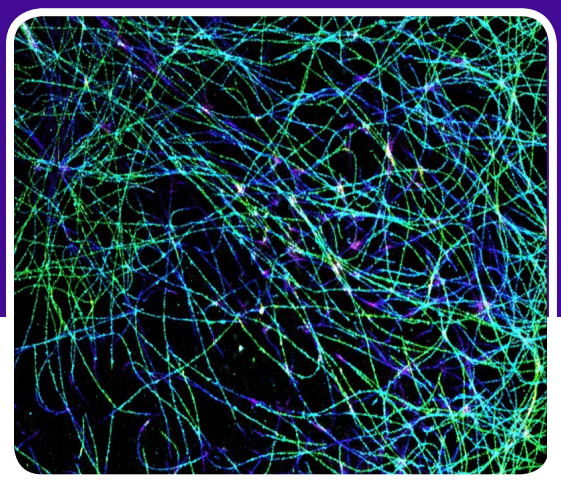

# Super-Resolution Microscopy Dyes

## CF™ Dyes for multicolor super-resolution microscopy

Recent publications comparing synthetic dyes for super-resolution imaging have shown CF™ dyes give the best performance for multiple methods. The superior brightness, photostability, and photochemical switching properties of certain CF™ dyes are ideal for 3-D SIM, 3-D STORM, and other super-resolution and single molecule imaging techniques. Biotium's CF™405M has been found to be the brightest and most photostable short wavelength fluorescent dye for SIM. Several of our CF™ dyes spanning the visible red, far-red, and near-infrared spectra have been validated for STORM, including three color imaging with CF™568, CF™647, and CF™680. See Lehmann et al. 2015, and a full list of references for CF™ dye single-molecule imaging applications on the other side. Biotium offers a wide selection of CF™ dye labeled secondary antibodies, other conjugates, and labeling kits.

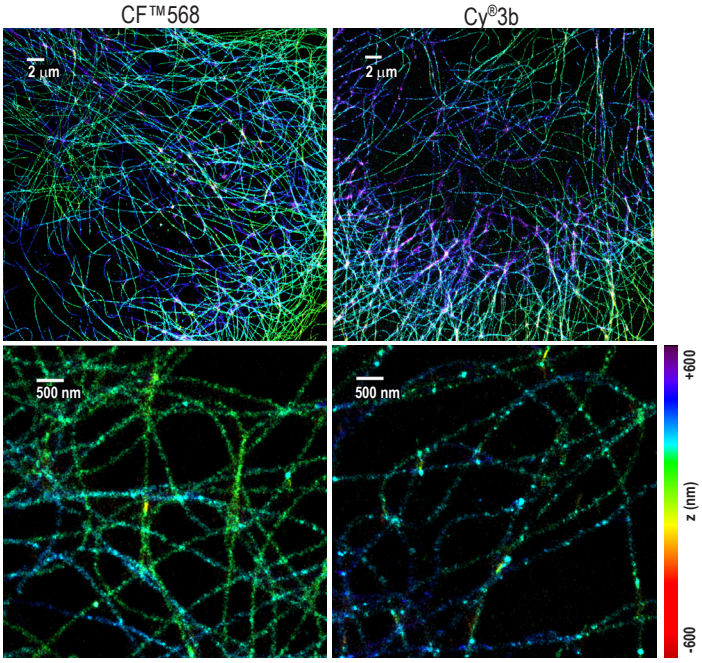


Figure 1. CF™568 (left) produces better images than Cy®3b (right) in 3-D STORM microscopy. Fixed cells were stained with mouse anti-tubulin antibody followed by dye-conjugated anti-mouse secondary antibodies. See Figure 1 legend for imaging conditions. Dye molecules were photoswitched and imaged using a 560 nm laser; a 405 nm laser was used to assist dye reactivation to the emitting state.

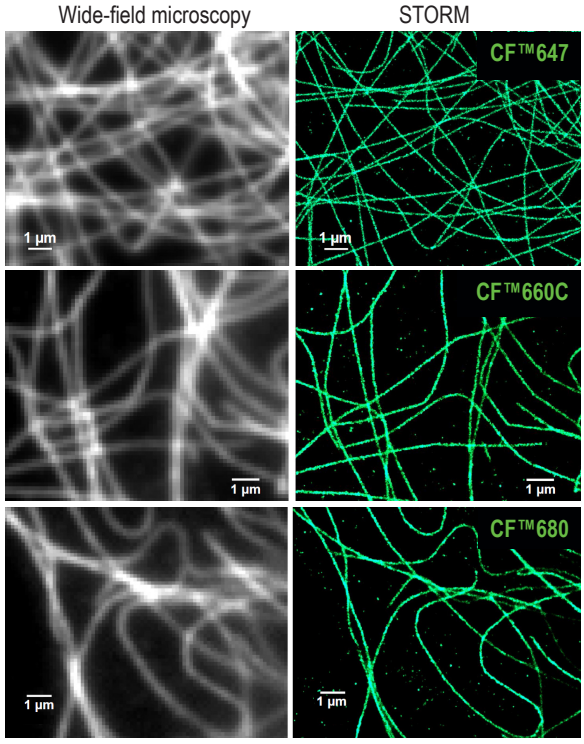


Figure 2. Comparison of conventional wide-field microscopy (left) with STORM (right) using CF™ dye conjugates. Fixed cells were stained with mouse anti-tubulin antibody followed by CF™ dye conjugated anti-mouse secondary antibody (top row: CF™647, middle row: CF™660C, bottom row: CF™680). For STORM, samples were sealed in buffer that contained 5% (w/v) glucose, 100 mM cysteamine, 0.8 mg/mL glucose oxidase, and 40 µg/mL catalase, in Tris-HCl (pH 7.5). Samples were imaged on a Nikon Ti-Eclipse w/ PFS microscope with a CFI Plan Apo Lambda 100x oil objective. Dye molecules were photoswitched and imaged using a 647 nm laser; a 405 nm laser was used to assist dye reactivation to the emitting state. Emission was collected with an Andor iXon Ultra 897 EMCCD camera for a total of 100,000 frames per image at a frame rate of 110 Hz.

## CF™ dyes and related products

- Primary antibody conjugates
- Labeled secondary antibodies
- Phalloidin and other bioconjugates
- Mix-n-Stain™ antibody labeling kits
- Protein labeling kits
- Reactive dyes with a full selection of functional groups
- Reactive dyes for bioorthogonal labeling
- Small ligand (SNAP-tag®, CLIP-tag™, HALO-tag®) labeling kits

*Microscopy images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.*

Biotium

**Super-resolution imaging techniques validated for CF™ dyes**

| CF™ Dye | Abs/Em maxima (nm) | Extinction coefficient | Super resolution application | References |
|---|---|---|---|---|
| CF™405M | 408/452 | 41,000 | SIM | Markaki, Y. et al. (2013). Fluorescence In Situ Hybridization Applications for Super-Resolution 3D Structured Illumination Microscopy. Methods Mol Biol 950, 43-64. |
| CF™488A | 490/515 | 70,000 | TIRF | Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200. |
| CF™535ST | 535/568 | 95,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. |
| CF™555 | 555/565 | 150,000 | Multicolor STORM | Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119 |
| CF™568 | 562/583 | 100,000 | TIRF, multicolor STORM | 1) Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119. 2) Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200. 3)Zhang, M. et al. (2015). Translocation of interleukin-1β into a vesicle intermediate in autophagy-mediated secretion. eLife 2015;10.7554/eLife.11205 |
| CF™594ST | 593/614 | 115,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. Please note: CF™594ST is a unique dye designed specifically for STORM. Our original CF™594 dye is not suitable for STORM. |
| CF™633 | 630/650 | 100,000 | TIRF, FIONA, gSHRImP | 1) Bosch, P. J. et al. (2014). Evaluation of fluorophores to label SNAP-tag fused proteins for multicolor single-molecule tracking microscopy in live cells. Biophys J 107, 803-814. 2)Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200. 3)Kim, H. J., and Selvin, P. R. (2013). Fluorescence Imaging with One Nanometer Accuracy. SpringerReference Encyclopedia of Biophysics. 4) Simonson, P. D.,Rothenberg, E., and Selvin, P. R. (2011). Single-molecule-based super-resolution images in the presence of multiple fluorophores. Nano Lett 11, 5090-5096. DOI:10.1021/nl203560r |
| CF™640R | 642/662 | 105,000 | TIRF, FLImP | 1) Bosch, P. J. et al. (2014). Evaluation of fluorophores to label SNAP-tag fused proteins for multicolor single-molecule tracking microscopy in live cells. Biophys J 107, 803-814. 2) Martin-Fernandez, M. L. et al. (2013). A 'pocket guide' to total internal reflection fluorescence. J Microsc 252, 16-22. 3) Zanetti-Domingues, L.C. et al. (2013). Hydrophobic Fluorescent Probes Introduce Artifacts into Single Molecule Tracking Experiments Due to Non-Specific Binding. PLoS ONE 8(9): e74200. 4) Needham, S.R.,et al. (2015). Determining the geometry of oligomers of the human epidermal growth factor family on cells with <10 nm resolution. Biochem Soc Trans 43, 309–314. |
| CF™647 | 650/665 | 240,000 | Multicolor STORM | Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119<br>Olivier, N. et al. (2013). Simple buffers for 3D STORM microscopy. Biomed Opt Express 4, 885-899. |
| CF™660C | 667/685 | 200,000 | Multicolor STORM | Zhang, Z., et al. (2015). Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. Nature Methods doi:10.1038/nmeth.3528 |
| CF™680 | 681/698 | 210,000 | Multicolor STORM, dual-Color 3D Superresolution Microscopy | 1) Früh, S.M. et al. (2015). Molecular architecture of native fibronectin fibrils. Nature Communications 6, 7275. 2) Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119. 3) Platonova, E. et al. (2015). A Simple Method for GFP- and RFP-based Dual Color Single-Molecule Localization Microscopy. ACS Chem. Biol.10(6),1411–1416. 4) Platonova, E. et al. (2015). Single-molecule microscopy of molecules tagged with GFP or mRFP derivatives in mammalian cells using nanobody binders. Methods doi: http://dx.doi.org/10.1016/j.ymeth.2015.06.018. 5) Salvador-Gallego, R. et al. (2016). Bax assembly into rings and arcs in apoptotic mitochondria is linked to membrane pores. EMBO J. DOI 10.15252/embj.201593384. 6) Venkataramani, V. et al. (2016). SuReSim: simulating localization microscopy experiments from ground truth models. Nature Methods 13, 319-321. doi:10.1038/nmeth.3775. 7) Winterflood, C.M. et al. (2015). Dual-Color 3D Superresolution Microscopy by Combined Spectral-Demixing and Biplane Imaging. Biophys J. 109, 3–6. 8) Zhang, Z., et al. (2015). Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. Nature Methods doi:10.1038/nmeth.3528 |
| CF™680R | 680/701 | 140,000 | STED, single-molecule spectroscopy | Görlitz, F. et al. (2014). A STED Microscope Designed for Routine Biomedical Applications. Progress Electromagnetics Res 147, 57-68.<br>König, I. et al. (2015). Single-molecule spectroscopy of protein conformational dynamics in live eukaryotic cells. Nature Methods doi:10.1038/nmeth.3475 |
| CF™750 | 755/777 | 250,000 | STORM | Collaborator communication; contact techsupport@biotium.com for more information. |

FIONA: Fluorescence Imaging with One Nanometer Accuracy; FLImP: Fluorophore localization imaging with photobleaching; SHRImP: Single-molecule high-resolution imaging with photobleaching; SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastical optical reconstruction microscopy; TIRF: Total internal reflection fluorescence



www.biotium.com
General Inquiries: order@biotium.com
Technical Support: techsupport@biotium.com
Phone: 800-304-5357