

Fluorescent probes for plant imaging

Invitrogen tools for plant cell biology

Introduction

Fluorescence imaging in plants has unique challenges and methodologies. Plant staining is complicated by endogenous autofluorescence of plant tissues, along with the impermeability of the plant cell wall to protein-based labels. Autofluorescence arises from a variety of plant biomolecules, including chlorophyll, carotene, and xanthophyll. Chlorophyll, the major contributor to autofluorescence, has an absorption band in the blue region of the visible spectrum with a high extinction coefficient, and produces a significant amount of fluorescence above 600 nm when excited with wavelengths between 420 and 460 nm. In addition, the impermeability of cellulose cell walls to protein-based probes such as antibodies can pose significant challenges for plant imaging.

Fortunately, methods have been developed to overcome these challenges, and successful imaging of plant cells and tissues is fully possible with the right tools. To allow large molecule access to intracellular contents, the cell wall can be enzymatically digested. In fixed samples, autofluorescence can be removed by several methods. Chemical treatments including sodium borohydride and copper sulfate can be used, and embedding procedures using paraffin and methacrylate have also been reported.¹ Removing autofluorescence frees up the red wavelengths (550–700 nm) and allows the use of long-wavelength stains such as Alexa Fluor® 647.

If autofluorescence removal techniques are not feasible in your experimental system, the use of dyes with emission spectra outside the autofluorescence region can be used. Blue dyes (e.g., DAPI), yellow/orange dyes (e.g., rhodamines), and long-wavelength stains (emission >650 nm) are not affected by autofluorescence, depending on the tissue source. Green stains such as fluorescein or Alexa Fluor® 488 also have a strong use history in plants without published background problems. Alternatively, the use of microscope filters that block excitation in the 420–460 nm range is sufficient to remove the background autofluorescence signal due to chlorophyll.²

The following sections describe the use of fluorescent technologies in a wide range of plants, for a variety of applications. Invitrogen Molecular Probes® reagents were used in the majority of the studies. See the ordering information and associated references for a complete list of Invitrogen products available for plant imaging.

Histological applications

Immunohistochemistry

Immunohistochemistry exploits the binding of antibodies to specific targets to determine the localization of biomolecules within tissues. The technique is routinely used in plants, and has been used to label diverse plant tissues and organelles including cell walls, vacuoles, chromatin, nuclei, nuclear membranes, and chloroplasts.^{3–8} Invitrogen offers a comprehensive selection of dyes suitable for immunohistochemistry in plants, including the bright and stable Alexa Fluor® dyes and conjugates that span the entire visible spectrum. In addition, our unique Zenon® immunolabeling technology provides an easy, versatile method of labeling antibodies with a broad selection of Molecular Probes® premier dyes, haptens, and enzymes. See Figures 1–4 for examples.

Qdot® nanocrystals in plants

Qdot® nanocrystals are a novel class of fluorophores that combine revolutionary fluorescence performance inherent in their nanocrystal structure with a highly customizable surface for directing their bioactivity, producing a fluorescent probe that outperforms traditional dyes in many applications. The use of Qdot® nanocrystals has been reported in plants as a highly sensitive method for immunostaining.⁹

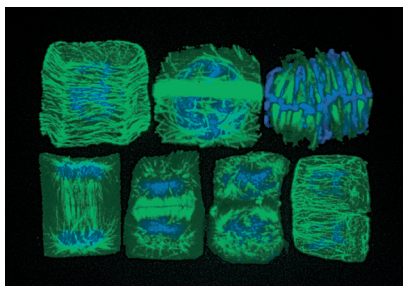


Figure 1—Dividing wheat root cells. Panel of confocal micrographs showing cells from wheat root tips in seven stages of the cell cycle. DNA was stained with 7-aminoactinomycin D (Cat. no. A1310), and microtubules were labeled with an anti- β -tubulin antibody in conjunction with a fluorescein-labeled secondary antibody. Image contributed by B.E.S. Gunning, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University. Used with permission from Gunning, B.E.S. and Steer, M.W., *Plant Cell Biology—Structure and Function*, Jones and Bartlett Publishers (1995).

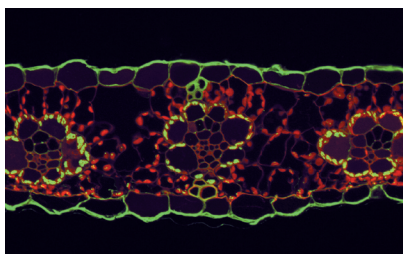


Figure 2—Rubisco localization in maize leaf section. Rubisco was localized using a rabbit anti-rubisco antibody and visualized using the highly cross-adsorbed Alexa Fluor® 488 goat anti-rabbit IgG antibody (Cat. no. A11034). The 2.0 μ m maize leaf section illustrates the immunolocalization of the enzyme ribulose biphosphate carboxylase (rubisco) in the chloroplasts of the bundle sheath cells surrounding the vascular bundles. The red fluorescence, localized to the mesophyll plastids, is due to background autofluorescence of chlorophyll. Lignin appears dull green and is localized to the xylem of the vascular bundle; cutin appears bright green and is localized to the cuticle outside the epidermis. Image contributed by Todd Jones, DuPont.

TC-FIAsh™ expression technology

TC-FIAsh™ expression technology utilizes a small 6–amino acid tag and the FIAsh reagent for fast, easy, and specific detection of tagged proteins. With the TC-FIAsh™ Expression Analysis Detection Kits, a TC tag expression construct is generated, which is transformed into host cells for subsequent detection of the protein upon incubation with the labeling reagent. The technique has been used successfully for protein visualization in *Arabidopsis* and tobacco.¹⁰

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) technology in plants uses the same methodology employed in animal cells. FISH is a powerful technique for localizing specific nucleic acid targets within fixed tissues and cells, allowing you to obtain temporal and spatial information about gene expression and genetic loci.^{11–14} Using spectrally distinct fluorophore labels for each different hybridization probe enables analysis of multiple targets within a single sample. Invitrogen offers a comprehensive selection of FISH kits and reagents.

Cell wall stains

Plant cell wall stains are diverse and include Hoechst 33258 (aka bis-benzimide), lectin conjugates, Calcofluor (available from Sigma and a component of Invitrogen's Yeast Viability Kit), and histochemical stains with incidental fluorescent properties.^{15–19} All of these products localize to the polysaccharide components in cell walls. In addition, the NanoOrange® dye, a reagent used to quantitate proteins in solution, has also been used to label plant cell walls.

Membrane stains

The water-soluble FM® dyes, which are nontoxic to cells and virtually nonfluorescent in aqueous media, are believed to insert into the outer leaflet of the membrane where they become intensely fluorescent. The styryl dye FM® 4-64 has been reported to selectively stain yeast vacuolar membranes with red fluorescence, and its use has been reported in plants.^{20,21} Anecdotal evidence from Invitrogen customers also supports the use of Dil variants (SP-Dil, Cat. no. D7777) and FM® 1-43 for plant membrane staining.

Endoplasmic reticulum stains

While not tested in our labs, the drug glibenclamide (glyburide) shows activity in plants.²² This drug binds to the sulfonylurea receptors of ATP-sensitive K⁺ channels which are prominent on the endoplasmic reticulum (ER); thus, the pharmacological activity of glibenclamide potentially affects ER function. The ER-Tracker™ Green and ER-Tracker™ Red stains are conjugates of glibenclamide with BODIPY® fluorophores, and are highly selective for the ER. These dyes are likely to effectively label the ER in plants.

Mitochondrial stains

Although conventional fluorescent stains for mitochondria, such as tetramethylrosamine and rhodamine 123, are readily sequestered by functioning mitochondria, these stains are easily washed out of cells once the mitochondria experience a loss in membrane potential. This characteristic limits the use of such conventional stains in experiments that require cells to be treated with aldehyde fixatives or with other agents that affect the energetic state of the mitochondria. Many of cell-permeant MitoTracker® probes are not subject to this limitation, and are retained in the mitochondria after fixation. MitoTracker® Green FM and MitoTracker® Red FM are good live-cell options. Another option is JC-1, a novel cationic carbocyanine dye that accumulates in mitochondria, used as a sensitive marker for mitochondrial membrane potential.^{23–28}

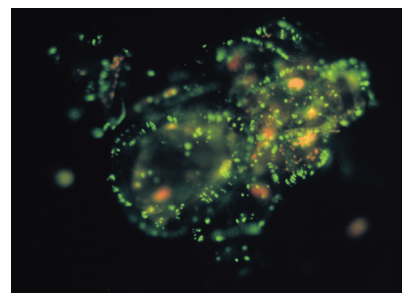


Figure 3—Pectin associated with plasmodesmata pit fields of kiwifruit cells. Pectin, a component of the cell wall matrix and the main constituent of the middle lamella that forms between daughter cell walls, was tagged with an anti-pectin monoclonal antibody, JIM 5. The primary antibody was detected and visualized with Alexa Fluor® 488 goat anti-rat IgG (Cat. no. A11006). The primary antibody was a gift from Dr. Paul Knox, University of Leeds, U.K. Image contributed by Paul Sutherland, The Horticulture and Food Research Institute of New Zealand, Ltd., Mt. Albert Research Centre.

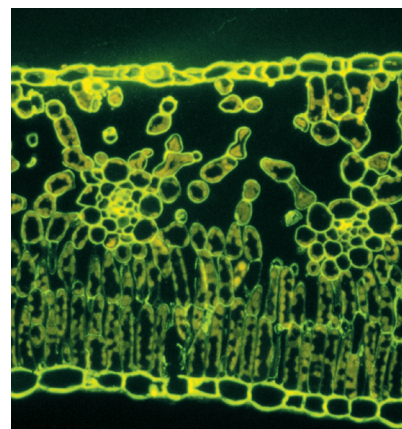


Figure 4—Pectin localization in apple leaf cell walls. Primary cell walls in an apple leaf section (500 nm thick) were identified with an antibody to the methyl-esterified regions of pectic polysaccharides or pectin, and visualized with green-fluorescent Alexa Fluor® 488 goat anti-rabbit IgG antibody (Cat. no. A11008). The orange regions inside the cells are due to the autofluorescent properties of chlorophyll localized in the chloroplasts. Image contributed by Paul Sutherland, EM Unit, Mt. Albert Research Centre, Auckland, New Zealand.

Nucleic acid stains and nucleotide incorporation

DNA staining in plants is commonly used for estimation of nuclear or chloroplast DNA content using flow cytometry.^{29–46} Other applications include viability assays, cell cycle analysis, spatial and temporal organization of DNA, and base content analysis. Commonly used stains for nucleic acids in plants are 7-AAD (Figure 1), DAPI, Hoechst 33258, YOYO[®]-1 iodide, YO-PRO[®]-1 iodide, acridine orange, ethidium bromide, and propidium iodide. For ultrasensitive detection of double-stranded nucleic acids, the YO-PRO[®]-1 iodide stain is one of the most sensitive fluorescent probes available for DNA staining.

For nucleotide incorporation studies, BrUTP is an excellent substrate for RNA polymerase and has been used to monitor nucleolar transcription *in situ*. BrUTP can be detected with anti-BrdU antibodies.

Cytoskeletal stains

The use of phalloidins to stain actin filaments is common in plants.^{47–49} Fluorescent and biotinylated phallotoxins stain F-actin at nanomolar concentrations. They are extremely water-soluble, and have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phallotoxin molecule per actin subunit in muscle and nonmuscle cells from many different species of plants and animals.

Cell biology applications

AM ester dye loading

The acetoxymethyl (AM) ester derivatives of fluorescent indicators and chelators make up one of the most useful groups of compounds for the study of live cells. Modification of carboxylic acids with AM ester groups results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a charged form that leaks out of cells far more slowly than its parent compound.

AM ester dyes can be used to fluorescently label plant cells for cell biology applications and functional studies. The Calcium Green[™]-1 dye, a calcium-sensitive AM ester, can be loaded into guard cells for analysis of calcium-dependent pathways regulating stomatal aperture.⁵⁰ This technique is likely to be applicable to other plant cell types. The fluorescent dye carboxyfluorescein diacetate (5(6)-CFDA) has been used as an indicator of the intactness of isolated chloroplasts, and may potentially be useful for protein import assays.⁵¹

Endocytosis and transport studies

A diverse selection of fluorescent probes, including Qdot[®] nanocrystals, can be used for endocytosis and transport studies in plant cells.^{52–55} Examples include FM[®] 1-43, Lucifer Yellow, Texas Red[®] dye, carboxyfluorescein, and sulforhodamine G. These dyes are taken up by endocytosis and may be distributed to distinct intracellular compartments.

Glucose metabolism

2-NBDG is a fluorescent glucose analog that can be used to monitor glucose uptake in live cells, and as an indicator of cell viability. In plants, 2-NBDG has been used to study the mechanism of glucose uptake, in conjunction with Texas Red[®] and Alexa Fluor[®] 488 dyes.⁵⁶

Viability staining

Fluorescein diacetate (FDA) has been used as a viability indicator for pollen grains and cultured plant cells.^{57,58} Although not tested in our labs, calcein AM, a cell-permeant dye that has been tested in many eukaryotic cells, is likely to work in plants as well. In addition, other AM ester dyes have the potential to be used in plants for viability staining.

For viability analysis in whole plant tissues, it has recently been reported that the SYTOX[®] Green, Blue, and Orange nucleic acid stains can be used to selectively stain nonviable cells within living plant tissues, including embryos and roots.⁵⁹ These dyes bind selectively to nucleic acids in cells with compromised membranes—a common feature of nonviable plant cells. Because the SYTOX[®] dyes cover a broad range of the spectrum, they can be easily combined with other stains or fluorescent-labeled proteins for colocalization studies.

Glutathione detection

Monochloromobinane (mBCl) has been used to quantify glutathione (GSH) *in vivo* in several plant cell types, including poplar leaves, and *Arabidopsis* single cells or cell culture populations.^{60,61} mBCl is essentially nonfluorescent until conjugated and readily reacts with several low molecular weight thiols including glutathione, N-acetylcysteine, and mercaptopurine.

Calcium/ion imaging

Chemical fluorescent dyes are widely used for *in vivo* Ca²⁺ imaging in plants.^{62–66} These studies take advantage of fluorescence intensity increases upon the binding of the dye to intracellular Ca²⁺. Fura dextran conjugates tend to remain in the cytosol without compartmentalization or leakage, and are less likely to bind to cellular proteins, making them useful for long-term Ca²⁺ mea-

surements (Figure 5). Other available options are fluo-3, Fura Red™, fura-2, PBFI AM, and calcein AM. For potassium imaging, PBFI AM is a potassium indicator that is ratiometric and UV light–excitable. This acetoxymethyl (AM) ester form is useful for noninvasive intracellular loading.

Reactive oxygen sensing

Invitrogen offers several probes that either generate or detect various reactive oxygen species, including singlet oxygen, hydroxyl radicals, and various peroxides and hydroperoxides. For plant studies, most fluorescent dyes for the detection of reactive oxygen species are versions of the cell-permeant H₂DCFDA indicator.^{67–74} H₂DCFDA is nonfluorescent until the acetate groups are removed by intracellular esterases, and oxidation occurs within the cell. Improved versions of this dye include 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate.

Another option is Singlet Oxygen Sensor Green, a highly selective, cell-impermeant detection reagent for singlet oxygen.⁷⁵ Unlike other available fluorescent and chemiluminescent singlet oxygen detection reagents, Singlet Oxygen Sensor Green does not show any appreciable response to hydroxyl radical or superoxide. This indicator initially exhibits weak blue fluorescence, but in the presence of singlet oxygen, it emits a green fluorescence (excitation/emission maxima ~504/525 nm) similar to that of fluorescein.

The Amplex® Red Glutamic Acid/Glutamate Oxidase Assay Kit provides an ultrasensitive method for continuously detecting glutamic acid or for monitoring glutamate oxidase activity in a fluorescence microplate reader or fluorometer.⁶⁷ XTT is used to assess cell viability as a function of redox potential.⁶⁹ Actively respiring cells convert the water-soluble XTT to a water-soluble, orange colored formazan product.

pH sensors

Fluorescent dyes provide the sensitivity required for optical pH measurements inside live cells. Invitrogen offers a variety of fluorescent pH indicators, several of which have published usage in plant cells. For successful quantitation of pH, it is essential to match the indicator's pK_a to the pH of your experimental system. The cell-permeant, dual-excitation ratiometric pH indicator BCECF AM is ideal for measuring changes in the cytosolic pH of most cells.⁷⁶ Intracellular pH measurements with BCECF are made by determining the pH-dependent ratio of emission intensity (detected at 535 nm) when the dye is excited at ~490 nm versus the emission intensity when excited at its isosbestic point of ~440 nm.

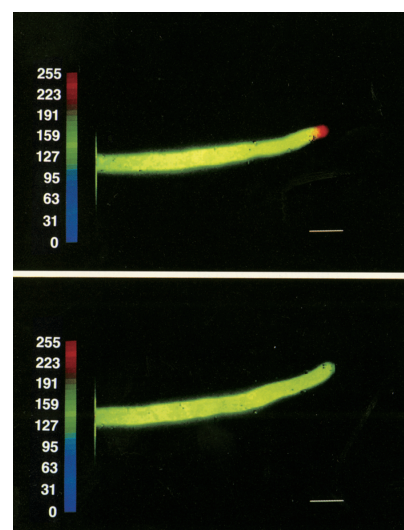


Figure 5—Ca²⁺ gradient in elongating lily pollen tube. Top panel: Pseudocolored image of a pollen tube of *Lilium longiflorum* injected with fura dextran (Cat. no. F3029). The cell continues elongating and clearly shows a Ca²⁺ gradient. Bottom panel: The same pollen tube after injection with dibromo BAPTA (Cat. no. D1211) remains healthy but is no longer elongating. Images contributed by Debra Miller, Dale Callahan, David Gross, and Peter Hepler, University of Massachusetts.

References

General

1. Kronenberger J et al. (1993) A methacrylate embedding procedure developed for immunolocalization on plant tissues is also compatible with in situ hybridization. *Cell Biol Int* 17:1013–1021.
2. Fricker MD, White NS (1992) Wavelength considerations in confocal microscopy for botanical specimens. *J Microscopy* 166:29.

Immunohistochemistry

3. Kukkola EM et al. (2004) The dibenzodioxin lignin substructure is abundant in the inner part of the secondary wall in Norway spruce and silver birch xylem. *Planta* 218:497–500.
4. Park M et al. (2004) Identification of the protein storage vacuole and protein targeting to the vacuole in leaf cells of three plant species. *Plant Physiol* 134:625–639.
5. Shibata F et al. (2004) Differential localization of the centromere-specific proteins in the major centromeric satellite of *Arabidopsis thaliana*. *J Cell Sci* 117:2963–2970.
6. Kramer VL et al. (2004) The promotion of gravitropism in *Arabidopsis* roots upon actin disruption is coupled with the extended alkalization of the collemella cytoplasm and a persistent lateral auxin gradient. *Plant J* 39:113–125.
7. Goodin M et al. (2005) Live-cell imaging of rhabdovirus-induced morphological changes in plant nuclear membranes. *Mol Plant Microbe Interact* 18:703–709.
8. Gao H et al. (2006) FZL, an FZO-like protein in plants, is a determinant of thylakoid and chloroplast morphology. *Proc Natl Acad Sci U S A* 103:6759–6764.

Qdot® nanocrystals in plants

9. Muller F et al. (2006) Quantum dots—a versatile tool in plant science? *J Nanobiotechnology* 4:5.

FIAsH expression technology

10. Estevez JM et al. (2006) FIAsH-based live-cell fluorescent imaging of synthetic peptides expressed in *Arabidopsis* and tobacco. *Biotechniques* 41:569–570.

Fluorescence in situ hybridization (FISH)

11. Leitch AR et al. (1994) The use of fluorochromes in the cytogenetics of the small grained cereals (Triticeae). *Histochem J* 26:471–479.
12. Guerra M (2001) Fluorescent in situ hybridization in plant polytene chromosomes. *Methods Cell Sci* 23:133–138.
13. Ribeiro T et al. (2004) Evidence for ‘cross-talk’ between A and B chromosomes of rye. *Proc Biol Sci Suppl* 6: S482–484.
14. Kato A et al. (2005) Advances in plant chromosome identification and cytogenetic techniques. *Curr Opin Plant Biol* 8:148–154.

Cell wall stains

15. Decreux A et al. (2005) Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. *J. Plant Cell Physiol* 46:268–278.
16. Hernandez LF et al. (1988) Fluorescent staining of primary plant cell walls using bis-benzimide (33258 Hoechst) fluorochrome. *Stain Technol* 63:190.
17. Meadows MG et al. (1984) A batch assay using Calcofluor fluorescence to characterize cell wall regeneration in plant protoplasts. *Anal Biochem* 141:38–42.
18. Hogetsu T et al. (1990) Detection of hemicelluloses specific to the cell wall of tracheary elements and phloem cells by fluorescein-conjugated lectins. *Protoplasma* 156:67.
19. Robin JB et al. (1986) Rapid visualization of three common fungi using fluorescein-conjugated lectins. *Investigative Ophthalmol Visual Sci* 27:500.

Membrane stains

20. Fukada A et al. (2004) Function, intracellular localization and the importance in salt tolerance of a vacuolar Na⁽⁺⁾/H⁽⁺⁾ antiporter from rice. *Plant Cell Physiol* 45:146–159.
21. Arvinte T et al. (1988) Characterization of the pH-induced fusion of liposomes with the plasma membrane of rye protoplasts. *Biochemistry* 27:5671.

Endoplasmic reticulum stains

22. Leonhardt N et al. (1999) ATP binding cassette modulators control abscisic acid-regulated slow anion channels in guard cells. *Plant Cell* 11:1141–1152.

Mitochondria stains

23. Eisfelder BJ et al. (2004) The mitochondrion—an organelle commonly involved in programmed cell death in *Arabidopsis thaliana*. *Plant J* 40:596–610.
24. Arimura S et al. (2004) Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proc Natl Acad Sci U S A* 101:7805–7808.
25. Arimura S et al. (2004) *Arabidopsis* dynamin-like protein 2a (ADL2a), like ADL2b, is involved in plant mitochondrial division. *Plant Cell Physiol* 45:236–242.
26. Obara K et al. (2002) The use of multiple transcription starts causes the dual targeting of *Arabidopsis* putative monodehydroascorbate reductase to both mitochondria and chloroplasts. *Plant Cell Physiol* 43:697–705.
27. Petit PX (1992) Flow cytometric analysis of rhodamine 123 fluorescence during modulation of the membrane potential in plant mitochondria. *Plant Physiol* 98:279.
28. Liu Z et al. (1987) Potentiometric cyanine dyes are sensitive probes for mitochondria in intact plant cells. *Plant Physiol* 84:1385.

DNA stains and nucleotide incorporation

29. Rowan BA et al. (2007) A high-throughput method for detection of DNA in chloroplasts using flow cytometry. *Plant Methods* 3:5.
30. Dolezel J et al. Estimation of nuclear DNA content in plants using flow cytometry. *J. Nat Protoc* 2:2233–2244.
31. Chamovitz DA et al. (1996) The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* 86:115.
32. Sawitzky H et al. (1995) Phragmoplast of the green alga *Spirogyra* is functionally distinct from the higher plant phragmoplast. *J Cell Biol* 130:1359.
33. Noueir AO et al. (1994) Two proteins of a plant dna virus coordinate nuclear and plasmodesmal transport. *Cell* 76:925.
34. Hasezawa S et al. (1992) Okadaic acid as a probe to analyse the cell cycle progression in plant cells. *Bot Acta* 105:63.
35. Zhang G (1992) Flow cytometric characteristics of sperm cells isolated from pollen of *Zea mays* L. *Plant Physiol* 99:54.
36. Jordan EG (1992) Widely dispersed DNA within plant and animal nucleoli visualised by 3-D fluorescence microscopy. *Chromosoma* 101:478.
37. Villalba JM et al. Functional expression of plant plasma membrane H⁺-ATPase in yeast endoplasmic reticulum. *J Biol Chem* 267:12341.
38. Traas JA et al. (1989) The organization of the cytoskeleton during meiosis in eggplant (*Solanum melongena* (L.)): Microtubules and F-actin are both necessary for coordinated meiotic division. *J Cell Sci* 92:541.
39. Ulrich I et al. (1988) Application of DNA fluorochromes for flow cytometric DNA analysis of plant protoplasts. *Plant Sci* 55:151.
40. Ulrich I et al. (1986) Flow cytometric DNA-analysis of plant protoplasts stained with DAPI. *Naturforsch* 41:1052.
41. Levi M (1986) Determination of DNA content by static cytofluorimetry in nuclei released from fixed plant tissue. *Protoplasma* 132:64.

42. Zilkah S et al. (1985) The effect of the plant cell inhibitor propachlor (alpha-chloro)-n-isopropyl-acetanilide) on the cell cycle of L1210 cells as evaluated by flow cytometry. *Life Sci* 36:2111.
43. Leemann U et al. (1982) Cytofluorometric determination of DNA base content in plant nuclei and chromosomes by the fluorochromes DAPI and chromomycin A3. *Exp Cell Res* 140:275.
44. Selliden G et al. (1981) Localization of DNA in mature and young wheat chloroplasts using the fluorescent probe 4'-6-diamidino-2-phenylindole. *Plant Physiol* 68:731.
45. Sodmergen et al. (1999) Application of YO-PRO-1 as an epifluorescent dye for in situ detection of small amount dna in plant cells. *J Plant Res* 112:117.
46. Bunney TD et al. (2000) Association of phosphatidylinositol 3-kinase with nuclear transcription sites in higher plants. *Plant Cell* 12:1679–1688.

Cytoskeletal stains

47. Paves H et al. (2004) Incorporation of mammalian actin into microfilaments in plant cell nucleus. *BMC Plant Biol* 4:7.
48. Yu YP et al. (2004) Two distinct distributions of F-actin are present in the hyphal apex of the oomycete *Achlya bisexualis*. *Plant Cell Physiol* 45:275–280.
49. Van Gestel K et al. (2001) A comparison of F-actin labeling methods for light microscopy in different plant specimens: multiple techniques supplement each other. *Micron* 32:571–578.

AM ester dye loading

50. <http://www-biology.ucsd.edu/labs/schroeder/protocols/calcium.html>
51. Schulz A et al. (2004) Uptake of a fluorescent dye as a swift and simple indicator of organelle intactness: import-competent chloroplasts from soil-grown *Arabidopsis*. *J Histochem Cytochem* 52:701–704.

Endocytosis and transport studies

52. Etxeberria E et al. (2006) Fluid phase endocytic uptake of artificial nanospheres and fluorescent quantum dots by sycamore cultured cells. *Plant Signal Behav* 1:196–200.
53. Hillmer S et al. (1989) Lucifer Yellow uptake in cells and protoplasts of *Daucus carota* visualized by laser scanning microscopy. *J Exp Bot* 40:417–423.
54. Oparka KJ et al. (1991) Uptake and compartmentation of fluorescent probes by plant cells. *J Exp Bot* 42:565.
55. Emans N et al. (2002) Uptake of a fluorescent marker in plant cells is sensitive to brefeldin A and wortmannin. *Plant Cell* 14:71–86.

Glucose metabolism

56. Etxeberria E et al. (2005) Existence of two parallel mechanisms for glucose uptake in heterotrophic plant cells. *J Exp Bot* 56:1905–1912.

Viability staining

57. Heslop-Harrison J et al. (1970) Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technol* 45:115–120.
58. Widholm JM (1972) The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. *Stain Technol* 47:189–194.
59. Truernit E et al. (2008) A simple way to identify non-viable cells within living plant tissue using confocal microscopy. *Plant Methods* 4:15–20.

Glutathione detection

60. Hartmann TN et al. (2003) Cell-specific measurement of cytosolic glutathione in poplar leaves. *Plant Cell Environ* 26:965–975.
61. Meyer AJ et al. (2001) Quantitative in vivo measurement of glutathione in *Arabidopsis* cells. *Plant J* 27:67–78.

Calcium/ion imaging

62. Plieth C et al. (2001) Plant calcium signaling and monitoring: pros and cons and recent experimental approaches. *Protoplasma* 218:1–23.
63. Walczysko P et al. (2000) Use of co-loaded Fluo-3 and Fura Red fluorescent indicators for studying the cytosolic Ca²⁺ concentrations distribution in living plant tissue. *Cell Calcium* 28:23–32.
64. Zottini M et al. (1993) The use of fura-2 fluorescence to monitor the movement of free calcium ions into the matrix of plant mitochondria (*Pisum sativum* and *Helianthus tuberosus*). *Plant Physiol* 102:573.
65. Karley AJ et al. (2000) Differential ion accumulation and ion fluxes in the mesophyll and epidermis of barley. *Plant Physiol* 122:835–844.
66. Thomas F et al. (1999) Calcein as a fluorescent probe for ferric iron. Application to iron nutrition in plant cells. *J Biol Chem* 274:13375–13383.

Reactive oxygen sensing

67. Ashtamker C et al. (2007) Diverse subcellular locations of cryptogein-induced reactive oxygen species production in tobacco Bright Yellow-2 cells. *Plant Physiol* 143:1817–1826.
68. Ahn JW et al. (2006) Depletion of UDP-D-apiose/UDP-D-xylose synthases results in rhamnogalacturonan-II deficiency, cell wall thickening, and cell death in higher plants. *J Biol Chem* 281:13708–13716.
69. Rodriguez AA et al. (2002) Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. *Plant Physiol* 129:1627–1632.
70. Schopfer P et al. (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol* 125:1591–1602.
71. Yamasaki H et al. (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. *FEBS Lett* 468:89–92.
72. Kawano T et al. (2000) Aromatic monoamine-induced immediate oxidative burst leading to an increase in cytosolic Ca²⁺ concentration in tobacco suspension culture. *Plant Cell Physiol* 41:1251–1258.
73. Kashulin PA et al. (2000) Extremely rapid effects of polyunsaturated fatty acids and N-acetylglucosamine on free-radical metabolism in cultured potato plant cells. *Biochem Soc Trans* 28:865–867.
74. Simontacchi M et al. (1993) Oxidative stress affects α-tocopherol content in soybean embryonic axes upon imbibition and following germination. *Plant Physiol* 103:949.
75. Flors C et al. (2006) Imaging the production of singlet oxygen in vivo using a new fluorescent sensor, Singlet Oxygen Sensor Green. *J Experimental Botany* 57:1725–1734.

pH sensors

76. Song CP et al. (2004) A probable Na⁺(K⁺)/H⁺ exchanger on the chloroplast envelope functions in pH homeostasis and chloroplast development in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 101:10211–10216.

Ordering information

Product	Quantity	Cat. no.	References
Immunohistochemistry			
Alexa Fluor® dyes	For ordering information, visit www.invitrogen.com/alexa		3, 5, 7
Zenon® immunolabeling technology	For ordering information, visit www.invitrogen.com/zenon		5
Qdot® Nanocrystals			
Qdot® 565 streptavidin conjugate, 1 µM solution	200 µL	Q10131	9
TC-FIAsh™ expression technology			
TC-FIAsh™ II In-Cell Tetracysteine Tag Detection Kit, green fluorescence, for live-cell imaging	1 kit	T34561	10
TC-ReAsH™ II In-Cell Tetracysteine Tag Detection Kit, red fluorescence, for live-cell imaging	1 kit	T34562	10
Fluorescence <i>in situ</i> hybridization (FISH)			
FISH Tag™ DNA Far Red Kit, with Alexa Fluor® 647 dye, 10 reactions	1 kit	F32950	
FISH Tag™ DNA Green Kit, with Alexa Fluor® 488 dye, 10 reactions	1 kit	F32947	
FISH Tag™ DNA Multicolor Kit, Alexa Fluor® dye combination, 10 reactions	1 kit	F32951	
FISH Tag™ DNA Orange Kit, with Alexa Fluor® 555 dye, 10 reactions	1 kit	F32948	
FISH Tag™ DNA Red Kit, with Alexa Fluor® 594 dye, 10 reactions	1 kit	F32949	
Cell wall stains			
NanoOrange® Protein Quantitation Kit	1 kit	N6666	15
Hoechst 33258, pentahydrate (bis-benzimide)	100 mg	H1398	16
Calcofluor White (component of LIVE/DEAD® Yeast Viability Kit)	1 kit	L7009	
Latrunculin B	100 µg	L22290	6
Wheat germ agglutinin, fluorescein conjugate	5 mg	W834	19
Concanavalin A, fluorescein conjugate	10 mg	C827	
Membrane stains			
1,1'-dioctadecyl-6,6'-di(4-sulfophenyl)-3,3',3'-tetramethylindocarbocyanine (SP-DiIC ₁₈ (3))	5 mg	D7777	
1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate ('DiI'; DiIC ₁₈ (3))	100 mg	D282	
1,1'-didodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC ₁₂ (3))	100 mg	D383	
1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC ₁₆ (3))	100 mg	D384	
1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid (DiIC ₁₈ (3)-DS)	5 mg	D7776	
1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate ('DiI'; DiIC ₁₈ (3)) *crystalline*	25 mg	D3911	
1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid (DiIC ₁₈ (5)-DS)	5 mg	D12730	
CellTracker™ CM-Dil	1 mg	C7001	
1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (FAST DiI™ oil; DiIΔ9,12-C ₁₈ (3), ClO ₄)	5 mg	D3899	
1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine, 4-chlorobenzenesulfonate (FAST DiI™ solid; DiIΔ9,12-C ₁₈ (3), CBS)	5 mg	D7756	
1,1',3,3,3',3'-hexamethylindocarbocyanine iodide (DiIC ₇ (5))	100 mg	H14700	
FM® 1-43	1 mg	T3163	
FM® 1-43	10 x 100 µg	T35356	
FM® 4-64	1 mg	T3166	20, 67
FM® 4-64	10 x 100 µg	T13320	
Calcein	100 mg	C481	21
Rhodamine DHP	5 mg	L1392	21

Product	Quantity	Cat. no.	References
Endoplasmic reticulum stains			
ER-Tracker™ Red	100 µg	E34250	22
ER-Tracker™ Green	100 µg	E34251	22
Mitochondrial stains			
MitoTracker® Orange CMTMRos *special packaging*	20 x 50 µg	M7510	24, 25, 26
MitoTracker® Red CMXRos *special packaging*	20 x 50 µg	M7512	67
Rhodamine 123	25 mg	R302	27, 28
Nigericin, free acid	10 mg	N1495	27
(DiOC ₃ (3)) 3,3'-dipentylloxycarbocyanine iodide	100 mg	D272	28
(DiOC ₇ (3)) 3,3'-diheptyloxycarbocyanine iodide	100 mg	D378	28
(DiOC ₂ (3)) 3,3'-diethylloxycarbocyanine iodide	100 mg	D14730	28
(JC-1; CBIC ₂ (3)) 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide	5 mg	T3168	23
Nuclear/nucleic acid stains			
BrUTP	25 µL	B21551	46
			7, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 43, 44, 67
(DAPI) 4',6-diamidino-2-phenylindole, dihydrochloride	10 mg	D1306	44, 67
ChromaTide® Alexa Fluor® 546-14-dUTP *1 mM in TE buffer*	25 µL	C11401	
CellTrace™ BODIPY® TR methyl ester *lipophilic counterstain for GFP* *solution in DMSO*	1 mL	C34556	
SYTO® 42 blue fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL	S11353	29
SYBR® Green I nucleic acid gel stain *10,000X concentrate in DMSO*	1 mL	S7567	29
Propidium iodide	100 mg	P1304MP	39, 42
Fluorescein-5-isothiocyanate (FITC 'isomer I')	1 g	F143	33
TOTO®-1 iodide (514/533) *1 mM solution in DMSO*	200 µL	T3600	33
Fluorescein diacetate (FDA)	1 g	F1303	35
Acridine orange	1 g	A1301	
Hoechst 33258, pentahydrate (bis-benzimide)	100 mg	H1398	39
YO-PRO®-1 iodide (491/509) *1 mM solution in DMSO*	1 mL	Y3603	45
YOYO®-1 iodide (491/509) *1 mM solution in DMSO*	200 µL	Y3601	
9-amino-6-chloro-2-methoxyacridine (ACMA)	100 mg	A1324	37

continued

Ordering information, continued

Product	Quantity	Cat. no.	References
Cytoskeletal stains			
Rhodamine phalloidin	300 units	R415	38, 47, 49
Fluorescein phalloidin	300 units	F432	32
Alexa Fluor® 488 phalloidin	300 units	A12379	48
Alexa Fluor® 568 phalloidin	300 units	A12380	48
Alexa Fluor® 594 phalloidin	300 units	A12381	48
Alexa Fluor® 350 phalloidin	300 units	A22281	48
Alexa Fluor® 532 phalloidin	300 units	A22282	48
Alexa Fluor® 546 phalloidin	300 units	A22283	48
Alexa Fluor® 633 phalloidin	300 units	A22284	48
Alexa Fluor® 660 phalloidin	300 units	A22285	48
Alexa Fluor® 680 phalloidin	300 units	A22286	48
Alexa Fluor® 647 phalloidin	300 units	A22287	48
Alexa Fluor® 635 phalloidin	300 units	A34054	48
AM ester dye loading			
Calcium Green™-1	10 x 50 µg	C3012	50
5-(and-6)-carboxyfluorescein diacetate (5(6)-CFDA) - mixed isomers	100 mg	C195	51
Endocytosis and transport studies			
FM® 1-43	1 mg	T3163	55
Alexa Fluor® 568 hydrazide	1 mg	A10437	55
Lucifer yellow CH, lithium salt	25 mg	L453	33
Glucose metabolism			
2-NBDG	5 mg	N13195	56
Viability staining			
SYTOX® Green nucleic acid stain	250 µL	S7020	59
SYTOX® Red dead cell stain	1 mL	S34859	59
SYTOX® Orange nucleic acid stain	250 µL	S11368	59
Fluorescein diacetate (FDA)	1 g	F1303	57, 58
Calcein AM	1 mL	C3099	
Glutathione detection			
Monochlorobimane (mBCl)	25 mg	M1381MP	60, 61
Monobromobimane (mBBr)	25 mg	M1378	61
Calcium/ion imaging			
Fura dextran, potassium salt, 10,000 MW, anionic	5 mg	F3029	62
Fluo-3, AM	1 mg	F1241	63
Fura Red™, AM	500 µg	F3020	63
Fura-2, AM	1 mg	F1201	64
PBFI, AM	20 x 50 µg	P1267MP	65
Calcein	100 mg	C481	66
Calcein, AM	1 mg	C1430	66

Product	Quantity	Cat. no.	References
Reactive oxygen sensing			
Singlet Oxygen Sensor Green	10 x 100 µg	S36002	75
Carbocyanine DiOC ₃ (3)	100 mg	D272	67
2',7'-dichlorodihydrofluorescein diacetate (2',7'-dichlorofluorescein diacetate; H ₂ DCFDA)	100 mg	D399	67, 68, 69, 70, 71, 72, 73, 74
6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester)	5 mg	C2938	
5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H ₂ DCFDA)	20 x 50 µg	C6827	
Amplex® Red reagent	5 mg	A12222	67
Amplex® UltraRed reagent	5 x 1 mg	A36006	67
XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide)	100 mg	X6493	69
pH sensors			
2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM)	1 mg	B1150	76