RECENTLY PUBLISHED

An in-depth look at fluorescent dyes for organelle labeling

A review of reagents for fluorescence microscopy of cellular compartments and structures, Parts I–III.

- "Part I: BacMam labeling and reagents for vesicular structures" Dolman NJ, Kilgore JA, Davidson MW (2013) *Curr Protoc Cytom* 65:12.30.1–12.30.27.
- "Part II: Reagents for non-vesicular organelles" Kilgore JA, Dolman NJ, Davidson MW (2013) *Curr Protoc Cytom* 66:12.31.1–12.31.24.
- "Part III: Reagents for actin, tubulin, cellular membranes, and whole cell and cytoplasm" Kilgore JA, Dolman NJ, Davidson MW (2014) *Curr Protoc Cytom* 67:12.32.1–12.32.17.

Choosing a fluorescent reagent for imaging cellular structures and organelles in live or fixed cells can be daunting. Not only must the excitation and emission spectra of the reagent match your imaging instrument, but often you also need to choose from among a number of reagents—including organic dyes, drug conjugates, and fluorescent protein constructs—that target the same structure. Compatibility with the cells, with other probes in the same experiment, and with the treatments and protocols you are using can lead to the question: How do I choose one fluorescent probe over another?

Each fluorescent reagent comes with its own set of characteristics that may prove to be beneficial or detrimental for a particular assay or environmental condition. Once a reagent is chosen, validated protocols are not always available, optimizations are often required, and image examples can be hard to locate. Moreover, when a problem arises during the course of the experiments, identifying a solution can be difficult due to a lack of troubleshooting notes in product manuals or published references.

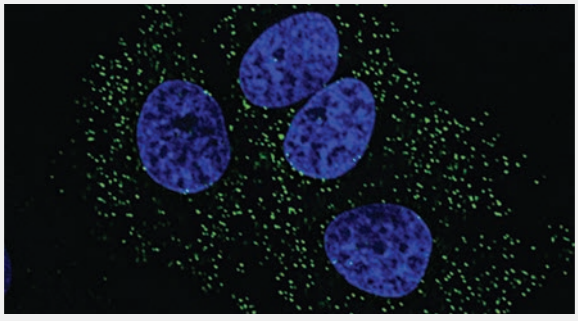

Figure 1. (Figure 12.30.4 from "Part I") Peroxisome staining in HeLa cells. HeLa cells were transduced with CellLight® Peroxisome-GFP to transiently express a GFP fusion protein in peroxisomes. Cells were counterstained with Hoechst® 33342 dye (blue, nuclei). Reprinted with permission from *Current Protocols in Cytometry*.

A three-part series of review articles that help to clarify these issues has recently been published in *Current Protocols in Cytometry*. Authored by our R&D scientists Jason Kilgore and Nick Dolman, who have developed and tested many Molecular Probes® organelle stains, as well as imaging guru Michael Davidson of Florida State University, this series pulls together a wealth of references and personal knowledge that covers the most common fluorescent reagents for nearly every cellular structure or organelle, focusing primarily on microscopic analysis.

Each article is broken into sections by target structure or organelle. The discussion includes a short history on the different reagents that have been used and their characteristics. A featured reagent is highlighted, along with a validated protocol for that reagent with an estimate on protocol time, a troubleshooting guide in table format, an image example, and relevant recipes for stock and final solutions.

In Part I the authors focus on the BacMam reagents, which have been used for transient transduction of fluorescent proteins targeted to specific cellular structures. They also discuss options for vesicular organelles such as endosomes (featuring pHrodo® dextran conjugates), lysosomes (featuring the organic dye LysoTracker® Red DND-99), peroxisomes (featuring the BacMam reagent CellLight® Peroxisome-GFP, Figure 1), and autophagosomes (featuring another BacMam reagent, the Premo™ Autophagy Sensor GFP-LC3B). →

In Part II the authors discuss reagents for labeling the endoplasmic reticulum and nuclear membranes (featuring the ER-Tracker™ dyes), Golgi apparatus (featuring dye-labeled ceramides), mitochondria (featuring the organic dye MitoTracker® Red CMXRos, Figure 2), and nucleoli (featuring the nucleic acid dye SYTO® RNASelect™ Green). The best nucleus-selective dyes for live- and fixed-cell labeling are also highlighted.

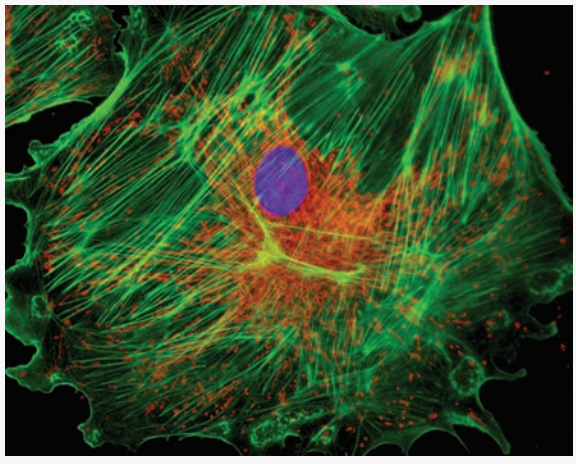

Figure 2. (Figure 12.31.6 from "Part II") Mitochondria and actin staining in a BPAE cell. A live BPAE (bovine pulmonary artery endothelial) cell was labeled with MitoTracker® Red CMXRos (red, mitochondria) and then fixed, permeabilized, and labeled with Alexa Fluor® 488 phalloidin (green, F-actin) and DAPI (blue, nuclei). Reprinted with permission from *Current Protocols in Cytometry*.

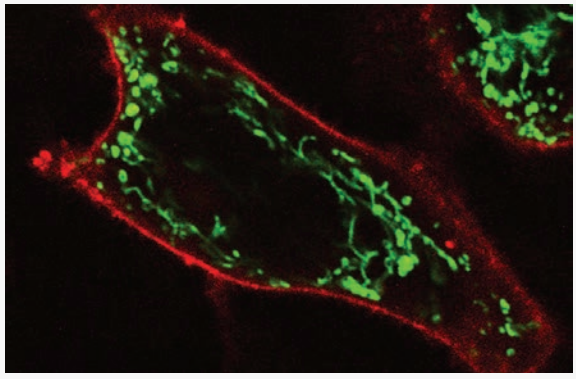

Figure 3. (Figure 12.32.3 from "Part III") Mitochondria and plasma membrane staining in HeLa cells. Live HeLa cells were transfected using pShooter™ vector pCMV/myc/mito/GFP and Lipofectamine® 2000 Transfection Reagent, stained with the Alexa Fluor® 594 conjugate of wheat germ agglutinin (WGA) (red, plasma membrane), and then imaged using confocal microscopy. Reprinted with permission from *Current Protocols in Cytometry*.

In Part III the authors describe reagents for actin (featuring Alexa Fluor® phalloidin conjugates, Figure 2), tubulin (featuring the drug conjugate Tubulin Tracker™ Green), cellular membranes (featuring plasma membrane labeling with fluorescent wheat germ agglutinin (WGA) conjugates, Figure 3), and whole-cell and cytoplasm reagents (featuring the organic dye CFSE, also called CFDA, SE).

All three articles in this *Current Protocols in Cytometry* series—Part I, Part II, and Part III—are available through the Wiley Online Library. ■

Selected products featured in this *Current Protocols in Cytometry* series	Quantity	Cat. No.
Part I: BacMam labeling and reagents for vesicular structures		
CellLight® Peroxisome-GFP, BacMam 2.0	1 mL	C10604
LysoTracker® Red DND-99	20 x 50 µL	L7528
pHrodo® Red Dextran, 10,000 MW	0.5 mg	P10361
pHrodo® Green Dextran, 10,000 MW	0.5 mg	P35368
Premo™ Autophagy Sensor LC3B-GFP, BacMam 2.0	1 kit	P36235
Premo™ Autophagy Sensor LC3B-RFP, BacMam 2.0	1 kit	P36236
Part II: Reagents for non-vesicular organelles		
7-Aminoactinomycin D (7-AAD)	1 mg	A1310
BODIPY® FL C5-Ceramide, complexed to BSA	5 mg	B22650
BODIPY® TR Ceramide, complexed to BSA	5 mg	B34400
DAPI	10 mg	D1306
ER-Tracker™ Blue-White DPX	20 x 50 µL	E12353
ER-Tracker™ Green (BODIPY® FL Glibenclamide)	100 µg	E34251
ER-Tracker™ Red (BODIPY® TR Glibenclamide)	100 µg	E34250
Hoechst® 33342, Trihydrochloride, Trihydrate	100 mg	H1399
MitoTracker® Red CMXRos	20 x 50 µg	M7512
NBD C6-Ceramide, complexed to BSA	5 mg	N22651
SYTO® 9 Green-Fluorescent Nucleic Acid Stain	100 µL	S34854
SYTO® 59 Red-Fluorescent Nucleic Acid Stain	100 µL	S11341
SYTO® RNASelect™ Green-Fluorescent Cell Stain	100 µL	S32703
SYTOX® Green Nucleic Acid Stain	250 µL	S7020
TO-PRO®-3 Iodide (642/661)	1 mL	T3605
Part III: Reagents for actin, tubulin, cellular membranes, and whole cell and cytoplasm		
Alexa Fluor® 488 Phalloidin	300 units	A12379
5-(and 6-)Carboxyfluorescein Diacetate, Succinimidyl Ester (CFSE; CFDA, SE)	25 mg	C1157
Tubulin Tracker™ Green (Oregon Green® Taxol, Bis-Acetate)	1 set	T34075
Wheat Germ Agglutinin, Alexa Fluor® 594 Conjugate	5 mg	W11262