DEMOCRATIZING MASS SPECTROMETRY

Credit: SCIEX

Mass spectrometry is often seen as a complicated, highly technical technique requiring years of experience to master. Jeffrey Perkel talks to users about the best ways for novices to approach mass spectrometry experiments.

As a postdoctoral researcher at the University of Wisconsin, Madison, Kevin Clark spent a lot of his time synthesizing peptides. Mass spectrometry (MS) was the key to Clark ensuring that those peptides were what he thought they were. “You have to do mass spectrometry to test your work,” he says.

Clark would end up using MS—specifically, matrix-assisted laser desorption ionization (MALDI)-time-of-flight (TOF) MS—for the bulk of 2 decades. Nevertheless, when he took up proteomics research at the University of Georgia in 2010, he felt like an MS newbie all over again.

“My experience was extremely limited to a certain type of mass spectrometry on a certain class of molecules, and that’s it,” he recalls. “The field of mass spectrometry is much, much bigger than that.”

It’s no wonder, then, that many researchers forego learning the technique altogether, choosing instead to simply drop samples off at the local core facility and wait for the results to arrive via e-mail. But is that necessary? Depending on the instrument, data, and project, MS can be “gratifyingly simple,” even for neophytes, according to Allis Chien, Director of the Stanford University Mass Spectrometry core facility.

“[New users] needn’t be intimidated,” Chien says. “But they need to show appropriate respect.”

Core options

Today, Clark works at the Proteomics and Mass Spectrometry Core Facility at the University of Georgia, where his primary

responsibility is helping students in the Department of Food Science and Technology run MS experiments. Located just south of Sanford Stadium in Athens, the 1500-square-foot laboratory houses 6 mass spectrometers, including a Bruker Esquire 3000 Plus ion trap, a “vintage” Hewlett-Packard GC-coupled single-quadrupole, a Bruker Autoflex reflectron MALDI-TOF, a Waters quadrupole-TOF, and 2 Thermo Fisher Scientific Orbitraps. With users coming from such disciplines as biochemistry, pharmacy, entomology, and plant science, these instruments are all in heavy use, says director Dennis Phillips, save one. “We don’t get a lot of low-boiling-point compounds submitted that are amenable to GC-MS.”

Like many facilities these days, the Georgia lab offers both open-access instruments and fee-for-service analysis. Phillips, runs samples and trains users on the “small molecule side,” while his co-director, Chau-Wen Chou, handles proteomics projects. Most of the users they interact with have relatively simple needs, such as monitoring organic syntheses. These experiments are sufficiently simple that Phillips is willing to train researchers if they want to learn to run the instruments themselves. Many, though, opt not to take him up on the offer. “One group discussed it and decided, ‘we don’t want to,’” Phillips recalls. “It’s quicker for them to drop [a sample] off, and we can usually turn it around the same day or next day.”

Proteomics is a different matter entirely; these experiments are sufficiently complex, and the hardware—including high-end Orbitraps—sufficiently expensive, that the

core insists upon doing the work in-house. “The MALDI or [the] Esquire, those were roughly \$250,000 each,” Phillips says. “But the instruments for proteomics can cost \$800,000 or more. If you get someone on the Orbi that does not know what they’re doing, that can cause a real headache.”

While at some level a mass spectrometer is a mass spectrometer, the complexity of their operation can vary widely. When performing MALDI-TOF MS, for instance, pretty much the only variables users need worry about once the instrument is calibrated and tuned are the types of molecules being analyzed and the desired resolution and mass range—for instance, whether to run in linear or “reflectron” mode. An Orbitrap, by contrast, contains up to three different mass analyzers, multiple fragmentation modes, both high- and low-resolution tandem mass capabilities, and more. “There’s a lot more to think about and to plan when you’re using an Orbitrap versus something like a MALDI-TOF,” Chien says.

Chien, who has been running the MS core at Stanford for 16 years, enforces similar rules to the Georgia core in her own lab. Stanford has 15 instruments available, from single-quads

Despite years of mass spectrometry experience, Kevin Clark still felt like a beginner when he switched to proteomics work in 2010. Credit: K. Clark.

to Orbitraps, but only the most basic hardware—3 single-quadrupoles and a MALDI-TOF—are available on an open-access basis. Those interested in using the open-access instruments must undergo training, Chien says, and currently about 250 users have done so.

Basic training takes only about 30 minutes and focuses mainly on sample preparation and basic instrument operation. Improperly filtered samples, for instance, can clog narrow fluidics, while samples that are too concentrated can leave residues inside the instrument that spoil downstream experiments. Samples in the wrong solvents can destroy delicate components. However, the operation itself is reasonably simple: The systems are pre-configured to run a limited set of experiments.

MALDI users require additional training, about 6 hours total over several sessions. “It’s not like you can put your plate in and press ‘Go,’” she says. It takes skill to prepare a good sample, mix it

with matrix, and spot it on the sample plate. And it takes art to learn where the “sweet spots” are, that is, where to focus the laser to achieve optimal sample desorption.

But even this more in-depth training hardly qualifies users as “experts.” “We can train you to run a certain protocol quickly,” Phillips says. “The hard part is knowing how to tweak it,” and what to do when something goes wrong. As Clark puts it, mass spectrometers are like cars. “I can teach you ... how to change the oil and change your tires. But as soon as you run into a problem, you’re lost. There’s so many levels of learning in a process like this.”

Beyond training courses

Recently, Tharan Srikumar, Manager of the Proteomics and Mass Spectrometry Core in Princeton University’s Department of Molecular Biology, began complementing his facility’s fee-for-service structure with open-access instruments. Researchers, he says, have embraced the concept. “It’s been overwhelming.”

Unlike the core labs at Georgia and Stanford, both ion traps and Orbitraps are available for researcher use at the

Mark Cafazzo, SCIE X Director of Academic Business. Credit: Shopper Photography.

Tharan Srikumar is the manager of the Proteomics and Mass Spectrometry Core in Princeton University's Department of Molecular Biology. Credit: Frank Wojciechowski, FWphoto.

Princeton lab, though training is required, Srikumar says. “We train on the ion trap instruments and graduate them up to the more sensitive instruments once we’re confident in their abilities.” The week-long process covers everything from sample preparation, nanoflow column packing, and calibration, to instrument operation and data analysis. The latter steps, Srikumar says, require the most attention, particularly for proteomics experiments.

“Companies have made leaps and bounds in terms of software and making the workflow more straightforward,” he says, but the process is still far from turnkey. Many vendors include default parameters that provide a good place to start, but, “There’s still a lot of thought that has to go into what parameters to use during the data-analysis step.”

Users of the Princeton facility tend to feel relatively comfortable, tackling on their own such tasks as intact mass analysis (which attempts to identify a molecule based only on its mass) and protein binding partner identification in affinity-purification experiments. More complicated studies, though, can easily trip up the uninitiated. Suppose a

researcher had expressed a eukaryotic protein in bacteria, purified it on a gel, and wanted to confirm its identity. Against which protein database should the user compare their results? That’s not immediately obvious, Srikumar says, though for the record, he advises a bacterial database supplemented with records for the eukaryotic protein of interest.

It’s not the instrument operation per se that’s difficult, says James Miller, a Research Chemist with the Proteomics and Mass Spectrometry Core Facility at the Pennsylvania State University; it’s the configuration. “There are software settings for voltages and things, where if you change those settings significantly then you would throw the instrument out of whack,”

he explains. “If somebody didn’t know what they were doing, they could potentially screw things up in a hurry—not damaging the hardware, but one could alter the settings to the point where you cannot get useful data.”

Tina Settineri, Senior Director of Applied Markets, Life Science Mass Spectrometry at Thermo Fisher Scientific, says complexity is greater with high-end mass spectrometers compared to low-end instruments, the better to enable experimental flexibility. Though it is possible to design “fit-for-purpose” instrumentation and software for specific applications—pesticide testing for food, for instance, or illicit drug testing in hospitals—academic life-science researchers typically need to perform many different kinds of analyses, on different samples and compounds, which necessarily requires a more

sophisticated system. Thus, Thermo Fisher’s high-end research mass spectrometers tend to be targeted to “expert to medium-expert” users while its newer mid-range triple-quadrupoles and Orbitrap systems target “medium-expert to non-expert” users.

What matters on high-end instruments is the range of applications users are able to run on them. A simple single-quadrupole delivers only a single nominal mass; thus, it can be used essentially to measure the molecular weight of one or more compounds. If those compounds are easily separated on a column and by mass, it’s a relatively simple matter to set up a simple workflow to test for them. “You pretty much just have to set it to ‘start,’” Settineri says. But to measure and quantify multiple overlapping molecules requires something like a triple-quadrupole, a more complicated instrument that allows researchers to smash ions of interest in a collision cell and measure the resulting fragment ions, identifying molecules by the unique pairing of parent and fragment. Those experiments are likewise more complicated, as users often must pro-

Allis Chien is the Director of the Stanford University Mass Spectrometry Core Facility. Credit: A. Chien.

gram “scheduling” software so it knows when to find molecules of interest, and identify the so-called “transitions” that uniquely identify those molecules in complex mixtures.

With their many bells and whistles, mid-range to higher-end systems, such as quadrupole-time-of-flights or Orbitraps, offer even greater experimental flexibility. As a result, scientists tend to use these systems for their most advanced experiments, which in turn tend to push the hardware to its limits. Where a triple-quad can quantify specific compounds, for instance, an Orbitrap can repeatedly capture and fragment ions to tease apart their structure, sequence large proteins through top-down analysis, and map post-translational modifications—all in addition to identifying thousands of proteins in a cell lysate.

Engineering simplicity

All of this is not to say simplicity cannot be built into the systems. Thermo Fisher’s high-end Orbitrap Fusion platform contains multiple components, each of which must be calibrated. But users need not do that manually, Settineri says; instead, there’s a simple point-and-click function that takes care of that process automatically and flags users when the system is not operating as it should. That, she says, is the “perfect example” of the kind of usability/complexity balance

Tina Settineri is the Senior Director of Applied Markets, Life Science Mass Spectrometry at Thermo Fisher Scientific. Credit: Thermo Fisher Scientific.

Thermo Fisher strives for in its high-end instruments. “We felt it would limit the burden of operation if we couldn’t simplify that,” she says.

Waters Corp. has likewise created a user-friendly self-calibrating and self-diagnosing compact single-quad called the QDa and is incorporating similar functionality in its new VION IMS QTOF, says James Langridge, the company’s Director of Health Sciences. The QDa is small—small enough to fit into Waters’ ACQUITY UPLC stack. That diminutive size may help put new users at ease. When he first started working with mass spec-

trometers, Langridge says, they “literally fill[ed] a room.” That kind of size can be intimidating, he notes. But, “when you see something that’s the size of a PC, you think, ‘that’s something I can use, it doesn’t look so difficult.’”

Vendors are also working to simplify MS through their acquisition and data analysis software interfaces. SCIEX, for instance, has bundled a quadrupole-time-of-flight mass spectrometer with user-friendly software to create a system intended for routine laboratory use, says Mark Cafazzo, the company’s Director of Academic Business. Launched in November 2015, the X500R QTOF System “is an accurate mass platform positioned for the food, environmental, and forensics [analysis] markets ... that balances the analytical power of a high-end mass spectrometer with the routine use that analysts need.”

Key to the X500R’s simplicity, is its newly designed operating software, SCIEX OS. “It is a complete departure from our traditional MS software control,” Cafazzo says. Among other things, the software includes preset parameters and rules that simplify specific use-cases for non-experts, for instance by preconfiguring chromatographic conditions and search libraries. “You can shield the user from the complexity of the instrument,” not by “dumbing down” the instrument, he emphasizes, but by automating common tasks.

Ultimately, says Phillips, MS is easier than ever, yet it’s far from easy. And that’s especially true for newbies, perhaps because they tend not to use the instruments every day. “If you don’t use it, you lose it,” he asserts.

Still, most agree it’s worth it to try. MS, says Stanford’s Chien, “is useful and informative and fun. But, as with all things, you’re not going to go climb [Yosemite’s] Half Dome if you’ve never climbed before. You have to work up to the complicated stuff.”

Written by Jeffrey Perkel, Ph.D.

BioTechniques 60:164-168 (April 2016)
doi: 10.2144/000114399

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