MOLECULAR IMAGING NO SWISS ARMY APPROACH

Imaging of living subjects is of growing importance in biomedical research, particularly in the field of molecular imaging, which represents the cutting edge of these technologies. Utilizing novel technologies and new imaging agents, molecular imaging is allowing scientists to noninvasively visualize specific molecular targets and dynamic events in living animals. Major advances have recently been made in the ability to detect multiple signals simultaneously, enabling visualization of the complex orchestra of molecular communications like never before. Multimodal imaging is gaining momentum, with fusion technologies that combine the power of individual structural and molecular approaches. Ultimately, the hope is that molecular imaging will yield critical information that accelerates the development of diagnostics and therapeutics.

By Ursula Calef

A big challenge for in vivo molecular imaging is the multiplexing of many signals simultaneously. Sanjiv Gambhir, director of the molecular imaging program at Stanford University, says that their latest work on Raman spectroscopy with Raman nanoparticles has helped to solve this problem. His group has made possible, for the first time, the use of Raman optical techniques for small animal imaging by modifying Raman cell microscopy instruments. Gambhir says, “This is, so far, one of the most multiplexable, if you will, strategies we’ve ever come up with.” While he says there is no “Swiss army knife of imaging—where one tool does it all—if you need multiplexing, Raman might be the right way to go.”

Raman imaging can be used to study the interplay of many cell populations, e.g., cancer cells and their neovasculature, pre- and posttreatment. Raman spectroscopy measures the inelastic scattering of light “where one out of every 10 million or so photons actually changes its wavelength when it bounces off the target area.” Gambhir and colleagues use nanoparticles that enhance this weak effect. The Raman particles “cause some light to shift to one wavelength, some light to shift to a different wavelength, creating unique spectra,” explains Gambhir. These spectra are very sharp and tight, and the composition of the particles can be modified to produce unique spectral signatures that are easily distinguished, thereby allowing high multiplexing even on the same cell.

Gambhir uses two kinds of Raman particles: SWNT nanoparticles, or single-walled carbon nanotubes, with intense intrinsic Raman peaks, and SERS-biotags, or surface enhanced Raman scattering active nanoparticles, with a gold core, commercialized by Oxonica as Nanoplex Biotags. The molecules with which these particles are functionalized may be varied, so that they can hone in on the specific molecular events being interrogated. Gambhir says that, compared to Raman, the use of different colors of fluorescent probes is more “limited in animals to colors that are red-shifted or in the infrared, because other colors don’t do well penetrating through tissue. Even quantum dots,” continues Gambhir, “with their many colors, have broad emission peaks. So, if you have four in the same place, it’s very hard to separate each of their concentrations.”

Raman imaging accomplishes straightforward multiplexing while maintaining exquisitely high sensitivity, and is also semiquantitative. Further, since natural Raman scattering is so low, it results in very little background. For these reasons, Gambhir expects Raman imaging, eventually including tomography, to be a huge future growth area. A main limitation of the approach is that “Raman particles are bigger and not all of them will reach the target site,” says Gambhir. While

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fluorescent probes might be very small (around 2 nm), “a Raman particle might be tens of nanometers big.”

**Multiplexing the Colors of the Rainbow**

Great strides have similarly been made in multiplexing fluorescent signals. Fluorescence-based imaging is especially useful for in vivo molecular imaging and can follow molecular targets on either a microscopic or macroscopic scale. In 2008, Roger Tsien was awarded the Nobel Prize in Chemistry in part for his work on developing the first of a whole color palette of fluorescent proteins. Additionally, there has been much work on activatable reagents that fluoresce when they react with specific targets.

Tissue autofluorescence has previously limited the sensitivity and utility of fluorescence methods, says James Mansfield, director of multispectral imaging systems at Cambridge Research & Instrumentation (CRI). However, recent advances in spectral imaging with CRI’s Maestro 2 system have enabled multiplexing of up to five markers in vivo. This technology breaks down light into its spectral components and reads the intensity, “kind of like spectral filtering,” explains Mansfield. Using a multispectral camera and sophisticated mathematics, signals are “unmixed,” thereby greatly improving contrast and quantitation. Mansfield believes that the Maestro systems are 50–300 times more sensitive than monochrome fluorescence systems, reducing detection limits and facilitating multicolor imaging.

Other multispectral imaging systems include Caliper Life Sciences’ IVIS Spectrum, which allows the multiplexing of several markers. To select the readable wavelength, IVIS uses large format fixed filters, while the Maestro from CRI uses a tunable filter, explains Stephen Oldfield, senior director of imaging marketing at Caliper Life Sciences. This difference allows for a more variable wavelength range for the Maestro 2 which is designed for fluorescence applications, and the reading of very low light levels by the IVIS Spectrum which provides enhanced sensitivity for bioluminescence. The IVIS can additionally “generate a 3D representation of a fluorescent or a bioluminescent signal using various tomographic techniques,” explains Oldfield. Such noninvasive, 3D imaging allows accurate quantitation and localization. Finally, Caliper’s IVIS Kinetic includes a real-time, fast imaging capability for studying millisecond events, e.g., perfusion rates of metastatic tumors, says Oldfield.

**Photothermal Molecular Imaging**

Gambhir and colleagues at Stanford University have also recently, for the first time, married photoacoustics to molecular imaging by developing nanoparticle imaging agents. This technique is ideally suited for high multiplexing, high sensitivity small animal imaging. In photoacoustics imaging, Gambhir explains, “Light goes in and interacts with the photoacoustic particle which absorbs the light, heats up slightly, and produces sound. The sound produced, unlike the light, has no trouble penetrating through tissue.” This approach therefore offers higher spatial resolution and allows deeper tissue imaging in 3D compared with most optical techniques. The main limitations are that the light going in only penetrates a shallow depth, and sound does not work well near bones or in the lungs. Still, clinical translation of photoacoustic molecular imaging has huge growth potential, e.g., in breast cancer diagnosis, and is currently being commercialized by Endra and Seno Medical.

**Multimodal Power**

While multiplexing lets scientists peek at intricate molecular interactions, advances in multimodal imaging promise to show them precisely where molecular signals are coming from inside animals. Researchers are seeking to combine the strengths of complementary approaches, especially focusing on techniques that combine detailed imaging of anatomy with sensitive imaging of molecular signals. Simon Cherry, director of the Center for Molecular and Genomic Imaging at University of California, Davis, and his colleagues have developed a preclinical positron emission tomography/magnetic resonance imaging (PET/MRI) hybrid that for the first time allows simultaneous acquisition of in vivo images. The PET scanner is built into the MRI magnet. PET/computed tomography (CT) and single photon emission computed tomography (SPECT)/CT are well-established approaches that provide sequential scans for molecular and structural information, respectively.

Cherry explains that MRI has major advantages over CT, providing better soft-tissue contrast and allowing you to get “lots of types of information in addition to structure.” Importantly, Cherry has put a lot of emphasis on “simultaneous imaging.” The challenge in building these integrated systems is to not degrade the performance of either. Fortunately, this is being overcome via better isolation of PET detectors/electronics and the development of newer photodetectors that are less sensitive to magnetic fields. Cherry suggests one good application of PET/MRI would be to study drug delivery into the brain. “You would label the drug so you can see it with PET and coinject gadolinium contrast agent to measure permeability by MRI” to simultaneously assess blood-brain barrier permeability and brain drug concentration, e.g., before and after a treatment to improve drug delivery. Clinical translation is also possible, and Siemens has now built the first human PET/MRI prototype systems that are undergoing testing at several sites in the United States and Europe.

One commercially available multimodal research system is Carestream Molecular Imaging’s Kodak Multispectral FX. It offers four modalities: luminescence, multispectral fluorescence allowing multiplexing of up to four markers, radioisotopic (nuclear), and radiographic (X-ray). This is the first multispectral system that enables “multimodal coregistration of molecular imaging (optical or nuclear) with high resolution X-ray anatomical imaging,” explains William McLaughlin, director of research and development at Carestream Molecular Imaging. “All four modes are captured at essentially the same focal plane, so the images can be very precisely overlaid, allowing researchers to more quickly and much more accurately identify the molecular targets of interest.” Further multimodal combinations are promised in the future. 

**In Vivo Imaging**

Tissue autofluorescence has previously limited the sensitivity and utility of fluorescence methods.
In Living Action

Chris Vega, product marketing manager of confocal microscopy at Leica Microsystems, asserts that in vivo imaging is exciting because “you can look at the organism living and whole, and watch the naturally occurring processes happening in real time, getting us closer to observing the dynamic processes of life.” To accomplish this, Leica Microsystems offers the Leica FCM1000 Confocal Microscope (previously the Cellvizio), which for the first time allows real-time acquisition of data at 12 frames per second. This focal laser device comes with various fibered microprobes to use externally, endoscopically via minor incision, or stereotactically. Its minimal invasiveness permits long-term experiments. Vega explains that with the Leica FCM1000 you can do functional imaging of neurons, cell migratory studies, and “anywhere where you need to actually look deeper into the animal; at a micro level, this cyber probe allows you to see micro structures in living action.”

Super Zoom

Some questions are best addressed with a system that allows you to “essentially start out with a world view and zoom in to the level of the house, or maybe even the people in the house,” describes Vega. He is referring to Leica Microsystems’ TCS Large Scale Imaging (LSI) Confocal with super zoom capability. The Leica TCS LSI is the first tool of its kind with “a true confocal scanning system on a macroscope,” says Vega. The macroscope provides the large field of view (up to 60 mm), while the optical and confocal zooms, plus additional microscope objectives, allow a range of magnifications from the whole animal down to the cell, all at high resolution. Advanced time lapse software enables longitudinal studies. These features, explains Vega, make LSI ideal for developmental biology applications. “You can watch an organism, such as a zebrafish, go through stages of development,” he says.

Molecular Toolbox for a Clearer View

In vivo images are only as good as the imaging agents and methodologies you are using, many of which have recently been added to the toolbox. CRI’s Dynamic Contrast Enhancement (DyCE) imaging methodology utilizes time series analysis of images of the injection of a near-infrared (NIR) dye, which allows for the first time the generation of all-optical anatomic maps by charting the path of the dye over time. Mansfield elucidates: “Each organ that has a different time signature can be put into a different color and we use those time signatures to pull out the anatomic information.” These anatomical surface maps of major internal organs can be readily coregistered with molecular data.

Other new products include LI-COR Biosciences’ NIR dyes, such as IRDye 800CW. This dye “excites and emits at the sweet spot for optical imaging” where tissue background interference is lowest, explains D. Michael Olive, vice president of science and technology at LI-COR, and produces a high signal-to-noise ratio with deeper penetration. Carestream Molecular Imaging has two novel small molecule dyes that also excite in the NIR range with very large Stokes shift. McLaughlin says that “excitation and emission peaks are about three times farther apart than typical dyes,” significantly improving signal over background. Additionally, Carestream offers fluorochrome-embedded nanospheres that are bright, organic, nontoxic, consistent in size, and have a large number of attachment groups. Finally, the realization of the increased power of fusion technologies is leading to the development of flexible, “multimodal imaging agents” for which different imaging modalities can be used to detect signal, notes McLaughlin.

When Money Matters

Remarkable advances in molecular imaging systems come at a premium. Cash-strapped scientists need cost-efficient alternatives. At under $75,000, “LI-COR’s Pearl Imaging System is the most affordable system out there,” declares Olive. The Pearl, used for NIR imaging, is a “laser-based system which gives deep tissue penetration and very high signal to background. The resolution of fine structures at the macroscopic level is really remarkable,” says Olive. This fluorescence reflectance system is suited for imaging events at the surface, obtaining 2D images, and doing relative quantitation experiments. While various companies do offer lower cost, manually operated base platforms for optical imaging, the Pearl “is truly a one button operation,” emphasizes Olive.

Toward the Clinic

Most advances in optical techniques described here are limited to small animal imaging, mainly because light only penetrates 1–2 cm. Also, to use them in humans, imaging agents would need to be clinically validated. Still, remarks CRI’s Mansfield, “Unlike bioluminescence, fluorescence imaging is potentially ‘translatable’ for any antibody label or any activatable reagent. Optical imaging can be used for anything where the surface can be imaged, using any available optics, including endoscopes.” McLaughlin notes, “Many are working toward new technologies that may make optical imaging in large animals and humans achievable at a greater depth.” Ultimately, the hope is that molecular imaging technologies allow earlier disease detection, separation of patient subgroups for treatment, and more rapid evaluation of therapeutic response.

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