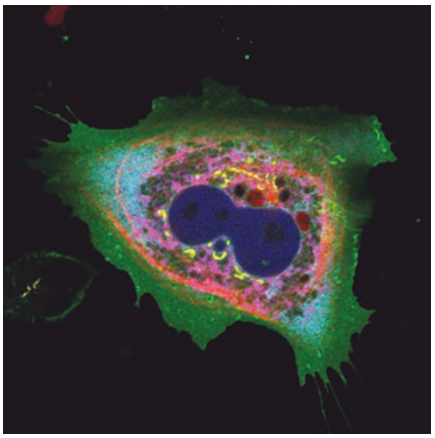
Helping cells to tell a colorful tale

A new generation of fluorescent reagents offers researchers unprecedented visual access to the inner workings of cells—and even live animals. Michael Eisenstein examines the latest tools for live-cell and *in vivo* optical imaging.

Although it was first identified in the early 1960s, it was not until 1992 that scientists finally cloned the gene encoding GFP from the jellyfish *Aequoria victoria*. After that, however, researchers quickly recognized the value of GFP both as a reporter of gene activity and, when fused to a protein of interest, as an effective label for monitoring synthesis, localization and degradation. In particular, GFP represented a considerable step forward from existing fluorescent dyes, which were bright but nonspecific unless conjugated to an antibody or other targeted ligand. Today, the original GFP is still available from Invitrogen and GE Healthcare, and remains a popular option for live-cell imaging.

But also there has been an explosion of new options as researchers have devoted considerable energy to isolating new fluorescent proteins from other marine organisms. Clontech offers a variety of alternatives with their Living Colors family of fluorescent proteins. "There are seven fluorescent proteins, two of them monomers—the monomeric DsRed and AcGFP—and the others are tetrameric or dimeric," says Michael Haugwitz, associate director of cell biology at Clontech. "And they cover an emission spectrum from 489 to 618 nanometers." Obligate multimerization is a consistent problem with newly isolated fluorescent proteins, requiring the engineering of monomeric variants if the proteins are intended for use as fusion tags. For example, their monomeric DsRed was derived from a tetrameric red fluorescent protein found in the coral *Discoma* sp. Conversely, the multimeric versions remain useful for nonfusion applications. "The tetrameric proteins are all extremely bright—especially the cyan and green—and really good to use as a readout for promoter activity," says Haugwitz.



African green monkey kidney (Vero) cell labeled with six different fluorescent proteins. (Courtesy of A. Miyawaki.)

Not all fluorescent proteins are simply 'on' all the time, and Atsushi Miyawaki's group at the RIKEN Brain Science Institute has identified several fluorescent proteins capable of changing color in response to certain excitation wavelengths. The first was Kaede, a coral-derived protein that irreversibly shifts from bright green to red fluorescence after exposure to ultraviolet or violet light. Miyawaki's team subsequently isolated other proteins with similar properties, such as Kikume Green-Red1, a brighter and more efficiently converted alternative to Kaede, and Dronpa, a coral protein whose green fluorescence can be rapidly and reversibly switched on and off by exposure to different wavelengths. These proteins have already proven useful for applications involving the marking of specific cells or tracking the localization behavior of proteins, and Miyawaki has made these and other fluorescent proteins commercially available through MBL International, as part of the CoralHue line.

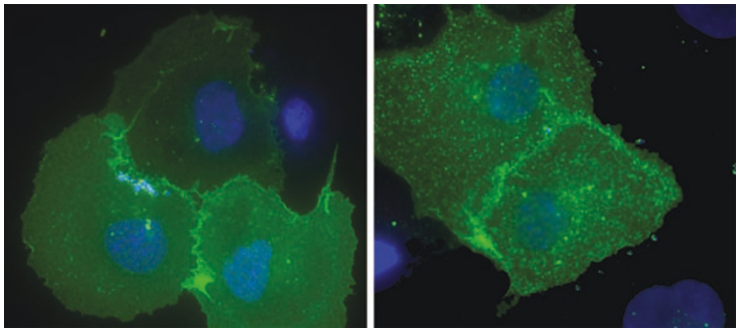
Sergey Lukyanov, of the Russian Academy of Sciences, has also worked to expand perceptions of what can be done with fluorescent proteins. His group recently isolated Dendra, a photostable monomeric fluorescent protein that undergoes a strong green-to-red shift in response to visible blue light, bypassing the need for potentially cytotoxic ultraviolet light. Lukyanov's team has also engineered fluorescent proteins with more specialized functions, such as KillerRed, which generates destructive reactive oxygen species upon photoactivation, allowing targeted cell-killing or inactivation of fusion proteins. More recently, the group described HyPer, a yellow fluorescent protein derivative that shows a shift in its excitation peak in response to environmental hydrogen peroxide. All of these proteins are available through the company Evrogen, of which Lukyanov is the scientific director.

A variety of other promising reagents are also waiting to make their way to the market (**Box 1**), and the demand for new fluorescent proteins continues to grow. Many researchers anticipate the generation of more proteins that, like HyPer or the calcium-detecting 'cameleons' developed by Miyawaki and Roger Tsien, provide real-time indicators for specific cellular processes. "Sensors of different colors and characteristics of their responses will be especially useful for simultaneous monitoring and correlation of several different events," concludes Lukyanov. Haugwitz also highlights a more pragmatic goal—engineering more monomeric variants from existing multimers. "Every time that you add a [monomer] to the portfolio," he says, "you also increase the number of proteins that you can use in the same experiment for multiplexing."

Pin the tag on the protein

Unfortunately, sometimes it simply is not practical to express a target protein as a GFP fusion. Besides adding considerable bulk—GFP adds a mass of 28 kilodaltons—fusions are often limited by delayed maturation and increased risk of misfolding or aggregation. Several researchers have therefore designed alternative strategies for live-cell labeling, working with genetically encoded fusion tags that are not themselves fluorescent but act as targets for labeling with chemical probes that incorporate fluorophores or other functional groups.

The first such system was developed in Roger Tsien's lab at the University of California–San Diego in 1998, based on the reaction of biarsenical dyes with a short peptide tag containing four cysteine residues, the tetracysteine motif. These dyes, FlAsH and ReAsH, are membrane-permeable, allowing labeling of intracellular targets, and fluorogenic, so no fluorescence is produced until the probe reacts with the tetracysteine. At the same time, there are also certain liabilities for this system, most notably issues relating to excessive background. FlAsH and ReAsH are currently available from Invitrogen, who have refined the system to address these concerns. "The new generation—FlAsH and ReAsH II, we call it—allows you to drop down that background so you get a much better signal-to-noise ratio," explains Brett Williams, business segment director for imaging and



Selective labeling of SNAP-tagged NK1 receptor in COS-7 cells, with a non–membrane permeable substrate (green; left). When these cells have are treated with Substance P, the tag makes it possible to visualize the endocytosed receptor (right). Nuclei counterstained with Hoechst 33342–blue. (Courtesy of Covalys.)

BOX 1 NOT AVAILABLE IN STORES—YET

Although a diverse array of fluorescent proteins can now be readily purchased with a few clicks of a mouse button, a number of promising reagents have not yet made it to the pages of any catalog.

The cloning of DsRed represented an important expansion of the fluorescence toolbox, but DsRed also suffers from some fundamental limitations. "The problem was that it was an obligate tetramer," explains Roger Tsien. "It was also slow to mature, a significant fraction stayed in an immature form, and there was just that one long-wavelength color." The subsequent development of a monomeric variant resolved some of these issues, but Tsien remained interested in pushing the potential of this protein. His group embarked on an extensive series of *in vitro* evolution experiments, generating a virtual fruit-basket of fluorescent proteins with emission spectra ranging from yellow-green (mHoneydew) to deep red (mCherry)[5]. Each of these addresses some limitations of previous DsRed derivatives, although tradeoffs are also required—for example, tdTomato offers high quantum yield but is a bulky tandem dimer, whereas mCherry matures rapidly and bleaches slowly but is less bright. Tsien's team subsequently expanded this collection further with the generation of mPlum, a monomeric protein that fluoresces in the far-red range[6].

Los Alamos National Laboratory investigators Geoff Waldo, Thomas Terwilliger and their colleagues also used directed evolution to tackle a major problem with existing fluorescent proteins. "We noticed that a lot of people at conferences had tried using GFP as a fusion reporter, and probably half the time it didn't fluoresce very well, regardless of how well-expressed it was," says Waldo. "[So] we tried to come up with a version of GFP that folded regardless of how poorly folded the fusion partner was." The resulting 'superfolder' contains mutations that confer greatly improved folding kinetics, producing fluorescent protein even when fused to a poorly soluble or insoluble partner, as well as enhanced tolerance for random mutation, which could prove invaluable for the engineering of more robust fluorescent protein functional variants[7]. Waldo and Terwilliger also collaborated with Stéphanie Cabantous in the engineering of a split-GFP system for detecting soluble proteins; in this system, the GFP chromophore is only capable of maturation and fluorescence after the assembly of two independently soluble and stable fragments[8]. These split-protein detectors work well for imaging protein colocalization and interaction, but Waldo sees this as only a first step: "I envision split GFPs that can be used to monitor three, four or five interacting proteins, and we're working in that area."

Since publishing their work, both groups have faced the challenge of dealing with overwhelming demand while struggling to get their inventions to the market, a process complicated by lengthy licensing negotiations and a complex intellectual property landscape. "At this point, we have more than several hundred requests for split GFP, which is more than we could possibly distribute alone," says Waldo. Tsien is likewise frustrated by ongoing delays in the commercialization of his group's fluorescent proteins. "We would desperately like to have it licensed," he says. "We have supplied about 3,000 other labs because of intense demand, but we don't want to charge for it. It's a very unsatisfactory state of affairs—it distracts my staff from their major duties, and we aren't in a position to provide detailed technical support."

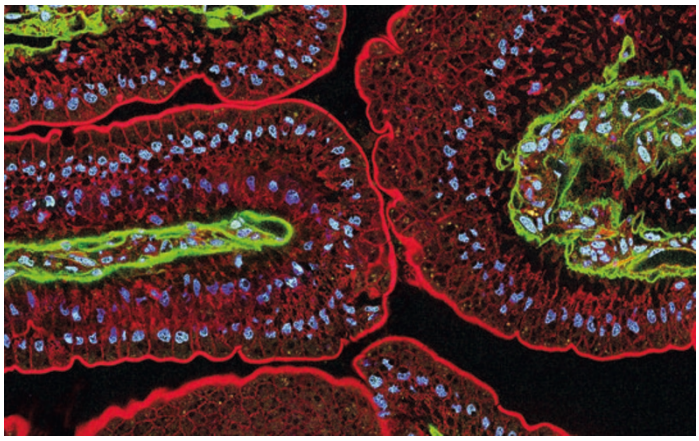
Both groups are happy about the interest generated by their inventions, but also hope to be relieved of the burdens of distribution so that more users can get access—and so that they can continue to work at innovating.

microscopy at Invitrogen. Tsien has also continued to develop this system, and his group recently described optimized tag sequences, which considerably enhance FlAsH and ReAsH fluorescence[1].

Two alternative approaches were recently developed by Kai Johnsson and colleagues at Ecole Polytechnique Fédérale de Lausanne, both of which are available through Covalys, a company cofounded by Johnsson. The SNAP tag is derived from the enzyme alkylguanine transferase (AGT), which reacts with benzylguanine-conjugated compounds to form a covalent linkage, allowing rapid and irreversible labeling of AGT-fusion proteins. "You can do labeling in a pulse-chase methodology, you can do selective labeling just on the outside of a cell or, with a little more effort, just on the inside of a cell," explains Covalys CTO Andreas Brecht. The SNAP tag is compatible with a diverse array of probes and target proteins, but is somewhat limited by the size of the tag, which is slightly smaller than GFP. To address this, Covalys will soon offer a complementary tagging system based on the smaller (~8 kDa) acyl carrier protein (ACP) tag. In this system, which is restricted to cell-surface proteins, modified coenzyme A–based probes are covalently linked to ACP-tagged targets by an engineered synthase enzyme. In addition to its more manageable tag size, the system also benefits from multiplexing capabilities. "You can have different synthase enzymes and different [ACPs]," says Brecht, "so you can go for multiple-color labeling."

With Promega's HaloTag system, the labeling activity is incorporated into the tag itself, which is derived from a prokaryotic hydrolase. The modified enzyme forms a covalent bond with chloroalkane-conjugated ligands and is compatible with a wide variety of labels for both intra- and extracellular use. "Using the same construct, we can assign different colors—red, green or blue—or environmentally sensitive dyes like pH sensors, all using the same construct," explains Georgyi Los, Promega's imaging group leader. Premade functional probes are available, but the company also offers kits for users to generate more specialized HaloTag substrates. The HaloTag is also large—33 kDa—but appears to be compatible with a wide variety of targets. "We have generated a number of fusions, including soluble proteins, ion channels [and] GPCRs," says Los, "and all of them are functional if your design is appropriate."

These commercially available systems are growing in popularity, but also illustrate an important compromise. "What you generally see is a trade-off between specificity and tag size," says Alice Ting of the Massachusetts Institute of Technology (MIT), who recently published an inventive alternative for live-cell labeling. "I thought, maybe the only way you can avoid that trade-off is if you bring in specificity through some different mechanism—and that's when I thought about using enzymes to mediate the labeling." Her team recently demonstrated the use of the *Escherichia coli* biotin ligase BirA to mediate the specific ligation of a ketone analog of biotin to cell-surface proteins tagged with a 15-residue



Multiplex imaging with QDots. Mouse intestine section immunostained with Qdot 655-conjugated antibodies against actin (red), and Qdot 525-conjugated antibodies against laminin (green). Nuclei counterstained with Hoechst 33342 (blue). (Courtesy of Molecular Probes; Invitrogen.)

BirA substrate[2]. The introduced ketone can then be selectively modified with hydrazide- or hydroxylamine-functionalized probes. This system combines small tag size with the high sequence specificity of an enzyme, although the ligase's strong preference for biotin has also posed a challenge, as BirA is incompatible with non-biotin-like substrates. Ting's team is now exploring the use of other enzymes as well as biotin ligases from other species, which could allow the use of more diverse probes.

These labeling platforms continue to improve with each new iteration, but according to Ting, the biggest refinement—getting past reliance on artificial genetic constructs for tagging—is still over the horizon. "Ultimately, we want to be able to label proteins that are endogenous and not recombinant," she says. "That's one of our Holy Grails."

Crystal-clear imaging

When Paul Alivisatos first began presenting his methods for improving the efficiency of semiconductor nanocrystal fluorescence in the mid-1990s, biophysicist Shimon Weiss quickly recognized the importance of his findings. "It was very clear to me that here we had a system that would allow me to do what I want to do, which was to excite many colors with one laser," recalls Weiss, who is now at the University of California–Los Angeles.

Shortly afterward, Weiss and Alivisatos teamed up with Marcel Bruchez and other researchers to launch the Quantum Dot Corporation (QDC), through which they made available their QDots, cadmium-selenium nanocrystals optimized with a specialized coating for water-solubility and biocompatibility. QDots and their successors quickly captured the research community's attention for several reasons—broad excitation and narrow emission spectra, which make them appealing for multiplexing, astonishing brightness, size-based tunability across a broad spectral range and resistance to photobleaching, among other benefits. Even balanced against their liabilities, which include relatively large size and the necessity of conjugating targeting factors to establish specificity, quantum dots are gaining acceptance as a powerful reagent for live-cell imaging applications.

QDC has since been integrated into Invitrogen's Molecular Probes division, which offers the full range of QDot products. According to Vicki Singer, business segment director for labeling and detection at Invitrogen, their top priorities include developing more targeted QDot-based reagents and expanding their potential for *in vivo* imaging. In this regard, Invitrogen's recent acquisition of quantum dot manufacturer BioPixel has proven beneficial. "With BioPixel, one of the things we acquired is a very nice coating technology," says Singer. "It's thinner and [we are] able to make smaller particles at any given wavelength... and we've made very small dots with this coating at a new wavelength that we're not currently selling, around 620 nanometers." These nanoparticles are now being tested in collaboration with Signalomics as potential tumor imaging agents.

Evident Technologies also has their eye on *in vivo* applications for quantum dots, and their EviDot nanocrystals were designed with this in mind. "We use a lipid coating as one of our coating technologies, which is a little more conducive to *in vivo* applications," says CEO Clint

BOX 2 FIT FOR INTERNAL USE?

An ongoing controversy and mystery regarding quantum dots relates to their safety for biological use—or potential clinical applications. The issue revolves in part around the use of cadmium, a highly toxic heavy metal, although the actual risk posed by the coated nanocrystals typically used in biological applications remains unclear.

"There is a lot of misconception in the literature," says Weiss, "because people try different quantum dots with different compositions and different coatings. One might expect that these would all behave differently." But he adds, "the point is—we don't know, and a very systematic study needs to be done." Invitrogen plans to embark on a large-scale toxicology analysis of quantum dots in the near future to address this issue, according to Singer. "We're starting with the cadmium selenide materials, and we have batches of those materials that we will put through a full toxicological study." At the same time, she adds, Invitrogen is also actively investigating noncadmium alternatives. Evident is also working with alternative nanocrystal composites, but the long-term biosafety of these materials for *in vivo* use also remains unclear.

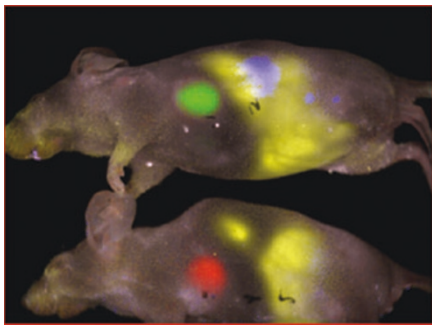
The debate over toxicity in humans may have little impact on the effective use of traditional quantum dots for small-animal imaging studies, but Evident's Ballinger also points out that there may be sound business reasons for making the move away from cadmium as well, pointing out that Japan and the European Union have already moved to restrict the import and use of cadmium in some contexts out of environmental safety concerns. "Scientifically, whether or not it's going to be toxic is almost a moot point," says Ballinger.

Ballinger. "We also have wavelengths that work really well for *in vivo* [applications], longer wavelengths that tend to penetrate tissue." EviDots are based on indium gallium phosphide nanocrystals, which exhibit strong fluorescence but allow researchers to avoid using cadmium, a compound associated with potential quantum-dot toxicity (**Box 2**). Further improvements will be seen in Evident's soon-to-be-released MP EviDots, which have been designed to fluoresce more brightly and exhibit greater target specificity.

Even as quantum dots grow in popularity, other promising nanoparticles are also emerging as potentially complementary imaging tools. Among the brightest possibilities—literally and figuratively—are the gold and silver nanoclusters recently described by Georgia Institute of Technology investigator Robert Dickson and his colleagues[3]. These particles are strongly fluorescent and tiny—each consists of only a handful of metal atoms encapsulated by dendrimers—raising the possibility of monovalent linkage to target molecules. The gold clusters exhibit the same size tunability as quantum dots, although they are less bright, and Dickson suggests they may prove useful for fluorescence resonance energy transfer (FRET) assays. In contrast, the silver particles are considerably brighter than quantum dots and feature especially narrow excitation spectra. Dickson is now exploring the biological potential of these nanoclusters, which were recently exclusively licensed by Invitrogen's Molecular Probes division for future development. "We have had some success at getting silver nanoclusters into cells," says Dickson, "and this is a very active area of research in my lab... but there is still much to do."

From molecules to mice

In recent years, fluorescence imaging has moved beyond single cells to offer a valuable complement to established platforms for whole-animal imaging. "What you get with computed tomography and magnetic resonance is exquisite resolution of anatomical features, but what you do not get with these modalities is molecular information." explains Wael Yared, vice president of imaging systems at VisEn Medical. "The biggest strength of optical imaging is the availability of targeted molecular probes that can go after key biological processes."



CRi's Maestro system allows multiplexed fluorescent imaging. Composite image shows mice injected with fluorescent dyes FITC (green), TRITC (blue), and Cy3.5 (red), as well as fluorescence from food (yellow). (Image reprinted from ref. 4 with permission from Wiley.)

BOX 3 SEEING (PAST) RED

Imaging deep within an animal takes more than just a bright fluorophore—it also requires compounds that are excited by and emit at the NIR wavelengths required for deep-tissue penetration. "Unless you're at 700 or 750 nm or beyond, you're limited to a centimeter at most," explains Levenson. There are several infrared- and NIR-fluorescing chemical dyes presently available for use as contrast reagents or for the synthesis of *in vivo* molecular probes, such as LI-COR Biosciences' IRDyes or VisEn Medical's VivoTag fluorochromes.

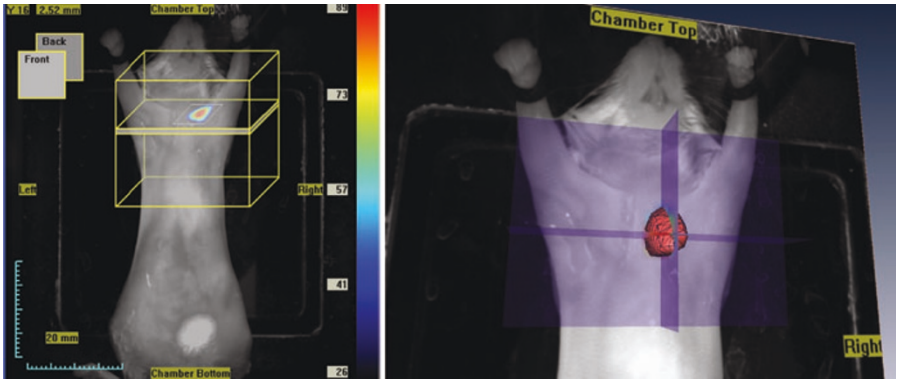
In contrast, engineering similarly redshifted fluorescent proteins has proven an onerous task; independent work from Tsien and Lukyanov has produced proteins that fluoresce well into the far-red, but these tend to be dim. Tsien believes that there are fundamental limitations on what existing fluorescent protein chromophores can achieve: "The mutagenesis gets harder and harder... and there's a limit to how much you can push the wavelength without changing the chromophore structure." His group's mPlum protein[6] has an emission maximum in the far-red, but requires shorter wavelengths for excitation—a fundamental limitation for *in vivo* work. Clontech's Haugwitz concludes, "I think that it might be easier to try to find endogenous fluorescent proteins in other organisms that are farther-shifted."

In this regard, quantum dots appear to hold more promise because of their brightness, tunability and resistance to bleaching. Unfortunately, standard cadmium-selenium dots are incapable of achieving NIR fluorescence, forcing researchers to explore alternative materials. Promising work in this regard has involved the development by MIT's Moungi Bawendi of so-called Type II quantum dots, which feature alternative core-shell architectures (for example, a cadmium-tellurium core with a cadmium-selenium shell) and are capable of NIR emission—in preliminary studies, these proved effective for mapping sentinal lymph nodes in live mice[9]. Shuming Nie's group at Emory University has likewise been experimenting with alloyed cadmium-tellurium-selenium semiconductors, which are tunable by composition rather than by size, and also operate in the NIR spectrum[10].

Kodak has an established track record in biological imaging, and their recently launched Image Station In-Vivo FX bridges the gap between optical and isotopic imaging in live animals. Users can image animals with fluorophores from across a broad spectral range, from the visible to the near-infrared (NIR); this fluorescence imaging data then can be correlated with radiographic imaging performed with an X-ray module. By combining these imaging modalities, it becomes possible to achieve extremely detailed anatomic localization of fluorescent markers.

Olympus America is likewise hardly a newcomer to microscopy, although the newly launched OV100 and IV100 systems represent their first foray into *in vivo* imaging. The OV100 benefits from innovative macro lenses with optimized light-gathering capabilities. "They've been designed to have a long working distance so that you can get an animal under the optics," says Angela Goodacre, group manager of applications systems at Olympus. "And in line with our other UIS2 microscope objectives, the glass and coatings have been optimized to go out to the near-infrared region," Conversely, the laser-scanning IV100 is designed for intravital imaging within the body cavity, and takes advantage of a set of specially designed microprobe lenses. The IV100 is outfitted with a set of lasers whose excitation capabilities span from green (488 nm) to NIR (748 nm), and it can be used to image three wavelengths simultaneously.

The Maestro In-Vivo Imaging System from Cambridge Research & Instrumentation (CRi) also allows simultaneous imaging at multiple spectra, with a tunable filter that can operate at wavelengths from 500 nm to 950 nm. Skin autofluorescence is a major obstacle to imaging at visible-light wavelengths, the Maestro features analytical software that allows users to detect and eliminate autofluorescence noise, and to accurately resolve fluorescence signals from spectrally similar fluorophores. Maestro's algorithm performs spectral unmixing procedures that take into account such factors as the tissue being imaged and its depth



VisEn Medical FMT V3.0 tomographic reconstruction of 4T1 lung metastasis with VisEn Medical ProSense bioprobe; axial slice (left) and 3D rendering (right). (Courtesy of VisEn Medical.)

within the animal, which can dramatically impact fluorescence detection—especially with easily scattered and absorbed photons from the visible range. "You can take a mouse that you know nothing about, stick it in the box and press a button, and it will tell you about how many spectral entities there are, calculate the spectra and unmix each signal into a different channel," says Richard Levenson, director of research for biomedical systems at CRi. "In fact, it can also go ahead and measure the signals that you get."

Accurate quantitation is a major selling point of VisEn Medical's Fluorescence Molecular Tomography (FMT) platform, which is based on the research of founder Ralph Weissleder, director of the Center for Molecular Imaging Research at Massachusetts General Hospital. Rather than using the planar epifluorescence approach employed by other systems, FMT uses transillumination—illuminating through the entire animal to capture fluorescence at any depth—at multiple near-infrared wavelengths to rapidly collect large data sets of measurements that are then processed by a tomographic reconstruction algorithm. This algorithm takes into account the optical properties of the tissue being imaged and of the probe being used. "The output is basically the three-dimensional distribution of fluorescence *in vivo*," says Yared. "What you get is a fully calibrated, physically meaningful readout of fluorochrome concentration in a way that makes sense to the end user."

The primary obstacle to effective *in vivo* imaging has now become the development of far-red and NIR reagents that can overcome the unique obstacles posed by living tissue (**Box 3**), and the hope is that advances in imaging agent development could help make this imaging technology human-friendly as well. "I think the strong hope is that in the future, optical molecular imaging will make the transition into the clinic," says Yared.

This would be an important shift for fluorescence imaging, and the idea that whole-body imaging—in animals and humans—might one day approach the resolution currently attainable by cell biologists is an appealing one. In the meantime, a growing number of researchers and companies will continue to pour their resources into the development of fluorescent reagents that promise to effectively integrate brightness and functional versatility for the robust illumination of biological processes.

Michael Eisenstein is technology editor for *Nature* and *Nature Methods*.

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SUPPLIERS GUIDE: COMPANIES OFFERING TOOLS FOR LIVE-CELL OR *IN VIVO* FLUORESCENT IMAGING

Company	Web address
Active Motif	http://www.activemotif.com
A.G. Scientific	http://www.agscientific.com
Amaxa	http://www.amaxa.com
American Dye Source	http://www.adsdyes.com
AnaSpec	http://www.anaspec.com
Applied Precision	http://www.api.com
BD Pharmingen	http://www.bdbiosciences.com/pharmingen
Biocat	http://www.biocat.de
BioPAL	http://www.biopal.com
Biotium	http://www.biotium.com
Cambridge Research & Instrumentation (CRi)	http://www.cri-inc.com
Carl Zeiss, Inc.	http://www.zeiss.com
Chemicon International	http://www.chemicon.com
Clontech	http://www.clontech.com
Covalys	http://www.covalys.com
Crystalplex	http://www.crystalplex.com
Dyomics	http://www.dyomics.com
Echelon Biosciences	http://www.echelon-inc.com
Evident Technologies	http://www.evidenttech.com
Evrogen	http://www.evrogen.com
GE Healthcare	http://www.amershambiosciences.com
Hamamatsu	http://www.hamamatsu.com
Immunochemistry Technologies, LLC	http://www.immunochemistry.com
Invitrogen/Molecular Probes	http://www.invitrogen.com
Kodak	http://www.kodak.com
Kreatech Biotechnology	http://www.kreatech.com
Leica Microsystems	http://www.leica-microsystems.com
LI-COR Biosciences	http://www.licor.com/bio
Lightools	http://www.lightools.com
LUX Biotechnology	http://www.luxbiotech.com
Mauna Kea Technologies	http://www.maunakeatech.com
MBL International	http://www.mblintl.com
Nanoco Technologies	http://www.nanocotechnologies.com
Nikon	http://www.nikon.com
NN-LABS, LLC	http://www.nn-labs.com
Olympus America	http://www.olympusamerica.com
OncoImmunin	http://www.phiphilux.com
Pierce Biotechnology	http://www.piercenet.com
Polysciences, Inc.	http://www.polysciences.com
Promega	http://www.promega.com
Roche Applied Sciences	http://www.roche-applied-science.com
Sigma-Aldrich	http://www.sigmaaldrich.com
Spherotech	http://www.spherotech.com
StemCell Technologies	http://www.stemcell.com
Stratagene	http://www.stratagene.com
TEF Labs	http://www.teflabs.com
Upstate	http://www.upstate.com
VisEn Medical	http://www.visenmedical.com