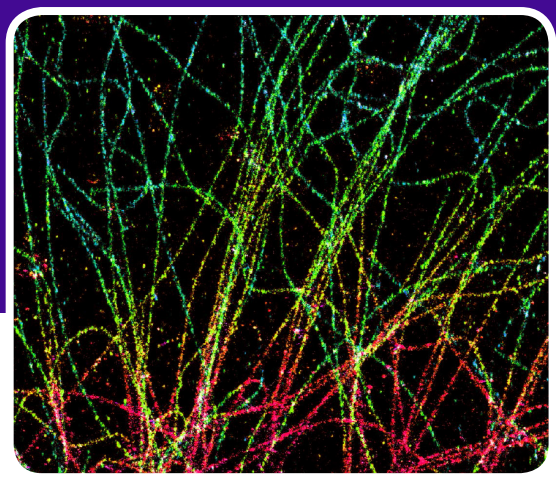

Super-Resolution Microscopy Dyes

CF® Dyes for multicolor super-resolution microscopy

Recent publications comparing synthetic dyes for super-resolution imaging have shown that 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 techniques. Biotium's CF®405M has been found to be the brightest and most photostable short wavelength fluorescent dye for SIM. Several 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 on the next page. Biotium offers a wide selection of CF® dye labeled antibodies, including single-label secondaries with low degree of labeling, which is reported to be optimal for STORM[1]. We also offer other conjugates, reactive dyes, and labeling kits.

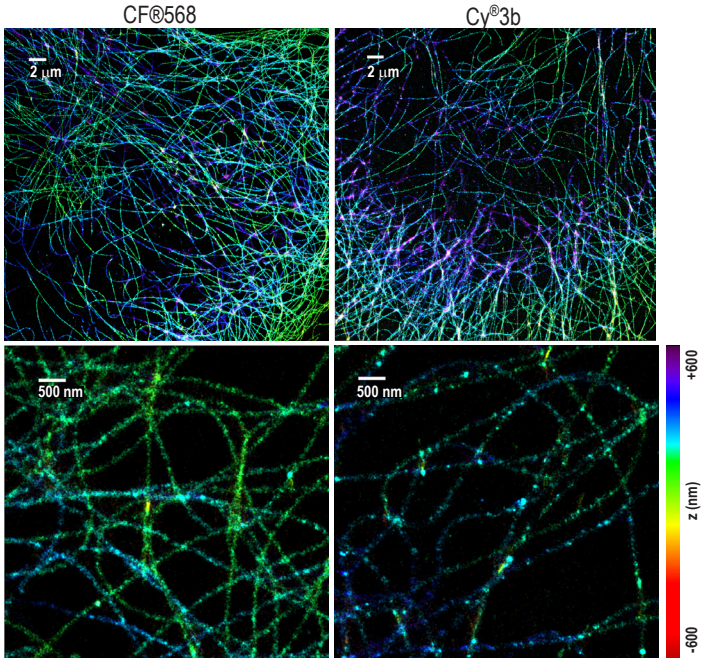

Figure 1. CF®568 (left) produces better images than Cy®3b (right) in 3-D STORM microscopy. Fixed cells were stained with mouse anti-tubulin antibody followed by dye-conjugated anti-mouse secondary antibodies. See Figure 2 legend for imaging conditions. Dye molecules were photoswitched and imaged using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

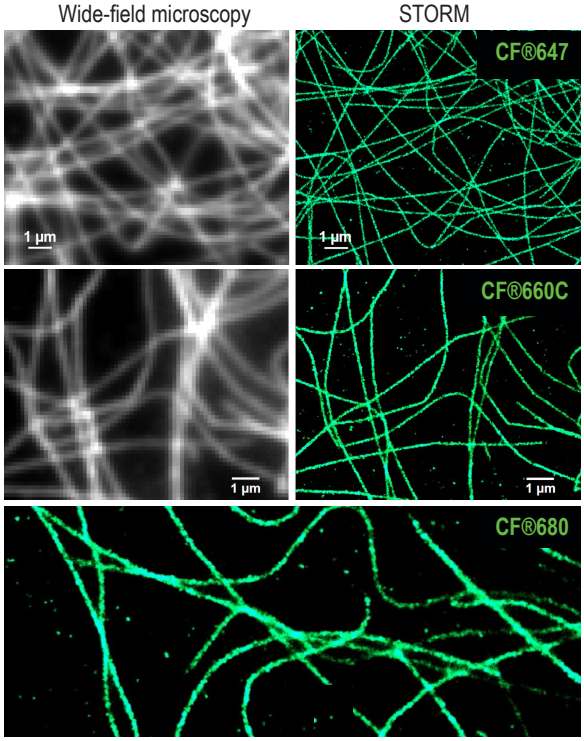

Figure 2. Comparison of conventional wide-field microscopy (left) with STORM (right) using CF® dye conjugates. Fixed cells were stained with mouse anti-tubulin antibody followed by CF® dye conjugated anti-mouse secondary antibody (top row: CF®647, middle row: CF®660C, bottom row: CF®680). For STORM, 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
- Single-label secondaries for STORM
- Phalloidin and other bioconjugates
- Mix-n-Stain™ antibody labeling kits
- Protein labeling kits
- Full selection of reactive dyes
- Dyes for bioorthogonal labeling
- Small ligand (SNAP-tag®, CLIP-tag™, HALO-tag®) labeling kits

[1] Bittel, A.M. et al. (2015). Proc. SPIE 9331, Single Molecule Spectroscopy and Superresolution Imaging VIII, 93310M. doi: 10.1117/12.2083209
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®405S	404/431	33,000	SIM	Essig et al. (2017). Immunity https://doi.org/10.1016/j.immuni.2017.11.008
CF®405M	408/452	41,000	SIM	Kraus, F. et al. (2017). Nat Protoc 12, 1011-1028. doi:nprot.2017.020 Markaki, Y. et al. (2013). Methods Mol Biol 950, 43-64. Miron, E. et. al. (2016). In: Mark C. Leake (ed.), Methods in Molecular Biology, vol. 1431, 127-140. Ohgomori, T. et al. (2017). Eur J Neurosci 46, 2001-2014. doi:10.1111/ejn.13650
CF®488A	490/515	70,000	TIRF	Angelov, B. & Angelova, A. (2017). Nanoscale 9, 9797-9804. doi:10.1039/c7nr03454g (STED) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF)
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). J Biophotonics DOI 10.1002/jbio.201500119
CF®568	562/583	100,000	Multicolor STORM, SIM, TIRF	Gong, Y.-N. et al. (2017). Cell Cycle, 1-13. doi:10.1080/15384101.2017.1371889 (STORM) Gorur, A. et al. (2017). J Cell Biol 216, 1745-1759. doi:10.1083/jcb.201702135 (STORM) Heller, J. (2017). OM&P 3, 48-58. doi:doi:10.20388/omp2017.002.0045 (STORM) Jorgans, D.M. et al. (2017). J Cell Sci 2017 130: 177-189. doi: 10.1242/jcs.190967 (STORM) Karanasios, E. et al. (2016). Nat Commun 7: 12420. DOI: 10.1038/ncomms12420 (STORM) Kraus, F. et al. (2017). Nat Protoc 12, 1011-1028. doi:nprot.2017.020 (SIM) Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 (STORM) Lim, A. et al. (2017). Mol Biol Cell doi:mbc.E16-12-0820 (SIM) Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 (STORM) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200.(TIRF) Zhang, M. et al. (2015). eLife 2015;10.7554/eLife.11205 (STORM)
CF®594ST	593/614	115,000	STORM	Collaborator communication; contact techsupport@biotium.com for more information. **Note:** CF®594ST is a unique dye designed for STORM. Our original CF®594 is not suitable for STORM.
CF®633	630/650	100,000	FIONA, gSHRImP, TIRF	Bosch, P. J. et al. (2014). Biophys J 107, 803-814. (TIRF) Kim, H. J., and Selvin, P. R. (2013). SpringerReference Encyclopedia of Biophysics. (FIONA) Simonson, P. D. et al. (2011). Nano Lett 11, 5090-5096. DOI:10.1021/nl203560r (gSHRImP) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF)
CF®640R	642/662	105,000	FLImP, SIM, TIRF	Bosch, P. J. et al. (2014). Biophys J 107, 803-814. (TIRF) Loh, L. N. (2017). MBio 8, doi:mBio.02030-16 (SIM) Martin-Fernandez, M. L. et al. (2013). J Microsc 252, 16-22. (TIRF) Needham, S.R. et al. (2015). Biochem Soc Trans 43, 309–314. (FLImP) Needham, S.R. et al. (2016). Nat Commun 7, 13307. doi:ncomms13307 (FLImP) Zanetti-Domingues, L.C. et al. (2013). PLoS ONE 8(9): e74200. (TIRF) Zanetti-Domingues, L.C. et al. (2015). Prog Biophys Mol Biol. doi:S0079-6107(15)00047-4 (FLImP)
CF®647	650/665	240,000	Multicolor STORM	Gong, Y.-N. et al. (2017). Cell Cycle, 1-13. doi:10.1080/15384101.2017.1371889 Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 Olivier, N. et al. (2013). Biomed Opt Express 4, 885-899. Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8
CF®660C	667/685	200,000	Multicolor STORM	Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 Zhang, Z., et al. (2015). Nature Methods doi:10.1038/nmeth.3528.
CF®680	681/698	210,000	Dual-Color 3D SMLM, Multicolor STORM	Früh, S.M. et al. (2015). Nature Communications 6, 7275. (STORM) Glebov, O.O. et al. (2017). Cell Rep 18, 2715-2728. doi:S2211-1247(17)30279-6 (STORM) Gorur, A. et al. (2017). J Cell Biol 216, 1745-1759. doi:10.1083/jcb.201702135 (STORM) Lehmann, M. et al. (2015). J Biophotonics DOI 10.1002/jbio.201500119 (STORM) Platonova, E. et al. (2015). ACS Chem. Biol.10(6),1411–1416. (STORM) Platonova, E. et al. (2015). Methods doi: http://dx.doi.org/10.1016/j.ymeth.2015.06.018. (STORM) Salvador-Gallego, R. et al. (2016). EMBO J. DOI 10.15252/embj.201593384. (STORM) Shrestha, R. L. et al. (2017). Nat Commun 8, 150. doi:10.1038/s41467-017-00209-z (STORM) Turkowyd, B. et al. (2016). Anal Bioanal Chem DOI 10.1007/s00216-016-9781-8 (SMLM) Winterflood, C.M. et al. (2015). Biophys J. 109, 3–6. (SMLM) Zhang, Z., et al. (2015). Nature Methods doi:10.1038/nmeth.3528. (STORM)
CF®680R	680/701	140,000	Single-molecule spectroscopy, STED	Görlitz, F. et al. (2014). Progress Electromagnetics Res 147, 57-68. (STED) König, I. et al. (2015). Nature Methods doi:10.1038/nmeth.3475 (Single molecule spectroscopy) Winter, F.R. et al. (2017). Scientific Reports 7, 46492. DOI: 10.1038/srep46492 (STED)
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; SMLM: Single molecule localization microscopy; STED: Stimulated emission depletion; STORM: Stochastical optical reconstruction microscopy; TIRF: Total internal reflection fluorescence

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