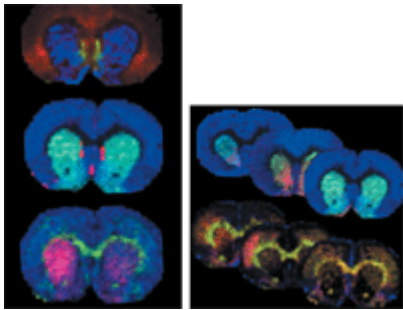


## Mass Spectrometry Targeting New Areas

Developments in any technological field tend to be driven by need, computing power, and often, new discoveries. Mass spectrometry researchers are constantly working to develop higher sensitivity or new applications for their technology.

**By Mark Terry**

Although mass spectrometry can safely be called a mature field, researchers are finding new applications or developing new spins on old techniques. Some of these techniques may find surprising clinical and diagnostic applications. In that respect, mass spec is in its infancy, but its use is growing in three areas: tissue imaging, cancer biomarker detection, and bioterrorism detection.



click the image to enlarge

In the figure on the left, above, one brain section of a rat was analyzed with imaging mass spectrometry. The substantia nigra in the right hemisphere was injected with a neurotoxin to produce Parkinson's disease on the right side of the brain. The protein ions exhibit specific regional distributions or display disease-induced changes in protein levels. Top panel: illustrated in green is a protein with  $m/z = 4801$  (septum); in red  $m/z = 7727$  (cortex); in blue  $m/z = 7416$  (striatum). Middle panel: in green  $m/z = 7383$  (striatum); in red  $m/z = 10659$  (ventricles); in blue  $m/z = 5488$  (gray matter). In the figure on the right, three rat brain sections, about 50  $\mu\text{m}$  apart, were analyzed with imaging mass spectrometry. Several unknown protein ions exhibit specific regional distributions. For example, in the top panel two proteins are mainly localized to the striatum, as illustrated in

Richard Caprioli, PhD, professor of biochemistry and director of the Mass Spectrometry Research Center at Vanderbilt University, Nashville, Tenn., works on tissue imaging using matrix-assisted laser desorption/ionization (MALDI) mass spec. "Basically, the technique is akin to assembling a digital photograph. We create pixels in a piece of tissue much like a digital photograph, but each pixel has 50,000 molecular channels in it, not just three as you would have in a color photograph."

This is achieved by applying a matrix onto the surface of a tissue sample. The matrix absorbs the energy of the laser and causes a micro-explosion at the surface that kicks off molecules. "Because they're charged, you extract them into the mass spectrometer and measure their mass. We use MALDI, with a raster of the laser beam to create an array of the pixels. For each x-y coordinate on a tissue, about 100 laser shots are used to ablate molecules followed by measurement of their mass to charge."

This process is repeated, moving the sample a micron scale, for each point on the tissue that needs to be analyzed. "In this kind of process," Caprioli says, "you can represent the intensity of any one of the many thousands of channels across the whole array of pixels. What you wind up with is a molecularly-specific photograph of that tissue section. And what you can see through this imaging process is the spatial arrangement of molecules in a piece of tissue." Because researchers can pinpoint where in the tissue small molecules, metabolites, and drugs may be active, the technique has applications for drug discovery and the pharmaceutical industry.

Currently, a tumor's edge is determined by histology. Using this imaging technology, a biopsy of histologically normal surrounding tissue can be investigated. "We can see molecular changes that are not obvious to any of the histological techniques. With this kind of technology you pick them up because they have unique signatures, and because you're looking at molecular presentations and an x-y coordinate, you can pick up signatures that extend out into that normal tissue."

green ( $m/z = 7383$ ) and red ( $m/z = 7416$ ); whereas the protein displayed in blue ( $m/z = 5488$ ) is more generally distributed in the gray matter of the cortex, septal nuclei, and striatum. (Source: Richard Caprioli, PhD)

The technique isn't without limitations. The process is slow. Original MALDI instruments operated at 3 Hz, or three laser shots per second, while modern instruments operate at 200 Hz. Sensitivity is also an issue. The amount of abundant signals makes it more difficult to see less abundant signals. "We need better detectors," says Caprioli, "and better algorithms to mine the rich data content in these spectra."

### SIMS

Nicholas Winograd, PhD, Evan Pugh professor of chemistry at Pennsylvania State University, State College, Pa., performs secondary ion mass spectrometry (SIMS). Instead of a focused laser beam, desorption is created using an energetic ion beam. "In the early days, people used projectiles like argon ions, so the bullet had some mass, and you could throw it into material at high energy. The resulting explosion throws off molecules," Winograd says. "The laser MALDI experiment came along later. The advantage of MALDI is that you could desorb much bigger molecules than you could with the ion beam, which tends to break apart big molecules."

SIMS has the advantage of not needing a matrix and is more useful for detecting molecules smaller than a few thousand Daltons. "The lateral resolution is much better than a sub-micrometer. In fact, people have reported lateral resolutions better than a tenth of a micrometer, whereas with the MALDI experiment, typically images are acquired at 20 to 50 micrometer resolution."

Winograd's laboratory uses clusters of atoms as projectiles instead of simple projectiles like argon. "We're using things like metal clusters, such as  $Au_3$ , which has three atoms instead of one. We're concentrating in our lab on using  $C_{60}$ —buckyballs—as a projectile. I think we've finally found a very good use for  $C_{60}$ ."

Like MALDI, the primary practical application for SIMS is drug localization in tissue, possibly as a replacement for autoradiography. Winograd also works on high-throughput screening. "We can look at small peptides on micron-sized polystyrene beads that are used in combinatorial library experiments, and we can array those into very dense arrays and screen tens of thousands of beads by MS in a matter of seconds. So there are some

#### Beating Biodefense into Plowshares

The federal government is providing a great deal of money for biodefense, and some of the technology being developed has potential applications in clinical medicine. In order to push industry to broaden its research into areas valuable to homeland security, the federal government has set up a system called the National Technology Alliance (NTA). "It is government reaching out to industry," says Andrew Feldman, PhD.

Feldman is a researcher at the Johns Hopkins University Applied Physics Laboratory Research & Technology Development Center, which is part of an NTA led by the 3M Corp. called the Chemical, Biological and Radiological Technology Alliance (CBRTA).

This bioterrorism detection research

possibilities in imaging of arrays in high-throughput screening."

Also, because of the high lateral resolution, they have been able to image single cells instead of tissue. "We've developed protocols in our laboratory for rapidly freezing cells and then fracturing them inside our mass spec because that's all done in a fairly high vacuum. Then we've done chemical imaging of the single cell surfaces. We published a paper in the journal *Science* [S.G. Ostrowski *et al.*, vol. 305, pp. 71-73 (2004)] that showed a correlation between the highly curved surfaces of membranes and the phospholipids chemistry that made up the membrane."

#### Cancer biomarker discovery

Sean Downing, PhD, a research fellow at the Dana-Farber Cancer Institute, Boston, is one of several researchers using mass spec to explore blood, where almost any health problem can be detected. However, it is very difficult to perform mass spec on blood. "The problem is the amount of proteins. The different types of proteins in the blood is huge; it boggles the mind. A lot of people believe that blood is really too complex to look at right now," says Downing, whose approach is to fractionate the blood.

He uses an anionic exchange column, basically a pH gradient, to separate the blood into six fractions before performing mass spec. "That still leaves a lot of complexity. People always question



A Pacific Northwest National Laboratory (PNNL) scientist works on the capillary electrophoresis electrospray ionization ion trap mass spectrometer (CE/ESI/MS). This instrument is designed to efficiently separate and detect a range of analytes. (Source: PNNL)

is showing signs of being useful for clinical medicine, Feldman adds. His lab has used its mass spec system to detect malaria. "You can detect a metabolite of malaria with very high sensitivity using direct laser desorption. The test's sensitivity is competitive with what's currently out there, but it has no consumables. "You literally take your blood sample, dilute it in water, let it dry, put it in the mass spec, and you can diagnose malaria."

The metabolite they test for is toxic to the parasite. The parasite developed a mechanism to envelop it in a tiny crystal to make it inert. "In order to detect things, you need to concentrate things into small places so you have enough to see it. But in this case, the parasite does it for you, which is why there's no sample preparation or consumables," Feldman says.

fraction and identify it." Downing admits it's not actually clear if the technology is up to the complexity of blood proteins. "I think the technology is on the cusp, and it's probably due to the complexity of the blood."

John Semmes, PhD, professor of microbiology and molecular cell biology at Eastern Virginia Medical School, Norfolk, Va., also works on blood serum and agrees with Downing on the complexities. "It's a technical nightmare. You have depletion strategies. By that I mean getting rid of the abundant proteins so you can see the less abundant proteins. However, they're complicated multistep processes that are not high throughput. The challenge is to make them high throughput. Some of the ways we do this are proprietary, and some of the ways are to develop antibodies to the highly abundant proteins, the top 10 or top 20, and pull them out."

These depletion techniques still leave a lot of abundant protein. Even if the technique is 99% accurate and effective at removing the abundant proteins present at  $10^{12}$ , the remaining 1% still amounts to  $10^9$  or  $10^{10}$  excess protein, when researchers might be looking for a protein that's only  $10^1$ . "You've only helped yourself through a few orders of magnitude. That's the basic dilemma. These techniques alone aren't going to get us to the depth we need to find biomarkers of disease. So we use depletion techniques in combination with other strategies such as targeting phosphorylated proteins or glycosylated proteins or membrane-secreted proteins to reduce complexity."

Semmes' lab is working on isolating T cells from blood. "In some aspects this is a simple solution to the complexity problem because it's easy to isolate T cells in leukemias. In other aspects it's much harder because any kind of cell amplification process results in loss of the original character of the tumor." Semmes says there are two major technical obstacles. One is speed. "It's still difficult to rapidly look at a lot of proteins and peptides and determine their post-translational modifications." The second is the need for technical improvements to better compare multiple spectra. "We're trying to combine things like profiling with tandem MS so we can look for differences in spectra and go back to the data or the sample again and determine what might be present, or whether to re-interrogate further."

### **Bioterrorism detection**

There are several challenges to detecting bioterror agents. One, the agent is typically unknown. Two, a detector for an unknown bioterror agent needs to be fast. Size is also an issue. A room-sized laboratory isn't practical on a street corner or in a major airport.

Researchers at the Johns Hopkins University Applied Physics Laboratory Research & Technology Development Center developed a MALDI time-of-flight (TOF) mass spectrometry instrument that fits into a suitcase

whether you should remove the more abundant proteins, such as albumin or transferrin. There are pros and cons to that." Removing the high abundant proteins theoretically makes the low abundant proteins more visible. Unfortunately, albumin, for instance, is a carrier protein, so removing it probably removes other proteins along with it.

Downing's work involves looking for biomarkers for prostate cancer detection. The current gold standard, the prostate-specific antigen test, is only about 30% accurate. When coupled with a digital rectal exam, the accuracy rises to 70% to 80%. Downing's work doesn't focus on detection, but prognosis.

Downing runs plasma through an anionic exchange column, splitting it into a stepwise pH gradient. Using a mass spectrometer from Ciphergen Biosystems Inc., Fremont, Calif., the fractions are placed onto chips. "It could be a hydrophobic chip or a metal affinity chip, like proteins that bind to copper, or do the reverse and do a cationic exchange. We're further reducing the complexity of the blood sample. Once we get the chips, we put them in the mass spec, we read the spectrum, we analyze those spectrum between the different groups, then basically there's a peak, a mass-to-charge ratio of something that is different. Then, using traditional biochemical and MS methods, we purify that protein out of the

measuring 17 inches by 10 inches by 7 inches. "We prototyped the system with a first responder in mind, so we also built an automated sample preparation station that allows somebody to literally collect the sample, insert the collection vial into a cartridge that has all the consumables prepackaged in it, and then put that into the sample prep station and out would come a metal ticket with the sample ready for MALDI analysis. We've demonstrated end-to-end in less than 15 minutes," says Andrew Feldman, PhD, principal scientist.



They initially focused on powder samples, although the system can handle liquids and air samples. Their major innovation is called the reflectron. The flight tube is folded in half, so instead of ions being detected at the end after being accelerated, they are folded around and return. "That allowed us to get the effect of a longer flight tube. The other innovation is a focusing mechanism that works across a wide range of masses without having to tune the system to focus a particular mass."

A suitcase-sized MALDI-TOF mass spec instrument can be used to detect bioterror agents. (Source: Andrew Feldman, PhD)

One of the cons of the system is slightly less mass accuracy and resolution. "You do get the advantage of a portable broad-band detection system. I think for a first response kind of application this should be just one of several tools you bring with you which will hopefully add to the confidence of your assessment. Some people want a definitive answer with whatever tool they have at their disposal. I just don't think that's realistic at this point."

David Wunschell, PhD, with the Pacific Northwest National Laboratory (PNNL), Richland, Wash., is working with TOF mass spec to identify bioterror agents. "The volatile markers are analyzed after two-dimensional gas chromatography and then ionized for mass spec. So there are three dimensions of data—two dimensions of separation and a mass spectra—to sort through to identify the markers."

Karen Wahl, PhD, staff scientist at PNNL, says they are also working on biomarkers for early exposure to biological and chemical agents. The samples, she says, could be blood or odors. "We're working on the techniques right now, developing them ideally for noninvasive types of applications—triage, if you will. We are trying to screen out the people who are already having a response if there was a bioterrorism event or natural outbreak, or the 'worried well' versus the 'actually sick.'"

If these examples prove anything, it's that mass spectrometry is still evolving and growing. "One thing that's occurred that has generated a lot of excitement is that the industry has responded to a different set of demands in mass spectrometry," says Semmes. "Traditionally the folks who did mass spec were interested in a lot of very cool ion gymnastics, how to get the most information out of fairly purified proteins and a lot of topics that didn't deal directly with the clinic or hunting for biomarkers in a massive way. But with folks coming into the field with new demands on MS, the industry has responded by giving us better and newer tools."

*Mark Terry is a freelance writer based in Oxford, Mich.*