Small animal imaging: Data that’s more than skin deep

To minimize complexity, researchers often study cellular proteins or nucleic acids in isolation. But sometimes—when testing a drug’s efficacy and safety, for instance, or monitoring tumor progression—ex vivo just won’t do. The only way to know how a compound or cells will behave in the body is to put them into an animal and watch what happens live. The results are easily recognizable in the pages of your favorite journal: the ghostly outline of a mouse, with a telltale multicolored heat bloom indicating where the action is. By Jeffrey M. Perkel

Molecular Imaging, a contract research organization (CRO) with locations in Ann Arbor, Michigan and San Diego, California, has a largely pharmaceutical clientele. Sometimes, says Chief Scientific Officer Patrick McConville, those clients are looking for drug screening services. More frequently, they have preliminary data but need help selecting the right candidate for clinical trials.

“It’s about trying to obtain information that cannot be obtained other ways,” he says.

How do Molecular Imaging’s scientists obtain such information? By getting under the skin of the problem. Literally.

Small-animal imaging allows researchers to test-drive the therapies and compounds they hope eventually to apply in the clinic. It provides a way to monitor disease progression and mechanisms for tracking various disease phenomena over time, often using methods that are available for human patients as well.

Suppose, for example, a researcher wants a comprehensive view of the disease progression and therapeutic response in an animal model of Parkinson’s disease, McConville says. One approach would be to obtain a set of genetically identical Parkinson’s disease animals, dose them all with either the candidate therapeutic or a placebo, sacrifice a few at each time point, and dissect out and analyze the appropriate brain regions. That requires a lot of mice, of course, but also poses the difficulty of trying to average the responses of different animals over time. And it can’t easily be applied to human patients.

The alternative is to exploit preclinical imaging to produce a multidimensional dataset in live animals. For instance, using [$^{18}$F]-fluorodeoxyglucose (FDG) positron emission tomography (PET), researchers can assess brain metabolic activity based on the rate at which different regions take up the radioactive probe. They can pinpoint functional dopaminergic activity in the cells that are affected in Parkinson’s disease—using a commercially available dopamine transporter-binding radiotracer called DaTscan (from GE), via single-photon emission tomography (SPECT) imaging. And they can align those data with anatomical features using computed tomography (CT) data. Other than anesthesia to keep the animal calm and unmoving, the mouse is unaffected by the experiment, meaning it can be imaged repeatedly for days, weeks, or months. Bottom line: fewer animals, richer data, and better decisions.

“The beauty of in vivo imaging is that you don’t need to sacrifice the animal,” says Hong Yuan, director of the Biomedical Research Imaging Center (BRIC) Small Animal Imaging Facility at the University of North Carolina, Chapel Hill, School of Medicine. “You can look at changes due to treatment or any other manipulation over time, and that is a very powerful method compared to traditional immunochemistry.”

The BRIC makes available to its users a broad suite of imaging modalities. These include a 9.4T Bruker magnetic resonance imager (MRI)—smaller in stature but more powerful than its clinical equivalent; a GE eXplore Vista PET/CT dual-modality imager; a GE eXplore speCZT SPECT/CT system; a Vevo 2100 ultrasound system from VisualSonics; and three standalone CTs—small animal analogs of equipment that would be at home in any (albeit extremely well-funded) medical facility. Also available are four optical imagers, including three In Vivo Imaging Systems (IVIS) and one fluorescence tomography system, all from PerkinElmer.

Generally speaking, imaging modalities fall into two categories, says Mario Bourdon, chief scientific officer at small animal imaging services provider BioLaurus: anatomic and molecular. Anatomic imagers includes X-ray, CT (which is essentially a 3-D X-ray), MRI (for soft tissue analysis), and ultrasound (for imaging blood flow, cardiac activity, and fetal development, for instance). Molecular imaging methods include nuclear medicine (PET and SPECT) and optical approaches, all of which allow researchers to track the abundance, location, and activity of specific molecular species in vivo.

The Small Animal Imaging Facility at The University of Texas MD Anderson Cancer Center sports three MRI scanners in its instrument stable, and they are “by far the most widely used” of the lab’s hardware, says John Hazle, professor of imaging physics and the facility director. Among other reasons, he says, MRI provides “excellent soft-tissue imaging and the ability to image some physiology and... continued>
metabolic processes," allowing researchers to determine, for instance, how tumors respond to therapy.

But, by most accounts, optical imaging is the most widely used preclinical modality overall. Capable of recording both fluorescent and bioluminescent signals, “Optical imaging has a couple advantages,” Yuan says. Among its virtues, optical imaging is relatively inexpensive and high throughput—researchers can image multiple animals simultaneously and rapidly, meaning it’s relatively easy to image statistically significant population sizes. By comparison, MRI scans, which provide soft tissue anatomy, are done one or a few at a time. “Our MRI scanner runs around the clock, but the throughput is much lower [than optical],” says Christopher Contag, director of the *Stanford Center for Innovation in In Vivo Imaging*, where optical imaging also is the most widely used modality. “On the optical system we process probably hundreds of mice [per day], versus a handful on the MRI or PET.”

Optical imaging also is technically easy to use. “We train users and they can handle the image acquisition and data interpretation pretty quickly,” Yuan says. Other modalities typically are handled by trained staff.

**Better optics**

Optical imaging comes in two flavors: fluorescent and bioluminescent. In fluorescence imaging, a fluorescent marker (such as a labeled antibody or genetically encoded fluorescent protein) is introduced or expressed in the animal and excited with an external light source. Bioluminescence involves genetically modifying the animal to produce a light-generating protein, such as luciferase, and produces light only in response to a supplied substrate. In either case, the abundance and distribution of a specific molecular target is measured by recording the resulting photoemission through the skin.

But therein lies the problem. As anyone who has put a flashlight behind their hands knows, light does not travel in a straight line through biological tissue; it scatters and is absorbed. Thus, some fraction of the excitation (in the case of fluorescence) and emission light (fluorescence and bioluminescence) is inevitably lost in optical imaging—though this is less true of near-infrared light. Also, the signal’s exact position is often difficult to work out. By contrast, PET and SPECT tracers can be localized precisely, as both emit high-energy particles that traverse the body unimpeded. As a result, optical imaging is rarely used on any animals other than mice, and even then, mostly for surface features such as subcutaneous tumors.

Researchers are working to change that, however. One approach generating considerable excitement, for instance, is photoacoustic tomography (PAT). Lihong Wang, the Gene K. Beare Distinguished Professor of Biomedical Engineering at *Washington University in St. Louis*, Missouri who pioneered the technique, says that while optical imaging loses resolution beyond about a millimeter, ultrasound maintains resolution at greater depths. PAT effectively combines the two, using light to excite a molecular target but reading out the ultrasonic pressure wave caused by the target’s heating and expansion. “It’s similar to lightning and thunder,” explains Andrew Needles, senior manager for product innovation at *FUJIFILM VisualSonics* in Toronto, Ontario, which has commercialized the technology. “The lightning causes the surrounding atmosphere to create a sound wave.”

In theory, says Wang, PAT can detect any molecule, provided the laser is tuned to the correct wavelength for molecular absorption. His lab has imaged everything from nucleic acids and carbohydrates to organic dyes and water. One common application, says Needles, is measuring blood oxygenation, functional data that can be overlaid over a traditional ultrasound image for anatomic detail. Now, Wang’s lab has been awarded BRAIN Initiative funding to image “the whole brain of a small animal in vivo and image action potentials.”

**TriFoil Imaging**, in Chatsworth, California, has developed another optical technology. Launched in September 2014, called fluorescence-emission computed tomography (FLECT) is to traditional planar imagers what CT is to X-ray. Laser energy strikes the animal through a thin slit while rotating around the whole animal in a helical scan. The resulting fluorescence emission is captured by a ring of 48 detectors positioned around the animal, which rotates through 360 degrees to produce a tomographic dataset, similar to PET.

Indeed, according to Staf Van Cauter, executive vice president for marketing and business development at TriFoil, FLECT “is a nonisotopic alternative to nuclear imaging techniques,” and using it, he says, researchers can detect tumors and other features smaller than a millimeter in size, and identify exactly where in the body they are located.

**A more accessible pet**

Still, since optical modalities are for the most part not approved for human use, researchers interested in clinical medicine must look elsewhere for molecular imaging. One popular option is PET.

PET, explains John Gore, director of the *Vanderbilt University Institute of Imaging Science*, provides molecular information with exceptional sensitivity. The tradeoff is resolution: Where MRI can image down to 100 μm, PET voxels are millimeter-sized.

“The value of PET is it’s a translational imaging modality,” says Patrick Phelps, president and chief executive officer of *Sofie Biosciences* in Culver City, California, a company that develops PET imaging systems and probes. A PET probe that works in mice can thus also, in theory, be applied in humans. But PET probes aren’t easy to work with. The most widely
The access to PET probes has been a huge barrier to access,” Phelps says.

Other isotopes, such as zirconium-89 (half-life 78.4 hours) and copper-64 (12.7 hours), open PET to researchers located at greater distances from synthesis laboratories. Or labs can generate their own probes, provided they have a cyclotron. But that isn’t a trivial undertaking.

The BRIC, for instance, installed a cyclotron in late 2014. According to Yuan, the hardware itself cost a couple million dollars. But infrastructure upgrades also were required. “The cyclotron we purchased is not self-shielding,” she explains, so the university had to install 6 feet of concrete underneath and lead-shielded walls surrounding the cyclotron. The room’s lead-shielded door weighs 11 tons. Adding in the cost for a radiochemistry-capable “hot cell” lab and quality-control equipment, she says, and the hardware cost spirals to “probably in the range of $20 million.”

Still, it takes more than access to isotopes to make PET practical. Given their short half-life, researchers also need a way to attach them to the desired molecule before they decay. Hidde Ploegh of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, has developed a method that could simplify that process, at least for labeling antibody fragments.

Using an 18F-labeled substrate and the sortase enzyme, Ploegh’s team has labeled camelid-derived antibody fragments containing a sortase recognition sequence to create an immunoo-PET probe. They then used that to observe myeloid cell infiltration of implanted tumors in vivo. The method provides a way to spy on tumors without knowing a priori where they are, Ploegh explains—just follow the macrophages. “It’s like, if you are on safari and you want to know where the lions are sleeping, just follow the other Land Rovers.”

New modalities

Despite the rich set of imaging tools now available, development continues on multiple fronts.

Magnetic Insight, for instance, is advancing a method called “magnetic particle imaging” (MPI). According to Patrick Goodwill, Magnetic Insight’s chief technical officer, MPI provides molecular contrast, like PET and optical imaging, but with magnets. “It’s nuclear medicine, but using an iron oxide nanoparticle.”

Researchers can, for instance, load mesenchymal stem cells with antibody-coated nanoparticles and track them in vivo, Goodwill says, detecting as few as 200 cells at any depth. “We’re using low-frequency magnetic fields, 20-kHz, that will pass through just about anything. If we had a whale-sized MPI scanner, we could see through a whale.”

Contag, with Stanford colleague Sam Gambhir, is studying ways to capture Raman spectral fingerprints in vivo. In one recent paper, his team described a molecular endoscope that could theoretically assess the molecular status of colon polyps in real time, rather than sending biopsies to a histopathologist. The technique relies on surfaced-enhanced Raman spectroscopy (SERS) nanoparticles, which dramatically boost the Raman signal molecules normally produce. “You can spray these SERS nanoparticles on the [colon’s] surface and as you withdraw the endoscope, it scans and searches for places where the particles stick,” he explains.

And Hazle’s lab has begun experimenting with new MRI contrast agents to better image metabolic activity—effectively blending MRI and molecular imaging—using “hyperpolarized” carbon 13 (13C)-MRI. Molecular imaging, Hazle explains, tells researchers where the probe is located, but not its chemical structure. But 13C-labeled pyruvate produces a different signal when converted into lactate, a transition that occurs at a different rate in cancerous tissues. “So not only do you know [the] tumor took up that sugar, but [also whether] it metabolized it in aerobic metabolism or anaerobic metabolism,” he says.

Whatever the fate of these technologies in preclinical core facilities or the clinic, most researchers agree existing modalities aren’t going anywhere. Different imaging methods, Wang says, exploit different contrast mechanisms and thus complement one another. “This is why you don’t want to say one technology will replace the rest,” Wang says. “I’ve found that never really happens, not in the medical-imaging world.”

Jeffrey M. Perkel is a freelance science writer based in Pocatello, Idaho. DOI: 10.1126/science.opms.p1500094