By Jeffrey M. Perkel

LAB TOOLS

Mass Spectacle

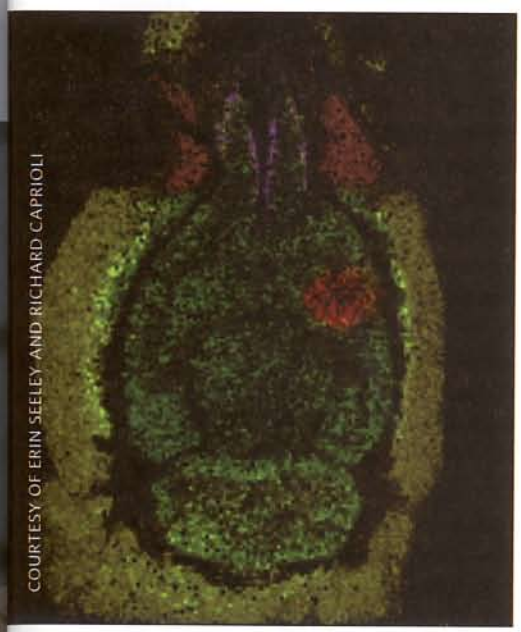
Making the most of mass spectrometry imaging.

Being able to identify a particular chemical or protein in a piece of tissue isn't always enough; sometimes you need to know exactly how it's distributed there. That question can be answered to some extent by path-lab standbys such as immunohistochemistry or immunofluorescence, but those techniques require specific antibodies, and are both biased and low-throughput.

Mass spectrometrists have an alternative. In MS imaging, tissue is mass-analyzed spot by spot in a raster pattern (across and down) to produce a two-dimensional dataset mapping chemical content to individual x,y coordinates ("pixels"). Overlay those data over an image of the tissue, and now you can map compounds to their biological locales.

Richard Caprioli of Vanderbilt University, who pioneered the first biologically relevant form of the technique, MALDI (matrix assisted laser desorption ionization) imaging, compares the process to digital photography. Just as a digital picture contains three channels—red, green, and blue—whose individual intensities define each pixel's color, MS images comprise thousands of channels, each corresponding to a particular spectral peak. "You can take any signal in the [mass] spectrum, from any one of those pixels, and express its relative intensity over all of the pixels in your image, and ... get a molecularly specific image."

Amenable to small metabolites, drug compounds, lipids, and proteins, the technique yields thousands of molecular channels relatively quickly, all without specific antibodies. Yet there's more than one way to scan a tissue, each with its own hardware requirements, molecular specificity, and resolution. *The Scientist* asked five leading MS imagers how they do it. Here's what they said.



COURTESY OF ERIN SEELEY AND RICHARD CAPRIOLI

The axial section of a mouse head showing molecular markers in different colors, including a protein uniquely found in the grey matter (bright green), a molecule uniquely found in growing tumors (red) and one specific for muscle (yellow-green).

PROTEIN CHALLENGE

Researcher: Richard Caprioli, Professor of Biochemistry & Director of the Mass Spectrometry Research Center, Vanderbilt University

Project: Characterizing the molecular microenvironment around tumor boundaries

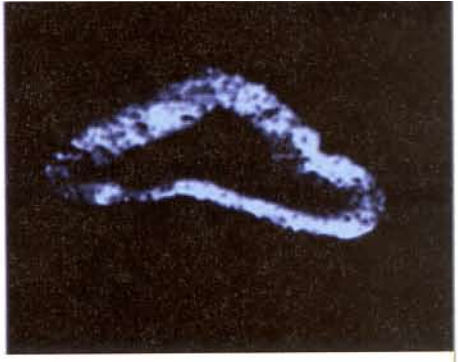
Problem: In a word: proteins. There are several ways to image small molecular weight molecules, but polypeptides are tougher because they're so big.

Solution: For this problem, the straightforward approach, MALDI imaging, works best. Caprioli placed sections of human clear cell renal cell carcinoma on MALDI target plates, applied a crystalline, organic matrix material, and imaged the tissue by rasterizing across the sample with a MALDI laser: zap, read, move the stage, repeat.

MALDI imaging might be the most common MS imaging approach, if only because MALDI time-of-flight mass spectrometers are so prevalent. Also, it's really the only approach capable of mapping proteins, as opposed to small molecules. "The strength of MALDI ... is the fact that the MALDI can do high molecular weights," says Caprioli, who has used the approach to map proteins up to 300 kDa in size.

Using such images of human kidney cancer sections, Caprioli's team was able to identify a group of proteins present only within the tumor histological boundary, and others that seeped beyond it. It was a molecular clue to why these tumors tend to recur so frequently at the site of surgical resection. "The [tumor] microenvironment was not normal," he explains. "It was fertile for cancer invasion."

Cost: Though matrix can be applied manually, Caprioli uses spotting robots for improved consistency. These can cost from five to six figures; one, the LabCyte Portrait, which uses sound waves to eject picoliter-sized droplets onto the tissue, costs "considerably over $100,000," he says. ▶



« Distribution of phosphatidylinositol, (PI 38:4), m/z 885, on the pig adrenal gland section.

BEDSIDE IMAGING

Researcher: R. Graham Cooks, Professor, Department of Chemistry, Purdue University

Project: Developing an MS imaging system for use during surgery

Problem: Mass spectrometers are not typically compatible with bedside applications: they are large and heavy, require lengthy sample preparation, and operate in a vacuum. Cooks wanted a system that was small, fast, and worked at atmospheric pressure, for instance to provide real-time guidance to surgeons regarding tumor boundaries.

Solution: Cooks and his team designed an approach called desorption electrospray ionization (DESI), which is similar to SIMS, but functions under atmospheric pressure. "DESI is a method in which one ionizes using fast-moving charged microdroplets of solvent [such as water or acetonitrile], which impact on a sample and carry away analyte," Cooks explains. Unlike MALDI, no sample preparation is required, making the process compatible with clinical work.

Here's what happens: Imagine a focused beam of charged droplets directed at a tissue slice. As the first droplets hit the sample, they form a thin film on the surface, which extracts both small molecules and proteins from the tissue below. Then, as subsequent droplets impact the surface at this same spot, the extract liquid splashes upwards into the mass analyzer.

"A DESI experiment is actually an extraction experiment," Cooks explains. "These little droplets extract compounds from the surface." In one case, his lab analyzed the lipid content of dog bladder tumors. "We can find the tumor margins using the distribution of the phospholipids," he says.

Cost: Purdue University has licensed DESI to Indianapolis-based Prosolia. The Omni Spray 2D Ion Source Kit costs $65,000.

EASY LIVING TISSUE

Researcher: Akos Vertes, Professor of Chemistry and of Biochemistry & Molecular Biology & Founder and Codirector, W.M. Keck Institute for Proteomics Technology and Applications, George Washington University

Project: Studying the primary and secondary metabolite distribution in a live leaf specimen

Problem: Depositing matrix is tricky, and the matrix itself often obscures the low molecular weight end of the spectra. In addition, MALDI mass spec is done in a vacuum, but vacuums and live specimens don't mix.

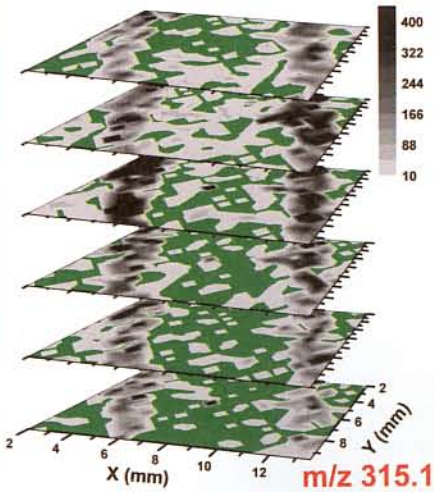
Solution: The matrix's purpose in MALDI mass spectrometry is to absorb laser light and thus vaporize the sample. But it turns out that biological samples can also absorb the energy directly: light of 2.94-μm wavelength excites water's OH bonds.

Vertes' solution combines two techniques. In the first, an atmospheric pressure infrared (APIR) MALDI laser excites the water in the tissue directly, vaporizing the sample in what literally is like a cell-sized nuclear explosion on the tissue surface. Ionized particles thus produced are then drawn into the mass analyzer for analysis. But, not all vaporized particles are charged, Vertes says; most are "neutrals" that are lost to APIR MALDI.

To capture these neutrals, Vertes uses a second method called LAESI (laser ablation electrospray ionization), which engulfs the particles in a plume of charged microdroplets, thereby re-ionizing them. Applied across the entire sample, the combined force of the two approaches, he says, provides greater molecular coverage and robustness.

Unlike most MS imaging processes, Vertes' method also adds depth to his imaging, allowing him to map metabolites in 3D. The technique's resolution—10 μm in diameter and 30 μm deep—also corresponds to biology's natural "voxel," the cell, meaning he can "build images the natural way, the way biology built its structures."

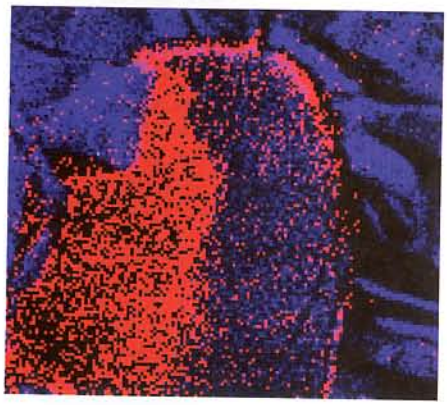
Cost: To build your own APIR MALDI/LAESI system, you'll need a mid-IR laser (available from Opotek of Carlsbad, Calif., for $40,000 to $60,000), an x/y/z translation stage (available from both Thor Labs and Newport, for $10,000 to $15,000), as well as a custom-built LAESI source.



« 3D image reconstruction of the m/z 315 ion from leaf epidermal cells recorded using LAESI.

TOP: COURTESY OF CHUNPING WU, ASTON LABS BOTTOM: COURTESY OF P. NEMES AND A. VERTES.

3D DRILLING



A total ion image of a paramecium cell, with information summed from all of the masses in the spectrum.

Researcher: Nicholas Winograd, Evan Pugh Professor of Chemistry, Penn State University

Project: Spatially mapping the chemical content of cells

Problem: Mapping that content in three dimensions, without significant molecular fragmentation

Solution: To solve his problem Winograd went back to MS imaging's origins. "Mass spec imaging has its roots in a technique called SIMS [secondary ion mass spectrometry], where molecules are desorbed from surfaces by focused energetic ion beams," Winograd says.

Researchers outside of biology have been imaging with SIMS for decades, says Winograd, for instance to understand the chemistry of integrated circuits. The technique has several benefits, including speed (~10,000 spectra per second), subcellular resolution (~100 nm), and lack of matrix. On the other hand, unlike MALDI, SIMS is not a "soft" technique; it can only image small molecules, often shattering then in the process.

Winograd tweaked the method with a new form of SIMS beam made of carbon-60 "buckyballs," which cause less chemical damage than do traditional SIMS beams. Nevertheless, 60 carbons striking the sample surface simultaneously, Winograd says, is akin to "a cluster bomb;" repeated bombardment enables his team to drill down into a sample to map molecules in three dimensions. He calls this process "molecular depth profiling."

Winograd's team has used this approach to study how changes in lipid chemistry relate to the curvature of the membrane in the protozoan *Tetrahymena*. "As the shape changes, the shape of the lipid molecule also changes," he says.

Cost: Imaging SIMS instruments cost "generally on the order of $1 million or more," Winograd says. But in a recent publication (*Anal Chem*, 80:7921-9, 2008) his team described converting a MALDI MS into a C60 imaging SIMS system, a process that is considerably less expensive, he says.

OLD HARDWARE, NEW USES

Researcher: Gary Siuzdak, Professor of Molecular Biology & Senior Director, Center for Mass Spectrometry, The Scripps Research Institute

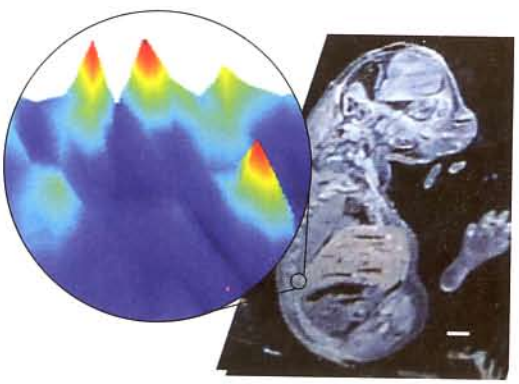
Project: Analyzing endogenous and exogenous metabolite distribution in mammalian tissue samples

Problem: Siuzdak wanted to avoid depositing MALDI matrix: matrix crystal size can lower resolution, and matrix ions obscure the low end of the mass spectra. But he still wanted to use MALDI hardware, one of the most prevalent MS systems in biological labs.

Solution: Siuzdak devised a novel technique called nanostructure initiator mass spectrometry, or NIMS, which relies on a fluorine polymer on a porous silicon surface rather than matrix to induce ionization (*Nat Protocols*, 3:1341-9, 2008). In NIMS, the polymer is deposited into etched holes in a custom-built silicon target plate, and the tissue sample is laid over the top of the polymer, ready for analysis. When this plate is hit with the nitrogen laser found in most MALDI systems, the fluorine polymer beneath the sample heats up and vaporizes, taking the sample with it.

Small molecules including lipids, sugars, and steroids are amenable to the technique, says Siuzdak, though each requires a slightly different fluorine polymer. "It's a one-step process. The beauty of it is, it's much simpler than MALDI because with MALDI, you put the tissue down, then have to deposit matrix onto the surface."

Because the fluorine polymer does not ionize well, it produces little spectral interference. And, because the ionization process is "soft," like MALDI, NIMS produces biological molecules that are ionized largely intact rather than in pieces. On the other hand, the technique's sensitivity for intact proteins is lower than MALDI's.



Nanostructure-Initiator Mass Spectrometry (NIMS) imaging of a mouse embryo section.

Cost: Required materials include silicon wafers ($18-$20 each, producing four chips per wafer), fluorine polymer (custom synthesis from Gelest, about $28/gm), and a custom Teflon cell to etch the silicon wafers. "The initial cost for the setup is probably going to be about $1,500, and then after that it's going to be on the order of $6 per chip," Siuzdak estimates.

TOP: COURTESY OF NICK WINOGRAD BOTTOM: COURTESY OF TRENT NORTHEN