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BioProbes 50

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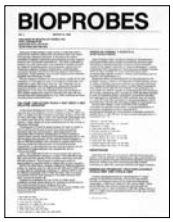
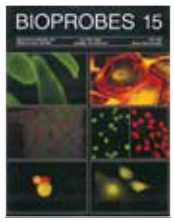
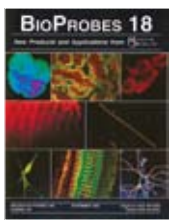
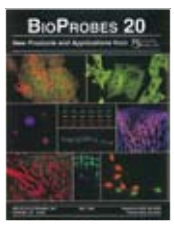


Celebrating 50 issues of *BioProbes*



When Richard Haugland, co-founder of Molecular Probes, pulled the first issue of *BioProbes* from his typewriter in 1979, he already envisioned the path that the company—and by extension, *BioProbes*—would take. As Molecular Probes grew from a garage-based business to eventually become an integral part of Invitrogen, *BioProbes* evolved from black-and-white mimeographed sheets describing a handful of products to a full-color magazine brimming with more than 75 new products and their applications. Throughout this evolution, our commitment to you has remained the same—to bring you the very latest information about Molecular Probes™ products and their applications.





We've reached an important milestone with our 50th issue of *BioProbes*. Thank you for taking the journey with us. Write to us anytime (**bioprobes@invitrogen.com**) and give us your opinion, ask us questions, or tell us what we could be doing better.

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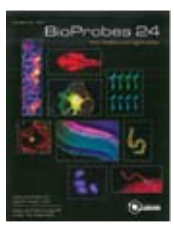

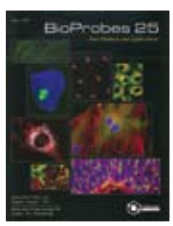

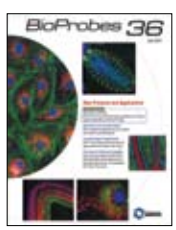

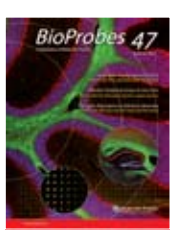

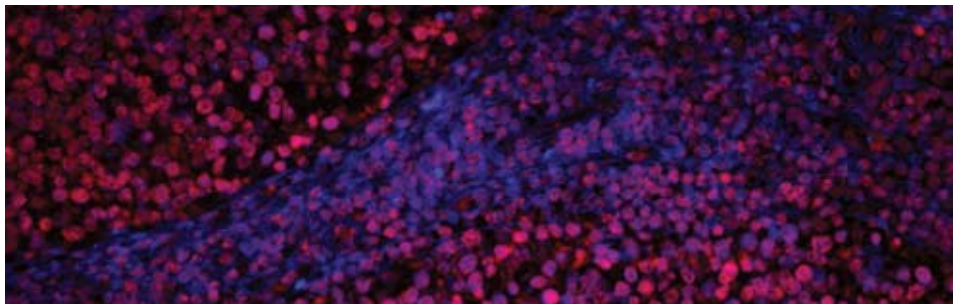

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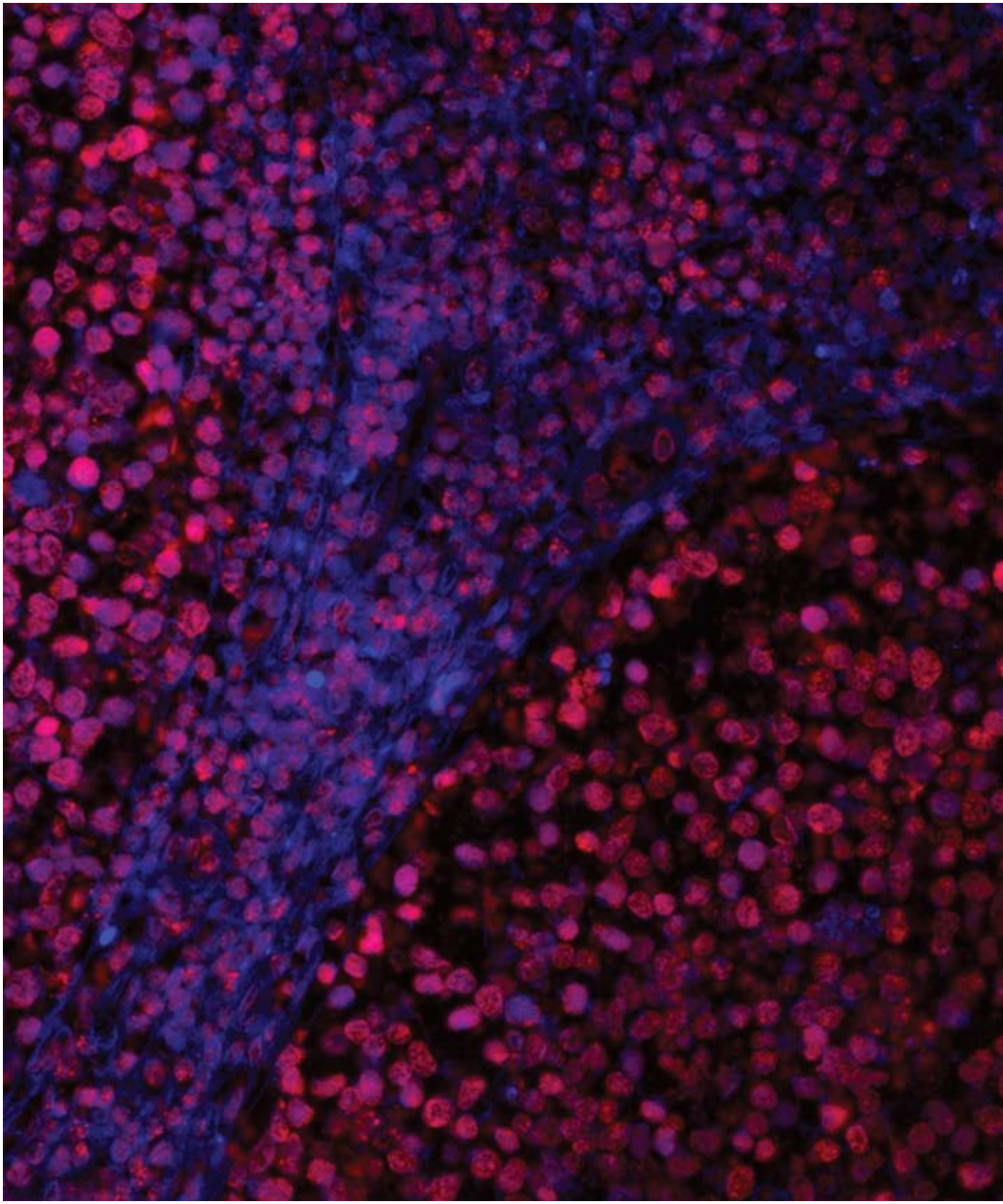

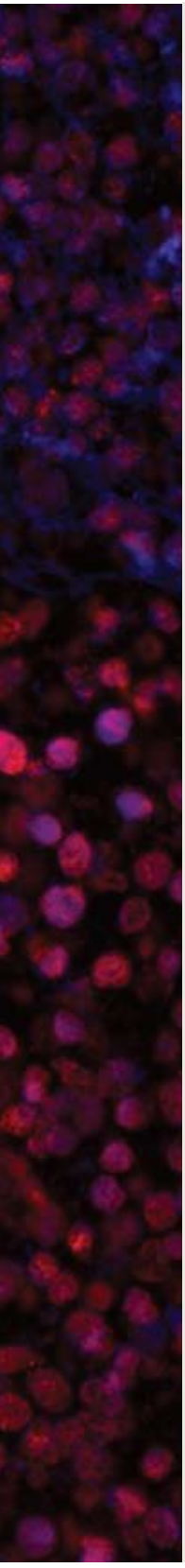

The coming revolution: nanocrystals in biomedical research

THINKING SMALL—IT'S THE NEW BIG.

Nanotechnology means more than miniaturization ad infinitum, more than smaller wheels, smaller levers, and smaller pulleys. The true promise of nanotechnology lies in the ability to manipulate materials on the same unimaginably small scale used by nature. From "goo" (used to describe a mass of microscopic, self-replicating robots) to "SmartDust" (microscopic sensors with onboard communications capability) to "spintronics" (computing devices based on electron spin "bits"), nanotechnological developments promise phenomenal improvements in devices and, presumably, an equally huge impact on the way we live our lives. Such is the definition of a disruptive technology—one that so significantly outperforms the status quo that it promises to consign current technologies to obsolescence.

Perhaps more than any other field of endeavor, biological research stands poised to reap exciting benefits from the advent of these nanomaterials. The language of modern biological understanding is filled with complex enzymatic and hormonal cascades, subtle regulation of gene expression, and the timely orchestration of molecular and cellular occurrences. Semiconductor nanocrystals represent a true nanomaterial with demonstrated promise in revealing this all-important context. At a basic level, nanocrystals are fluorophores—substances that absorb photons of light, then re-emit photons at a different wavelength. However, the similarity to traditional fluorophores, including small organic fluorescent dyes and naturally fluorescent proteins, ends there. →

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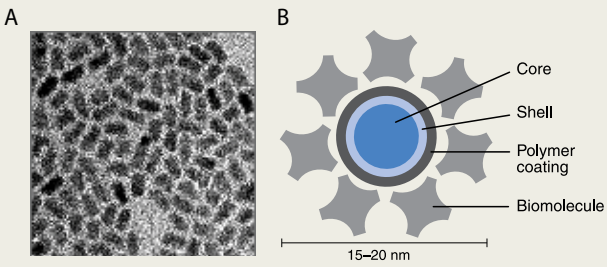

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(A) Transmission electron microscope image of core–shell Qdot® nanocrystals at 200,000x magnification. (B) Schematic of the overall structure of a Qdot® nanocrystal conjugate. The layers represent the distinct structural elements, and are roughly to scale.

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Semiconductor nanocrystals are nanometer-scale particles comprising a core, shell, and coating (Figure 1). The core is made up of a few hundred to a few thousand atoms of a semiconductor material (often cadmium mixed with sulfur, selenium, or tellurium). A semiconductor shell (typically ZnS) coats the core, improving the optical properties of the material. The inner coating is a layer of organic ligands covalently attached to the surface of the shell; the outer coating is a mixed hydrophobic/hydrophilic polymer with carboxylic acid derivatization. Together, the two layers of coating provide exceptional stability, solubility, and a surface to which a variety of biomolecules can be attached.

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Semiconductor nanocrystals are extremely efficient machines for generating fluorescence; their intrinsic brightness is often many times that observed for other classes of fluorophores. Also, nanocrystals fluoresce in a completely different way, without the involvement of π–>π^* electronic transitions. At the heart of the fluorescence of nanocrystals is the formation of excitons, or Coulomb-correlated electron-hole pairs. The exciton is analogous to the excited state of traditional fluorophores; however, excitons typically have much longer lifetimes (up to ~200 ns), a property that can be advantageous in certain types of time-gated detection studies.[1]

Another practical benefit of achieving fluorescence without involving conjugated double-bond systems is that the photostability of semiconductor nanocrystals is many orders of magnitude greater than that associated with traditional fluorescent molecules; this property enables long-term imaging experiments under conditions that would lead to the photo-induced deterioration of other types of fluorophores.[2,3]

Yet another distinction arises from the direct, predictable relationship between the physical size of the nanocrystal and the energy of the exciton[2] (therefore, the wavelength of emitted fluorescence) (Figure 2). This property has been referred to as "tuneability," and is being widely exploited in the development of multicolor nanocrystal-based assays.

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So how, exactly, are these novel properties being translated into a greater understanding of biological processes? Take, for example, a problem as fundamental as cellular signaling.

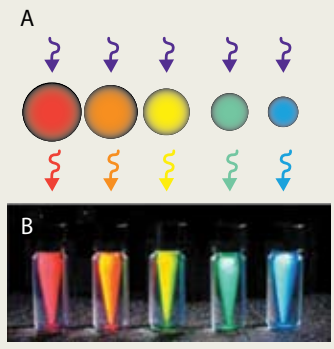

Figure 2—Different colors of nanocrystals

(A) Nanocrystals absorb light and then re-emit the light in a different color; the size of the nanocrystal determines the color. (B) Five different nanocrystal solutions are shown excited with the same long-wavelength UV lamp.

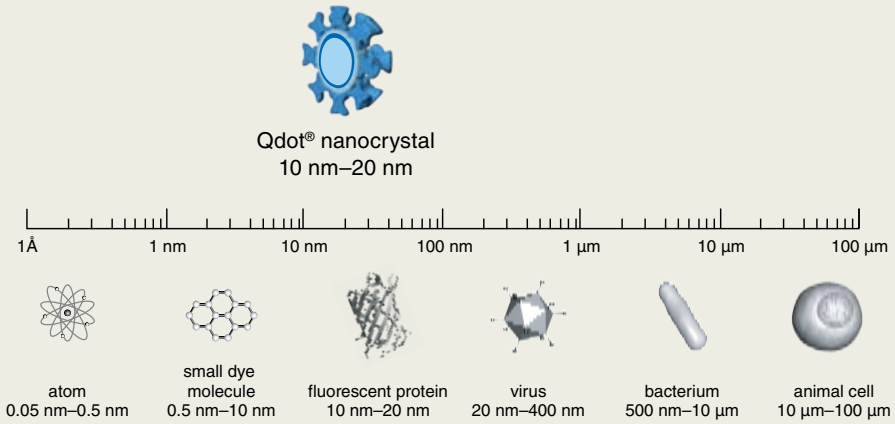

Figure 3—Relative size of Qdot® nanocrystals

Qdot® nanocrystals are protein-sized clusters of semiconductor material.

The ability of cells (and, by extension, multicellular organisms) to respond to chemical signals is central to their survival; similarly, the ability to develop drug therapies that take advantage of the signaling machinery is viewed as vital to the progress of medicine. Visualizing this dynamic, receptor-mediated process in live cells has been hampered by a lack of sensitivity of available reagents, which has in many cases precluded detailed knowledge of the way that a single effector molecule interacts with its receptor. Howarth and colleagues have achieved real-time imaging of the complex formed between a single ligand-labeled nanocrystal and its target receptor molecule on live neurons[4] (for details, see page 7). The authors tracked the movement of individual receptors and succeeded in identifying areas of high and low receptor mobility on the neuronal surface. They attribute the success of this approach in part to the brightness inherent in the nanocrystal particle.

Semiconductor nanocrystals have also shown tremendous promise in the realm of *in vivo* diagnostics.[2,3] Using bioconjugated nanocrystal probes comprising a nanocrystal encapsulated within a polymer coating studded with targeting ligands, Gao and coworkers have achieved detailed multicolor imaging of tumors in living mice.[5] By taking advantage of the long exciton lifetime of nanocrystals (roughly ten times as long as the excited state of organic dyes) through the application of fluorescence lifetime imaging techniques, the authors suggest there may be considerable room for improving the amount and quality of the information retrieved from such an approach. The group further predicts the development of nanocrystals that combine targeting, imaging, and therapeutic agents within a single multifunctional "smart nanostructure" for the rapid and highly specific diagnosis, imaging, and treatment of cancer and other diseases—simultaneously.

the advantages of Qdot® products

By bringing Quantum Dot Corporation into its Molecular Probes labeling and detection group, Invitrogen has demonstrated its commitment to this exciting fluorescence nanotechnology. Qdot® products are designed around the unique optical properties inherent in the nanocrystal structure. These protein-sized particles (Figure 3) provide bright and photostable fluorescence that can be observed for hours, and tissues stained with Qdot® nanocrystals can be archived permanently; re-analysis of archived samples remains as quantitative as it was during the

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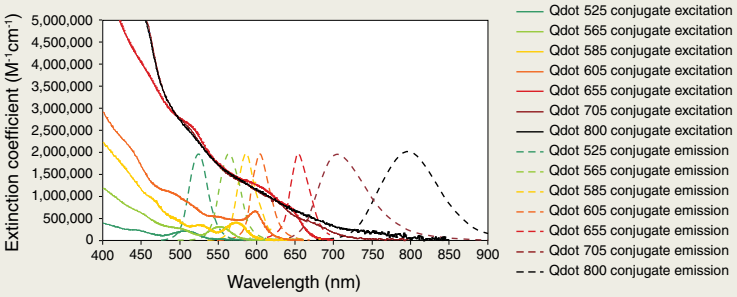

Figure 4—Excitation and emission profiles of Qdot® nanocrystals

Qdot® nanocrystals are characterized by broad excitation spectra and narrow, discrete emission profiles.

first assay. With their broad excitation and narrow emission properties (Figure 4), Qdot® nanocrystals require only a single excitation source,[3] enabling easy multiplex analysis of multiple targets or events in a single sample—simple color filtering can be used to resolve the individual signals. Because these nanocrystals are particle-based fluorophores, they have intrinsic electron and X-ray contrast, delivering powerful multimodality for correlative light and electron microscopy and for imaging studies that utilize both fluorescence and X-ray or CT.

multicolor analysis with Qdot® secondary antibodies

Qdot® secondary antibody conjugates (Table 1) combine the spectral characteristics of Qdot® nanocrystals with the best highly cross-adsorbed secondary antibodies, enabling highly sensitive multicolor analysis (Figure 5) and long-term sample stability in a broad range of applications. Qdot® secondary antibody conjugates are ideal for imaging experiments requiring very long integration to achieve the desired sensitivity (for example, when detecting very low-abundance targets), and for applications that call for prolonged storage and repeated analysis of the specimen.

Table 1—Qdot® secondary antibody conjugates

Product	525 nm	565 nm	585 nm	605 nm	655 nm	705 nm	800 nm
Anti–mouse IgG (200 µL)	Q11041MP	Q11031MP	Q11011MP	Q11001MP	Q11021MP	Q11061MP	Q11071MP
Anti–mouse IgG (100 µL)*		Q11032MP		Q11002MP	Q11022MP	Q11062MP	
Anti–rabbit IgG (200 µL)	Q11441MP	Q11431MP	Q11411MP	Q11401MP	Q11421MP	Q11461MP	Q11471MP
Anti–rabbit IgG (100 µL)*		Q11432MP		Q11402MP	Q11422MP	Q11462MP	
Anti–rat IgG		Q11631MP		Q11601MP	Q11621MP		
Anti–human IgG		Q11231MP		Q11201MP	Q11221MP		
Anti–goat IgG					Q11821MP		
Anti–chicken IgG		Q14431MP			Q14421MP		

*Specifically sized for 10 mini western blots.

Table 2—Qtracker® Cell Labeling Kits*

Product	Quantity	Cat. no.
Qtracker® 525	1 kit	Q25041MP
Qtracker® 565	1 kit	Q25031MP
Qtracker® 585	1 kit	Q25011MP
Qtracker® 605	1 kit	Q25001MP
Qtracker® 655	1 kit	Q25021MP
Qtracker® 705	1 kit	Q25061MP
Qtracker® 800	1 kit	Q25071MP

*Each kit provides sufficient reagents for 100 labelings.

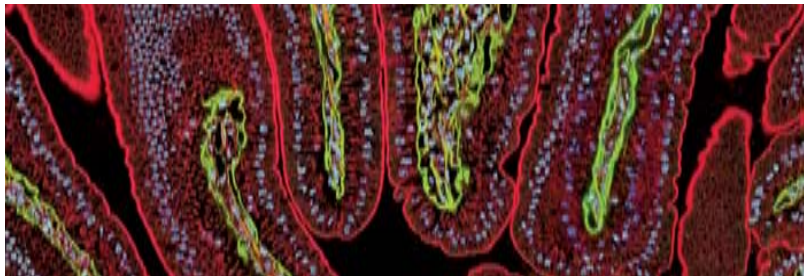

Figure 5—Multicolor fluorescence image of a mouse intestine section

Actin was detected with anti-actin mouse monoclonal antibody and visualized with Qdot® 655 goat F(ab')$_2$ anti–mouse IgG (red), laminin was detected with anti-laminin rabbit polyclonal antibody and visualized with Qdot® 525 goat F(ab')$_2$ anti–rabbit IgG (green), and nuclei were stained with Hoechst 33342 (blue). Image contributed by Thomas Deerinck and Mark Ellisman, The National Center for Microscopy and Imaging Research, San Diego, CA.

JOURNAL HIGHLIGHT

Targeting quantum dots to surface proteins in living cells with biotin ligase

Howarth, M., Takao, K., Hayashi, Y., and Ting, A.Y. (2005) *Proc. Natl. Acad. Sci. U S A* 102:7583

Can nanocrystals be used to track proteins in live cells? Proteins can be specifically labeled with nanocrystals through a three-step method, wherein the protein of interest is bound by a primary antibody, the primary is complexed with a biotinylated secondary antibody, and the complex is visualized with a quantum dot streptavidin conjugate. However, the large size of the resulting label complex can interfere with normal protein function, making it difficult to draw physiologically relevant conclusions in certain types of studies. By first tagging the AMPA receptor—a glutamate-activated ion channel involved in synaptic activity—with a 15-amino acid acceptor peptide (AP), the authors have demonstrated a simple method for directly biotinylating cell surface proteins via the activity of a bacterial biotin ligase enzyme (BirA), thereby allowing direct detection of the biotinylated receptor protein with a quantum dot streptavidin conjugate.

The authors used this method to examine trafficking of the AMPA receptor in live hippocampal neurons. Labeling was detected after only one minute of biotinylation with BirA, suggesting that the labeling method could be useful for detecting rapid events; control experiments demonstrated that labeling was restricted to AP-tagged proteins on the cell surface. The method enabled the observation of receptors labeled with single nanocrystals, allowing the authors to track receptor movement and to identify regions of high and low receptor mobility on the neuronal cell surface. Pulse–chase experiments using an Alexa Fluor® 488 streptavidin conjugate and the quantum dot streptavidin conjugate were used to demonstrate differential trafficking dynamics of AMPA receptors labeled with AP-GluR1 subunits versus AP-GluR2 subunits in response to treatment with glycine.

To view our wide selection of Qdot® and Alexa Fluor® conjugates, visit us at **probes.invitrogen.com**.

→

live-cell labeling with Qtracker® kits

The reagents in the Qtracker® Cell Labeling Kits (Table 2) provide a means to deliver fluorescent Qdot® nanocrystals into live cells by using a custom targeting peptide. Qtracker® Cell Labeling Kits are available with Qdot® nanocrystals in seven brilliant colors—emitting at 525 nm, 565 nm, 585 nm, 605 nm, 655 nm, 705 nm, or 800 nm—and are excellent tools for long-term studies of live cells and tissues, including migration, motility, morphology, and other cell function assays.

Using Qtracker® Cell Labeling Kits, researchers can observe labeled cells using continuous illumination, without photobleaching and degradation problems. Qtracker® labels

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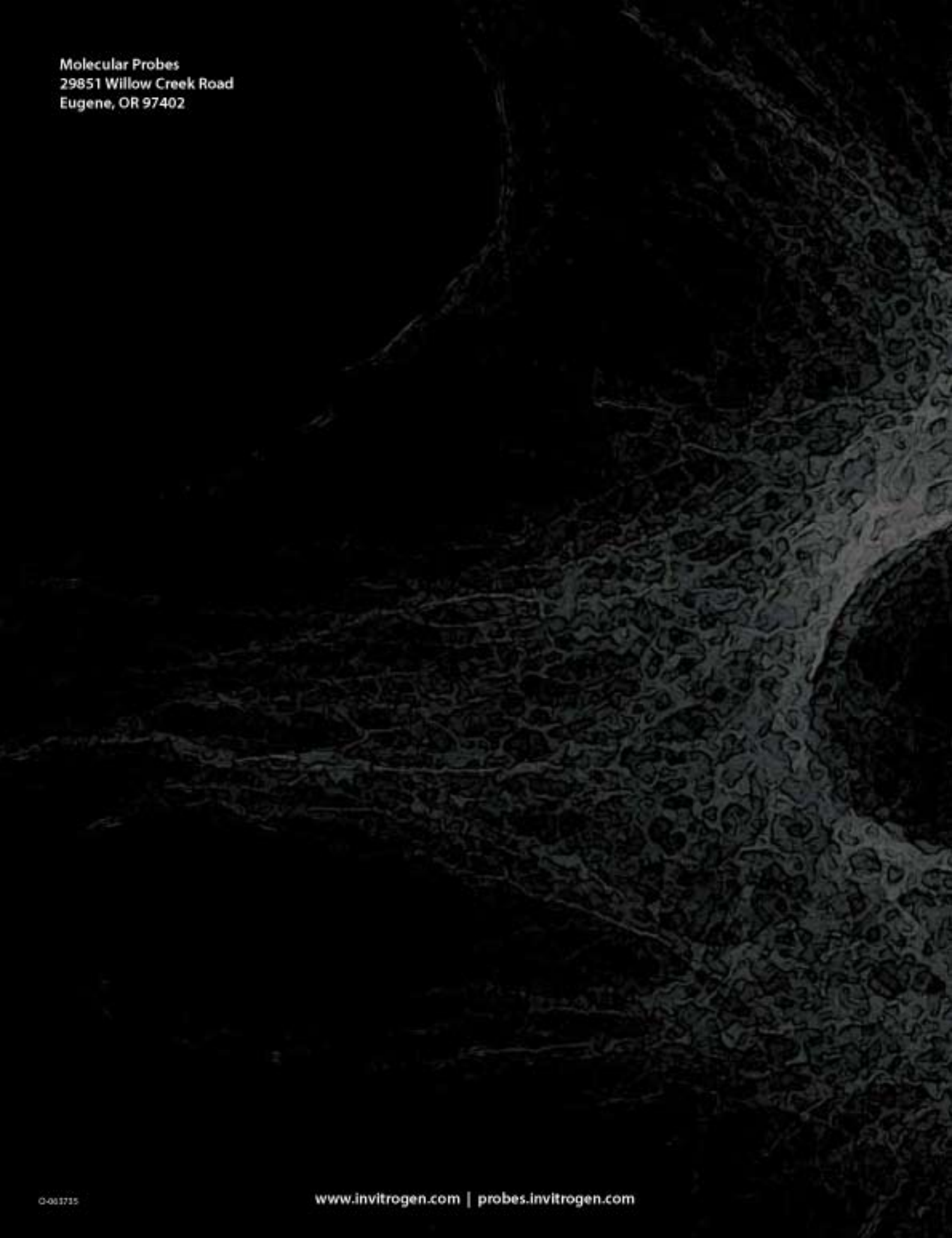

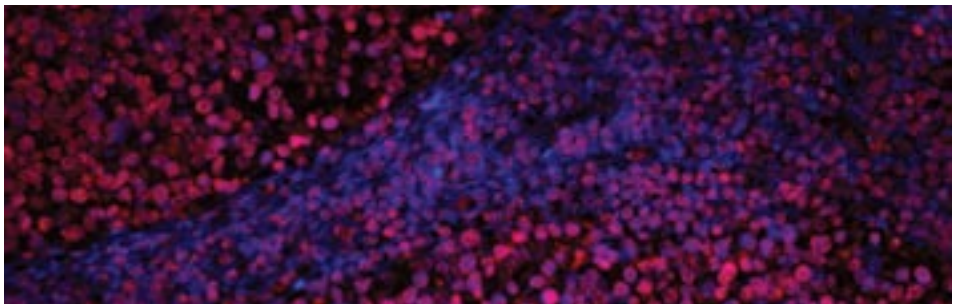

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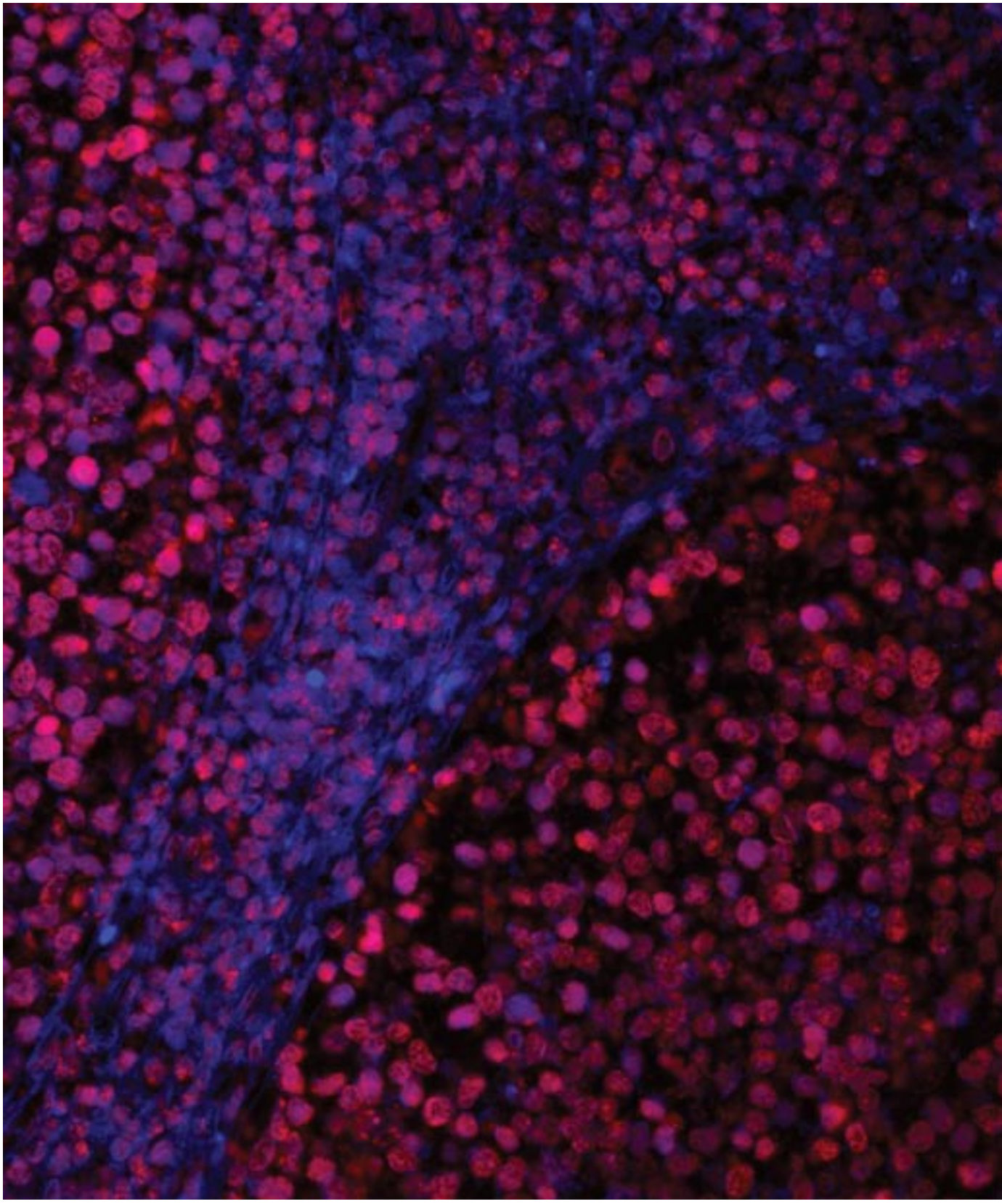

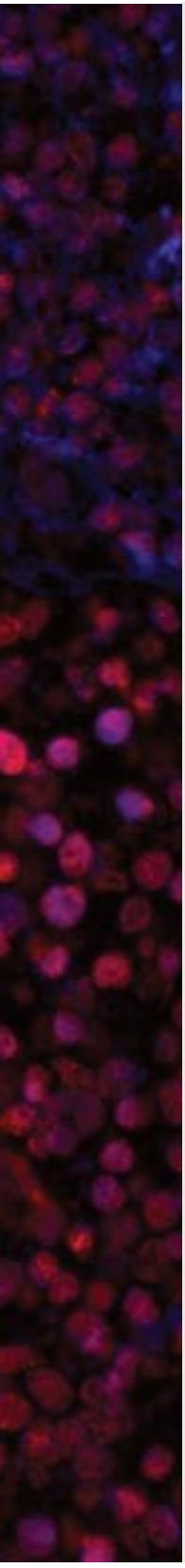

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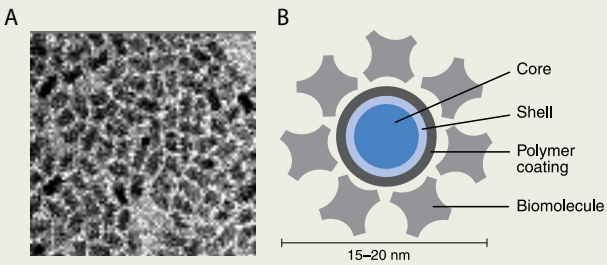

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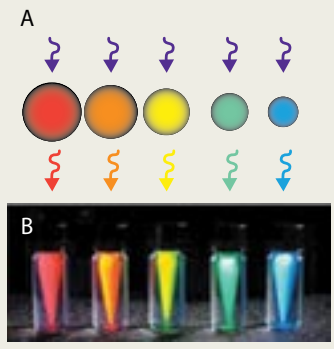
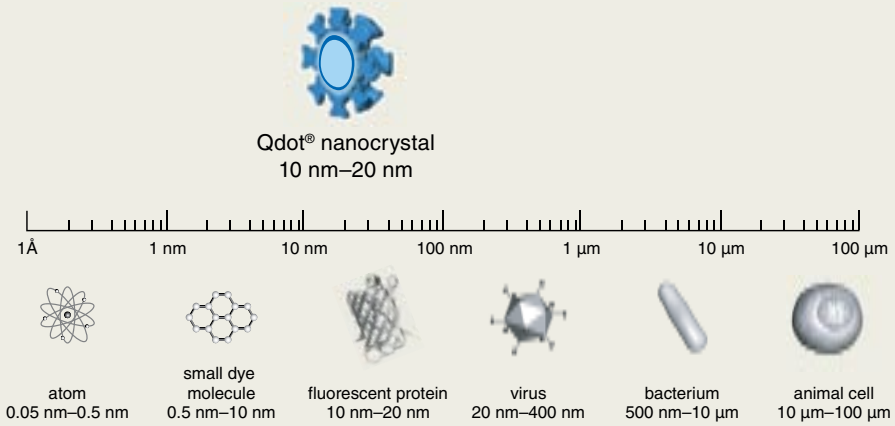


Figure 2—Different colors of nanocrystals

(A) Nanocrystals absorb light and then re-emit the light in a different color; the size of the nanocrystal determines the color. (B) Five different nanocrystal solutions are shown excited with the same long-wavelength UV lamp.



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Qdot® nanocrystals are protein-sized clusters of semiconductor material.

The ability of cells (and, by extension, multicellular organisms) to respond to chemical signals is central to their survival; similarly, the ability to develop drug therapies that take advantage of the signaling machinery is viewed as vital to the progress of medicine. Visualizing this dynamic, receptor-mediated process in live cells has been hampered by a lack of sensitivity of available reagents, which has in many cases precluded detailed knowledge of the way that a single effector molecule interacts with its receptor. Howarth and colleagues have achieved real-time imaging of the complex formed between a single ligand-labeled nanocrystal and its target receptor molecule on live neurons [4] (for details, see page 7). The authors tracked the movement of individual receptors and succeeded in identifying areas of high and low receptor mobility on the neuronal surface. They attribute the success of this approach in part to the brightness inherent in the nanocrystal particle.

Semiconductor nanocrystals have also shown tremendous promise in the realm of *in vivo* diagnostics.[2,3] Using bioconjugated nanocrystal probes comprising a nanocrystal encapsulated within a polymer coating studded with targeting ligands, Gao and coworkers have achieved detailed multicolor imaging of tumors in living mice.[5] By taking advantage of the long exciton lifetime of nanocrystals (roughly ten times as long as the excited state of organic dyes) through the application of fluorescence lifetime imaging techniques, the authors suggest there may be considerable room for improving the amount and quality of the information retrieved from such an approach. The group further predicts the development of nanocrystals that combine targeting, imaging, and therapeutic agents within a single multifunctional "smart nanostructure" for the rapid and highly specific diagnosis, imaging, and treatment of cancer and other diseases—simultaneously.

the advantages of Qdot® products

By bringing Quantum Dot Corporation into its Molecular Probes labeling and detection group, Invitrogen has demonstrated its commitment to this exciting fluorescence nanotechnology. Qdot® products are designed around the unique optical properties inherent in the nanocrystal structure. These protein-sized particles (Figure 3) provide bright and photostable fluorescence that can be observed for hours, and tissues stained with Qdot® nanocrystals can be archived permanently; re-analysis of archived samples remains as quantitative as it was during the

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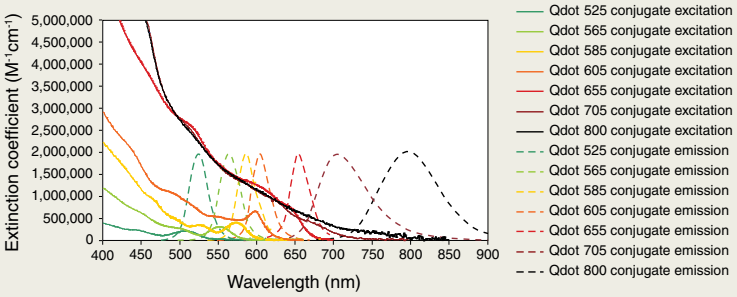

Figure 4—Excitation and emission profiles of Qdot® nanocrystals

Qdot® nanocrystals are characterized by broad excitation spectra and narrow, discrete emission profiles.

first assay. With their broad excitation and narrow emission properties (Figure 4), Qdot® nanocrystals require only a single excitation source,[3] enabling easy multiplex analysis of multiple targets or events in a single sample—simple color filtering can be used to resolve the individual signals. Because these nanocrystals are particle-based fluorophores, they have intrinsic electron and X-ray contrast, delivering powerful multimodality for correlative light and electron microscopy and for imaging studies that utilize both fluorescence and X-ray or CT.

multicolor analysis with Qdot® secondary antibodies

Qdot® secondary antibody conjugates (Table 1) combine the spectral characteristics of Qdot® nanocrystals with the best highly cross-adsorbed secondary antibodies, enabling highly sensitive multicolor analysis (Figure 5) and long-term sample stability in a broad range of applications. Qdot® secondary antibody conjugates are ideal for imaging experiments requiring very long integration to achieve the desired sensitivity (for example, when detecting very low-abundance targets), and for applications that call for prolonged storage and repeated analysis of the specimen.

Table 1—Qdot® secondary antibody conjugates

Product	525 nm	565 nm	585 nm	605 nm	655 nm	705 nm	800 nm
Anti–mouse IgG (200 µL)	Q11041MP	Q11031MP	Q11011MP	Q11001MP	Q11021MP	Q11061MP	Q11071MP
Anti–mouse IgG (100 µL)*		Q11032MP		Q11002MP	Q11022MP	Q11062MP	
Anti–rabbit IgG (200 µL)	Q11441MP	Q11431MP	Q11411MP	Q11401MP	Q11421MP	Q11461MP	Q11471MP
Anti–rabbit IgG (100 µL)*		Q11432MP		Q11402MP	Q11422MP	Q11462MP	
Anti–rat IgG		Q11631MP		Q11601MP	Q11621MP		
Anti–human IgG		Q11231MP		Q11201MP	Q11221MP		
Anti–goat IgG					Q11821MP		
Anti–chicken IgG		Q14431MP			Q14421MP		

*Specifically sized for 10 mini western blots.

Table 2—Qtracker® Cell Labeling Kits*

Product	Quantity	Cat. no.
Qtracker® 525	1 kit	Q25041MP
Qtracker® 565	1 kit	Q25031MP
Qtracker® 585	1 kit	Q25011MP
Qtracker® 605	1 kit	Q25001MP
Qtracker® 655	1 kit	Q25021MP
Qtracker® 705	1 kit	Q25061MP
Qtracker® 800	1 kit	Q25071MP

*Each kit provides sufficient reagents for 100 labelings.

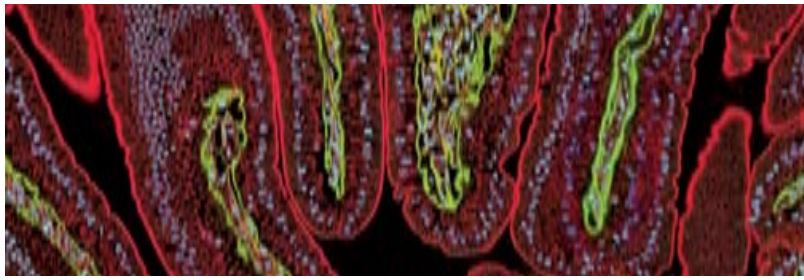

Figure 5—Multicolor fluorescence image of a mouse intestine section

Actin was detected with anti-actin mouse monoclonal antibody and visualized with Qdot® 655 goat F(ab')$_2$ anti–mouse IgG (red), laminin was detected with anti-laminin rabbit polyclonal antibody and visualized with Qdot® 525 goat F(ab')$_2$ anti–rabbit IgG (green), and nuclei were stained with Hoechst 33342 (blue). Image contributed by Thomas Deerinck and Mark Ellisman, The National Center for Microscopy and Imaging Research, San Diego, CA.

JOURNAL HIGHLIGHT

Targeting quantum dots to surface proteins in living cells with biotin ligase

Howarth, M., Takao, K., Hayashi, Y., and Ting, A.Y. (2005) *Proc. Natl. Acad. Sci. U S A* 102:7583

Can nanocrystals be used to track proteins in live cells? Proteins can be specifically labeled with nanocrystals through a three-step method, wherein the protein of interest is bound by a primary antibody, the primary is complexed with a biotinylated secondary antibody, and the complex is visualized with a quantum dot streptavidin conjugate. However, the large size of the resulting label complex can interfere with normal protein function, making it difficult to draw physiologically relevant conclusions in certain types of studies. By first tagging the AMPA receptor—a glutamate-activated ion channel involved in synaptic activity—with a 15-amino acid acceptor peptide (AP), the authors have demonstrated a simple method for directly biotinylating cell surface proteins via the activity of a bacterial biotin ligase enzyme (BirA), thereby allowing direct detection of the biotinylated receptor protein with a quantum dot streptavidin conjugate.

The authors used this method to examine trafficking of the AMPA receptor in live hippocampal neurons. Labeling was detected after only one minute of biotinylation with BirA, suggesting that the labeling method could be useful for detecting rapid events; control experiments demonstrated that labeling was restricted to AP-tagged proteins on the cell surface. The method enabled the observation of receptors labeled with single nanocrystals, allowing the authors to track receptor movement and to identify regions of high and low receptor mobility on the neuronal cell surface. Pulse–chase experiments using an Alexa Fluor® 488 streptavidin conjugate and the quantum dot streptavidin conjugate were used to demonstrate differential trafficking dynamics of AMPA receptors labeled with AP-GluR1 subunits versus AP-GluR2 subunits in response to treatment with glycine.

To view our wide selection of Qdot® and Alexa Fluor® conjugates, visit us at **probes.invitrogen.com**.

→

live-cell labeling with Qtracker® kits

The reagents in the Qtracker® Cell Labeling Kits (Table 2) provide a means to deliver fluorescent Qdot® nanocrystals into live cells by using a custom targeting peptide. Qtracker® Cell Labeling Kits are available with Qdot® nanocrystals in seven brilliant colors—emitting at 525 nm, 565 nm, 585 nm, 605 nm, 655 nm, 705 nm, or 800 nm—and are excellent tools for long-term studies of live cells and tissues, including migration, motility, morphology, and other cell function assays.

Using Qtracker® Cell Labeling Kits, researchers can observe labeled cells using continuous illumination, without photobleaching and degradation problems. Qtracker® labels

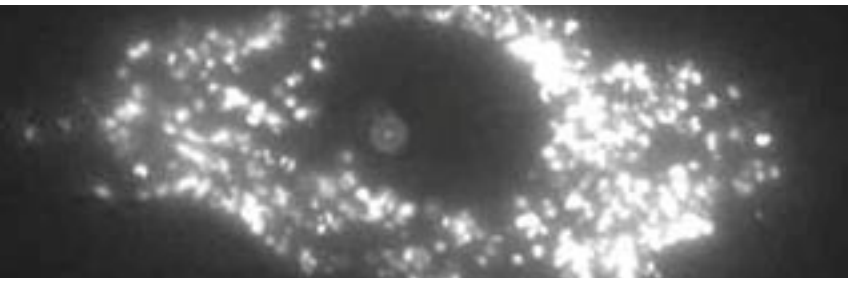

Figure 6—Distribution of Qtracker® labels in cytoplasm vesicles

HeLa cells were labeled with the Qtracker® 655 Cell Labeling Kit. A Leica TCS SP2 confocal microscope was used to observe the Qtracker® reagent in the cytoplasm (excitation at 488 nm).

are distributed in vesicles in the cytoplasm (Figure 6), and are inherited by daughter cells for at least six generations. Intense fluorescence is maintained in complex cellular environments and under various biological conditions including changes in intracellular pH, temperature, and metabolic activity. Qtracker® reagent–labeled live cells can be easily monitored on a variety of platforms, including flow cytometry, fluorescence/confocal microscopy, fluorescence microplate readers, and high-content imaging systems. Experiments indicate that labeling with the Qtracker® kit has no significant effect on cell proliferation and cellular enzyme activity.

design your own nanocrystal-based assay

Qdot® Innovator's Tool Kit (ITK™) nanocrystals (Table 3) enable researchers to achieve custom Qdot® nanocrystal labeling of nearly any material of interest. Qdot® ITK™ nanocrystals are available with three different surface chemistries—carboxyl groups, amino groups, or organic-soluble groups—for extremely versatile labeling options (Figure 7). These materials provide a remarkable platform for the development of nanocrystal–based assays; for example, they allow researchers to modify Qdot® nanocrystals in a controlled stoichiometric fashion, and to experiment with any number of functional surface modifications. The customizable surface of Qdot® ITK™ nanocrystals should prove particularly useful in the preparation of Qdot® nanocrystals with multiple surface functionalities for powerful, data-rich assays.

start using tomorrow's technology today

You can now order Qdot® nanocrystals products directly from Invitrogen by using our online ordering system at **www.invitrogen.com** or by calling our customer service representatives. In addition, we continue to work with Fisher Scientific as a distributor in the United States for Qdot® nanocrystals products. To inquire about commercial use of this exciting technology, please contact our business development department (**probesbusdev@invitrogen.com**). ■

references

1. Alivisatos, P. (2004) *Nature Biotech.* 22:47; **2.** Michalet, X. et al. (2005) *Science* 307:538; **3.** Alivisatos, P. et al. (2005) *Annu. Rev. Biomed. Eng.* 7:55; **4.** Howarth, M. et al. (2005) *Proc. Natl. Acad. Sci. U S A* 102:7583; **5.** Gao, X. et al. (2004) *Nature Biotech.* 22:969.

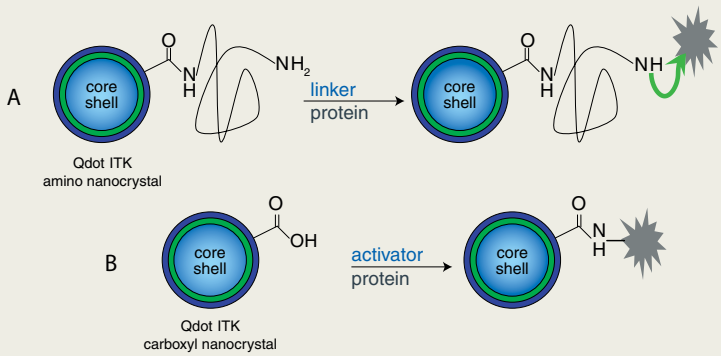

Figure 7—Coupling of Qdot® ITK™ nanocrystals

(A) Qdot® ITK™ amino (PEG) nanocrystals can be coupled by a wide variety of standard amine-reactive crosslinking chemistries. (B) Qdot® ITK™ carboxyl nanocrystals can be coupled using standard EDC (carbodiimide) activation and coupling chemistries.

Table 3—Qdot® nanocrystals

Product	Quantity	Cat. no.
Qdot® 525 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21541MP
Qdot® 545 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21591MP
Qdot® 565 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21531MP
Qdot® 585 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21511MP
Qdot® 605 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21501MP
Qdot® 655 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21521MP
Qdot® 705 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21561MP
Qdot® 800 ITK™ amino (PEG) quantum dots *8 µM solution*	250 µL	Q21571MP
Qdot® 525 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21341MP
Qdot® 545 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21391MP
Qdot® 565 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21331MP
Qdot® 585 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21311MP
Qdot® 605 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21301MP
Qdot® 655 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21321MP
Qdot® 705 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21361MP
Qdot® 800 ITK™ carboxyl quantum dots *8 µM solution*	250 µL	Q21371MP
Qdot® 545 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21791MP
Qdot® 565 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21731MP
Qdot® 585 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21711MP
Qdot® 605 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21701MP
Qdot® 655 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21721MP
Qdot® 705 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21761MP
Qdot® 800 ITK™ organic quantum dots *1 µM solution*	4 mL	Q21771MP

nanocrystals in recent publications

Small Animal Imaging

Ballou, B. et al. Noninvasive imaging of quantum dots in mice. (2004) *Bioconjug. Chem.* 15:79.

Live Cell Labeling and Assays

Lagerholm, B.C. et al. Multicolor coding of cells with cationic peptide coated quantum dots. (2004) *Nano. Lett.* 4:2019; Mattheakis, L.C. et al. Optical coding of mammalian cells using semiconductor quantum dots. (2004) *Anal. Biochem.* 327:200.

Ligand–Receptor Tracking

Lidke, D.S. et al. Reaching out for signals: Filopodia sense EGF and respond by directed retrograde transport of activated receptors. (2005) *J. Cell. Biol.* 170:619; Dahan, M. et al. Diffusion dynamics of glycine receptors revealed by single–quantum dot tracking. (2003) *Science* 302:442.

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Giepmans, B.N. et al. Correlated light and electron microscopic imaging of multiple endogenous proteins using quantum dots. (2005) *Nat. Methods* 2:743; Chan, P. et al. Method for multiplex cellular detection of mRNAs using quantum dot fluorescent *in situ* hybridization. (2005) *Nucleic Acids Res.* 33:161.

Biochemical Assays

Geho, P. et al. Pegylated, steptavidin-conjugated quantum dots are effective detection elements for reverse-phase protein microarrays. (2005) *Bioconjug. Chem.* 16:559.

Flow Cytometry

Telford, W.G. Analysis of UV-excited fluorochromes by flow cytometry using near-ultraviolet laser diodes. (2004) *Cytometry A* 61:9; Perfetto, S.P. et al. Seventeen-colour flow cytometry: unravelling the immune system. (2004) *Nat. Rev. Immunol.* 4:648.

Invitrogen flow cytometry—building choice

CALTAG, QUANTUM DOT, MOLECULAR PROBES, BIOSOURCE, AND DYNAL ARE INVITROGEN FLOW CYTOMETRY.

Invitrogen recognizes the importance of flow cytometry in all research settings—everything from basic research to clinical diagnostics and therapeutics. Our aim is to bring together the technologies and expertise required to create the next advances in research tools for flow cytometry.

Early evidence of this synergy includes several new antibody conjugates incorporating nanocrystal and violet-excitable fluorophores. Our combined product offering also includes research tools for the study of cell health and vitality, reagents for cytokine research, bead-based products for cell separation, beads for instrument calibration, and quality cell culture media and supplements (Figure 1).

With our broad product base, expertise, and support, you'll get more out of your experiments today, with the promise of new tools to solve tomorrow's problems. Take a look at the various technologies playing critical roles in this new endeavor—Invitrogen flow cytometry.

primary antibody conjugates (Caltag Laboratories)

With over 100 monoclonal antibodies to human antigens, over 100 monoclonal antibodies to mouse and rat antigens, and 19 different fluorochromes, Invitrogen's Caltag Laboratories is the technical leader in multicolor flow cytometry for basic research and clinical diagnosis. Caltag has FDA clearance for 16 IVDs (*in vitro* diagnostics) and more than 400 ASRs (analyte-specific reagents), and offers CE-marked antibodies in Europe, in addition to being an ISO 9001:2000 and ISO 13485:2003 facility. Invitrogen's Caltag™ antibody conjugates give you:

- GMP (good manufacturing practice) quality in every reagent
- the broadest range of fluorochromes available
- the most choices in violet antibody reagents

See the entire selection of primary antibodies at **www.caltag.com**.

Qdot® nanocrystals (Quantum Dot Corporation)

Invitrogen's Qdot® fluorescence labels represent the latest technology in fluorescence labeling. These nanometer-scale semiconductor particles have broad excitation and very narrow emission properties—a single excitation source can be used to generate very distinct emission signals from multiple Qdot® labels in one sample. These unique labels are very bright and withstand hours of illumination, making them ideal for applications that call for dynamic imaging, prolonged storage, and repeated interrogation of the specimen. Qdot® secondary antibody conjugates

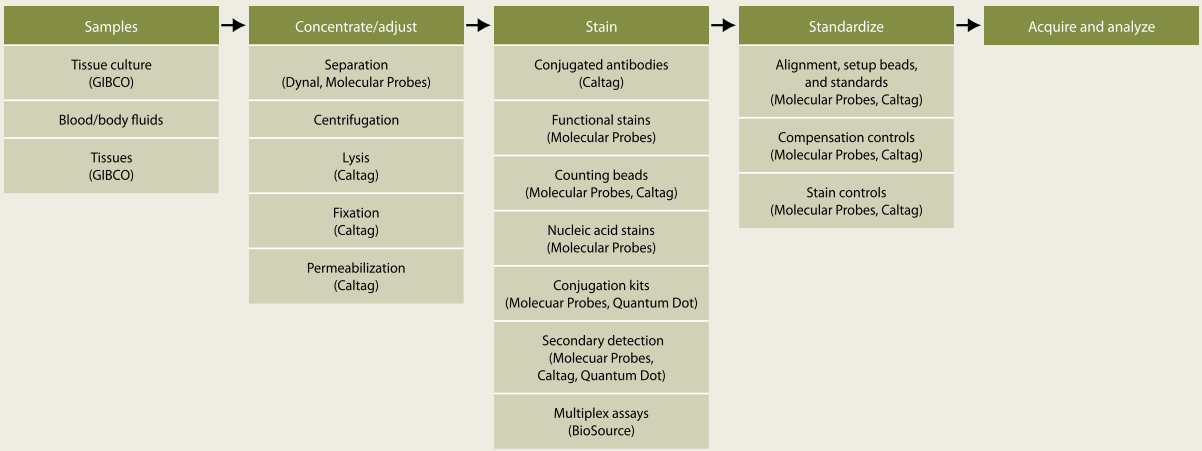

Figure 1—The flow cytometry workflow

Invitrogen flow cytometry offers tools for every stage of your workflow.

and Qtracker® Cell Labeling Kits are ideal for observations in living cells and animals, multicolor analysis of fixed tissues, violet laser flow cytometry (Figure 2, next page), and even multicolor western blotting. Qdot® nanocrystals from Invitrogen offer:

- stability for live-cell imaging and dynamics
- compatibility with paraformaldehyde fixation, allowing follow-up immunofluorescence from *in vivo* studies
- the option for permanent sample storage of pathology samples
- brilliant colors for single-excitation multicolor analysis

To learn more about this exciting technology, visit **probes.invitrogen.com/products/qdot**.

labeling and detection technologies (Molecular Probes)

The premier developer of organic dyes and fluorescence labeling technologies, Invitrogen's Molecular Probes, continues to deliver novel fluorescence-based assays for cell biology research. Our latest products include single-step dead-cell stains, apoptosis assay kits designed for use with violet laser excitation, dyes for cell-cycle analysis in live cells, and viability and vitality kits for prokaryotes. Look to Invitrogen's Molecular Probes technologies for:

- viability, vitality, cytotoxicity, and apoptosis assays
- Zenon® reagents for flexible, efficient labeling of your antibody samples
- novel dyes and kits compatible with violet laser excitation

To learn more about Molecular Probes products for flow cytometry, visit us at **probes.invitrogen.com/flowcytometry**.

cytokines and signaling (BioSource)

Invitrogen's BioSource™ products offer integrated solutions for the study of disease-related cellular pathways through an extensive selection of proteins, antibodies, and assays for cytokine, neurodegenerative, and signal transduction biomarkers. With over 280 phosphospecific antibodies available, BioSource offers a variety of choices for detecting phosphorylation events by flow cytometry. Invitrogen's BioSource™ phosphospecific and total protein antibodies give you:

- high specificity for the target of interest
- superior performance in a variety of immunodetection applications
- excellent lot-to-lot consistency

For more information about cytokine and signal transduction antibodies, visit **www.biosource.com**.

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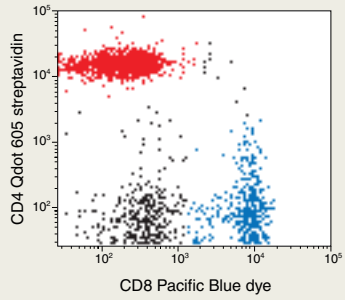


Figure 2—Multicolor analysis of CD4-positive and CD8-positive populations using Qdot® 605 streptavidin and a Pacific Blue™ dye–labeled conjugate

Human mononuclear cells were blocked with nonspecific goat IgG and then stained with a biotinylated mouse anti–human CD4 antibody (Caltag). Cells were incubated with Qdot® 605 streptavidin, then with mouse anti–human CD8 antibody conjugated to Pacific Blue™ dye (Caltag). Cells were analyzed on a flow cytometer equipped with a 405 nm violet diode laser and 450/50 and 605/20 nm bandpass filters. Compensation was performed using single-color controls. Cells were analyzed using a lymphocyte gate as determined by FSC/SSC. These reagents give clear separation of the CD4-positive and CD8-positive populations using a single excitation source, the violet diode laser.

bead separations (Dynal)

Invitrogen offers a number of Dynal® magnetic bead–based cell separation techniques with flow cytometrists in mind. For positive isolation protocols, Dynal has developed Dynabeads® products that will bind specific cell populations; the magnetic bead can then be detached prior to flow cytometric analysis. Alternatively, choose a negative isolation product to deplete your sample of unwanted material, leaving the target cells untouched (Figure 3). All Dynabeads® separation techniques are gentle to your target and offer consistent reproducibility and sensitivity. Invitrogen's Dynal® bead separation technologies give you:

- uniform, spherical, superparamagnetic bead particles for separation of cells, organelles, proteins, and nucleic acids
- the option to perform both positive and negative selection in mixed samples
- kits for specific separation tasks or activated beads for custom surface preparation

To learn more about how magnetic separation can assist you with up-front preparation of your flow cytometry sample, visit **www.invitrogen.com/dynal**.

cell culture solutions (GIBCO)

Invitrogen has a wide range of GIBCO® cell culture products that are ideal for use with flow cytometry. GIBCO® media are reliably consistent over time, from lot to lot and from bottle to bottle. These cell culture solutions give you better control, minimizing variables that can hinder your progress. Look to Invitrogen's GIBCO® media and reagents for a steady stream of new tools and technologies that keep pace with your research needs. GIBCO® cell culture products from Invitrogen offer:

- a comprehensive selection of media, reagent, and sera product selection
- new products to increase performance and simplify the process
- quality, reliability, and consistency

For all of your media and cell culture needs, visit us at **www.invitrogen.com/gibco**.

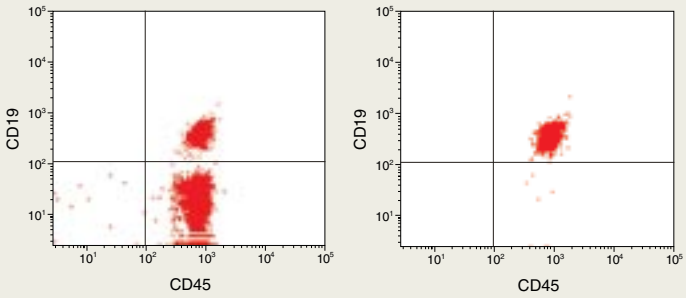
your new choice for flow cytometry

We've assembled an exciting group of flow cytometry technologies at Invitrogen, and we have some exciting things on the way in 2006. Check in at **www.invitrogen.com/flowcytometry** for the latest news and innovative products coming from Invitrogen flow cytometry. ■

tip Supplement your research at **www.invitrogen.com/antibodies**. You'll find a wealth of information about antibodies and immunodetection reagents and techniques, including detailed product information, application area support, and workflow solutions. And for information about Molecular Probes™ secondary antibodies, visit us at **probes.invitrogen.com/secondary detection**.

Figure 3—Negative isolation using the Dynal® B Cell Negative Isolation Kit

Untouched B cells negatively isolated from mononuclear cells (MNC) using the Dynal® B Cell Negative Isolation Kit and analyzed by flow cytometry. The graphs show B cell purity before (left) and after (right) isolation from MNC.



JOURNAL HIGHLIGHT

Safer, faster susceptibility testing of *Mycobacterium tuberculosis* using SYTO® 16 stain

Pina-Vaz, C., Costa-de-Oliveira, S., and Rodrigues, A.G. (2005) *J. Med. Microbiol.* 54:77

Mycobacterium tuberculosis is the causative agent of tuberculosis and has recently re-emerged as a major health hazard. The development and testing of antimycobacterial treatments for tuberculosis is of primary importance, particularly now that many strains have become resistant to the drugs used to treat them. With current susceptibility testing methods taking weeks to complete, assessing the effectiveness of antimycobacterial agents in a timely fashion is critical to ensure that the patient receives effective treatment and that the number of people he or she has contacted while in an infective state is minimized.

In a recent publication, Pina-Vaz et al. report the development of a rapid, accurate method for susceptibility testing of *M. tuberculosis*. The study compared the existing BACTEC MGIT 960 test (a fluorometric assay; median wait period for results is 7.3 days) with a SYTO® 16 stain flow cytometry assay on 16 strains of *M. tuberculosis* challenged with the antimicrobials streptomycin, isoniazid, rifampicin, and ethambutol (SIRE). The SYTO® 16 susceptibility protocol involved incubating the test strains with SIRE for 72 hours at 37°C, heat-inactivating the cells by autoclaving for 20 minutes at 121°C, and staining with 10 µM SYTO® 16 stain for 20 minutes. SYTO® 16 nucleic acid stain is unable to penetrate healthy cells but will cross the membrane of cells that have sustained damage caused by an antimycobacterial compound. Thus, the effectiveness of the compound is correlated to the intensity of the staining. Comparing the staining intensity of SYTO® 16 stain in the treated and control samples made it possible to distinguish sensitive, low-level resistant, and resistant phenotypes. There was excellent correlation between the flow cytometry results based on SYTO® 16 stain obtained after 72 hours and the BACTEC 960 results obtained after 12–15 days. If applied in a clinical setting, this efficiency would allow the correct therapy to be delivered more quickly to the patient and, in turn, reduce the amount of time he or she is potentially exposing others to the disease. In addition, because the SYTO® 16 protocol involves heat inactivation of the sample, the technician who performs the flow cytometric analysis is also protected from transmission.

Product	Quantity	Cat. no.
SYTO® 16 green fluorescent nucleic acid stain *1 mM solution in DMSO*	250 µL	S7578

flow cytometry resources on the web

Practical Flow Cytometry, Fourth Edition

by Howard M. Shapiro

- It's all here. From the mysteries of the photon to the rigors of good data analysis—what more can be said about a book that so thoroughly treats the subject of flow cytometry? Enjoy free online access to the entire contents of the fourth edition of Shapiro's important reference work, *Practical Flow Cytometry*, only from Invitrogen.
- Simply fill out a form at **probes.invitrogen.com/practicalflowcytometry** for complete access to *Practical Flow Cytometry*. You will be given a link to access the entire book online. (Only your name and email address are required.) *Practical Flow Cytometry*, Fourth Edition, has been made available by arrangement with John Wiley & Sons, Inc.

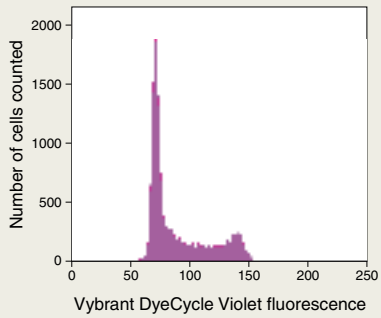


Figure 1—Clear visualization of mammalian cell cycle phases

Live Jurkat cells were stained with Vybrant® DyeCycle™ Violet stain to show DNA content distribution. G_0/G_1 and G_2/M phase histogram peaks are separated by the S-phase distribution. Cells were analyzed on a flow cytometer equipped with a 405 nm violet diode laser and a 440/40 nm bandpass filter.

Violet-excitable flow cytometry reagents

RECENT MOLECULAR PROBES™ PRODUCTS DESIGNED FOR THE VIOLET LASER PROVIDE MORE OPTIONS FOR YOUR FLOW CYTOMETRY RESEARCH.

Violet lasers are becoming increasingly common as a second or third laser on flow cytometers. New Molecular Probes™ and Caltag™ violet-excitable reagents allow you to move common assays off your 488 nm excitation line to make room for other markers of interest. This lets you use your violet source for cell cycle analysis, viability assays, and up to two colors of immunophenotyping.

analyze cellular DNA content and cell-cycle distribution in live cells

Vybrant® DyeCycle™ stains are DNA-selective stains that exhibit emission signals proportional to DNA mass. Analysis of a stained population by flow cytometry is then used to produce a frequency histogram that reveals the various phases of the cell cycle (Figure 1). Vybrant® DyeCycle™ stains offer:

- cell-cycle data on live cells
- simple protocol with no washing required
- three fluorescent colors, including one for the violet laser of your flow cytometer

single-step dead-cell stain

SYTOX® Blue dead-cell stain is a simple and quantitative single-step dead-cell indicator for use with violet laser–equipped flow cytometers. After brief incubation with SYTOX® Blue stain, the nucleic acids of dead cells fluoresce bright blue (~480 nm) when excited with 405 nm violet laser light. Emission at this wavelength has minimal spectral overlap with the emission of commonly used green and red dyes. SYTOX® Blue stain provides:

- compatibility with violet laser excitation
- simple protocol—no fixing, no washing
- easy incorporation into experiments using green and red dyes (emission maxima ~470 nm)

fixable dead-cell stain

The live/dead discrimination reported by the LIVE/DEAD® Fixable Dead Cell Stain Kits

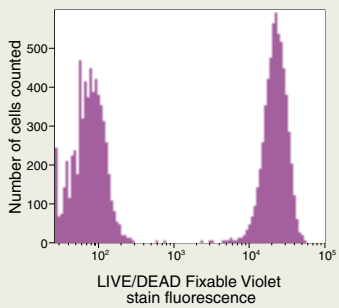


Figure 2—Clear discrimination between live and dead cells

The LIVE/DEAD® Fixable Violet Dead Cell Stain Kit was used to differentially stain a mixture of live and heat-treated Jurkat cells. Following the staining reaction, the cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry. The live-cell population is easily distinguished from the killed population; nearly identical results were obtained using unfixed cells (data not shown).

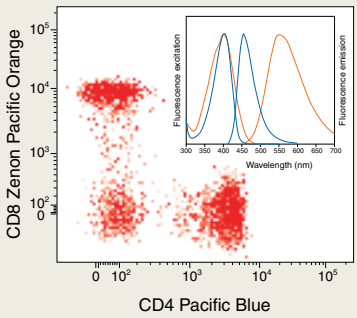


Figure 3—Multiparameter analysis with the violet laser

Human mononuclear cells labeled with CD4 Pacific Blue™ (Caltag Laboratories) and mouse anti–human CD8 complexed to Zenon® Pacific Orange™ labeling reagent. The plot is gated on lymphocytes using FSC/SSC gate. Analysis was performed on a flow cytometer using 405 nm excitation and 450/50 nm and 585/42 nm emission filters. Inset shows spectra for Pacific Blue™ dye (blue lines) and Pacific Orange™ dye (orange lines).

is based on the interaction between the fluorescent reactive dye and cellular amines. The staining pattern is completely preserved following formaldehyde fixation. This single-color assay uses only one channel of a flow cytometer, leaving the other channels available for multicolor experiments. LIVE/DEAD® Fixable Dead Cell Stain Kits offer:

- blue-, green-, red-, and violet-fluorescent (Figure 2) versions
- staining that is retained following formaldehyde fixation
- easy incorporation into experiments using other fluorescent dyes

two-color immunophenotyping from the violet laser

With an emission maximum at ~551 nm, Pacific Orange™ dye is fully compatible with Pacific Blue™ dye (emission maximum ~455 nm), giving you the option of multiparameter analysis with the violet laser (Figure 3). Invitrogen offers many Caltag™ primary antibody conjugates of Pacific Blue™ dye. Other reagents incorporating Pacific Orange™ and Pacific Blue™ dyes include Zenon® antibody labeling kits for mouse IgG, secondary antibody conjugates against mouse IgG or rabbit IgG, and streptavidin conjugates. For researchers interested in preparing their own conjugates, Pacific Orange™ dye and Pacific Blue™ dye are available in protein and monoclonal antibody labeling kits that conveniently include all of the reagents needed for dye labeling and purification. Reactive forms of Pacific Orange™ and Pacific Blue™ dyes are also available in 1 mg and 5 mg quantities, respectively. Pacific Orange™ dye–labeled conjugates offer:

- excitation with the violet laser
- easy incorporation into experiments using green and red dyes; best read with a 575 nm bandpass filter ■

Product	Quantity	Cat. no.
LIVE/DEAD® Fixable Blue Dead Cell Stain Kit *for flow cytometry* *200 assays*	1 kit	L23105
LIVE/DEAD® Fixable Violet Dead Cell Stain Kit *for flow cytometry* *200 assays*	1 kit	L34955
Mouse anti-human CD4 antibody, conjugated to Pacific Blue™ dye	0.5 mL	MHCD0428 *
Pacific Orange™ $F(ab')_2$ fragment of goat anti-mouse IgG (H+L) *2 mg/mL*	250 µL	P31585
Pacific Orange™ goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL	P31586
SYTOX® Blue dead cell stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL	S34857
Vybrant® DyeCycle™ Violet Stain *5 mM in water* *200 assays*	200 µL	V35003
Zenon® Pacific Orange™ Mouse IgG_1 Labeling Kit *50 labelings*	1 kit	Z25256
Zenon® Pacific Orange™ Mouse IgG_{2a} Labeling Kit *50 labelings*	1 kit	Z25257

Related products

LIVE/DEAD® Fixable Green Dead Cell Stain Kit *for flow cytometry* *200 assays*	1 kit	L23101
LIVE/DEAD® Fixable Red Dead Cell Stain Kit *for flow cytometry* *200 assays*	1 kit	L23102
Vybrant® DyeCycle™ Green Stain *5 mM solution in DMSO* *200 assays*	400 µL	V35004
Vybrant® DyeCycle™ Orange Stain *5 mM solution in DMSO* *200 assays*	400 µL	V35005

* For more information on primary antibody conjugates of Pacific Blue™ or Pacific Orange™ dyes, visit **www.caltag.com**. For current prices, please visit **probes.invitrogen.com**

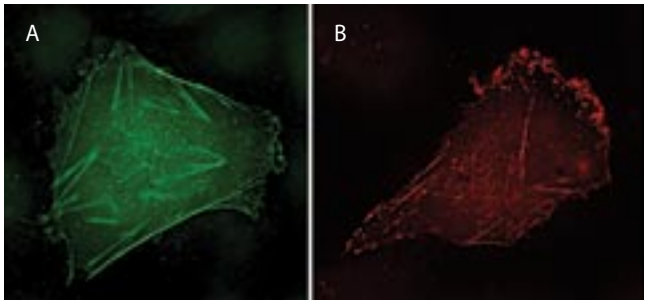


Figure 1—Choice of fluorescent colors using the TC-FlAsH™ TC-ReAsH™ In-Cell Tetracysteine Tag Detection Kit

An actin–TC-Tag fusion protein was used in a transient transfection experiment of live CHO cells. Separate samples of transfected cells were stained with FlAsH-EDT_2 labeling reagent (A) and ReAsH-EDT_2 labeling reagent (B). These images show that a single tagged protein can be detected with either green or red fluorescence labels.

New releases

HIGHLIGHTS OF OTHER NEW PRODUCTS AND TECHNOLOGIES FROM OUR R&D LABS.

secondary detection

TC-FlAsH™ TC-ReAsH™ In-Cell Tetracysteine Tag Detection Kits

TC-FlAsH™ TC-ReAsH™ labeling technology, based on the interaction of a tagged protein with a biarsenical labeling reagent, provides flexible and specific fluorescent detection of recombinant proteins in live cells.[1–3] An expression construct comprising the protein of interest fused to a tetracysteine tag (TC-Tag, CCPGCC) is used to transform the host cell line. The tagged protein is detected by the addition of one of two labeling reagents: FlAsH-EDT_2 labeling reagent, which generates green fluorescence, or ReAsH-EDT_2 labeling reagent, which generates red fluorescence. TC-FlAsH™ TC-ReAsH™ labeling technology gives you:

- a very small tag that is less likely to interfere with the protein of interest
- labeling reagents that are fluorescent only in the presence of the tagged protein
- choice of green or red fluorescence from the same tagged protein (Figure 1)

Recent publications highlight some of the unique applications of the TC-FlAsH™ TC-ReAsH™ In-Cell Tetracysteine Tag Detection Kit technology:

- **monitoring protein turnover and trafficking**—sequential application of the two labeling reagents can be used to follow temporal changes in the protein of interest [4]
- **visualizing cellular structure at high resolution**—ReAsH-EDT_2 labeling reagent is compatible with fluorescence microscopy and electron microscopy [4]
- **detecting receptor activation and protein-protein interaction**—FlAsH-EDT_2 labeling reagent provides a superior alternative to Yellow-Fluorescent Protein (YFP) when coupled with Cyan-Fluorescent Protein (CFP) for FRET-based cellular analysis [5]

secondary detection

Alexa Fluor® 555 dye–labeled products

Alexa Fluor® 555 dye–labeled conjugates are the ideal Cy3 dye replacements. Red-orange–fluorescent Alexa Fluor® 555 dye has excitation and emission maxima similar to those of tetramethylrhodamine (TMR) and Cy3 dyes, but it is significantly brighter (Figure 2) and more photostable, so you have more time to capture the perfect image—without changing any of your instrumentation and fluorescence parameters. Table 1 lists many of these new conjugates—for a full list, visit us at **probes.invitrogen.com/secondarydetection.**

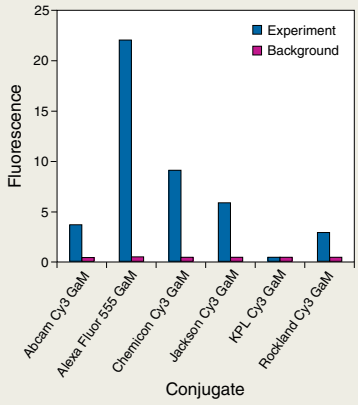


Figure 2—Brightness comparison of Alexa Fluor® 555 goat anti–mouse IgG antibody and Cy3 goat anti–mouse IgG antibody conjugates

Human blood was blocked with normal goat serum and incubated with an anti-CD3 mouse monoclonal antibody; cells were washed, resuspended and incubated with either the Alexa Fluor 555 or Cy3 goat anti–mouse IgG antibody at equal concentrations. Red blood cells were lysed and the samples were analyzed with a flow cytometer equipped with a 488 nm argon-ion laser and a 585 ± 21 nm bandpass emission filter.

secondary detection

Alexa Fluor® 680 dye–labeled products

Alexa Fluor® 680 dye–labeled conjugates can replace Cy5.5 labels in any of your experiments. Alexa Fluor® 680 dye has near-IR excitation/emission maxima (~679/702 nm) well separated from other common red fluorophores and thus provides a valuable option for multicolor applications. Its spectral similarity to Cy5.5 means that it is compatible with common equipment platforms, including flow cytometers equipped with 633 or 647 nm laser lines and the LI-COR Odyssey imaging system. Table 2 (next page) lists many of these new conjugates—for a full list, visit us at **probes.invitrogen.com/secondarydetection**.

Table 1—Alexa Fluor® 555 products equivalent to tetramethylrhodamine conjugates and derivatives

Fluorophore derivative	TMR derivative cat. no.	Alexa Fluor® 555 derivative cat. no.
Secondary antibodies		
Goat anti–mouse IgG	T2762	A21127, A21137, A21147, A21422, A21424, A21425, I37152
Goat anti–rabbit IgG	T2769	A21428, A21429, A21430, I37157
Other conjugates for cell structure and function		
Avidin and streptavidin	S870, A6373	S21381, S32355, I37162
Transferrin	T2872	T35352
Epidermal growth factor	E3481	E35350
Phalloidin	R415	A34055
Bovine serum albumin	A23016	A34786
Wheat germ agglutinin	W849	W32464
Dextran	D1816, D1817, D1868	D34679
α-Bungarotoxin	T1175	B35451
Reactive labels		
Amine-reactive	T490, T668, C1171, T1480, T1481, C2211, T6105, C6123	A20009, A20109, A32755, A32756
Thiol-reactive	T6006, T6027, T6028	A20346
Amine-containing	A1318	A30677
Protein labeling kits	F6163	A20174, A20187, A30007, Z25005, Z25105, Z25205, Z25305, Z25405, Z25605

enzyme assays

cardiovascular research tools

Myeloperoxidase (MPO) is a unique enzyme that has been detected in human atherosclerotic lesions and may be the source of oxidants of low-density lipoprotein (LDL), the major carrier of blood cholesterol. Therefore, MPO is considered a potentially important cardiac biomarker.[6,7] The EnzChek® Myeloperoxidase (MPO) Activity Assay Kit is designed to assay for MPO in solution and cell lysates, and is the only assay that measures both peroxidation and chlorination activities. The →

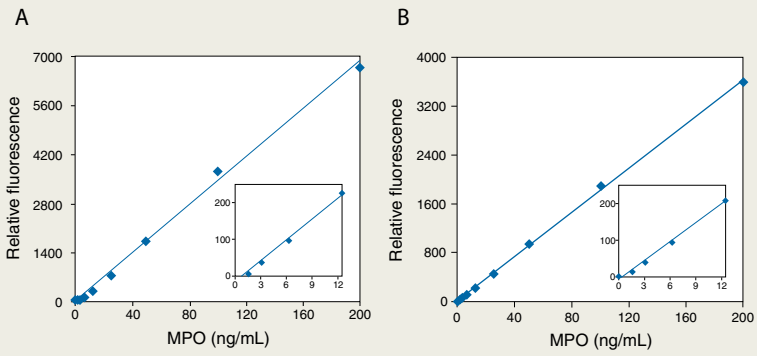


Figure 3—Typical standard curves using the EnzChek® Myeloperoxidase (MPO) Activity Assay Kit

(A) 3′-(*p*-aminophenyl)fluorescein (APF)-based chlorination assay, with fluorescence measured using excitation at 485 nm and emission at 530 nm. (B) Amplex® UltraRed substrate–based peroxidation assay, with fluorescence measured using excitation at 530 nm and emission at 590 nm. For both assays, reactions were incubated at room temperature for 30 minutes.

simple, homogeneous assays can be continuously monitored for up to 30 minutes, and will detect as little as 1.5 ng/mL of human MPO. Figure 3 shows typical standard curves for this assay. The EnzChek® MPO kit is designed for use with a fluorescence microplate reader and provides sufficient materials for 200 chlorination assays and 200 peroxidation assays in 96-well microplate format.

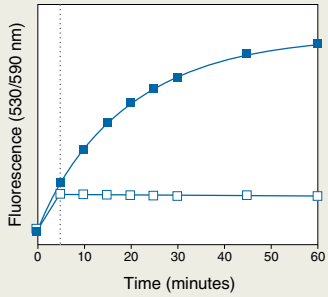
The Zen™ Myeloperoxidase (MPO) ELISA Kit takes sensitive MPO detection a step further and allows assays in a variety of biological samples, including serum. The assay uses a horseradish peroxidase (HRP)–amplified ELISA format and Amplex® UltraRed reagent, which reacts with H_2O_2 to produce a highly fluorescent, strongly absorbing product (excitation/emission maxima ~568/581 nm). In contrast to commonly used reagents such as ABTS and TMB, Amplex® UltraRed reagent is exceptionally resistant to auto-oxidation, making it a superior choice for HRP-amplified ELISAs. With a high extinction coefficient and good quantum efficiency, the fluorescence-based Amplex® UltraRed reagent is also more sensitive than standard colorimetric reagents

Table 2—Alexa Fluor® 680 conjugates and labeling products

Product	Application	Cat. no.
Antibodies		
Goat anti–mouse IgG	Secondary detection in western blots, flow cytometry, imaging	A21048, A21057, A21058, A21059
Goat anti–rabbit IgG	Secondary detection in western blots, flow cytometry, imaging	A21076, A21077, A21109
Rabbit anti–goat IgG	Secondary detection in western blots, flow cytometry, imaging	A21088
Rabbit anti–mouse IgG	Secondary detection in western blots, flow cytometry, imaging	A21065
Goat anti–rat IgG	Secondary detection in western blots, flow cytometry, imaging	A21096
Donkey anti–goat IgG	Secondary detection in western blots, flow cytometry, imaging	A21084
Donkey anti–sheep IgG	Secondary detection in western blots, flow cytometry, imaging	A21102
Goat anti–mouse IgG_1, IgG_{2a}, IgG_{2b}	Selective detection of mouse IgG_1, IgG_{2a}, IgG_{2b}	A31562, A31563, A31564
Other conjugates for cell structure and function		
Wheat germ agglutinin	Selective binding to *N*-acetylglucosamine (gram-positive bacteria)	W32465
Transferrin	Endosomal pathways	T35357
Annexin V	Detection of apoptosis, phosphatidylserine exposure	A35109
α-Bungarotoxin	Acetylcholine receptors	B35452
Bovine serum albumin	Tracing for endocytosis and cell morphology	A34787
Phalloidin	Cytoskeletal marker of actin	A22286
Streptavidin	Detection of biotinylated conjugates	S21378
Dextrans	Neuronal tracers, endocytosis, pinocytosis	D34680, D34681

Figure 4—Application of the Amplex® Red/UltraRed stop reagent to control H_2O_2/peroxidase-coupled detection reactions

Two parallel reactions containing 0.5 mU/mL horseradish peroxidase in 50 mM sodium phosphate buffer, pH 7.4, were initiated by addition of 50 µM Amplex® Red reagent + 1 mM H_2O_2. Reaction progress was monitored by detection of the fluorescent product resorufin at 37°C in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. After five minutes (⋮), one of the reactions (□) was terminated by addition of Amplex® Red/UltraRed stop reagent. The fluorescence signal in the stopped reaction remained at the constant level shown for 3 hours (data not shown).



and provides a broader measurement range. The versatile Amplex® UltraRed reagent can be detected using either fluorescence- or absorbance-based instrumentation, and the assay will detect as little as 0.1 ng/mL of human MPO. The Zen™ MPO ELISA Kit provides sufficient reagents for 200 assays in microplate format.

Lipases play an essential role in the transfer of lipids in cell signaling and metabolism. The triacylglycerol-based EnzChek® lipase substrate offers higher throughput and better sensitivity than chromogenic (TLC or HPLC) assays, and a visible wavelength–detection alternative to pyrene-based fluorescent substrates. This substrate produces a bright, green-fluorescent product for the accurate and sensitive detection of lipase activity in solution. The EnzChek® lipase substrate also offers:

- better sensitivity than colorimetric lipase substrates
- pH-insensitive spectra in the physiological pH range
- compatibility with fluorescein optics (excitation/emission maxima ~505/515 nm)

The EnzChek® epoxide hydrolase substrate is ideal for studying the epoxide hydrolase family of enzymes, including the arachidonic epoxide hydrolases (implicated in the regulation of inflammation and blood pressure) and microsomal epoxide hydrolases (reported to detoxify epoxides into diols), and their respective inhibitors. The EnzChek® epoxide hydrolase substrate produces a bright, blue-fluorescent product for the accurate and sensitive detection of epoxide hydrolase activity in solution. The EnzChek® epoxide hydrolase substrate also provides:

- better sensitivity than colorimetric epoxide hydrolase substrates
- pH-insensitive spectra in the physiological pH range
- compatibility with optics used for coumarin detection in most fluorometers and UV-Vis spectrophotometers (excitation/emission maxima ~358/452 nm

enzyme assays

Amplex® Red/UltraRed stop reagent

The Amplex® Red/UltraRed stop reagent provides convenience and control in any hydrogen peroxide (HRP)-coupled assay that uses our versatile, sensitive Amplex® Red or Amplex® UltraRed reagents. Amplex® Red/UltraRed stop reagent allows you to terminate the fluorescence-generating reaction at any time point (Figure 4), offering:

- a stable fluorescent signal for at least 3 hours after the addition of the Amplex® Red/UltraRed stop reagent
- the ability to terminate reactions containing up to 0.1 units/mL HRP and 5 µM H_2O_2

Amplex® Red/UltraRed stop reagent is compatible with all Amplex® Red assays and Amplex® Red and Amplex® UltraRed standalone reagents—for details about these products, visit **probes.invitrogen.com/microplateassays**.

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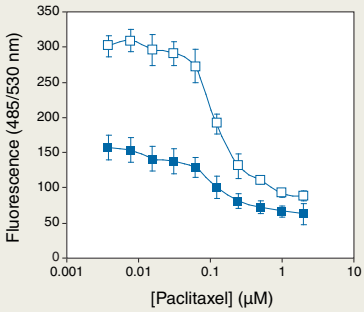

Figure 5—Cell growth inhibition by paclitaxel monitored using the CyQUANT® NF Cell Proliferation Assay Kit

CHO cells (M1WT3 ATCC CRL-1985) were plated at 5000 per well in 96-well poly-D-lysine–coated microplates. Cell samples were incubated with concentrations of paclitaxel from 4 nM to 2 µM. After 48 hours (■) or 72 hours (□) incubation, paclitaxel-containing growth medium was removed, and cell numbers (represented by linearly proportional fluorescence intensities) were measured using a fluorescence microplate reader and the reagents and protocols provided in the CyQUANT® NF Cell Proliferation Assay Kit. Plotted data points represent averages ± 1 standard deviation for hexaduplicate samples.

enzyme assays

EnzChek® Peptidase/Protease Assay Kit

EnzChek® cellulase substrate

EnzChek® assays (Table 3) offer simple protocols, usually requiring minimal cell or lysate preparation and only one or two reagents to be added to your samples. The newest addition, the EnzChek® Peptidase/Protease Assay Kit, provides a FRET (fluorescence resonance energy transfer)-based method for simple and accurate quantitation of a wide range of protease activities. The EnzChek® peptidase/protease substrate comprises a fluorophore and a quencher moiety separated by a short amino acid sequence. Upon cleavage of the sequence by a protease, the fluorophore separates from the quencher and is free to fluoresce (excitation/emission maxima ~502/528 nm). The magnitude of the fluorescent signal is directly proportional to the degree of substrate cleavage, and can therefore be used to quantitate enzyme activity. The EnzChek® Peptidase/Protease Assay Kit provides sufficient reagents for 200 assays in 96-well microplate format, and also offers:

- a simple mix-incubate-read assay protocol
- rapid results in just an hour
- easy adaptation to diverse pH requirements

The EnzChek® cellulase substrate was developed for simple and rapid quantitation of cellulase. Compared to other more complex, multistep assays, the fluorescent EnzChek® cellulase assay is great for a high-throughput environment. This substrate can also be used in a colorimetric assay, albeit with reduced sensitivity. The EnzChek® cellulase substrate offers:

- a simple mix-incubate-read assay method
- fast results in 30 minutes or less
- sensitive detection—as low as 40 µU/mL cellulase

Table 3—EnzChek® assay kits

Kit	Limit of detection*	Ex/Em†	Number of assays	Cat. no.
EnzChek® Gelatinase/Collagenase Assay Kit	2×10^{-3} U/mL	494/515	250–2000	E12055
EnzChek® Lysozyme Assay Kit	20 U/mL	494/518	400	E22013
EnzChek® Paraoxonase Assay Kit	50 mU/mL	360/450	100	E33702
EnzChek® Peptidase/Protease Assay Kit	NA‡	502/528	100	E33758
EnzChek® *Ultra* Amylase Assay Kit	2×10^{-3} U/mL	502/512	500	E33651
EnzChek® *Ultra* Phytase Assay Kit	0.001 FTU/mL	568/581	500	E33701
EnzChek® *Ultra* Xylanase Assay Kit	1.5–200 mU/mL	358/455	500	E33650

*Unit definitions for enzymes are available in product manuals—please visit **probes.invitrogen.com**. †Fluorescence excitation (Ex) and emission (Em) maxima, in nm. ‡Detection limits may vary with instrumentation and enzyme source. NA=Not available.

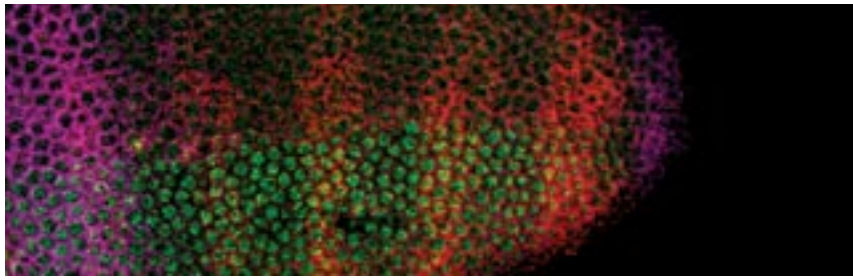

Figure 6—RNA targets labeled in a *Drosophila melanogaster* embryo

Simultaneous detection of expression of three genes in a whole mount *Drosophila melanogaster* embryo by fluorescence *in situ* hybridization (FISH) using the FISH Tag™ RNA Multicolor Kit. Green: *sog* (short gastrulation) labeled with Alexa Fluor® 488 dye; red: *ftz* (fushi tarazu) labeled with Alexa Fluor® 594 dye; magenta: *Kruppel* labeled with Alexa Fluor® 647 dye. The slide was mounted using *SlowFade®* Gold antifade reagent.

microplate assays

CyQUANT® NF Cell Proliferation Assay Kits

Methods for cell proliferation analysis are generally based on the incorporation of thymidine analogs such as ^{3}H-thymidine or bromodeoxyuridine (BrdU) during DNA synthesis, or on measurement of metabolic activity indices such as oxidoreductase activity or ATP levels. The new CyQUANT® NF assay is based on measurement of cellular DNA content via fluorescent dye binding. Because cellular DNA content is highly regulated, it is closely proportional to cell number. The extent of proliferation is determined by comparing cell counts for samples treated with drugs or other compounds of interest with untreated controls [8,9] (Figure 5).

The original Molecular Probes™ CyQUANT® assay [10] requires a freeze-thaw lysis step to facilitate the interaction of the CyQUANT® GR dye with nuclear DNA. The CyQUANT® NF assay avoids this freeze-thaw step by using a cell-permeant DNA binding dye in combination with a plasma membrane permeabilization reagent. The CyQUANT® NF assay can be completed in under an hour—just remove growth medium (for adherent cells), replace with dye binding solution, incubate for 30–60 minutes, and measure fluorescence. The CyQUANT® NF assay is available as either a 200 or 1000 assay kit (based on a sample volume of 100 µL in 96-well microplate format). CyQUANT® NF assay kits offer:

- a high-throughput protocol
- analysis of adherent or nonadherent cells in 96- or 384-well microplates
- linear response from at least 100 to 10,000 cells per well in a 96-well microplate
- no need for radioisotopes, enzymes, or antibodies

imaging analysis

FISH Tag™ DNA and FISH Tag™ RNA Kits

FISH Tag™ DNA and FISH Tag™ RNA Kits (Table 4, next page) are optimized for multiplex fluorescence *in situ* hybridization (FISH) applications (Figure 6). Each kit provides all the tools needed for synthesizing, labeling, and purifying your probe, then imaging your labeled specimen.

FISH Tag™ kits employ a two-step labeling technology for optimized labeling efficiency.[11] Nick translation (for DNA probes) or *in vitro* transcription (for RNA probes) is used to enzymatically incorporate an amine-modified nucleotide—aminoallyl dUTP for DNA or aminoallyl UTP for RNA—followed by chemical labeling with amine-reactive Alexa Fluor® dyes. Lacking bulky dye groups, the aminoallyl-modified nucleotides are consistently incorporated at high levels compared with dye-labeled nucleotides. PureLink™ nucleic acid purification technology is then used to rapidly and efficiently purify the labeled probe, providing high yields of DNA or RNA. *SlowFade®* Gold antifade reagent is included →

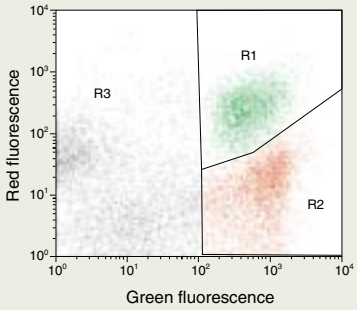

Figure 7—Distinguishing healthy and unhealthy bacterial cells using the *Bac*Light™ RedoxSensor™ CTC Vitality Kit

E. coli were stained with 5 mM CTC and 10 nM SYTO® 24 nucleic acid stain, then analyzed on a flow cytometer with 488 nm excitation and with 530 nm bandpass and 670 nm longpass filters. Healthy cells show red fluorescence; unhealthy cells show green fluorescence.

in the kits for superior photostability during imaging. FISH Tag™ kits provide:

- a complete workflow solution for FISH applications
- exceptional signal intensity and photostability
- immediate results—no need for secondary detection methods
- multiplexing capabilities—spectrally distinct dyes allow you to view multiple targets simultaneously

flow cytometry

BacLight™ RedoxSensor™ CTC Vitality Kit

The *Bac*Light™ RedoxSensor™ CTC Vitality Kit contains 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) for evaluating the respiratory activity of many bacterial populations (Figure 7). Healthy cells respiring via the electron transport chain will absorb and reduce CTC to an insoluble, red-fluorescent formazan product. Counterstaining with green-fluorescent SYTO® 24 or blue-fluorescent DAPI nucleic acid stain enables you to differentiate cells from debris and to calculate total cell numbers.

The *Bac*Light™ RedoxSensor™ CTC Vitality Kit offers:

- an effective assay for both gram-positive and gram-negative bacteria
- an ultrapure, reactive form of CTC
- reliable staining that is compatible with formaldehyde fixation

Table 4—FISH Tag™ kits

Kit	Fluorophore(s)	Ex/Em *	Cat. no. †
FISH Tag™ DNA Green Kit	Alexa Fluor® 488	490/520	F32947
FISH Tag™ DNA Orange Kit	Alexa Fluor® 555	555/565	F32948
FISH Tag™ DNA Red Kit	Alexa Fluor® 594	590/615	F32949
FISH Tag™ DNA Far Red Kit	Alexa Fluor® 647	650/670	F32950
FISH Tag™ DNA Multicolor Kit	Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 594, Alexa Fluor® 647	See above	F32951
FISH Tag™ RNA Green Kit	Alexa Fluor® 488	490/520	F32952
FISH Tag™ RNA Orange Kit	Alexa Fluor® 555	555/565	F32953
FISH Tag™ RNA Red Kit	Alexa Fluor® 594	590/615	F32954
FISH Tag™ RNA Far Red Kit	Alexa Fluor® 647	650/670	F32955
FISH Tag™ RNA Multicolor Kit	Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 594, Alexa Fluor® 647	See above	F32956

* Fluorescence excitation and emission wavelengths, in nm. † All FISH Tag™ kits provide sufficient reagents for 10 reactions of 1 µg each.

flow cytometry

CountBright™ absolute counting beads

CountBright™ absolute counting beads are a suspension of fluorescent microspheres of known concentration. These beads are designed to be mixed with a cell sample and assayed via flow cytometry; absolute numbers of cells in the sample can be calculated from the ratio of bead events to cell events. CountBright™ absolute counting beads offer:

- single-platform testing for determination of absolute cell number
- excitation from UV to 635 nm and emission from 385 to 800 nm, all from the same bead suspension
- utility with any sample type, including no-wash/lysed whole blood testing

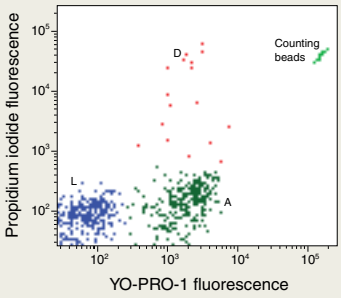

Figure 8—Determination of live, dead, and apoptotic cells and absolute cell count

Plot of YO-PRO®-1 fluorescence collected with 530/30 nm bandpass filter vs. propidium iodide fluorescence collected with 585/42 nm bandpass filter, showing apoptotic (A), live (L), and dead (D) cells, as well as counting beads. Jurkat cells (human T-cell leukemia) were treated with 10 µM camptothecin for four hours. Cells were then treated with the reagents in the Vybrant® Apoptosis Assay Kit #4. Counting beads were added and the sample was analyzed by flow cytometry using 488 nm excitation.

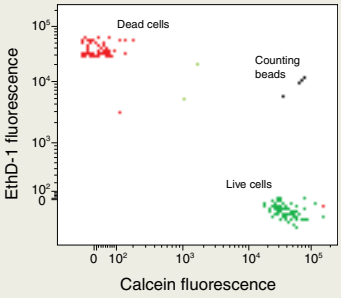

Figure 9— Determination of live and dead cells and absolute cell number

Plot of calcein fluorescence collected with 530/30 nm bandpass filter vs. ethidium homodimer-1 fluorescence collected with 610/20 nm bandpass filter, showing clear separation of live and dead cells, as well as counting beads. A mixture of live and heat-killed Jurkat cells (human T-cell leukemia) was treated with the reagents in the LIVE/DEAD® Viability/Cytotoxicity Kit, counting beads were added, and the sample was analyzed by flow cytometry using 488 nm excitation.

For convenient cell culture monitoring, add CountBright™ absolute counting beads to cell samples and analyze the results—no need for a wash step. Examples of the use of CountBright™ beads with our Vybrant® Apoptosis Assay Kit #4 and our LIVE/DEAD® Viability/Cytotoxicity Kit are shown in Figures 8 and 9, respectively. ■

references

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Product	Quantity	Cat. no.
Amplex® Red/UltraRed stop reagent *500 tests* *set of 5 vials*	1 set	A33855
*Bac*Light™ RedoxSensor™ CTC Vitality Kit *for flow cytometry and microscopy*	1 kit	B34956
CountBright™ absolute counting beads *for flow cytometry* *100 tests*	5 mL	C36950
CyQUANT® NF Cell Proliferation Assay Kit *200 assays*	1 kit	C35007
CyQUANT® NF Cell Proliferation Assay Kit *1000 assays*	1 kit	C35006
EnzChek® cellulase substrate *blue fluorescent, 339/452*	1 mg	E33953
EnzChek® epoxide hydrolase substrate	100 µg	E33956
EnzChek® lipase substrate *green fluorescent, 505/515*	100 µg	E33955
EnzChek® Myeloperoxidase (MPO) Activity Assay Kit *400 assays* *for myeloperoxidase chlorination and peroxidation activity*	1 kit	E33856
EnzChek® Peptidase/Protease Assay Kit *100 assays*	1 kit	E33758
TC-FIAsH™ In-Cell Tetracysteine Tag Detection Kit *green fluorescence* *for live-cell imaging*	1 kit	T35359
TC-FIAsH™ TC-ReAsH™ In-Cell Tetracysteine Tag Detection Kit *green and red dual fluorescence* *for live-cell imaging* *with mammalian TC-Tag Gateway® expression vectors*	1 kit	T35358
TC-ReAsH™ In-Cell Tetracysteine Tag Detection Kit *red fluorescence* *for live-cell imaging*	1 kit	T35360
Zen™ Myeloperoxidase (MPO) ELISA Kit *200 assays*	1 kit	Z33857

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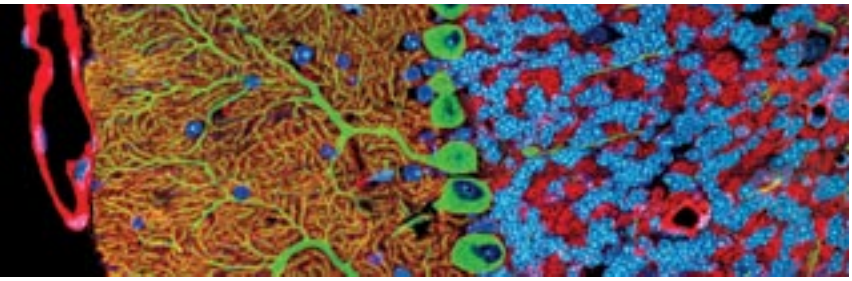

Figure 1—Multicolor fluorescence image of a mouse cerebellum section

IP3 receptors were labeled with anti-IP3R antibody and visualized using Qdot® 525 goat F(ab')$_2$ anti–rabbit IgG (green), actin was labeled with rhodamine phalloidin (red), and nuclei were stained with DAPI (blue). Image contributed by Thomas Deerinck and Mark Ellisman, The National Center for Microscopy and Imaging Research, San Diego, CA.

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This summary of Molecular Probes' neurobiology products covers only the most commonly used tracers for dissecting neural networks and their functions. For questions about these or other probes for neurobiological research, please contact the team of scientists in our Technical Support Department or visit us at **probes.invitrogen.com**. ■

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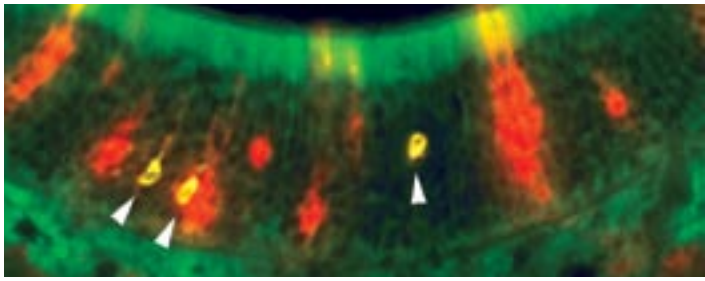

Figure 2—Double labeling with DiI and Alexa Fluor® 488 anti-GFP antibody

The olfactory epithelium of a *MOL2.3-IGITL* mouse was cross-sectioned two weeks after DiI deposition onto the dorsal glomerulus. The section was also probed with Alexa Fluor® 488 anti-GFP antibody because the intrinsic GFP fluorescence of *MOL2.3* cells was no longer detectable after the tracing period. Retrogradely DiI-labeled cells are visible in red fluorescence. *MOL2.3* cells containing GFP and DiI are yellow fluorescent (arrowheads) because of an overlay of the red and green fluorescence. Image contributed by Joerg Strotmann and Olga Levai, University of Hohenheim, Germany, and reproduced with permission from *J Comp Neurol* 458:209 (2003).

Table 1—Probes for neural anatomy and neural functions

	Fluorescence Emission*						
Probe Class	Blue	Green to Yellow	Orange to Red	Far-Red	Biotin Probes	Related Probes	Highlights and Neurobiology References
Neural Anatomy: Lipophilic Probes for Retrograde and Anterograde Tracing							
DiI and *FAST* DiI			D282 D3899 D3911 D7756 N22880			L7781 N22884	Lipophilic dialkylcarbocyanines, including DiI, DiO, DiD, and DiR, are widely used retrograde and anterograde tracers in live and fixed tissues. Ready-to-use NeuroTrace® DiI and DiO tissue-labeling pastes can be applied directly to tissues using the tip of a needle, allowing dye penetration into bundled neurons for labeling axons both on and below the surface. Also available are the NeuroTrace® Multicolor Tissue-Labeling Kit, which contains DiI, DiO, and DiD pastes, and the Lipophilic Tracer Sampler Kit, which contains DiI, DiO, DiD, DiR, and DiA analogs.
DiO and *FAST* DiO		D275 D3898 N22881					
DiD and DiR			D307 D7757	D12731			
CellTracker™ CM-DiI			C7000 C7001 N22883				CellTracker™ CM-DiI, also available as a NeuroTrace® tissue-labeling paste, is a fixable DiI analog containing a chloromethyl moiety that allows the dye to covalently bind cellular thiols.[1,2]
DiA and FAST DiA		D3883 D7758					DiA is often preferred over DiO for multicolor labeling with DiI.[3,4]
Neural Anatomy: Neuron-Selective Probes for Identifying and Localizing Neuronal Cells							
NeuroTrace® Nissl stains	N21479	N21480 N21481	N21482	N21483			Nissl staining is a standard histochemical method for visualizing neurons in the brain and spinal cord.[5,6]
Isolectin GS-IB_4		I21411	I21412 I21413	I32450	I21414		Isolectin GS-IB_4 has a particularly strong affinity for brain microglial and perivascular cells.[7,8]
Anti-GFAP antibody		A21294	A21295			A21282†	Anti-GFAP antibodies recognize the intermediate filament protein GFAP, a major structural component of astrocytes and some ependymal cells.[9] Anti-Hu antibodies recognize the HuC, HuD, and Hel-N1 RNA-binding proteins of the ELAV family, present exclusively in neuronal cells.[10] Anti–NMDA receptor antibodies recognize the NMDA receptor channel, which is gated by the neurotransmitter l-glutamate.[11] Anti-synapsin I antibodies recognize the actin-binding protein synapsin I, which is unique to synaptic vesicles.[12] A list of primary antibodies for neurobiology research is available at **probes.invitrogen.com**. Additional antibodies can be found at **www.invitrogen.com/antibodies**.
Anti-HuC/HuD neuronal protein antibody					A21272	A21271†	
Anti–NMDA receptor subunit-specific antibodies						A6473† A6474† A6475†	
Anti–synapsin I antibody						A6442†	
Neural Anatomy: Myelin-Selective Stains for Studying Myelin Distribution, Identifying Brain Structures, and Investigating Demyelinating Diseases							
FluoroMyelin™ Green and FluoroMyelin™ Red myelin stains		B34650 F34651	F34652				The BrainStain™ Imaging Kit enables three-color combinatorial labeling of myelin, neurons, and nuclei in brain cryosections in a single 20-minute staining step plus washes.

*Fluorescence emission colors are based on the fluorescence emission maximum of each probe, regardless of how broad the emission spectrum is; selective filters can be used to limit the emission wavelengths detected. †Unlabeled probe.

1. Cramer, K.S. et al. (2000) *Dev. Biol.* 224:138; **2.** McLean, P.J. et al. (2000) *J. Biol. Chem.* 275:8812; **3.** deAzevedo, L.C. et al. (2003) *J. Neurobiol.* 55:288; **4.** Vercelli, A. et al. (2000) *Brain Res. Bull.* 51:11; **5.** Hunot, S. et al. (2004) *Proc. Natl. Acad Sci. U S A* 101:665; **6.** Ikegaya, Y. et al. (2004) *Science* 304:559; **7.** Wurmser, A.E. et al. (2004) *Nature* 430:350; **8.** Dailey, M.E. and Waite, M. (1999) *Methods* 18:222; **9.** Martinac, J.A. et al. (2001) *Neurobiol. Aging* 22:195; **10.** Marusich, M.F. et al. (1994) *J. Neurobiol.* 25:143; **11.** Loftis, J.M. and Janowsky, A. (2000) *J. Neurochem.* 75:2040; **12.** Ryan, T.A. et al. (1993) *Neuron* 11:713.

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Table 1—Probes for neural anatomy and neural functions, continued

Probe Class	Fluorescence Emission * Blue	Green to Yellow	Orange to Red	Far-Red	Biotin Probes	Related Probes	Highlights and Neurobiology References
Neural Anatomy: Small Polar Dyes for Tracing Neurons and for Studying Gap Junctional Communication							
Alexa Fluor® hydrazides	A10439	A10436 A10440	A10437 A10438 A10441 A10442 A20501MP	A20502 A30634			Alexa Fluor® hydrazides are extremely bright, photostable polar tracers for neuronal tracing [13,14] and gap junctional communication [15,16] studies. Alexa Fluor® 488, Alexa Fluor® 568, and Alexa Fluor® 594 hydrazides are available as filtered 10 mM solutions in 200 mM KCl, ready for direct microinjection into cells. Anti–Alexa Fluor® 488 dye antibodies are available.
Cascade Blue® hydrazide	C687 C3221 C3239						Cascade Blue® hydrazide is a fixable, blue-fluorescent retrograde tracer. Anti–Cascade Blue® dye antibodies are available.
Lucifer yellow CH		L453 L682 L1177 L12926					The fixable, yellow-fluorescent retrograde tracer lucifer yellow CH is available as a 100 mM solution in water for loading by microinjection. Anti–lucifer yellow dye antibodies are available.
Biocytins		A12924 L6950 O12920	A12922 A12923 T12921		A12924 L6950 O12920 A12922 A12923 T12921 B1603		Fluorescent biocytins are fixable, biotinylated retrograde and anterograde tracers that can be further amplified with fluorescent and horseradish peroxidase (HRP) avidins.
Hydroxystilbamidine Aminostilbamidine		H22845 A22850					Hydroxystilbamidine is the active component of Fluoro-Gold and a well-established, fixable retrograde tracer.[17,18]
Sulforhodamine 101			S359				Sulforhodamine 101 is a nonfixable, red-fluorescent retrograde tracer and a selective astroglia marker in the neocortex.[19]
True blue chloride	T1323						UV-excitable, cationic retrograde tracer [17,18]
Neural Anatomy: Protein Conjugates for Retrograde and Anterograde Tracing							
Cholera toxin, subunit B (CTB) conjugates		C22841 C34775	C22843 C22842 C34776 C34777	C34778	C34779	C34780	Fluorescent CTB conjugates, which bind glycosphingolipid moieties, serve as lipid raft markers [20,21] and as fixable retrograde tracers.[22,23] The HRP conjugate of CTB is also available.
Wheat germ agglutinin (WGA) conjugates	W11263	W834 W6748 W11261	W849 W11262 W21405 W32464	W21404 W32465 W32466		W7024	WGA conjugates undergo axonal transport and have been shown to cross from axonal nerve endings into adjacent neurons. A WGA Sampler Kit containing four fluorescent conjugates is available.
Lectin PHA-L conjugates		L11270	L32456	L32457			Lectin PHA-L is a widely used anterograde neuronal tracer that is not degraded over long periods.[24,25]
Neural Anatomy: Dextran Conjugates for Retrograde and Anterograde Tracing							
3000 MW dextrans	D7132	D3306 D7156‡	D3308 D3328 D7162‡		D7135 D7156‡ D7162‡		3000 MW to 70,000 MW fluorescent and biotinylated dextrans are routinely employed to trace neuronal projections. Attachment of multiple labels (fluorophores, biotin, and fixable lysine groups, designated by ‡) to a single, water-soluble dextran allows tracers such as 3000 MW micro-emerald and micro-ruby and 10,000 MW mini-emerald and mini-ruby to be visualized using both fluorescence imaging and standard avidin–biotin methods. Increased fluorescence output and photostability make our 10,000 MW Alexa Fluor® dextrans [26,27] superior alternatives to traditionally labeled fluorescent dextrans. The NeuroTrace® BDA-10,000 Neuronal Tracer Kit provides BDA-10,000, HRP avidin, and DAB for neuroanatomical tracing; smaller and larger molecular weight BDA tracers are also available.[28]
10,000 MW dextrans	D1976	D1820 D1825 D7153 D7168 D7171 D7178‡ D22910	D1817 D1863 D1868 D3312‡ D22911 D22912 D22913 D34679	D22914 D34680	D1956 D3312‡ D7178‡ N7167		
40,000 MW dextrans		D1845					
70,000 MW dextrans		D1822 D7173	D1818 D1864		D1957		

* Fluorescence emission colors are based on the fluorescence emission maximum of each probe, regardless of how broad the emission spectrum is; selective filters can be used to limit the emission wavelengths detected. ‡ Dextran conjugates in which multiple labels (fluorophores, biotin, and fixable lysine groups) are covalently linked to a single, water-soluble dextran.

13. Ohki, K. et al. (2005) *Nature* 433: 597; **14.** Wilson, R.I. et al. (2004) *Science* 303:366; **15.** Weber, P.A. et al. (2004) *Biophys. J.* 87:958; **16.** Romualdi, A. et al. (2002) *Cell Tissue Res.* 307:315; **17.** Nogueira M.I. et al. (2000) *Anat. Anz.* 182:35; **18.** King, M.S., and Bradley, R.M. (2000) *Brain Res.* 866:237; **19.** Nimmerjahn, A. et al. (2004) *Nat. Methods* 1:31; **20.** Foerg, C. et al. (2005) *Biochemistry* 44:72; **21.** Hammond A.T. et al. (2005) *Proc. Natl. Acad. Sci. U S A* 102:6320; **22.** Pfeiffenberger, C. et al. (2005) *Nat. Neurosci.* 8:1022; **23.** Torborg, C.L. et al. (2005) *Nat. Neurosci.* 8:72; **24.** Vercelli, A. et al. (2000) *Brain Res. Bull.* 51:11; **25.** Gerfen, C.R. and Sawchenko, P.E. (1984) *Brain Res.* 290:219; **26.**Gerachshenko, T. et al. (2005) *Nat. Neurosci.* 8:597; **27.** Briggman K.L. et al. (2005) *Science* 307:896; **28.** Reiner, A. et al. (2000) *J. Neurosci. Methods* 103:23.

Table 1—Probes for neural anatomy and neural functions, continued

Probe Class	Fluorescence Emission* Blue	Green to Yellow	Orange to Red	Far-Red	Biotin Probes	Related Probes	Highlights and Neurobiology References
Neuron and Neural Network Functions: Membrane Potential Probes for Following Depolarization and Hyperpolarization							
DiBAC (bis-oxonol) dyes		B438 B24570	B413 B436				DiBAC dyes are useful for detecting changes in neuronal membrane potential induced by receptor agonists and channel modulators.[29,30]
ANEP (styryl) dyes			D1199 D3167 D6923 D6927				These fast-response potential-sensitive probes change their electronic structure, and thus their fluorescence properties, in response to a change in the surrounding electric field. Their optical response is sufficiently fast to detect transient (millisecond) potential changes in excitable cells, including neurons, cardiac cells, and intact brains. Protocols for imaging neuron activity using JPW 1114 and RH 414 have recently been published.[31]
RH (styryl) dyes				R649 S1108 S1109 T1111			
Neuron and Neural Network Functions: Calcium Indicators for Monitoring Calcium Fluxes							
Fura-2 and fura-2 AM §		F1200 F1201 § F1221 § F1225 § F14185 §				F3029	Fura-2, with a K_d for Ca^{2+} of 0.15 µM, remains a popular ratiometric indicator for quantitative calcium measurements. Fura-2 is also available as the 10,000 MW fura dextran.
Fluo-4 and fluo-4 AM §		F14200 F14201 § F14217 § F23917 §				F14240 F36250	Fluo-4, Oregon Green® BAPTA-1, and Oregon Green® BAPTA-2, with a K_d for Ca^{2+} of 0.35 µM, 0.17 µM, and 0.58 µM, respectively, have become the probes of choice for two-photon excitation microscopy of calcium fluxes in brain.[32,33] Low-affinity and high-affinity 10,000 MW dextran conjugates of fluo-4, as well as 10,000 MW and 70,000 MW dextran conjugates of Oregon Green® 488 BAPTA-1, are also widely used for calcium imaging of neural networks.[34,35]
Oregon Green® 488 BAPTA-1 and its AM §		O6806 O6807 §				O6798	
Oregon Green® 488 BAPTA-2 and its AM §		O6808 O6809 §					
Neuron and Neural Network Functions: Membrane Probes for Studying Synaptic Vesicle Cycling							
FM® 1-43 and the fixable analog FM® 1-43FX			F35355 T3163 T35356				FM® 1-43 and FM® 1-43FX are proven tools for identifying actively firing neurons and studying synaptic vesicle cycling.[36] FM® 2-10 is a more hydrophilic analog of FM® 1-43, requiring higher concentrations for vesicle staining.
FM® 2-10			T7508				
FM® 4-64 and the fixable analog FM® 4-64FX				F34653 T3166 T13320			The more hydrophobic analogs of FM® 1-43—FM® 4-64 and FM® 5-95—exhibit long-wavelength fluorescence emission (690–730 nm in cells) that can be distinguished from the green fluorescence of GFP reporter fusions.[37-39]
FM® 5-95				T23360			
Neuron and Neural Network Functions: Neurotransmitter Receptors and Their Ligands							
Nicotinic acetylcholine receptors: α-Bungarotoxin		B13422 F1176	B13423 B35451 T1175	B35450 B35452	B1196	A12217 B1601 † C13654	Fluorescent α-bungarotoxins[40,41] and caged carbamylcholine[42,43] are available, as well as the Amplex® Red Acetylcholine/Acetylcholinesterase Assay Kit.
$GABA_A$ receptors: caged GABA						A7110	Photolabile caged GABA has proven useful for kinetic and neuronal circuit mapping studies.[43,44]
Glutamate receptors: caged glutamate						A12221 C7122 G7055	Photolabile caged glutamates are useful for kinetic studies of receptor binding and channel opening.[43,45] The Amplex® Red Glutamic Acid/Glutamate Oxidase Assay Kit is also available.
Neurokinin receptors: Substance P		S13427	S13428				Oregon Green® 488 and tetramethylrhodamine conjugates of substance P are important probes for studying binding and activation of neurokinin receptors.[46,47]
$P2X_7$ purinoceptors: YO-PRO-1		Y3603					Pore dilation of $P2X_7$, an ATP-gated channel that is highly expressed in astrocytes, can be assessed using YO-PRO-1.[48]

*Fluorescence emission colors are based on the fluorescence emission maximum of each probe, regardless of how broad the emission spectrum is; selective filters can be used to limit the emission wavelengths detected. † Unlabeled probe. § Cell-permeant acetoxymethyl ester (AM) form of the indicator. BDA = biotin dextran amine. DAB = 3,3'-diaminobenzidine. ELAV = embryonic lethal abnormal vision. GABA = γ-aminobutyric acid. GFAP = glial fibrillary acidic protein. HRP = horseradish peroxidase. NMDA = *N*-methyl-D-aspartic acid. UV = ultraviolet light. X = 7-atom aminohexanoyl spacer.

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JOURNAL HIGHLIGHT

Imaging input and output of neocortical networks *in vivo*

Kerr, J.N., Greenberg, D., and Helmchen, F. (2005) *Proc. Natl. Acad. Sci. U S A* 102:14063

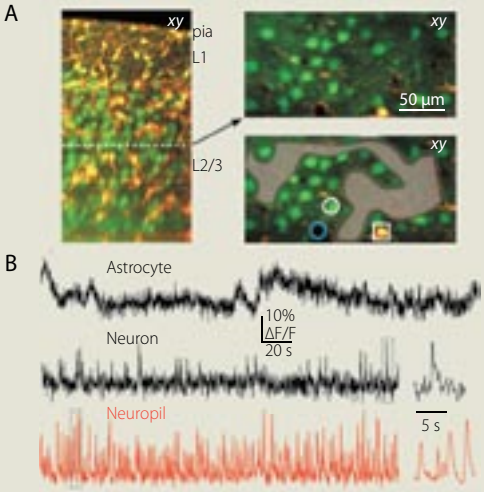
Information processing within the cortex cannot be fully understood at the level of the single neuron, as a neuronal network contains complex communities of cells that function synchronously as well as in series to achieve the high level of sensory processing required of the animal brain. To obtain a more comprehensive description of population activity in a neocortical circuit with single-neuron and single-spike resolution, Kerr, Greenberg, and Helmchen used two-photon excitation microscopy to image and optically dissect calcium transients *in vivo* in a rat neocortex that had been bulk-labeled with the acetoxymethyl ester (AM) of the Oregon Green® 488 BAPTA-1 Ca^{2+} indicator (see figure below).

The recently developed multicell bolus loading method [1-3] allowed these researchers to target the neuronal circuit of interest by microinjecting the cell-permeant AM ester into the corresponding neocortical layers of the exposed rat brain. Because of the nonspecific nature of this type of labeling, the calcium signals arising from astrocytes and neurons had to be subsequently distinguished using the red-fluorescent sulforhodamine 101 dye, which selectively labels the astrocyte population.[4] These researchers confirmed that the astrocytes were responsible for the slow calcium oscillations (on the minute time scale), whereas the neurons exhibited the spontaneous but infrequent calcium transients of short duration (less than 1 second). These neuron-derived calcium transients were confirmed using targeted electrical recordings of action potential–evoked calcium influx through voltage-dependent calcium channels. The researchers were then able to optically extract the action potential–evoked activity ("output") with single-cell and single-spike resolution from the total calcium signals emitted by the bulk-labeled neocortical tissue, and to assign this action-potential activity to specific neurons in the local neuronal circuit.

In addition to the astroglial and neuronal calcium transients, Kerr and coworkers used two-photon calcium imaging combined with electrocorticogram and whole-cell recordings to explore the prominent axonal-based calcium signal arising from the surrounding neuropil. They found that these spontaneous calcium signals were tightly correlated with the electrocorticogram and subthreshold membrane potentials of neurons within the field of view and that they provided a volume-averaged measure of synaptic-potential activity ("input").

Using these measures of output and input, the researchers characterized spontaneous activity during cortical Up states. They concluded that spontaneous activity in the neocortex is sparse and heterogeneously distributed spatially and temporally across the neuronal population. Their techniques for optically dissecting input and output activities in bulk-labeled tissue should enable further studies of signal transmission within cortical networks and a more complete understanding of neocortical activities.

1. Sullivan, M.R. et al. (2005) *J. Neurophysiol.* 94:1636; **2.** Ohki, K. et al. (2005) *Nature* 433:597; **3.** Stosiek, C. et al. (2003) *Proc. Natl. Acad. Sci. U S A* 100:7319; **4.** Nimmerjahn, A. et al. (2004) *Nat. Methods* 1:31.



Spontaneous calcium transients in cell somata and neuropil of bulk-loaded neocortical layer 2/3

(Panel A, left) Side projection of Oregon Green® BAPTA-1–loaded cells in the motor cortex. Astrocytes (yellow) were counterstained with sulforhodamine 101. (Panel A, top right) Two-photon image 250 µm below pial surface showing neurons (green), astrocytes (yellow), and surrounding neuropil loaded with Oregon Green® 488 BAPTA-1. (Panel A, bottom right) Same area as top right showing regions of interest: neuron (white circle), astrocyte (white square), neuropil (shaded gray), and blood vessel lumen for background (blue circle). (Panel B) Simultaneous calcium transients from identified astrocyte (top), neuron (middle), and neuropil (bottom) recorded over several minutes. (Inset) Note the sharp transients with fast onset and exponential decay (black) and ongoing neuropil signal (red) on expanded time scale (from boxes). Image contributed by Jason N.D. Kerr and Fritjof Helmchen, Max Planck Institute for Medical Research, Germany, and reproduced with permission from *Proc. Natl. Acad. Sci. U S A* 102:14063 (2005).

Product	Quantity	Cat. no.
Oregon Green® 488 BAPTA-1, AM *cell permeant* *special packaging*	10 × 50 µg	O6807
sulforhodamine 101	25 mg	S359