# An evaluation of spectrally distinct succinimidyl ester dyes for tracking cell proliferation by flow and image cytometry

McDonald D, Clarissa A, Bradford J, Meeson A, Filby A (2017) Poster presented at: CYTO 2017, 32nd Congress of the International Society for Advancement of Cytometry; July 10–14, 2017; Boston, Massachusetts, USA.

The study of cell proliferation has widespread applications in fields as diverse as embryogenesis, immunology, and cancer biology. In *BioProbes 73*, we highlighted the methods developed by Filby and coworkers to evaluate amine-reactive fluorogenic dyes used for tracking cell proliferation by flow and image cytometry [1–3]. At the CYTO 2017 meeting, they presented their work with two recently introduced amine-reactive dyes—the UV-excitable Invitrogen™ CellTrace™ Blue dye and the 532 nm– or 561 nm—excitable Invitrogen™ CellTrace™ Yellow dye. When used in conjunction with flow or image cytometry, fluorogenic succinimidyl ester (SE) dyes, such as the CellTrace proliferation dyes, provide a means of following the proliferative history of a single cell *in vivo* or *in vitro*.

The CellTrace proliferation dyes are designed to pass freely through cell membranes, where they are cleaved by intracellular esterases and spontaneously react with intracellular proteins through lysine side chains and other available amine groups, producing highly fluorescent, cell-impermeant proteins (see **thermofisher.com/celltrace**). These fluorescent proteins are distributed equally between daughter cells during cell division, such that each daughter cell exhibits half the fluorescence intensity of the parent cell, which is readily detectable by flow cytometry, fluorescence microscopy, or a fluorescence microplate reader. To be effective, dyes for tracking proliferation should: 1) exhibit intense and homogeneous fluorescent labeling of the parent cell that can be followed through successive cell divisions, 2) be available in a choice of excitation and emission wavelengths to facilitate multiparametric measurements and reduce compensation requirements, and 3) not perturb cell proliferation throughout the observation period.

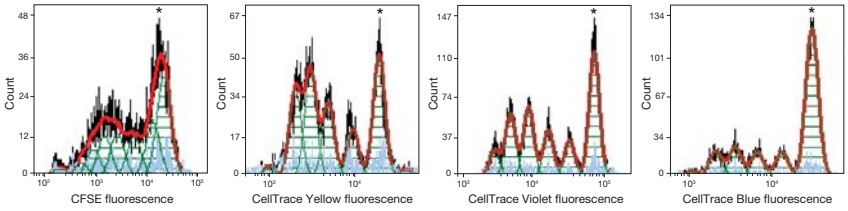


**Figure 1. Data showing the proliferative performance of human peripheral blood mononuclear cells (PBMCs) stained with four different CellTrace proliferation dyes.** *Peak corresponding to undivided cells. Reprinted with permission from Andrew Filby, Newcastle University, United Kingdom.

In their CYTO 2017 poster, McDonald and coworkers describe their flow cytometric evaluation of CellTrace proliferation dyes using either Jurkat cells or human peripheral blood mononuclear cells (PBMCs) activated with phytohemagglutinin (PHA) in the presence of interleukin-2 (IL-2); DAPI or propidium iodide staining was used to exclude dead cells. For each CellTrace dye and cell type, the dye was first titrated to find the optimal labeling concentration before conducting the cell proliferation analysis. For the Jurkat T cells and PBMCs, optimal concentrations of 8 µM were determined for CellTrace Blue and CellTrace Yellow dyes, and 4 µM for CellTrace Violet and CellTrace Far Red dyes. Labeled cells were then analyzed every 24 hours over a 96-hour period for percentage divided, proliferation index, and mean division metrics by flow cytometry (Figure 1). McDonald and coworkers confirmed earlier findings that CellTrace CFSE proliferation dye—the original fluorescein derivative—negatively affected cell proliferation and produced poor division peak quality and resolution, which hindered observation of successive generations. They conclude that, while all SE dyes are not equivalent in terms of proliferative performance, the CellTrace proliferation dyes, including the recent CellTrace Blue and CellTrace Yellow additions, provide useful and reliable tools for tracking cell division by flow and image cytometry. ■

## References

1. Begum J, Day W, Henderson C et al. (2013) *Cytometry A* 83:1085–1095.
2. Filby A, Begum J, Jalal M et al. (2015) *Methods* 82:29–37.
3. *BioProbes 73 Journal of Cell Biology Applications* (May 2016) Journal Club: Appraising the suitability of succinimidyl and lipophilic fluorescent dyes to track proliferation in non-quiescent cells by dye dilution. **http://www.thermofisher.com/bp73**

| Product | Laser for excitation | Ex/Em* | Quantity† | Cat. No. |
|---|---|---|---|---|
| CellTrace™ Blue Cell Proliferation Kit | UV | 355 or 375/410 | 180 reactions | C34568 |
| CellTrace™ Violet Cell Proliferation Kit | 405 nm | 405/450 | 180 reactions | C34557 |
| CellTrace™ CFSE Cell Proliferation Kit | 488 nm | 495/519 | 180 reactions | C34554 |
| CellTrace™ Yellow Cell Proliferation Kit | 532, 561 nm | 546/579 | 180 reactions | C34567 |
| CellTrace™ Far Red Cell Proliferation Kit | 633, 635 nm | 630/661 | 180 reactions | C34564 |

*Excitation (Ex) and emission (Em) maxima in nm, after hydrolysis. †CellTrace kits are also available for 20 reactions.