



June 2009

Technology Feature

In vivo molecular imaging: the inside job

Nathan Blow

Abstract

In a short period of time, *in vivo* molecular imaging systems have become indispensable research tools in many clinical and basic research laboratories. But developers are now pushing the technology further in the hopes of making a new generation of platforms with greater accuracy and sensitivity for a wider array of applications.

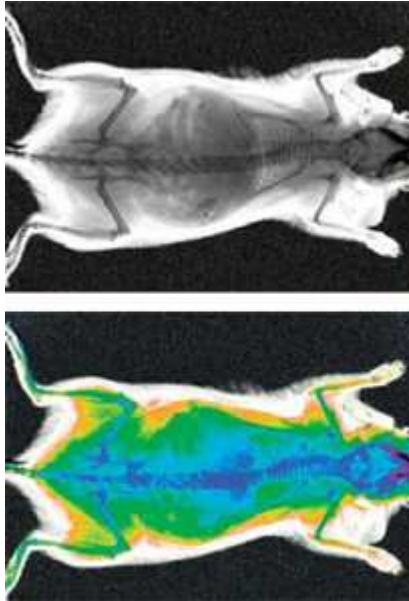
Introduction

Walking through the animal imaging department at Millennium: The Takeda Oncology Company, director Matthew Silva points out the different imaging instruments his group uses for imaging small animals. Both the positron emission tomography (PET) system and the magnetic resonance scanner look like smaller clones of their human counterparts that you might encounter at most major metropolitan hospitals. "We have every major anatomical and molecular imaging technology for small animals," Silva notes as we pass by four optical imaging systems next to a computerized tomography scanner.

Silva's group is not alone these days when it comes to applying whole-animal imaging technologies to address complicated research questions. A growing number of scientists are using anatomical and *in vivo* molecular imaging approaches to explore everything from how and where tumors grow, to locating the places where infectious organisms hide in the body to avoid therapeutics. And the number of fields in which *in vivo* imaging is being applied continues to steadily increase.

"Imaging stem cells *in vivo* is now an area of intense interest among researchers," notes Stephen Oldfield, who is senior director of marketing for imaging products at Caliper Life Sciences, a company working to advance optical imaging techniques based on tomography.

For their part, developers are pushing the envelope of imaging sensitivity and resolution through improvements in instrument design, as well as in the development of novel imaging agents, in the hopes of providing researchers even clearer pictures from inside live animals.



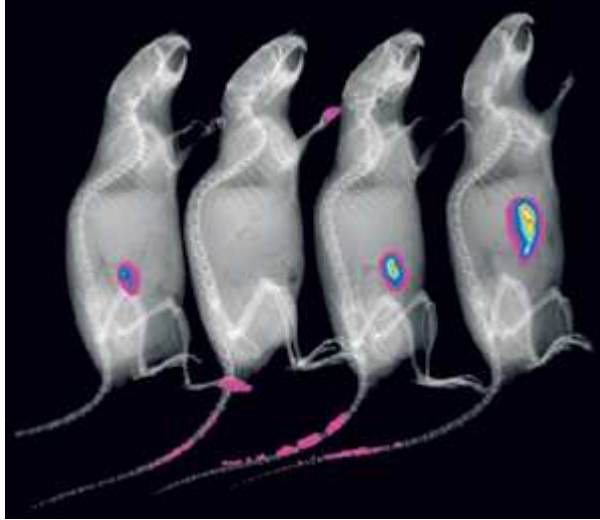
Carestream Molecular Imaging

Anatomical imaging can be used to assist researchers in locating signals from molecular imaging studies.

Seeing is believing

Similar to anatomical imaging, where different approaches such as magnetic resonance scanning and computerized tomography can be used to generate pictures of organs and tissue throughout the body, there are different strategies for performing molecular imaging *in vivo*. But according to Ralph Weissleder, who is the director of the Center for Systems Biology at Massachusetts General Hospital, each *in vivo* imaging approach has its own advantages and disadvantages, so a researcher's particular needs at a given time should be the critical criteria when choosing an imaging modality. "The question should be: what is the fastest, simplest and most powerful method to come to an answer and get on with life?" he says.

At the moment *in vivo* molecular imaging essentially falls into two main areas of development: PET scanning approaches and optical imaging modalities. PET instruments were first described in the mid 1960s, and they work by detecting the gamma radiation released from radiolabeled compounds or reporters, such as radiolabeled glucose, to image metabolic processes taking place in the body. It can also be used to track the location and distribution of labeled molecules. Optical approaches rely on the vast knowledge accumulated on bioluminescence and fluorescent agents from microscopic imaging, for *in vivo* macroscopic imaging of molecular and cellular processes.



Carestream Molecular Imaging

An example of multimodal imaging where a combination of anatomical and molecular imaging is used to improve signal localization in live mice.

"Bioluminescence and fluorescence actually give us the opportunity to target particular cellular processes occurring *in vivo*," says Silva. The development of fluorescence and bioluminescence imaging approaches started in the 1990s with fluorescence reflectance imaging. More recent advances in optical imaging assays have allowed Silva's group to develop additional readouts that help identify targets of cellular pathways modulated by drug treatment, enabling a more complete understanding of the activity of a potential therapeutic.

One method for *in vivo* optical imaging uses a two-dimensional planar approach, akin to *in vitro* epifluorescence microscopy, which allows researchers to explore surface tissues and bones of an animal with fluorescence or bioluminescence. Carestream Molecular Imaging developed a series of planar imaging systems capable of detecting not only bioluminescence and fluorescence but also radioisotopic signals. Additionally, one of the platforms is now capable of performing X-ray imaging as well to obtain anatomical information about the animal to accompany optical and isotopic data. "The approach is very easy, and the co-registration of the *in vivo* signal with the anatomical images is outstanding," says Bill McLaughlin, director of research and advanced applications at Carestream. Other companies including CRi, UVP and Lighttools Research also offer planar imaging systems for both bioluminescence and fluorescence work.



S. Cherry

Simon Cherry is working to create instrumentation that combines the functional capabilities of PET imaging with anatomical imaging modalities such as computerized tomography and MRI.

The deeper you go

"The challenge with fluorescence is that the signal is so strongly depth-dependent," says Simon Cherry, a professor of Biomedical Engineering at the University of California, Davis. According to Cherry, how deep the fluorescence source is in the body is a real issue because light tends to scatter greatly as it passes through tissues and other biological materials. So for imaging deeper fluorescence signals developers need to work around light scattering and absorbance issues to be able to localize and quantify their signals. This is where many researchers think fluorescence tomography can help. Fluorescence tomography systems use a transillumination mode and sophisticated computer algorithms to reconstruct three-dimensional (3D) maps of fluorophores inside living animals.

In 2007, Caliper Life Sciences acquired Xenogen and has since worked on advancing what Caliper calls a fluorescence imaging tomography approach in the IVIS optical tomography systems. "The way to pinpoint fluorescence quantitatively in a calibrated fashion is with 3D imaging using a tomographic technique, so then you are looking at the absorption of different wavelengths to tell you where the source location is and how many picomoles of that dye are present," explains Oldfield.

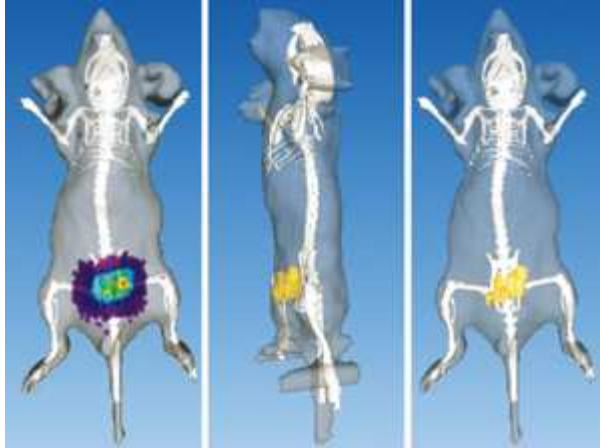


Carestream Molecular Imaging

A new generation of instruments, such as Carestream's In-Vivo Multispectral system FX, is now combining anatomical and molecular imaging capabilities.

VisEn Medical is advancing fluorescence molecular tomography (FMT) for researchers with their FMT2500 system. Kirtland Poss, president and chief executive officer at VisEn Medical, sees the FMT platform as enabling the process of early-stage drug discovery in the pharmaceutical industry more than other molecular imaging modalities such as PET. "FMT is really directed toward wide adoption in preclinical research and drug development at the moment," he says, although he is also quick to add that the FMT platform is designed to be applicable from *in vitro* to preclinical settings and even in the clinic.

Others agree fluorescence tomography offers a nice alternative to PET especially when it comes to higher-throughput, preclinical studies. "With optical instruments we can scan four animals at the same time in less than a minute," says Silva about his facility at Millennium, adding that although their lab is one of the higher-throughput labs using PET scanning approaches, they still can only image two animals at a time and at most 32 animals in a day.



Caliper Life Sciences

Prostate tumor imaged using bioluminescence and 3D reconstructions.

Weissleder, who was also a co-founder of VisEn Medical, and his group have been working to merge FMT with the anatomical modalities of computerized tomography and magnetic resonance imaging (MRI) to further the potential applications of optical imaging. "FMT- [computerized tomography] allows you to fuse fluorescence information onto [computerized tomography] scans so you do not have to deal with isotope probes that decay. You can use fluorescent proteins as well as reactive and sensing probes; all the stuff that you cannot do with PET probes."

But there is also a feeling that fluorescence tomography approaches still have a way to go before they see more widespread usage. "In general, the concept of injecting a fluorescent probe that goes everywhere in the body and having some concentration where there is a molecular target that has to be recovered and quantified is very hard to do with light," says Cherry, who has developed new instrumentation that combines magnetic resonance and computerized tomography imaging capabilities with PET scanning. Although he says fluorescence approaches work well in very specific and highly controlled situations, he worries about the robustness of algorithms used to reconstruct the 3D fluorescence image. "Sometimes they will work and give you the right answers when you have these nice focused distributions, and other times they just fail, and how does the user know?"

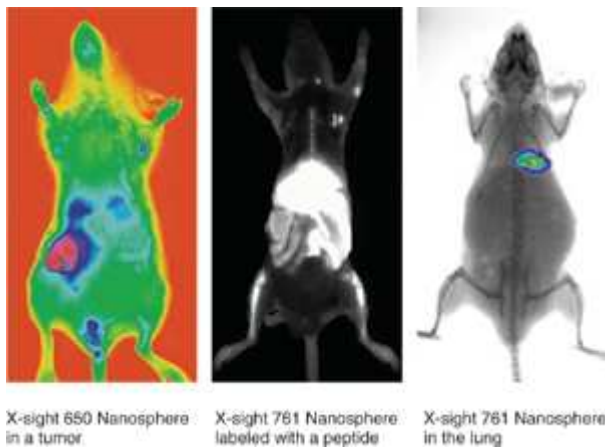
And for translating probes and imaging approaches to the clinic, optical is still very much on the starting line. "On the PET side, from a pharma perspective, the concept of translational imaging is very important," says Silva. And because traditional PET imaging can be performed in humans, the process of translating probes and assays from animals to humans is a more rapid process with PET.

Still, Weissleder thinks in time FMT approaches will catch on among researchers and clinicians alike. "There is this knee-jerk response where researchers dismiss FMT because they only think of epifluorescence and its limitations," says Weissleder, "but they need to realize there is this new powerful technology out there."

Probing the issues

A hurdle in the use of PET or optical approaches for molecular imaging is the development of new probes and imaging agents. Although in theory common fluorescent probes such as green fluorescent protein or red fluorescent protein could be used to track small molecules or work as reporters *in vivo*, the reality is that working in an *in vivo* environment creates new requirements on probe development.

"Say you put GFP into a mouse and wanted to image [it]; you would get a lot of fluorescence in the form of autofluorescence from the gut and lymph nodes and all the natural fluorophores that are in there," explains Weissleder. Finding ways of eliminating autofluorescence and amplifying the correct signal have been key areas of research for developers.



Carestream Molecular Imaging

Advances in nanoparticles and fluorescent dyes, such as the Kodak X-Sight Nanospheres shown here, are creating more possibilities for researchers when it comes to *in vivo* molecular imaging.

At the center that Weissleder directs they are taking a high-throughput library approach to the identification of better fluorescent agents for *in vivo* imaging. "The bottleneck in making probes that work *in vivo* is that it is very difficult to rationally design things that actually work the exact way you design them," says Weissleder. For *in vivo* agents, factors including pharmacokinetics, clearance rates, distribution and pH-sensitivity have to be taken into account and are often difficult to predict. Weissleder's center has decided to let biology sort it out by taking compound library screening *in vivo* in their search for new imaging agents. But there are some basic properties of fluorescent probes that have emerged as being important for *in vivo* imaging.

"Operating in the near-infrared range of the spectrum greatly reduces autofluorescence," notes Wael Yared, chief technology officer at VisEn Medical. VisEn offers researchers near-infrared fluorochrome labels with emission maxima at 690 and 773 nanometers for small-molecule and ligand coupling, along with targeted near-infrared fluorescent probes for looking at integrins, cathepsin activity and metalloprotease activity. Evrogen recently announced a new generation of the monomeric far-red fluorescent protein TagFP635, called mKate2, which can be used to label

proteins for detection in living tissues. And LI-COR offers near-infrared dyes for labeling proteins, nucleic acids and antibodies along with bone-tagging dyes.

For PET, the probe situation is much more complex. "PET probe development requires a committed radiochemist along with a commitment to performing associated validation and biology experiments," says Silva. For this reason he says that the number of PET probes generally available is quite limited. Still, one of Silva's hopes is that this need for new and improved PET probes to enhance translational research will lead to better relationships between the academic and pharmaceutical worlds. "[Academics] often have the probe development expertise and we have the therapeutics, models and instruments to validate their probes," he notes.

***In vivo* molecular imaging has turned the corner, emerging as a preclinical research tool in many labs around the world, which, according to Oldfield, is an important development because some biological events might be best studied using *in vivo* molecular imaging. "Watching processes such as the engraftment pattern of stem cells is visual to the point where it really draws scientists in." See [Table 1](#).**