919
Francis Aston produces a velocity focusing mass spectrograph.



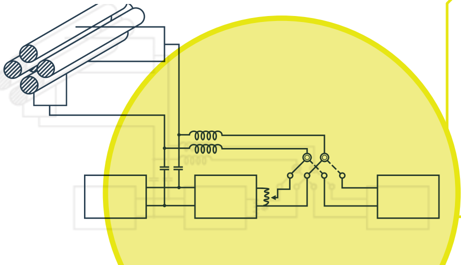
1946
William Stephens reveals the concept of a time-of-flight mass analyzer.

1969
The American Society for Mass Spectrometry (ASMS) is formed.

1898 1910 1919 1943 1946 1953 1969 1974

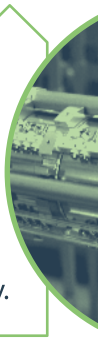
1910
J.J. Thomson separates particles with different mass-to-charge ratios.

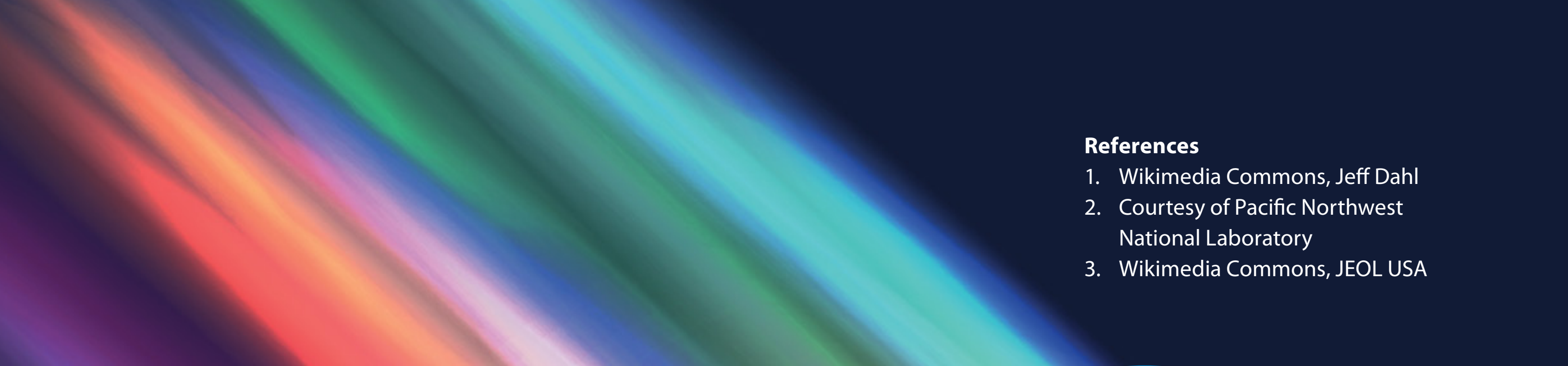
1943
Consolidated Engineering Corporation (CEC) becomes the first organization to successfully market mass spectrometers.



1953
Wolfgang Paul and Helmut Steinwedel present the concept of a quadrupole mass filter.

1974
Melvin Comisarow and Alan Marshall present Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry.





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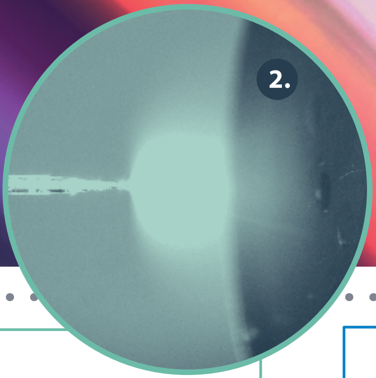
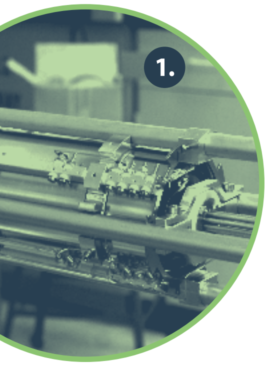
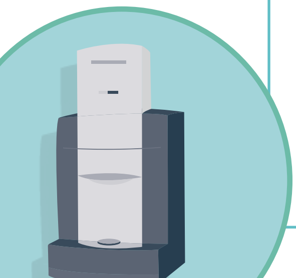
1. Wikimedia Commons, Jeff Dahl
2. Courtesy of Pacific Northwest National Laboratory
3. Wikimedia Commons, JEOL USA

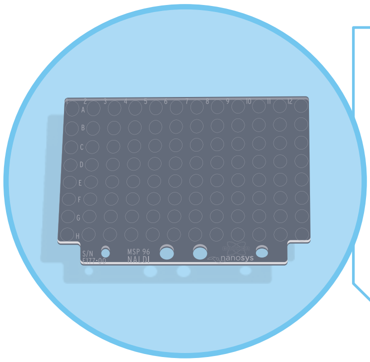


1984
John Bennett Fenn and his team ionize biomolecules using electrospray.

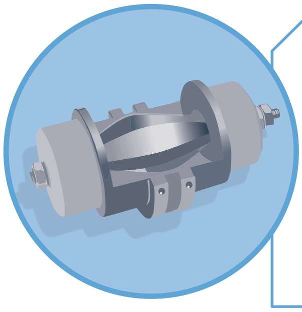




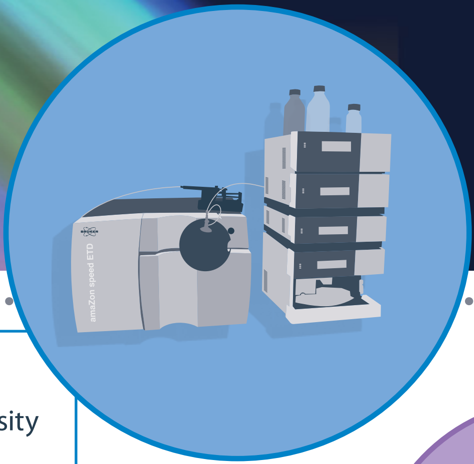
1985
Franz Hillenkamp, Michael Karas, and their colleagues present matrix-assisted laser desorption/ionization (MALDI).



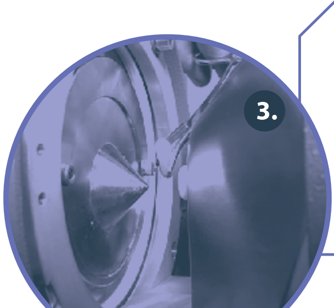
1987
Koichi Tanaka ionizes intact proteins using a method later known as soft laser desorption (SLD).



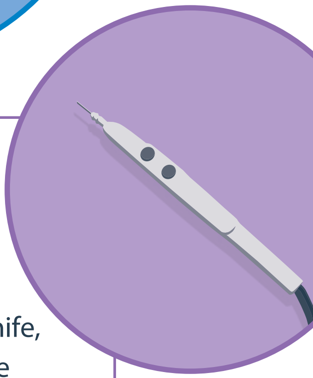
1999
Alexander Makarov presents the concept of the Orbitrap™ mass analyzer at the ASMS annual conference.



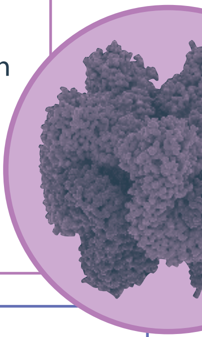
2004
A research group at the University of Virginia, presents electron transfer dissociation (ETD).



2005
Direct Analysis in Real Time (DART) is presented by Robert B. Cody at the January 2005 ASMS Sanibel Conference.



2013
Professor Zoltan Takats and his team present the concept of the iKnife, an intelligent knife that can analyze tissue during surgery.



2014
Two separate research groups publish draft maps of the human proteome based on mass spectrometry.

1984 1985 1987 1999 2004 2005 2013 2014

Advances in Single Cell Proteomics

The Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer provides a major boost to single cell proteomic studies

by Thermo Fisher Scientific

Dr Erwin Schoof is Associate Professor, Department of Biotechnology and Biomedicine, Section for Protein Science and Biotherapeutics, Cell Diversity Lab, at the Technical University of Denmark.

Q: Could you please tell us about yourself?

A: I've spent the last five or six years implementing single cell proteomics workflows. We have this biological need to understand cell heterogeneity and how different cell types interact, in my case, both in the normal blood system and very much so in a cancer setting.

Q: Why do we need to do single cell proteomics? How is it different than general proteomics?

A: For the last 20 years or so, we've been trying to do global proteomics, bulk proteomics from which we get an average of a biological system. We can take a tumor, mash it up, and read out what proteins were expressed, what was upregulated, and what was downregulated. But it lacks this resolution of what's happening at the subcellular level. Which cell populations within a tumor are responding to treatment? Which ones are not? How much tumor cell material is there? Is there immune cell infiltration? We miss out on all these things by taking a bulk proteomics approach. And to this end, single cell proteomics will give us more revolutionary insights.

Q: How does the Thermo Scientific Orbitrap Astral mass spectrometer change the ability to do single cell proteomics?

A: The Orbitrap Astral mass spectrometer presents a new horizon for the field of single cell proteomics, not only because of the high scan speed but also the added sensitivity compared to any other generation mass spectrometer I've worked with before. We are getting deeper proteomes and double the throughput in terms of single cells.

Q: What are the advantages of having both an Orbitrap and Astral mass analyzer?

A: We really like having multiple mass analyzers in our instrument. In the case of the Orbitrap Astral mass spectrometer, we rely on the Orbitrap analyzer for MS1 quantitation, very high resolution, and mass accuracy combined with reading out the MS/MS in the Astral analyzer, which is a super sensitive and very fast analyzer. The combination of the two allows us to achieve the deepest proteome coverage while retaining accurate and precise quantitation in single cells.

Q: What does the singe cell proteomics data look like from the Orbitrap Astral MS?

A: The first time I opened up a raw file from the Orbitrap Astral MS, I couldn't believe my eyes. We collected so many scans, more than 9,000 in just a 12-minute run. I had never seen such high intensities of MS2 scans coming from a single cell combined with the high resolution we got from the Astral analyzer. It was simply astonishing that the spectra looked so much clearer and less noisy.

Q: What single cell proteomics results have you been able to achieve with the Orbitrap Astral MS?

A: Not only were we able to increase the number of cells per day to 80, effectively doubling our throughput, but we also increased the number of proteins per cell from 2,000 to 3,000. So we really got a massive boost on both fronts. The deeper proteome coverage means we start picking up more key cellular decision making proteins like transcription factors, epigenetic regulators, and kinases that we were not measuring very well before. So we get more of the important cellular decision making proteins while also being able to increase our cell throughput. Being able to run 80 cells per day in a label free fashion with data-independent acquisition (DIA), we start to be able to run larger cohorts of cells in a relatively short time.

Q: How does your research combined with the Orbitrap Astral MS change your view of what is possible with single cell proteomics?

A: The research in my lab focuses a lot on making the world a healthier place. I think that the Orbitrap Astral MS will really allow us to study in more detail how protein signaling is causing disease and causing specific treatment responses. For example, in leukemia patients, we very clearly see that some patients respond to treatment while others do not and need to undergo additional therapy. This is where the Orbitrap Astral MS will allow us to measure the protein landscapes that are driving these biological phenotypes with single cell proteomics. The throughput and sensitivity that the Orbitrap Astral MS brings to single cell proteomics means that we can get much closer to the clinic. Single cell proteomics could eventually enter the clinic since we will have the throughput required to be able to make these real-time, real-life decisions, and that is how we're going to have a much more positive impact on human health.

Navigating the Complexities of IMS-MS Data Analysis

Scientists are leveraging a range of tools to tackle escalating IMS-MS data processing challenges.

by Aimee Cichocki

Ion mobility spectrometry–mass spectrometry (IMS-MS) is an increasingly popular tool, particularly in the omics fields such as proteomics and metabolomics. However, the large and complex data sets generated by IMS-MS applications present significant challenges in terms of data processing and analysis.

One scientist who is all too familiar with these challenges is Erin Baker, a bioanalytical chemist and Associate Professor at the University of North Carolina at Chapel Hill. Baker was awarded the 2022 ASMS Biemann Medal, recognized for her work in the application and development of IMS-MS technologies. Key achievements in her impressive career include significant contributions to improving drift tube IMS (DTIMS) platforms and leveraging techniques to enable high-throughput and highly sensitive measurements.

Much of Baker's recent work revolves around the various omics, areas that produce vast amounts of data. This has led to some of her other notable achievements, including creating one of the first collision cross section (CCS) databases for metabolites and xenobiotics and developing a cheminformatic toolbox dubbed SCOPE (short for structural-based connectivity and omic phenotype evaluations). SCOPE enables scientists to assess and visualize lipidomic associations in clinical and environmental studies.

We spoke to Baker to find out about some of the data processing challenges she's facing in her work and how those challenges are being overcome.

Could you provide some background on the types of IMS-MS projects you've been involved with in recent years?

We have become involved in many different omic analyses, from proteomics to lipidomics, and we are really getting into exposomics now. The exposomic studies allow us to investigate the presence of xenobiotic molecules and their transformation products that cause changes in the endogenous molecules in your body. However, performing multi-omic studies becomes highly complex since we must use multiple methods to analyze the different molecular types. Furthermore, we perform multidimensional separations, which means we combine a chromatography step with ion mobility spectrometry and mass spectrometry to obtain information on the polarity, size, and mass of the detected features. Thus, multi-omics and multi-dimensional analyses can be complex to understand, so figuring out how to analyze all the data and fully understand what is going on is a huge component of our research. Currently, we often perform each omic analysis individually, try to find out what was most significant, and then bring the omic studies together. However, that is not the best way to do things, and it would be great to understand how the different molecules are changing up and down in the various studies. Therefore, we are trying to use machine learning techniques to bring the multiple omics together and evaluate the different features at the same time.

> ***"Multi-omics and multi-dimensional analyses can be complex to understand, so figuring out how to analyze all the data and fully understand what is going on is a huge component of our research."***

Are the analytical tools available for you to process all of the data you produce?

There is still a lot of work to be done in this area. I am lucky because many of my students are interested in

Baker is also noted for her role in setting up Females in Mass Spectrometry (FeMS), an initiative that provides a support network for women who work in the field of mass spectrometry. FeMS was started in early 2019 and has already grown to a group of more than 5,000.

coding, so we try to find current open-source tools that meet some of our needs and then modify them. However, if nothing exists, we write the tools ourselves or work with others in the same area. If we create a new tool, we try to release it as quickly as possible on GitHub so that others can use and modify it.

Are there any particular tools shining right now?

We are using Skyline for our targeted data analysis so that we can manually check each identification and possible biomarker. Skyline has been fundamental in our studies as it enables us to validate our identification. Plus, working with the Skyline software team has been amazing, and they have helped us add new analysis capabilities that we could not do on our own. We also employ pmartR from Pacific Northwest National Laboratory (PNNL) and MetaboAnalyst for our statistical analyses. And we are looking into other ways to carry out the non-targeted analyses. Much of that has so far involved more of our open source, homebuilt software where we incorporate information such as ion mobility collision cross sections and masses and mass defects for the precursor and fragment ions for the analyses.

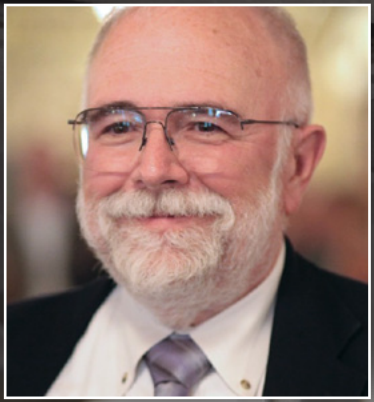
> ***"Many people are pushing our capabilities in data processing, such as those creating large standard libraries, building machine learning tools, and growing molecular networking capabilities."***

Do you see work being done to mitigate potential future challenges in IMS-MS data processing?

There will certainly continue to be challenges in terms of all the data we can create and what we can do with it. Many people are pushing our capabilities in data processing, such as those creating large standard libraries, building machine learning tools, and growing molecular networking capabilities. Social media is also playing a large role in advancing the field as it provides an open forum for researchers to ask questions and experts to weigh in quickly or point people to resources that they might not have known existed. Since information sharing is key for our field to address and conquer the difficult clinical and environmental problems we study, it is exciting to imagine what advances may be made in the next decade.

__Aimee Cichocki__ is the senior editor for Separation Science. She can be reached at acichocki@sepscience.com.

Chromatography Forum: A Hub for the Analytical Chemistry Community



An online forum where analytical scientists find expert answers and connect with like-minded peers.

by David Hills

Chromatography Forum (CF) was established in 1999 (shortly after the launch of Google) by Tom Jupille as part of LC Resources Inc. As Jupille describes, it's a forum where users and experts can come together and communicate. And unlike many early online forums, this one has stood the test of time. Originally launched to gain exposure for LC Resources, the forum now has close to 50,000 members and houses more than 150,000 posts.

CF has played a significant role in the development of the analytical chemistry field since its inception, providing a platform for users to discuss their problems and share knowledge. The forum provides a wealth of information and resources for the analytical chemistry community and is still diligently moderated by Jupille himself. Its growth and continued success was enhanced following its purchase by Separation Science in 2012, a move that opened up significant global user channels.

Jupille reveals that in the forum's early days, there was a real paucity of resources for chromatography. "The St. Louis Chromatography Discussion Group had an online forum, and one of the American Laboratory editors ran a listserv mailing list discussing chromatography issues. Both stopped being maintained within a couple of years, so we were the only site left standing."

The forum's early audience was mainly chromatographers (and some mass spectrometrists) whose professional interest was in separation techniques. However, Jupille notes that over time, that has shifted to today's audience, which has a higher proportion of chromatography and mass spectrometry users, people whose professional interest is in something else (pharmaceutical chemistry, environment, biopharma, or other fields) and for whom separation technique is simply a tool.

He acknowledges that this parallels the evolution of instrumentation. "In the past, a fair amount of expertise was required to use a chromatograph or mass spectrometer." Today, Jupille explains, the instruments are largely appliances. "The user has to follow the "recipe" but doesn't really have to understand how the oven works." Jupille also notes that while early forum content focused almost entirely on liquid chromatography, over the years, gas chromatography and mass spectrometry have become increasingly prevalent.

However, some of the most important aspects have remained the same. "One thing that has not changed is the level of civility in our user base," acknowledges Jupille. "I can count the number of "flame wars" on the fingers of one hand and the number of people I've had to ban on the other."

That said, CF has faced hurdles, in particular, the arms race with spammers. To address this issue, Jupille and his team set up a registration quiz, which has helped. Another challenge has involved resisting the temptation to subdivide the forum too much. "It's a case of balance between having enough subforums to let users find topics of interest while maintaining a critical mass," says Jupille.

So what is it about CF that keeps users subscribing and coming back for more? Jupille shares that several factors contributed to the forum's survival, including his own continued interest in the project, the recruitment of an "advisory board" (a group of Jupille's friends) to post answers to queries, and strategic partnerships with prominent industry figures and organizations. "For users, you can discuss your problems anonymously and get advice from experienced chromatographers," explains Jupille. "For us old-timers, well, many people provided help and expertise to help fix my dumb mistakes when I was starting out. The least I can do is provide the same to the newbies!"

Want to have your questions answered or join the advisory board? Visit www.chromforum.org today.

__David Hills__ is the scientific director for Separation Science. He can be reached at david.hills@sepscience.com.

Future Trends

Leading Lights in Mass Spectrometry

Discover the technologies shaping the field of mass spectrometry.

By Aimee Cichocki

The field of mass spectrometry has undergone a remarkable transformation over the years, with scientists continually leveraging research and technological advancements to push the boundaries of possibility. As we look ahead, it is important to recognize the trailblazing technologies and techniques at the forefront of these advancements.

We asked several mass spectrometry experts to weigh in on the technologies set to have the greatest impact moving forward. They explore some of the leading lights in the field, highlighting not only the ground-breaking work of researchers but also the implications and potential applications of new innovations.

Data Analysis Through Machine Learning

One result of modern advances in mass spectrometry is the creation of vast amounts of data. But unlocking the power of big data requires effective techniques for analysis and interpretation. Erin Baker, a bioanalytical chemist and Associate Professor at the University of North Carolina at Chapel Hill, predicts that machine learning will continue to play an increasingly important role in this area.

“Tasks such as reliably predicting molecules and mechanisms are gradually becoming attainable,” says Baker. “By leveraging machine learning to analyze all the data we have available, we expect to find a wealth of novel insights that were once beyond our reach. That is just one reason why it is so important to deposit data and make it available to the scientific community.”

Baker also provided her insights into some of the researchers performing pioneering work in this field. “Pieter Dorrestein (University of California, San Diego) is using GNPS, a web-based MS environment, to mine data and discover new molecules. I’ve been fortunate enough to work with him on a project where we identified new molecules and confirmed that they were components of real biological systems,” reveals Baker. “In addition, Gary Siuzdak (Scripps Research Institute) is doing incredible work with his METLIN resource—his group is currently working on an ion mobility spectrometry database housing collision cross sections for 27,000 standards, which is extraordinary.”

Fortunately, these exciting advancements are not happening behind closed doors, and part of the magic of the mass spectrometry community is the vast amount of collaboration and data-sharing among novice and experienced users. “Social media has been amazing for helping advance research. For example, using the right hashtags (such as #TeamMassSpec) on Twitter can lead to experts like Peter, Gary, Susan Abbatiello (Waters), Benjamin Neely (National Institute of Standards and Technology), or John Yates (Scripps Research Institute), and many others providing quick responses to questions that might have remained unanswered or taken months to resolve.

Direct Analysis in Real Time (DART)

Open air mass spectrometry is revolutionizing real-time sample analysis, enabling researchers to quickly and efficiently identify substances in various contexts. “Open air mass spectrometry involves introducing and ionizing a sample in the open air,” explains David Sparkman, Adjunct Professor of Chemistry at the University of the Pacific. “And one of the leading technologies within that is direct analysis in real time (DART).” He adds that this technique really shines because it puts the word “quick” in the term “quick and dirty.”

As an example, Sparkman describes an early use of DART to detect melamine in adulterated pet food. “Melamine is a monomer and is used to adulterate certain food because it has a high nitrogen content,” says Sparkman. “It can indicate that you have higher protein content than you actually do.” He explains that extracting the melamine from wet pet food samples typically took several days. “We showed that using DART technology, we were able to stick a melting point capillary into a can of dog food, hold it in the interface of the instrument, and instantly determine whether or not melamine was present.”

“US customs laboratories use DART instruments regularly,” says Sparkman. “These laboratories are charged with determining if certain banned pesticides are present in imported fruits and vegetables. This normally involves an extremely tedious analytical lab testing process, but with DART, you can take a peach out of the crate, hold it in front of the interface, and instantly determine whether or not those pesticides are present.” Sparkman adds that another use for this technique is to quickly analyze seized substances to determine whether or not they contain illegal drugs.

With the possibilities DART technology opens up, Sparkman thought that it would take off quickly—but this wasn’t the case. He suggests the lack of momentum arose from a combination of insufficient promotion and inadequate technology performance due to incompatibilities with certain mass spectrometer models. That said, he is hopeful that more organizations will realize the potential of this technology and we’ll see a renewed interest in the near future.

Ion Spectroscopy

While mass spectrometry instruments have come a long way, Alexander Makarov, Director of Global Research LSMS at Thermo Fisher Scientific, explains that there is still work to be done in certain areas. In particular, he suggests the integration of ion spectroscopy with mass spectrometry is poised to significantly enhance our understanding of molecular structures. “This could just be action spectroscopy or something similar where we could complement mass measurement by structural information,” explains Makarov.

“I also see a lot of interesting potential in a completely tangential direction such as the use of mass spectrometry for soft landing of ions, for example, to prepare a sample for cryo-electron microscopy (cryo-EM),” adds Makarov. “You could take a protein complex and perform quality control in an Orbitrap analyzer to check whether you obtain what you expect or if it’s already fallen apart. Then, if you have what you expect, you could just select this particular protein complex and deposit it softly on the target.” Makarov explains that this way, you know you are transferring intact complexes instead of some dissociated fragments, which can save a significant amount of time.

Makarov also notes that the mass spectrometry landscape lends itself to continued innovation. “There are still a lot of competitors within the industry, each banking on a different technology,” explains Makarov. “This makes it exciting and keeps the field developing. Hopefully, we will see further improvements in terms of sensitivity, resolution, and speed. Even in competition against emerging single molecule techniques in areas such as proteomics, I think high-resolution mass spectrometry shows huge potential.”

***Aimee Cichocki** is the senior editor for Separation Science. She can be reached at acichocki@sepscience.com.*

Mastering Analytical Techniques Through Online Courses

On-demand programs help scientists unlock their potential in mass spectrometry and beyond.

by Janet Kelsey

There's no doubt that mass spectrometry plays an increasingly pivotal role in pharmaceuticals, biotechnology, environmental science, and other key industries. However, it's a complex field, and users of mass spectrometry and allied techniques require specialized training to fully understand and utilize these tools effectively.

Analytical Training Solutions (ATS) is a leading training provider that offers specialized courses in the field of analytical science. This educational platform boasts a wide range of video-based programs designed to equip professionals with the skills and knowledge needed to excel in their roles.

ATS's key courses include *Advanced Method Development Using QbD Principles*, *LC-MS/MS for Chromatographers*, and *GC-MS for Operators & Developers*. These programs are delivered by renowned experts such as John Dolan and Matthew Klee and cover basic techniques as well as some advanced applications. Each course is designed to be engaging and interactive, and all modules include a quiz component to help validate the user learning experience.

While these courses cover the theory behind the methods, an essential feature of all ATS tutorials is a focus on practical applications, including tips and tricks to help master techniques. This ensures that every learner walks away with the confidence to apply these teachings effectively in their everyday work.

Another standout feature of ATS courses is their flexibility. Each program is delivered entirely online, which means that professionals can receive training from anywhere in the world, at a time that is convenient for them. Courses can be purchased individually, although site licenses are available for single or multiple courses, or for the entire series of topics. The latter option is ideal for organizations wishing to provide employees with full access to expert training.

Aside from mass spectrometry, ATS courses cover a range of basic and more advanced chromatography topics, for example, *Introduction to GC*, *HPLC & UHPLC Troubleshooting*, and *Principles of HPLC Validation*. This comprehensive approach means individuals can develop a broad range of skills that are highly valued in their respective industries.

If you'd like to elevate your analytical skills in a specific area or equip your team with access to a suite of world-class training programs, visit www.analytical-training-solutions.com to learn more.

***Janet Kelsey** is the editor for Separation Science. She can be reached at janet.kelsey@sepscience.com.*

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Meet the Experts

John Dolan, a world expert in HPLC and related techniques, has written over 300 troubleshooting articles and more than 100 peer-reviewed technical articles. He has also co-authored three books and taught training classes to more than 10,000 students around the world.

Dr Matthew S. Klee has a PhD in analytical chemistry and is a recognized authority in the area of GC analysis and instrumentation. During 23 years at Agilent, Dr Klee was involved in the invention and commercialization of many of the company's GC and GC-MS products.

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