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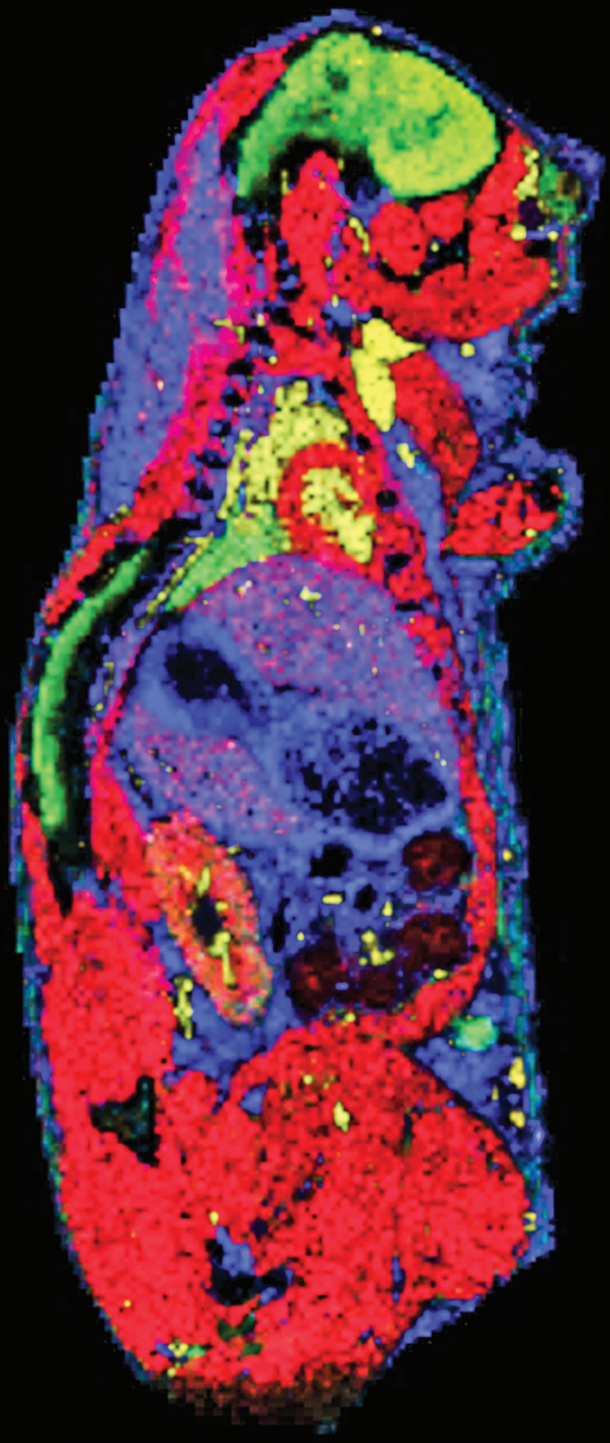

Imaging mass spec

Pharma gets the picture on where drugs go in tissues

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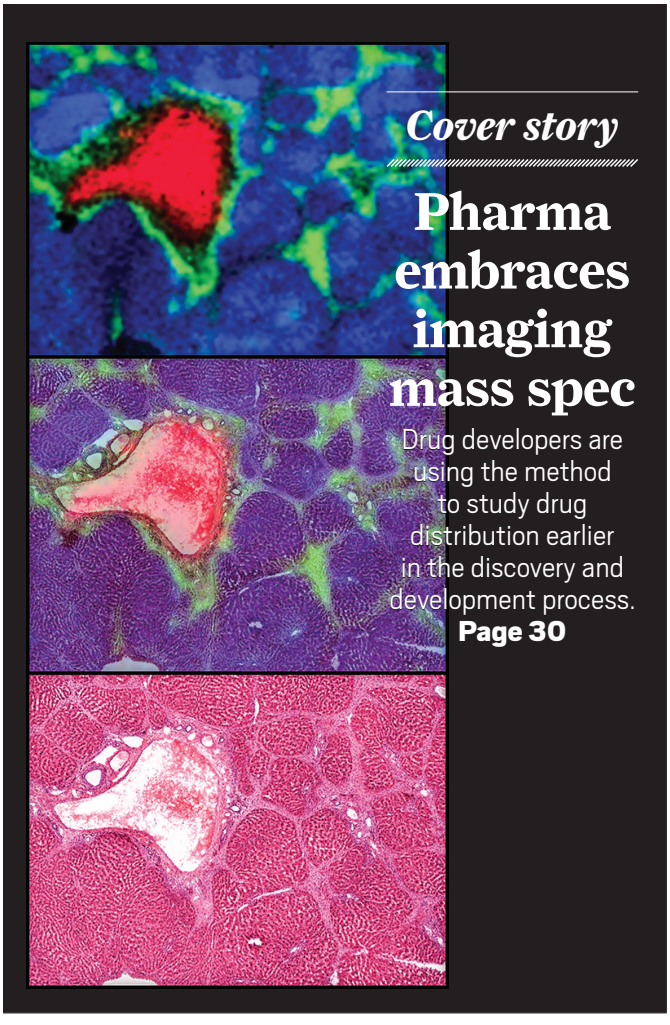

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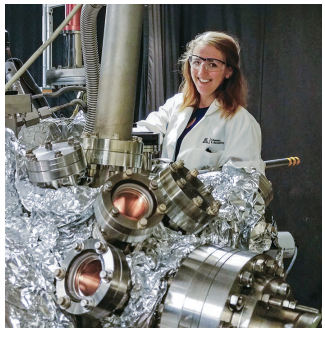

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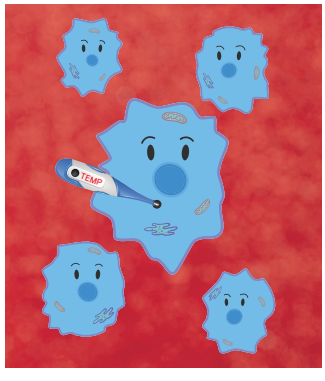

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On the cover
A mass spec image of the distribution of a drug (green) in a mouse. The other colors represent lipids that were used to visualize organs. Micrograph by ImaBiotech

Quote of the week

"Imagine a place with floating displays, information at the tip of a finger, and few barriers to analysis, synthesis, or creativity."

—Michael P. Conroy,
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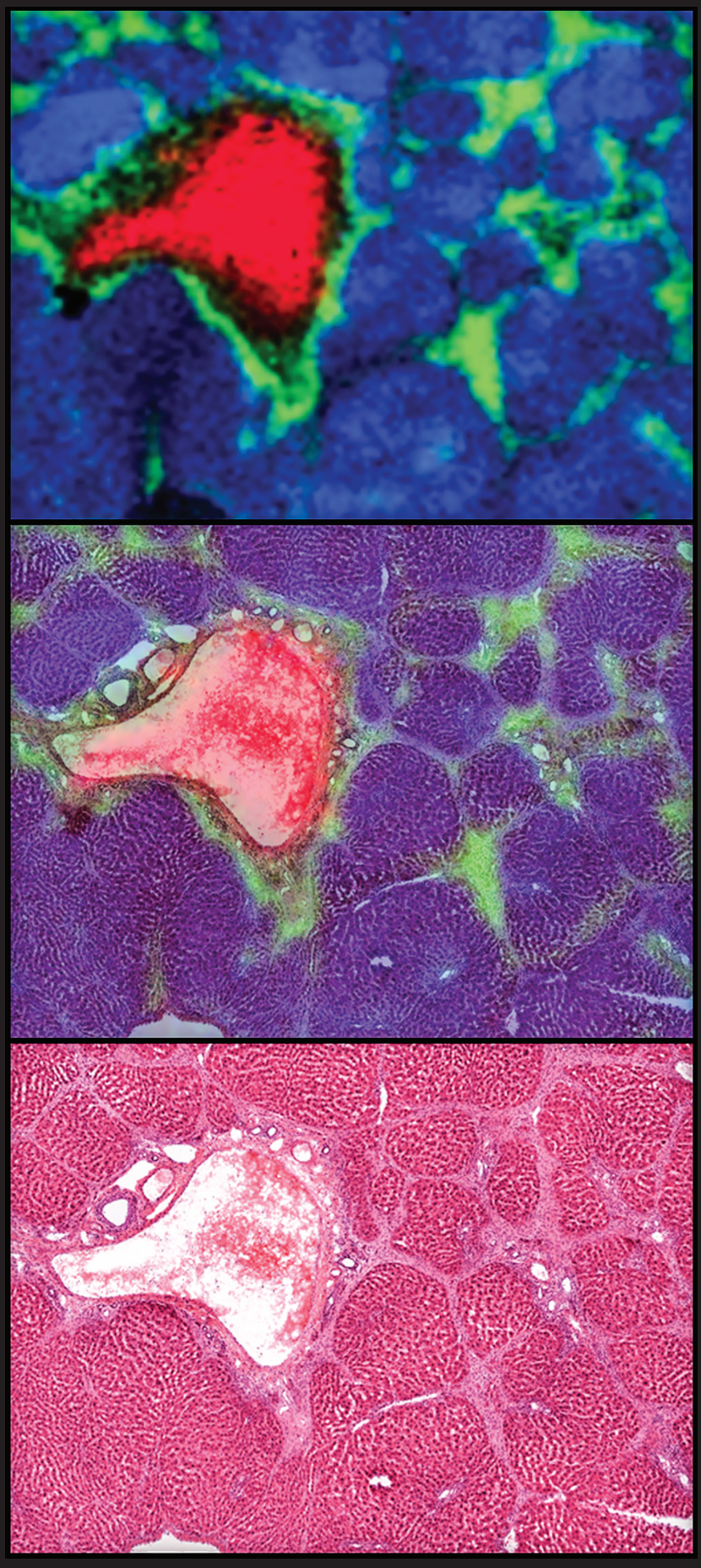


MS imaging helps visualize early-stage fibrosis in a rat liver (top) by showing the location of hepatocytes (blue), fibrotic bands (green), and the portal vein (red). In the center image, the MS data are overlaid on the conventional histology image (bottom).

In brief

Sometimes it's not enough to know how much drug has reached a particular tissue. Sometimes you need to know where in that tissue the drug has gone. To answer that and other questions, pharmaceutical companies are turning to imaging mass spectrometry. It allows pharma scientists to collect information about drug distribution much earlier in the discovery process than previously possible. The resulting pictures are helping drug developers prevent toxicity and off-target effects.

Pharma embraces imaging mass spec

Drug developers are using the method to study drug distribution earlier in the discovery and development process

CELIA HENRY ARNAUD, C&EN WASHINGTON

Genentech was testing a drug candidate targeting lung tissue, and the data didn't add up. Data from cell-based assays suggested that the drug was potent, and analyses of ground-up tissue suggested that plenty of drug was reaching the lung. But the agent wasn't consistently producing the expected effect in animals.

So the project team asked Sheerin K. Shahidi-Latham, head of imaging mass spectrometry at Genentech, for help. Her team used matrix-assisted laser desorption ionization (MALDI) MS to acquire images of the compound's distribution in lung tissue and find out where the drug was going. They took images at different time points with different drug formulations and different routes of administration. They even tried different drugs to make sure the problem wasn't compound-specific.

"We found that the route of administration and the formulation influenced the distribution of the drug in the lung and whether it reached the target tissue," Shahidi-Latham says. When administered through the nose, the drug didn't always reach the target tissue. Inhalation through the mouth turned out to be a better option. "Imaging MS was the key piece of data we could give the team to help get them back on track," she says.

The realization that compound distribution—not just to different organs but also to different tissues within a specific organ—can affect the efficacy and safety of drugs has spurred pharma companies to adopt imaging MS as a regular part of their tool kit. They are using imaging MS to answer questions at points along the development timeline from early-stage drug discovery to preclinical development.

In imaging MS, researchers use a spatially defined ionization method to collect mass spectra from a small region of a sample. That ionization method could be MALDI, with its laser beam, or desorption electrospray ionization, with its solvent stream. After collecting a mass spectrum at one spot, researchers move to another spot and repeat the process. They choose a peak in the resulting spectra that corresponds to their compound of interest, such as a drug or metabolite, and use the MS data to map its distribution across the tissue sample. In this way, researchers build pictures of the spatially resolved distribution of a compound in tissue pixel by pixel. Each data set contains

CREDIT: GLAXOSMITHKLINE

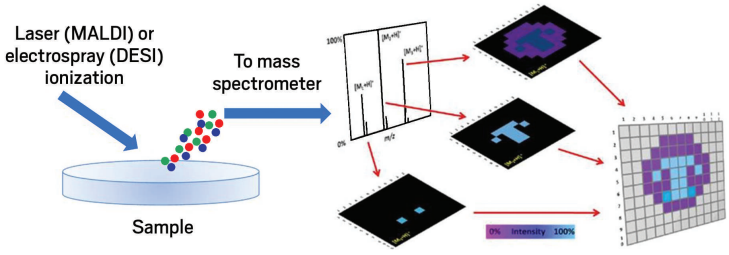
a veritable gallery of pictures because any peak in each spectrum can be spatially mapped.

Imaging MS has been around for 20 years, but now it's past a tipping point in the pharmaceutical industry, according to Richard M. Caprioli, a mass spectrometrist at Vanderbilt University who in the 1990s was the first to use MS for tissue imaging. "Why it took so long I couldn't tell you," he says.

But, Caprioli says, advances in the technology and informatics have helped increase the technique's popularity. Imaging is much faster now, for example. In the 1990s, Caprioli started acquiring MALDI images with lasers that fired only three shots per second. The fastest MALDI systems today come with lasers that fire 10,000 shots per second. With such speedy lasers, images take just seconds to minutes to collect, depending on the desired spatial resolution.

Imaging MS 101

In imaging MS, a laser beam or solvent stream ionizes molecules from a defined spot on a sample such as a piece of tissue. A complete mass spectrum is acquired at each spot. Software then constructs an image by mapping the intensity of an individual mass peak at different spots in the tissue.



Signs of the times

One indicator of the ascendance of imaging MS in pharma is the rapid growth of ImaBiotech, a contract research organization focused solely on the technique. Jonathan Stauber, the firm's chief executive officer, founded the company in 2010 with only two clients. The firm, which is based in Loos, France, now has 250 clients, most of which are pharma companies.

Companies come to ImaBiotech for a variety of reasons, Stauber says. Some companies are testing out the technique before setting up their own imaging labs. Other companies already have their own imaging labs but have more projects than they can handle. And some started as the first type and have become the second.

"We have hundreds of samples per week," Stauber says. To deal with all those samples, "we need to have a fast and robust technology."

Some of that technology ImaBiotech develops itself, especially in informatics. The company has been a pioneer in using imaging MS quantitatively. "The data sets are very large," Stauber says. "We are able to manage a few terabytes of data per project."

ImaBiotech is opening a location in the Boston area in partnership with the instrument maker Bruker, which is a leader in imaging MS. "We are going to be an extension of Bruker's demo lab," Stauber says. "We're going to codevelop and comarket mass spec imaging."

The first pharma companies to explore imaging MS started more than a decade ago. Some of those early forays were through academic collaborations. AstraZeneca, for example, started collaborating with Per Andrén at Uppsala University by funding postdoctoral researchers in his lab, according to Richard Goodwin, head of imaging MS at the company. When the collaboration reached a point that two postdocs were working full-time on AstraZeneca projects, Goodwin started making a case for bringing the technology in-house.

"We were showing the power of the technique, which means we were starting to get more demand," Goodwin says. "We had a shift from generating data that was pretty to supporting project decisions in a timely manner." In 2014, AstraZeneca brought the technology in-house, he says. But the company still collaborates with academic researchers to evaluate new imaging technologies.

Finding off-target effects

At GlaxoSmithKline, Stephen Castellino, director of imaging MS in the U.S., was inspired to explore the technology when he heard Vanderbilt's Caprioli give a presentation about using MALDI imaging for determining tumor margins in oncology.

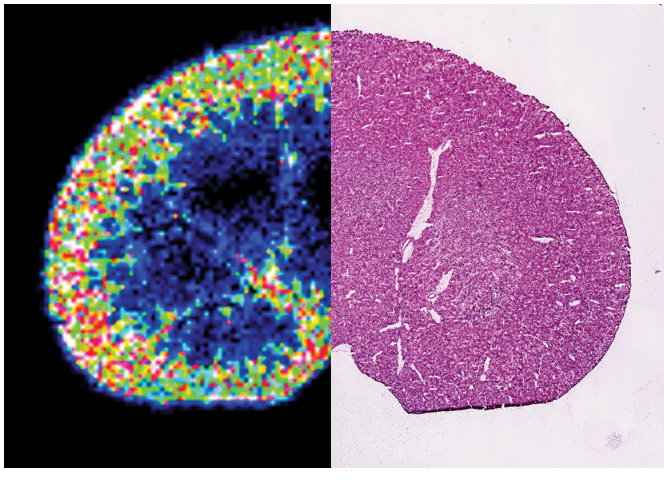
Castellino recognized the potential for the technology in drug metabolism and pharmacokinetic studies in late-stage development, which his lab focused on. "I came out of that seminar going 'if we could do this for drugs and drug metabolites, it would be a game changer for us,'" he says. At nearly the same time, Castellino's colleagues across the Atlantic also started using imaging MS at the other end of the pipeline, in early-stage drug discovery. Recently, the two groups merged to form a single bioimaging department at GSK.

Castellino's team initially used imaging MS for investigative toxicology. The mass spectra and resulting pictures gave them a way to link a drug to biological changes in tissue morphology. Before, they had no way of knowing whether drugs or metabolites were actually associated with adverse changes seen in tissue samples. They often used extracts from homogenized tissues to get an overall tissue concentration.

But those overall concentrations could be misleading. The homogenate could have low concentrations of drug per gram of tissue, even though the drug was concentrated in specific regions of the tissue. "Imaging mass spec has brought us the realization that drugs and metabolites more times than not are not evenly distributed within tissues," Castellino says. For example, GSK used imaging MS to show that an off-target effect of a drug was the result of a metabolite accumulating in the choroid layer of the eye.

To enhance the information obtained from conventional pathology, Castellino's team integrates the MS images with images obtained by histology staining. This allows them to connect locations of molecules of interest with known features of a given tissue.

Typically, the researchers take consecutive thin slices and use one for MALDI imaging MS and the next for histology.



CREDIT: WATERS (SCHEME); GENENTECH (KIDNEY)

Imaging MS (left) shows that the distribution of a drug in a rat kidney is not uniform. The drug distribution can't be seen with conventional pathology staining (right).

"When you look at our data, you can see the distribution in space, but because it's coregistered with the histology section, you can localize within the biological tissue."

Instrument maker Bruker has collaborated closely with GSK to improve matching the MS image with histology, says Shannon Cornett, an applications development manager for the instrument company. "Within our software, you can see both the ion images and the pathology images," he says. They take a picture of the sample before it goes in the instrument. They find three noticeable features in the tissue slices and use them to overlay the MS and histology images.

Sometimes, Castellino's team can even perform imaging MS and histology on the same tissue slice. "After the MALDI data are collected, we can wash off the matrix and stain the tissue," Castellino says. "But the quality of the histology from that section isn't as good as from a serial section. You can see gross features in the histology but not the fine detail."

GSK has had multiple cases where imaging MS helped explain off-target effects that ended up shutting down programs. In one case, an HIV nonnucleoside reverse transcriptase inhibitor triggered seizures in patients after at least four weeks of treatment during a Phase IIb clinical trial. With a combination of liquid chromatography/MS analysis of human cerebrospinal fluid and imaging MS of brain tissue in animals, they were able to show that a particular metabolite, which was able to cross the blood-brain and cerebrospinal fluid barriers or was formed in the brain, persisted in the central nervous system long after the last dose had been administered (*Chem. Res. Toxicol.* 2013, DOI: 10.1021/tx3004196). When levels of this metabolite built up, it sometimes triggered the seizures.

In another case, GSK was investigating whether it could treat children with a particular cancer drug. With imaging MS, the GSK researchers demonstrated that the drug triggered calcium phosphate deposits in the kidneys of the youngest juvenile rats (*J. Am. Soc. Mass Spectrom.* 2015, DOI: 10.1007/s13361-015-1103-4). "The compound was not getting eliminated through the liver, which is the normal pathway, because of the immaturity of the pups," Castellino says. "The drug was physically overwhelming the kidney and disrupting the tissue." Those findings provided safety guidance for treating future pediatric patients.

Ionization choices

Although MALDI is the most common ionization method used for imaging, it is not the only one. Other spatially resolvable ionization methods, such as desorption electrospray ionization (DESI) and liquid extraction surface analysis (LESA), are also used in pharmaceutical applications. Both of these methods involve using a solvent to extract and ionize molecules from a defined region of tissue.

Ron M. A. Heeren, an imaging MS expert at Maastricht University who collaborates with pharma researchers, was originally skeptical of DESI's suitability for imaging. "Developments in DESI sources have made them more stable," he says. "You can tweak the solvent composition for specific molecules you're interested in. It's a mix of targeted and discovery-based mass spec imaging."

Selecting an ionization method involves trade-offs between spatial resolution, sensitivity, and the molecules that can be analyzed. MALDI's spatial resolution is limited by the size of the laser spot and the size of the crystals of matrix material needed to help ionize analytes. It can achieve spatial resolution as good as 5 μm but is more frequently used at 10- to 20-μm resolution. LESA, on the other hand, has spatial resolution on the millimeter scale. In that sense, LESA serves more as a profiling method than a true imaging method.

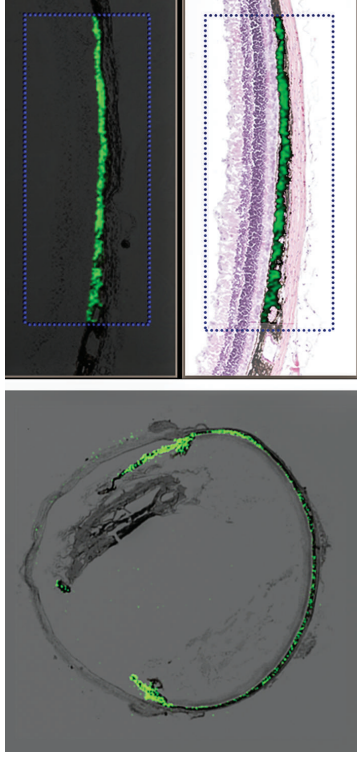
"Profiling imaging gave us the ability to have greater sensitivity when we needed to detect compounds we couldn't detect by MALDI," AstraZeneca's Goodwin says. "By opening up a suite of techniques, we are able to balance spatial resolution with sensitivity and speed. We can now pretty much guarantee we'll be able to provide drug distribution information for any project." As recently as 2014, Goodwin says, he and his colleagues wouldn't have dared make such a promise. Before they had a suite of ionization methods, they had only a 50-50 chance of detecting every early-stage drug molecule, he says.

A place in the tool kit

Imaging MS is starting to challenge existing methods for studying drug distribution in the body. One such method is quantitative whole-body autoradiography, in which radiolabeled compounds are used to track the distribution of drugs and their metabolites in lab animals. Such tests are usually reserved for only the most promising drug candidates because synthesizing radiolabeled versions of candidates is expensive and laborious.

There are other drawbacks too, Genentech's Shahidi-Latham says. "These studies can only provide information on the total drug-related distribution," she says. "They can't distinguish between drug and metabolite that has retained the label."

Imaging MS, in contrast, is label-free. "We rely on the mass spectrometer to provide our specificity. Now we can distinguish between a drug and its metabolites while maintaining the spatial distribution," she says.



MS imaging shows that a drug (green) accumulates in the melanin-containing layer of this mouse eye. The images shown here are a slice of the eye (bottom) and close-ups of an MS image alone (top left) and overlaid on a conventional histology image (top right).

CREDIT: GLAXOSMITHKLINE

Shahidi-Latham's team produces a lot of whole-body MS images of rats. In such studies they're usually looking for which tissues the drug accumulates in. These tests are particularly good for choosing between candidates that are similar on paper. By comparing MS images head-to-head, the researchers can determine if one drug's tissue distribution has a lower potential for causing off-target effects than the other compound, she says.

Because they are cheaper and easier, imaging MS studies can be done much earlier in the discovery process than autoradiography. "It's revolutionized how early we can study compound distribution and accumulation," AstraZeneca's Goodwin says.

But Shahidi-Latham doesn't anticipate imaging MS replacing whole-body autoradiography just yet. The other purpose of autoradiography studies is to help determine safe doses of radioactive tracers for human studies of drug absorption, metabolism, and excretion that are required by the U.S. Food & Drug Administration.

Despite such growing applications of imaging MS, even some of the early adopters have not yet made it part of their routine workflows for drug and metabolite distribution analysis. Novartis, which hired Markus

Stoeckli in 2000 to set up and run its imaging MS lab, was one of the earliest adopters of imaging MS. Although Novartis regularly uses the method, it is still not yet part of the normal workflow, Stoeckli says. Instead, "we selectively apply it to analytical questions where it's the only feasible technique to get a result and therefore will have great impact on drug discovery and development," he says.

It hasn't become part of Novartis's regular workflow because of sensitivity limitations, especially at high spatial resolution, Stoeckli says. "With LC/MS, we can measure very low compound concentrations in tissue, concentrations that we cannot see with imaging MS," Stoeckli says. "A lot of substances we dose too low to result in a good signal." So, he says, they use imaging MS for applications in which they have significant exposure, such as tests of formulation and safety.

Beyond telling drug developers how an agent and its metabolites move through the body, imaging MS also provides information about how those compounds affect the body's own molecules. "We've observed that in many dosed tissues, there are significant changes in endogenous compounds compared to control tissues," GSK's Castellino says. Goodwin agrees, saying that the imaging MS techniques collect data on these endogenous molecules and analysis of those data could make imaging MS a game changer.

> "It's the coming-of-age of mass spec imaging."
>
> —**Richard Goodwin,** head of imaging MS, AstraZeneca

Future work will involve figuring out what's causing those changes—whether they are part of the drug's pharmacology or the disease itself. Castellino anticipates that imaging MS in pharma will involve a metabolomics approach that encompasses not just the drug and its metabolites but also the body's response to the drug.

Pharma researchers are also interested in creating three-dimensional images of tissues. Shahidi-Latham's team recently reported the 3-D reconstruction of a whole mouse lung using MALDI imaging MS (*J. Am. Soc. Mass Spectrom.* 2017, DOI: 10.1007/s13361-017-1658-3). They used masses of various lipids found in the lung for the reconstruction.

At AstraZeneca, "we're using mass spec imaging to understand complex tumor and tissue microenvironments," Goodwin says. Goodwin and another of his AstraZeneca colleagues are part of a consortium that won a five-year grand challenge grant from Cancer Research UK. That team, which is led by Josephine Bunch of the U.K.'s National Physical Laboratory, is using imaging MS to study tumor metabolism all the way from the single cells through 3-D cell culture, xenografts, and patient biopsies.

"We think it's the coming-of-age of mass spec imaging," Goodwin says.

For now, imaging MS in pharma lives mostly in labs staffed by MS experts. But instrument company Waters Corp. hopes to change that. Michael Batey, senior business development manager for the company, would like to move imaging MS to the point where it doesn't require a specially trained person to run the analyses. "I'll be regretful if in five years' time we don't have a black-box solution where you load your slides, press the button, go have a cup of tea, come back, and get the answer," he says.

It's an exciting time to be involved with imaging MS in the pharma industry, says Shahidi-Latham. "The more examples you see in the literature of how it's been impactful, the more folks are going to embrace and use this technology," she says. "We're really just scratching the surface of its potential." ■