

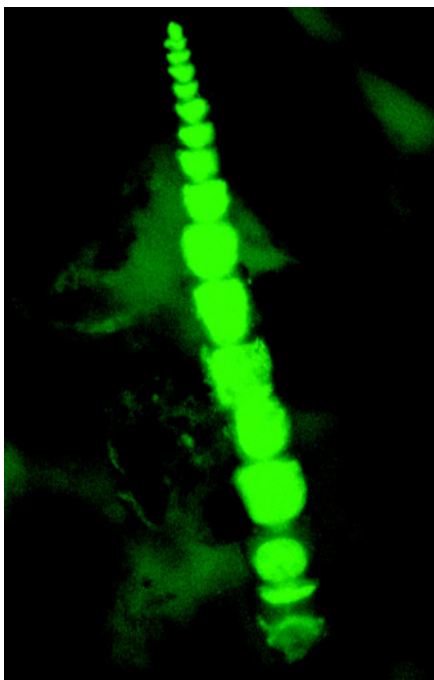
**Figure 14.3** A multicolored mixture of Molecular Probes' FluoSpheres fluorescent microspheres photographed through a DAPI longpass optical filter set. Molecular Probes applies its proprietary fluorescent dye technology to produce a range of intensely fluorescent FluoSpheres microspheres labeled with biotin, streptavidin and NeutrAvidin biotin-binding protein, providing important tools for improving the sensitivity of flow cytometry applications and immunodiagnostic assays.

of molecular weights makes them useful as size-exclusion probes for determining pore sizes in membranes.

- **Fluorescent microspheres** (Section 14.6). Molecular Probes' FluoSpheres and Trans-FluoSpheres fluorescent microspheres — which contain  $\sim 10^2$  to  $\sim 10^{10}$  fluorescent dyes per bead — are the most intensely fluorescent tracers available (Table 6.5, Table 6.6, Figure 14.3). Although other multiply labeled particles such as our BioParticles fluorescent bacteria (Section 16.1) may be used as tracers, they are often not biologically inert nor are they as physically durable as fluorescent microspheres. These properties make fluorescent beads particularly useful as long-term markers for transplantation studies. Submicron microspheres can be injected into cells or taken up by phagocytosis (Section 16.1). Much larger (10–15  $\mu\text{m}$ ) beads provide an alternative to radioactive microspheres for determination of organ blood flow, and intermediate-sized (1–5  $\mu\text{m}$ ) microspheres are useful for studies that trace inhaled particles.
- **Proteins and protein conjugates** (Section 14.7). Our fluorescent protein tracers have molecular weights ranging from  $\sim 12,000$  (cholera toxin subunit B conjugates) to  $\sim 240,000$  daltons (B- and R-phycoerythrin). Their applications are sometimes similar to those of the fluorescent dextrans; however, unlike the polydisperse dextrans, fluorescent protein tracers have molecular weights that are reasonably well defined.

### References

1. Brain Res Bull 51, 11 (2000);
2. Methods Cell Biol 29, 153 (1989);
3. Cytometry 21, 230 (1995);
4. Cell 84, 381 (1996);
5. Biochim Biophys Acta 988, 319 (1989).



**Figure 14.4** Detection of organisms in marine sediments by incubating an intact sediment core sample with the fixable, cell-permeant CellTracker Green CMFDA (C-2925, C-7025). The core sample was subsequently embedded, sectioned and examined for fluorescently labeled organisms. The micrograph reveals the microorganism *Leptohalysis scotti*, a marine benthic foraminifera. Image contributed by Joan M. Bernhard, Wadsworth Center, New York State Department of Health.

## 14.2 Membrane-Permeant Reactive Tracers for Long-Term Cell Labeling

### Thiol-Reactive CellTracker Probes

Molecular Probes' CellTracker reagents are fluorescent chloromethyl derivatives that freely diffuse through the membranes of live cells (Figure 14.4, Figure 14.5, Figure 14.6). Once inside the cell, these mildly thiol-reactive probes undergo what is believed to be a glutathione *S*-transferase-mediated reaction to produce membrane-impermeant glutathione-fluorescent dye adducts, although our experiments suggest that they may also react with other intracellular components (Figure 14.7). Regardless of the mechanism, many cell types loaded with the CellTracker probes are both fluorescent and viable for at least 24 hours after loading and often through several cell divisions. Most other cell-permeant fluorescent dyes, including the acetoxymethyl (AM) esters of calcein and BCECF (Section 15.2), are retained in viable cells for no more than a few hours at physiological temperatures (Figure 15.3). Furthermore, unlike the free dye, the peptide-fluorescent dye adducts contain amino groups and can therefore be covalently linked to surrounding biomolecules by fixation with formaldehyde or glutaraldehyde. This property permits long-term storage of the labeled cells or tissue and, in cases where the anti-dye antibody is available (see below), amplification of the conjugate by standard immunohistochemical techniques. Fixation without loss of the tracer also facilitates the safe handling and analysis of cells containing pathogens.

### CellTracker Probes in a Variety of Fluorescent Colors

Molecular Probes' patented CellTracker product line includes reactive chloromethyl derivatives of:

- Blue-fluorescent 7-aminocoumarin (CellTracker Blue CMAC, C-2110, Figure 14.8)
- Blue-fluorescent 7-hydroxycoumarin (CellTracker Blue CMHC, C-2111, Figure 14.9)

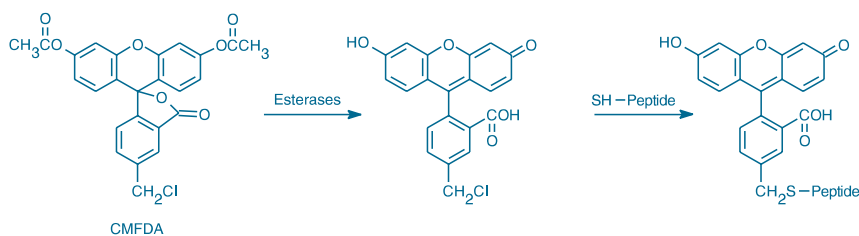
- Blue-fluorescent 6,8-difluoro-7-hydroxycoumarin (CellTracker Blue CMF<sub>2</sub>HC, C-12881, Figure 14.10)
- Green-fluorescent fluorescein diacetate (CellTracker Green CMFDA, C-2925 and C-7025, Figure 14.7)
- Green-fluorescent BODIPY derivative (CellTracker Green BODIPY, C-2102, Figure 14.11)
- Orange-fluorescent tetramethylrhodamine (CellTracker Orange CMTMR, C-2927, Figure 14.12)

CMFDA freely diffuses into the cell, where cytosolic esterases cleave the acetate groups, releasing the fluorescent product. The coumarin, BODIPY and tetramethylrhodamine CellTracker probes do not require enzymatic cleavage to activate their fluorescence. Our related MitoTracker probes (Section 12.2) and other mitochondrial stains may be similarly useful for tracing actively metabolizing cells. We also offer BODIPY 493/503 methyl bromide (B-2103, Figure 14.13), a green-fluorescent dye, and BODIPY 630/650 methyl bromide (B-22802, Figure 14.14), a dark-red-fluorescent dye, which have slightly greater thiol-reactivity than do chloromethyl derivatives. BODIPY 630/650 methyl bromide is our longest-wavelength cell-permeant tracer and its fluorescence can be excited by either the 633 nm spectral line of the He-Ne laser or the 647 nm spectral line of the krypton-ion laser used in many confocal laser-scanning microscopes and flow cytometers.

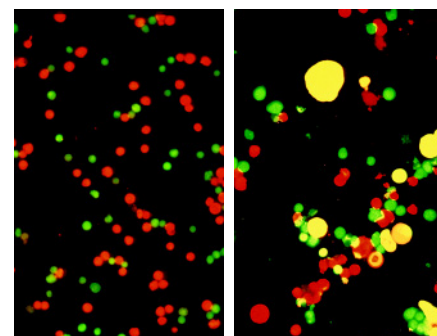
### Applications for CellTracker Probes

The thiol-reactive CellTracker Green CMFDA — which is particularly easy to use as a tracer — is suitable for long-term cell labeling and has been used in a wide variety of applications, including studies of:

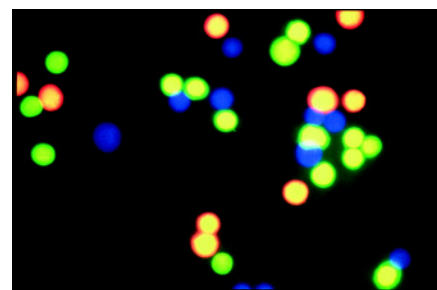
- Cell adhesion,<sup>1,2</sup> including adhesion on prosthetic surfaces<sup>3</sup>
- Cell communication through gap junctions<sup>4</sup>
- Cell migration (chemotaxis)<sup>5</sup> (Section 15.6)
- Cell tracing in mixed cultures<sup>1,6</sup> (Figure 14.5)
- Platelet labeling without activation and their *in vivo* tracing for days<sup>7</sup>
- Long-term viability and cytotoxicity assays<sup>8,9</sup> (Section 15.2)
- Transplantation<sup>10</sup>
- Multidrug resistance and transport of the glutathione adduct by the multispecific organic anion transporter<sup>11,12</sup> (Section 15.6)
- Tumor cell death in culture<sup>13</sup>
- Keratocytes in intact cornea<sup>14</sup>
- Glutathione content in cells<sup>4,15–17</sup> (Section 15.6)
- Fungal growth and differentiation<sup>18</sup>



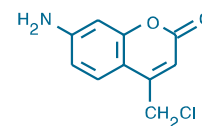
**Figure 14.7** Intracellular reactions of our fixable CellTracker Green CMFDA (5-chloromethylfluorescein diacetate; C-2925, C-7025). Once this membrane-permeant probe enters a cell, esterase hydrolysis converts nonfluorescent CMFDA to fluorescent 5-chloromethylfluorescein, which can then react with thiols on proteins and peptides to form aldehyde-fixable conjugates. This probe may also react with intracellular thiol-containing biomolecules first, but the conjugate is nonfluorescent until its acetates are removed.



**Figure 14.5** HL60 cells that have been stained with CellTracker Orange CMTMR (C-2927) and then mixed with WEH17.1 cells stained with CellTracker Green CMFDA (C-2925, C-7025) (left panel). Several minutes after initiating cell-cell electrofusion, a CMTMR-stained HL60 cell is observed fusing with a CMFDA-stained WEH17.1 cell; cytoplasmic mixing is evident by the appearance of yellow fluorescence. After electrofusion is complete, dual-fluorescing (yellow) hybrids can be easily distinguished (right panel). Images contributed by Mark J. Jaroszeski, Department of Surgery, University of South Florida.



**Figure 14.6** Individual populations of mouse myeloma (P3X) cells stained with our reactive tracers — CellTracker Orange CMTMR (C-2927), CellTracker Green CMFDA (C-2925) and CellTracker Blue CMAC (C-2110). Each of three cell populations was stained with a different tracer and then the populations were mixed, demonstrating that these tracers allow simultaneous long-term monitoring of different groups of cells in transplantation and other assays.



**Figure 14.8** C-2110 CellTracker Blue CMAC.

*Our CellTracker dyes permit easy labeling of live cells in any of several colors for use as tracers; CellTracker probe-labeled cells that have fused yield a mixture of their original colors.*

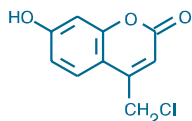


Figure 14.9 C-2111 CellTracker Blue CMHC.

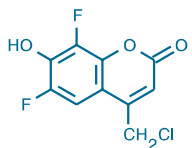


Figure 14.10 C-12881 CellTracker Blue CMF<sub>2</sub>HC.

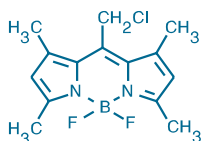


Figure 14.11 C-2102 CellTracker Green BODIPY.

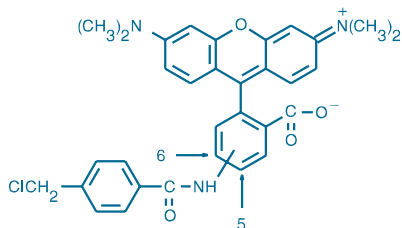


Figure 14.12 C-2927 CellTracker Orange CMTMR.

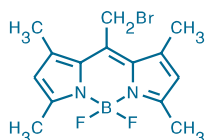


Figure 14.13 B-2103 BODIPY 493/503 methyl bromide.

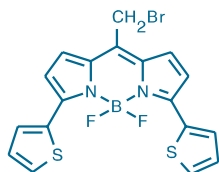


Figure 14.14 B-22802 BODIPY 630/650 methyl bromide.

- Ingestion of live stained protists by mixotrophic dinoflagellates<sup>19</sup>
- Cell–cell and cell–liposome fusion<sup>20–24</sup>
- Vacuolar morphology in yeast<sup>25</sup>

Virtually any of the other CellTracker probes could be used for the same applications as above and combinations of two (or more) CellTracker probes or use of CellTracker probes in conjunction with the lipophilic tracers described in Section 14.4 often permit simultaneous, multicolor applications. We particularly recommend the combination of CellTracker Green CMFDA and our chloromethyl SNARF pH indicator (C-6826, see below) for two-color tracing. Both dyes can be excited near 490 nm and the green-fluorescent CellTracker Green CMFDA–stained cells can be distinguished from the red-fluorescent chloromethyl SNARF dye–stained cells, provided that the fluorescence intensities of the stained cells are similar. Our deep red-fluorescent BODIPY 630/650 methyl bromide dye (B-22802, see below) can be utilized as a third dye for triple staining and the blue-fluorescent CellTracker dyes (C-2110, C-2111, C-12881) or monobromobimane (M-1378, see below) can be added as a fourth label.

CellTracker Green CMFDA was used to track wild-type and myosin II mutant *Dictyostelium discoideum* cells within aggregation streams during early multicellular morphogenesis; differentiation and morphogenesis pathways were reportedly unaffected in labeled cells imaged over several days by confocal laser-scanning microscopy.<sup>26,27</sup> CellTracker Green CMFDA and some other CellTracker dyes have been utilized to selectively label intracellular *Chlamydia psittaci* in infected cells.<sup>28</sup> This unexpected application of the CellTracker dyes could be important for *in vivo* labeling of obligate intracellular bacteria and parasites for which no genetic tools exist. Additionally, intracellular bacteria accumulated BODIPY FL C<sub>5</sub>-ceramide (D-3521, B-22650; Section 12.4) in their inclusion membranes rather than labeling the Golgi of the host cells.<sup>28,29</sup>

CellTracker Orange CMTMR has been used to stain the cytoplasm of *engrailed*-expressing *Drosophila* cells in an *in vitro* reconstruction experiment,<sup>30</sup> to follow T-cell differentiation and maturation<sup>31</sup> and to trace implanted tumor cells for at least two weeks.<sup>32,33</sup> Chlamydia labeled with CellTracker Orange CMTMR continued to grow and differentiate, and labeled chlamydiae isolated from infected cells remained infectious.<sup>28</sup> Both the CellTracker Green CMFDA and CellTracker Orange CMTMR probes have been employed in a flow cytometry method for quantitating cell–cell electrofusion products<sup>22,23</sup> (Figure 14.5) and in two-color imaging to detect cell–cell association.<sup>2,34</sup>

Metabolic activity and drug-induced cytotoxicity were measured with CellTracker Blue CMAC in a fluorescence-based microplate assay.<sup>35</sup> Peptidase substrates derived from CellTracker Blue CMAC yield blue-fluorescent products that are well retained in live cells<sup>36</sup> (Section 10.4). Our CellTracker Blue CMF<sub>2</sub>HC (C-12881) has a low pK<sub>a</sub> that ensures that the dye's conjugates will be ionized and have bright-blue fluorescence in the cytoplasm. CellTracker Blue CMAC was used in combination with calcein AM (C-1430, C-3099, C-3100; Section 15.6) and the lipophilic tracer DiI (D-282, D-3911, V-22885; Section 14.4) to identify lipid mixing and cytoplasm mixing between labeled effector and labeled target cells.<sup>21</sup>

The ability to fix the intracellular products of CellTracker Green CMFDA, CellTracker Green BODIPY and CellTracker Orange CMTMR in permeabilized cells permits the stained cells to be probed with our anti-fluorescein/Oregon Green, anti-BODIPY FL and anti-tetramethylrhodamine antibodies, respectively (Section 7.4, Table 7.13). After antibody staining, these cells can be developed for electron microscopy using standard immunohistochemical techniques or for fluorescence amplification by our ultrasensitive tyramide signal-amplification (TSA) and Enzyme-Labeled Fluorescence (ELF) technologies (Section 6.2, Section 6.3).

## Other Thiol-Reactive Tracers

### PFB Aminofluorescein Diacetate

The green-fluorescent intracellular hydrolysis product of our patented pentafluorobenzoyl aminofluorescein diacetate (PFB-FDA, P-12880, Figure 14.16) — PFB aminofluorescein<sup>37</sup> (PFB-F, P-12925; Section 14.3) — localizes in the cytoplasm, is well-retained in cells with intact cell membranes (even through cell division) and can be fixed in cells

by formaldehyde or glutaraldehyde. PFB-F appears to be retained in viable cells by two mechanisms: 1) retention of the relatively lipophilic PFB group in the cell membrane, and 2) glutathione *S*-transferase-catalyzed reaction of PFB-F with intracellular glutathione. In addition, the relatively high lipophilicity of PFB-FDA facilitates passive cell loading: cells are simply incubated at 37°C in 1–10 μM substrate in standard culture medium. Thus, like our CellTracker Green CMFDA (C-2925, C-7025), PFB-FDA may prove useful for long-term cell labeling.

### Chloromethyl Derivatives of SNARF-1, a Dihydrofluorescein and a Microsomal Dealkylase Substrate

Although they were designed for other purposes, the chloromethyl derivatives of our SNARF-1 pH indicator (C-6826, Figure 14.17) and of 2',7'-dichlorodihydrofluorescein diacetate, acetate ester<sup>38</sup> (CM-H<sub>2</sub>DCFDA, C-6827, Figure 14.18) possess some unique properties for tracking cells. As with the CellTracker probes, cytoplasmic enzymes hydrolytically remove the acetate groups from the membrane-permeant probes, and the chloromethyl moieties become conjugated to intracellular thiols. With its long Stokes shift, the SNARF-1 derivative is the only cell tracking dye that has bright, easily distinguished red-orange fluorescence in the cytoplasm when excited at the same wavelengths used for the green-fluorescent CellTracker Green CMFDA (Figure 14.19, Figure 21.13). This property may permit simultaneous or sequential tracking of two cell populations by either microscopy or flow cytometry, provided the cells are stained to similar intensities. The broad visible absorption of the SNARF-1 dye (Figure 21.13) also permits cells stained by this dye to be excited using filters optimized for tetramethylrhodamine (Table 24.8) and detected independently from any CellTracker Green CMFDA-stained cells.

As with other dihydrofluorescein derivatives (Section 19.2), CM-H<sub>2</sub>DCFDA requires an additional oxidation step before becoming fluorescent. This probe is potentially useful for following stimulation of oxidative activity by external agents or natural killer (NK) cells over extended periods, as well as for passively labeling cells that lack appropriate oxidative activity and then following their ingestion by scavengers such as neutrophils. CM-H<sub>2</sub>DCFDA has been used to measure intracellular reactive oxygen species (ROS) in cardiac myocytes.<sup>39</sup>

The chloromethyl fluorescein ether (C-6533, Section 10.6) becomes fluorescent only in cells that have the required microsomal dealkylase activity to remove its ethyl ether moieties. Its thiol-reactive chloromethyl group ensures retention of its fluorescent products in live cells.

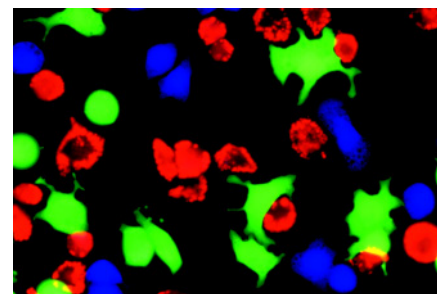
### Bimanes: Blue-Fluorescent Reactive Tracers

The bimeane derivatives, monobromobimane (mBBr, M-1378; FluoroPure Grade, M-20381), monochlorobimane (mBCl, M-1381) and monobromotrimethylammoniumbimane (qBBr, M-1380), are important thiol-derivatization reagents (Section 2.3). The essentially nonfluorescent mBBr and mBCl dyes are known to passively diffuse across the plasma membrane into the cytoplasm, where they form blue-fluorescent adducts with intracellular glutathione and thiol-containing proteins (Section 15.6). Although the blue fluorescence of their thiol adducts is much weaker than that of our CellTracker Blue dyes (CMAC, C-2110; CMHC, C-2111; CMF<sub>2</sub>HC, C-12881), mBBr and mBCl have been shown to serve as effective tracers of gap-junctional communication.<sup>4</sup> Likewise, the bimeane qBBr is a water-soluble quaternary ammonium salt that may be suitable as a microinjectable tracer.

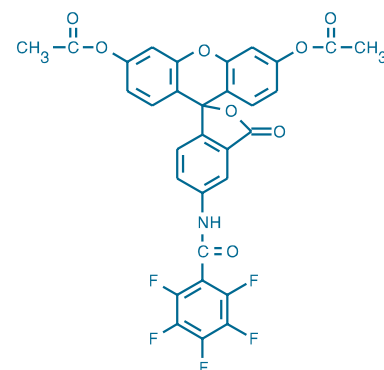
## Amine-Reactive Tracers

### Amine-Reactive Fluorescein Probes

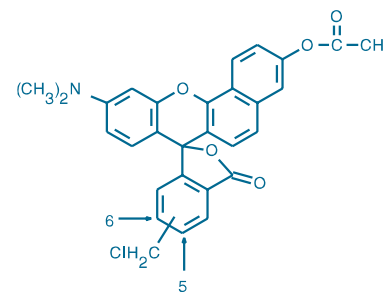
The carboxyfluorescein diacetate succinimidyl esters (C-1157, C-1165) and the carboxyeosin diacetate succinimidyl ester (C-22803) may have applications similar to the CellTracker probes.<sup>41</sup> These amine-reactive reagents can passively diffuse into cells and are colorless and nonfluorescent until their acetate groups are cleaved by intracellular esterases to yield the highly fluorescent, amine-reactive fluorophores. Upon reaction with amine-containing residues of intracellular proteins, these probes form dye-protein adducts that are well-retained in cells as they move and divide during embryonic develop-



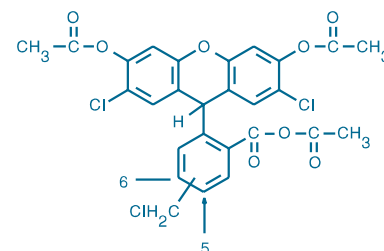
**Figure 14.15** Rat basophilic leukemia (RBL) cells labeled in suspension with CellTracker Blue CMAC (C-2110), CellTracker Green CMFDA (C-2925, C-7025) or CellTracker Orange CMTMR (C-2927) prior to plating. The image was acquired using optical filter sets appropriate for DAPI, fluorescein and the Texas Red dye.



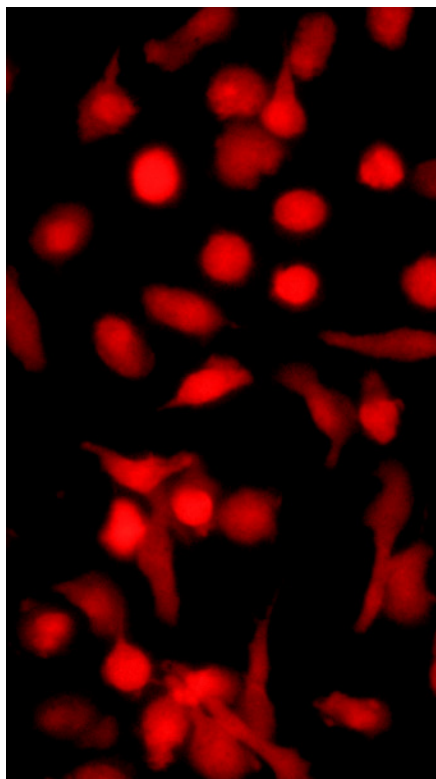
**Figure 14.16** P-12880 PFB-FDA.



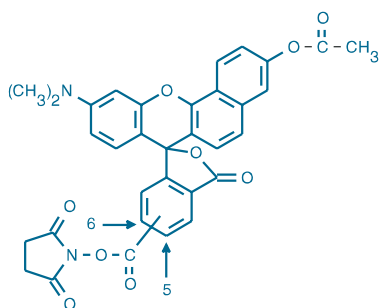
**Figure 14.17** C-6826 5-(and-6)-chloromethyl SNARF-1, acetate.



**Figure 14.18** C-6827 CM-H<sub>2</sub>DCFDA.



**Figure 14.19** Human neutrophils loaded with 5-(and -6)-chloromethyl SNARF-1 acetate (C-6826).



**Figure 14.20** S-22801 SNARF-1 carboxylic acid, acetate, succinimidyl ester.

*The red fluorescence of the SNARF tracer dyes and the green fluorescence of CellTracker Green CMFDA can be simultaneously excited by the argon-ion laser at 488 nm for two-color labeling of cell populations.*

ment.<sup>41,42</sup> In addition, the probes survive subsequent fixation with formaldehyde or glutaraldehyde. Because it is intrinsically more reactive, the succinimidyl ester of CFDA is more likely to react at sites on the extracellular surface than is CMFDA. If that is undesirable, it may be possible to quench much of the fluorescence of outer-membrane fluorescein conjugates by acidifying the extracellular medium, by addition of trypan blue (Figure 16.23) or by incubating with anti-fluorescein/Oregon Green antibody<sup>43</sup> (A-889, Section 7.4).

The succinimidyl ester of carboxyfluorescein diacetate (5(6)-CFDA, SE; C-1157) — which is frequently but inappropriately termed CFSE — is a particularly useful probe for tracing transplanted cells.<sup>42,44</sup> Once incorporated into cells, CFDA SE remains there — even through cell division — thus preventing transfer to unlabeled cells.<sup>45</sup> Homing and proliferation of CFDA SE-labeled thymocytes has been extensively studied.<sup>46–48</sup> The feasibility of using cell-permeant fluorescent tracers to follow asynchronous cell division of natural killer (NK) cells, B cells, T cells, thymocytes, lymphocytes, fibroblasts and hematopoietic cells has been demonstrated with CFDA SE<sup>46,49–58</sup> (Figure 15.53). Lymphocytes labeled with CFDA SE were detected up to eight weeks after injection into mice during lymphocyte migration studies,<sup>59,60</sup> and similarly labeled hepatocytes were easily located by fluorescence microscopy even 20 days after intrahepatic transplantation.<sup>61</sup> CFDA SE has also been successfully employed to quantitate adhesion of neutrophils<sup>62</sup> and leukocytes,<sup>63</sup> to assay T-cadherin-mediated cell aggregation,<sup>64</sup> to follow neurite growth in an *in vitro* bioassay<sup>65</sup> and to trace fetal cells in culture.<sup>66</sup> Different loading concentrations of CFDA SE can be used to produce cells that can be distinguished by their relative brightness.<sup>67</sup> CFDA SE has been utilized for tracing the transport of viable bacterial cells in groundwater under no-growth conditions for periods of at least 28 days.<sup>68</sup> Carboxyeosin diacetate succinimidyl ester (C-22803) has applications that are expected to be similar to CFDA SE; however, its fluorescence can be excited and detected at longer wavelengths, possibly permitting two-color tracing experiments of mixed-cell populations. Eosin derivatives are also effective reagents for photoablation of cells (Section 1.5).

CFDA SE is also available conveniently packaged for cell-tracing applications in our Vybrant CFDA SE Cell Tracer Kit (V-12883). The fluorescent CFDA SE product has excitation/emission maxima of ~495/525 nm and can be detected using a fluorescence microscope, flow cytometer or fluorescence microplate reader. Each kit includes ten single-use vials of CFDA SE, as well as high-quality anhydrous DMSO and a complete protocol.

Unlike CFDA SE, fluorescence of the intracellular hydrolysis products of the succinimidyl ester of 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (C-1165) is relatively insensitive to fluctuations in pH. This amine-reactive tracer was reported to be more useful than the lipophilic marker DiI (D-282, Section 14.4) in an investigation of palatal fusion in rodent embryos.<sup>69</sup>

#### **Amine-Reactive SNARF-1 Probe**

To permit simultaneous long-term tracing of mixed-cell populations using different fluorescent colors, we have developed an amine-reactive probe whose applications should be similar to those of CFDA SE. Cells labeled with our succinimidyl ester of SNARF-1 carboxylic acid, acetate (S-22801, Figure 14.20) have a red-orange fluorescence that can easily be distinguished from that of cells loaded with green-fluorescent tracers such as CFDA SE. However, the fluorescence intensity of cells loaded with this SNARF-1 derivative will not be as high as that of cells loaded with the same concentration of CFDA SE. Thus, it is necessary to adjust the reagent concentration and/or select optical filters to appropriately balance the fluorescence intensities when doing two-color experiments.

## References

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**Data Table — 14.2 Membrane-Permeant Reactive Tracers for Long-Term Cell Labeling**

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
B-2103	341.00	F,D,L	DMSO, MeCN	533	62,000	561	CHCl <sub>3</sub>	1
B-22802	449.14	F,D,L	DMSO, MeCN	658	73,000	678	CHCl <sub>3</sub>	2
C-1157	557.47	F,D	DMF, DMSO	<300		none		3
C-1165	626.36	F,D	DMF, MeCN	<300		none		4
C-2102	296.55	F,D,L	DMSO	522	72,000	529	MeOH	
C-2110	209.63	F,D,L	DMSO	353	14,000	466	pH 9	5
C-2111	210.62	F,D,L	DMSO	372	16,000	470	pH 9	6
C-2925	464.86	F,D	DMSO	<300		none		3
C-2927	554.04	F,D,L	DMSO	541	91,000	565	MeOH	
C-6826	499.95	F,D	DMSO	<350		none		7
C-6827	577.80	F,D,AA	DMSO	287	9,100	none	MeOH	8
C-7025	464.86	F,D	DMSO	<300		none		3
C-12881	246.60	F,D,L	DMSO	371	16,000	464	pH 9	6
C-22803	873.05	F,D	DMSO	<300		none		9
M-1378	271.11	F,L	DMF, MeCN	398	5,000	see Notes	pH 7	10
M-1380	409.12	L	H <sub>2</sub> O	378	5,500	see Notes	pH 7	10, 11
M-1381	226.66	F,L	DMSO	380	6,000	see Notes	MeOH	10
M-20381	271.11	F,L	DMF, MeCN	398	5,000	see Notes	pH 7	10, 12
P-12880	625.46	F,D	DMSO	<300		none		13
S-22801	592.56	F,D	DMSO	<350		none		7

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

### Notes

- B-2103 spectra are for the unreacted reagent. The thiol adduct has Abs = 493 nm, Em = 503 nm in MeOH.
- B-22802 spectral data are for the unreacted reagent. The thiol adduct has Abs = 629 nm, Em = 647 nm in dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>).
- This compound is converted to a fluorescent product with pH-dependent spectra similar to C-1904 (Section 14.3) after acetate hydrolysis.
- C-1165 is converted to a fluorescent product with spectra similar to C-368 (Section 14.3) after acetate hydrolysis.
- C-2110 in MeOH: Abs = 364 nm (EC = 16,000 cm<sup>-1</sup>M<sup>-1</sup>), Em = 454 nm.
- Spectra of hydroxycoumarins are pH dependent. Below the pK<sub>a</sub>, Abs shifts to shorter wavelengths (325–340 nm) and fluorescence intensity decreases. Approximate pK<sub>a</sub> values are: 7.8 (C-2111) and 4.9 (C-12881).
- C-6826 and S-22801 are converted to fluorescent products with spectra similar to C-1270 (Section 21.2) after acetate hydrolysis.
- Acetate hydrolysis and subsequent oxidation of C-6827 generate a fluorescent 2',7'-dichlorofluorescein derivative with spectra similar to C-368 (Section 14.3).
- Acetate hydrolysis of C-22803 yields a fluorescent product with spectra similar to 5-carboxy eosin, Abs = 519 nm (EC = 100,000 cm<sup>-1</sup>M<sup>-1</sup>), Em = 542 nm in pH 9 buffer.
- Bimanes are almost nonfluorescent until reacted with thiols. For monobromobimane conjugated to glutathione, Abs = 394 nm, Em = 490 nm (QY ~0.1–0.3) in pH 8 buffer (Methods Enzymol 143, 76 (1987); Methods Enzymol 251, 133 (1995)).
- Unstable in water. Use immediately.
- This product is specified to equal or exceed 98% analytical purity by HPLC.
- P-12880 is converted to a fluorescent product (P-12925, Section 14.3) after acetate hydrolysis.

Cat #	Product Name	Unit Size
B-22802	8-bromomethyl-4,4-difluoro-3,5-bis-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene (BODIPY® 630/650 methyl bromide) .....	1 mg
B-2103	8-bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY® 493/503 methyl bromide) .....	5 mg
C-1165	5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate, succinimidyl ester *mixed isomers* .....	25 mg
C-22803	5-(and-6)-carboxy eosin diacetate, succinimidyl ester *mixed isomers* .....	5 mg
C-1157	5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (5(6)-CFDA, SE, CFSE) *mixed isomers* .....	25 mg
C-2110	CellTracker™ Blue CMAC (7-amino-4-chloromethylcoumarin) .....	5 mg
C-12881	CellTracker™ Blue CMF <sub>2</sub> HC (4-chloromethyl-6,8-difluoro-7-hydroxycoumarin) .....	5 mg
C-2111	CellTracker™ Blue CMHC (4-chloromethyl-7-hydroxycoumarin) .....	5 mg
C-2102	CellTracker™ Green BODIPY® (8-chloromethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene) .....	5 mg
C-2925	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) .....	1 mg
C-7025	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) *special packaging* .....	20 x 50 µg
C-2927	CellTracker™ Orange CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl)amino)tetramethylrhodamine) *mixed isomers* .....	1 mg
C-6827	5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H <sub>2</sub> DCFDA) *mixed isomers* *special packaging* .....	20 x 50 µg
C-6826	5-(and-6)-chloromethyl SNARF®-1, acetate *mixed isomers* *special packaging* .....	20 x 50 µg
M-1378	monobromobimane (mBBr) .....	25 mg
M-20381	monobromobimane (mBBr) *FluoroPure™ grade* .....	25 mg
M-1380	monobromotrimethylammoniumbimane bromide (qBBr) .....	25 mg
M-1381	monochlorobimane (mBCI) .....	25 mg
P-12880	5-(pentafluorobenzoylamino)fluorescein diacetate (PFB-FDA) .....	5 mg
S-22801	SNARF®-1 carboxylic acid, acetate, succinimidyl ester *special packaging* .....	10 x 50 µg
V-12883	Vybrant® CFDA SE Cell Tracer Kit .....	1 kit

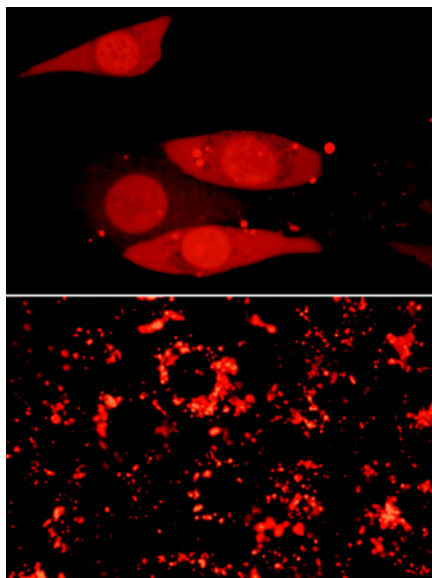
## 14.3 Polar Tracers

### Fixable Polar Tracers

Molecular Probes prepares a wide variety of highly water-soluble dyes and other detectable probes that can be used as cell tracers. Polar tracers can also be incorporated into liposomes to generate polymeric fluorescent tracers or antibody-labeling reagents.<sup>1-5</sup>

In most cases, the polarity of these water-soluble probes is too high to permit them to passively diffuse through cell membranes. Consequently, special methods for loading the dyes into cells must be employed, including microinjection, pinocytosis or techniques that temporarily permeabilize the cell's membrane<sup>6</sup> (Table 14.1). Our Influx pinocytic cell-loading reagent (I-14402, Figure 14.44; see below and Section 20.8) is particularly useful for loading many of the polar tracers in this section — as well as the dextrans and fluorescent proteins described in Section 14.5 and Section 14.7 — into many types of cells (Figure 14.21, Figure 14.43). Permeabilization of cells with staphylococcal  $\alpha$ -toxin<sup>7</sup> or the saponin ester  $\beta$ -escin is reported to make the membrane of smooth muscle cells permeable to low molecular weight (<1000 daltons) molecules, while retaining high molecular weight compounds.<sup>8-10</sup> Electroporation has been used to transport several of the polar tracers through the skin<sup>11</sup> and into cells.<sup>12,13</sup> Many of these tracers can also be loaded into cells noninvasively as their cell-permeant acetoxymethyl (AM) esters, which are discussed in more detail in Section 15.2.

For ultrasensitive detection of polar tracers that are based on fluorescein, Oregon Green, Cascade Blue, lucifer yellow, tetramethylrhodamine or BODIPY FL dyes in fixed-cell preparations, we recommend use of our TSA Kits (Section 6.2, Table 6.1) that contain an Alexa Fluor, Oregon Green 488 or Pacific Blue tyramide. The TSA kits that utilize anti-mouse IgG or anti-rabbit IgG conjugates of horseradish peroxidase (HRP) can be combined with an antibody to any of these fluorophores (Section 7.4, Table 7.13) to yield excellent spatial resolution and extremely bright signals that should be detectable in the finest biological structures. Following fixation, biotinylated tracers such as biocytin



**Figure 14.21** Adherent CRE BAG 2 cells passively loaded with the membrane-impermeant polar tracer Alexa Fluor 594 hydrazide (A-10438, A-10442). The image in the top panel illustrates the relatively uniform, cytoplasmic labeling one can obtain with the Influx pinocytic cell-loading reagent (I-14402), compared to the punctate labeling that results from pinocytic uptake in normal growth medium (bottom panel). Both images were acquired using a bandpass filter set appropriate for rhodamine dyes.