CF™ Dyes for Multicolor Super-Resolution Microscopy

Biotium

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. Six 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.

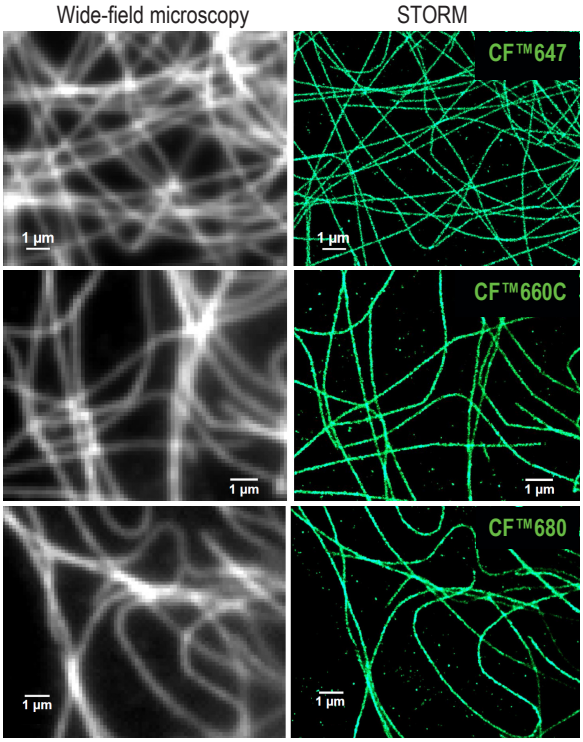

Figure 1. Comparison of conventional wide-field microscopy (left) with STORM (right) using CF™ dye conjugates. Fixed cells were stained with mouse anti-tubulin antibody followed by CF™ dye conjugated anti-mouse secondary antibody (top row: CF™647, middle row: CF™660C, bottom row: CF™680). For STORM, 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.

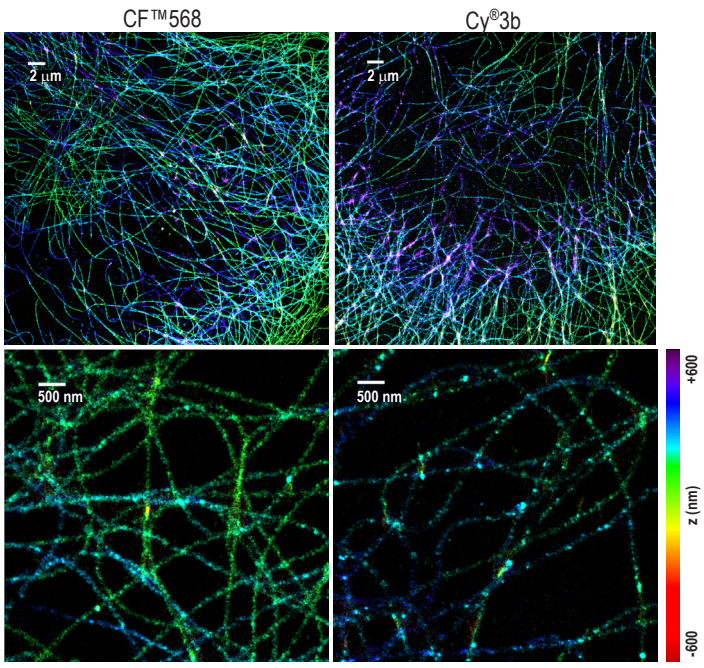

Figure 2. CF™568 (left) produces better images than Cy®3b (right) in 3-D STORM microscopy. Fixed cells were stained with mouse anti-tubulin antibody followed by dye-conjugated anti-mouse secondary antibodies. See Figure 1 legend for imaging conditions. Dye molecules were photoswitched and imaged using a 560 nm laser; a 405 nm laser was used to assist dye reactivation to the emitting state.

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

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CF™ Dyes for Super-Resolution Microscopy

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	Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119 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. 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.
CF™633	630/650	100,000	TIRF, FIONA, gSHRImP	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. 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. Kim, H. J., and Selvin, P. R. (2013). Fluorescence Imaging with One Nanometer Accuracy. SpringerReference Encyclopedia of Biophysics 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	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. Martin-Fernandez, M. L. et al. (2013). A 'pocket guide' to total internal reflection fluorescence. J Microsc 252, 16-22. 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. 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 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	Früh, S.M. et al. (2015). Molecular architecture of native fibronectin fibrils. Nature Communications 6, 7275. Lehmann, M. et al. (2015). Novel organic dyes for multicolor localization-based super-resolution microscopy. J Biophotonics DOI 10.1002/jbio.201500119 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. 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 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 Winterflood, C.M. et al. (2015). Dual-Color 3D Superresolution Microscopy by Combined Spectral-Demixing and Biplane Imaging. Biophys J. 109, 3–6. 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. 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

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