

Super-Resolution Microscopy

Fluorescent dyes and stains ideal for STORM and other super-resolution techniques

Superior Dyes for Super-Resolution Microscopy

Super-resolution microscopy (SRM) encompasses diverse techniques that all rely on extremely precise control over the excitation, emission, and image acquisition of fluorescently labeled cells and tissues. Consequently, the quality and efficiency of SRM imaging relies heavily on the properties of the fluorescent dyes used. Biotium's CF® Dyes have met this demand with industry-leading dyes developed and validated for STORM and other SRM techniques (see page 3). CF® Dyes are offered in a wide array of products useful for super-resolution microscopy including labeled primary and secondary antibodies, antibody labeling kits, reactive dyes, single-label secondary antibody conjugates for STORM, and CF® Dye conjugates of phalloidin, lectins, nucleotides, and more (see page 2 for more details). Biotium also offers a variety of stains for cell nuclei, extracellular vesicles, and other organelles that have been validated for use in SRM (see page 4).

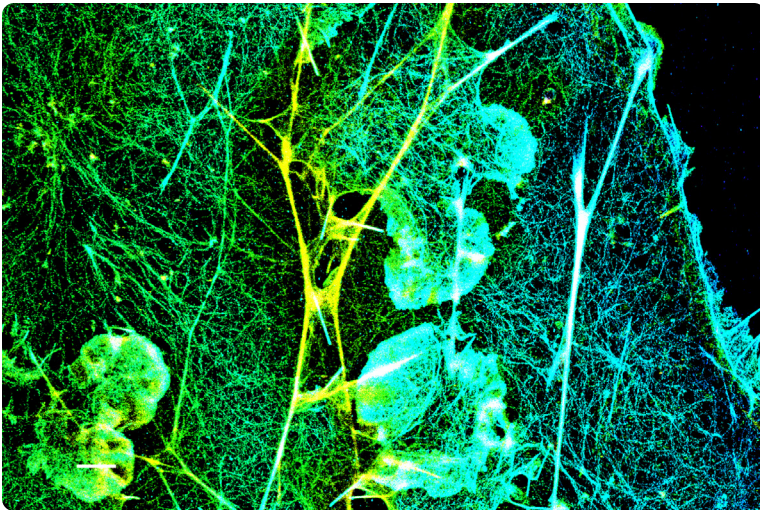


Figure 1. Typical (d)STORM image of the actin cytoskeleton in a fixed COS-7 cell labeled by phalloidin-CF®583R. (d)STORM was performed under standard conditions using a Tris-based (d)STORM buffer containing 100 mM cysteamine and an oxygen scavenger. Image courtesy of Bowen Wang, Michael Xiong, and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

CF® Dye Advantages for Super Resolution

- Superior brightness and photoswitching properties
- Validated for STORM, 2-photon, TIRF, and more
- Selection of reactive dyes, including click chemistry
- Many color options available from blue to near-IR
- Unique dye options designed specifically for STORM

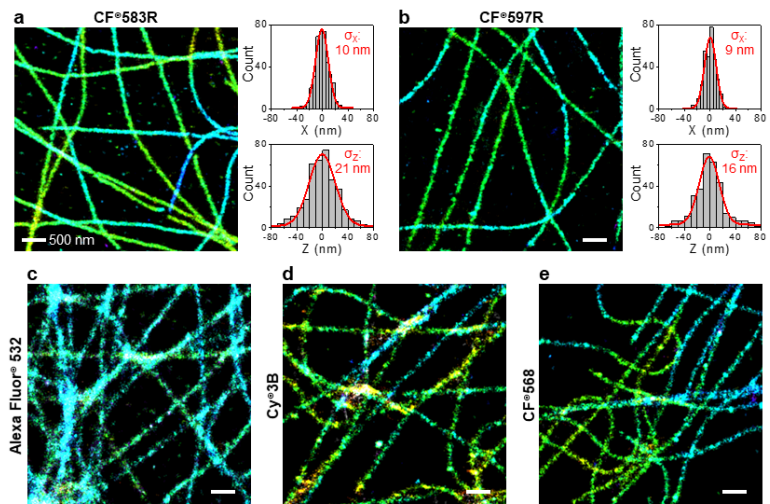


Figure 2. Comparison of typical (d)STORM images obtained in fixed COS-7 cells for microtubules immunolabeled by different dyes. (d)STORM was performed under standard conditions using a Tris-based (d)STORM imaging buffer containing 100 mM cysteamine and an oxygen scavenger. (a) CF®583R. (b) CF®597R. (c) Alexa Fluor® 532. (d) Cy®3B. (e) CF®568. Localization distributions are further given for single CF®583R and CF®597R molecules in the sample, in the X (in-plane; top) and Z (depth; bottom) directions, respectively. Gaussian fits (red curves) give standard deviations of ~10 and ~20 nm in the two directions, respectively. Color denotes depth (Z) values. Images and figures courtesy of Bowen Wang, Michael Xiong, and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

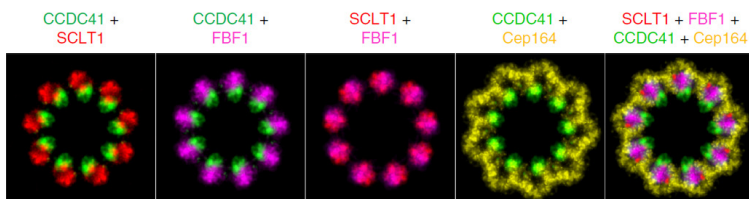


Figure 3. STORM analysis of centriole assembly. RPE-1 Centrin 1-GFP cells were labeled using primary antibodies for the indicated proteins and Biotium's CF[®]647 F(ab')₂ secondary antibody conjugate then imaged using 3D STORM (pseudo-colored for display purposes). For scale, image edges represent approximately 650 nm across. Credit: Bowler *et al.* doi.org/10.1038/s41467-018-08216-4 reproduced under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

CF[®] Dyes Validated for STED and FLIM Imaging

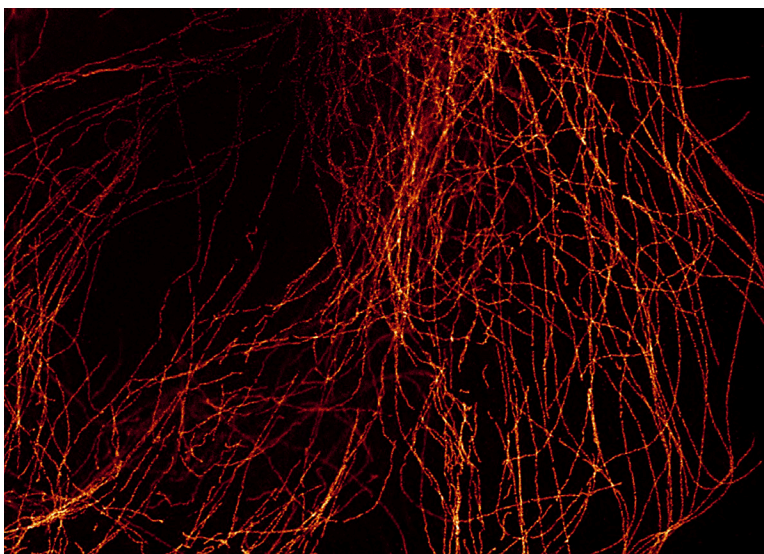


Figure 4. STED imaging of microtubules in U-2 OS cells. Microtubules were labeled with mouse anti-tubulin (DM1a) and anti-mouse CF[®]640R secondary antibody. Image acquired on a STELLARIS 8 STED FALCON confocal microscope, courtesy Leica Microsystems GmbH, Germany.

CF[®] Dye Fluorescence Lifetime Data

Measurements were made on a Stellaris 8 STED FALCON microscope courtesy of Leica Microsystems, Germany.

Dye	τ (ns) /free acid in PBS pH 7.4, ϵ (ns)	τ (ns) /S.Ab [§]
CF [®] 405S	3.88 ± 0.05	–
CF [®] 488A	4.11 ± 0.05	1.705
CF [®] 568	3.66 ± 0.05	1.539
CF [®] 594	–	1.746
CF [®] 633	3.39* (in water)	3*
CF [®] 640R	2.38 ± 0.05	1.557
CF [®] 647	1.07 ± 0.05	1.195
CF [®] 680	1.23 ± 0.05	1.277
CF [®] 680R	1.22 ± 0.05	1.6
CF [®] 750	0.58 ± 0.05	0.636
CF [®] 790	0.39 ± 0.05	0.54

§ Fluorescence lifetime measurements of CF[®] Dye labeled anti-mouse secondary antibodies used for immunostaining microtubules in U2OS using mouse anti-tubulin (DM1a) and mounted in ProLong[™] Diamond.

*Lifetime data obtained via customer communication under different experimental conditions and imaging setup.

CF[®] Dye Products Useful for Super-Resolution

Antibody Labeling Kits Designed for STORM

Our Mix-n-Stain[™] STORM CF[®] Dye Antibody Labeling Kits allow you to label 50 ug of antibody in your own lab with one of Biotium's STORM CF[®] Dyes to produce an antibody conjugate with a low 1-2.5 DOL (degree of labeling, or number of dye molecules per antibody molecule) optimal for STORM. Labeling takes just 30 minutes, with minimal hands-on time and no purification step needed.

Mix-n-Stain[™] STORM CF[®] Dye Antibody Labeling Kits

- **Low DOL:** Optimized to yield 1-2.5 degree of labeling
- **Rapid:** Only 30 minutes total reaction time
- **Validated Labels:** 7 CF[®] Dye colors ideal for STORM

Single-Label Secondary Antibody Conjugates for STORM

Secondary antibodies with a low DOL have been reported to be optimal for STORM. Biotium offers single-label secondary antibody conjugates with an average DOL of 1 for STORM applications.

Reactive CF[®] Dyes

CF[®] Dyes in a wide array of reactive chemistries for your preferred labeling technique can be found in our catalog. Succinimidyl esters (SE) are ideal for efficiently labeling antibodies, proteins, or other amine-containing molecules, while maleimide and MTS CF[®] Dyes selectively label thiols. We also offer aminoxy and hydrazide reactive CF[®] Dyes for labeling carbonyl groups or carbohydrates, and azides, alkynes, as well as BCN CF[®] Dyes for highly specific bioorthogonal labeling.

Reactive CF[®] Dyes

- **High efficiency SE Dyes:** For labeling amines in antibodies and other proteins
- **Maleimide and MTS Dyes:** For labeling thiols
- **Click Chemistry:** Alkynes, azides, and BCN for bioorthogonal labeling

Probes for Cytoskeleton and Other Cellular Structures

Biotium offers a wide variety of CF[®] Dye bioconjugates for labeling specific cellular structures. This includes phalloidin conjugates for F-actin labeling available in 16 CF[®] Dye options; Con A, WGA, and PNA lectin CF[®] Dye conjugates for labeling glycoproteins and cell surfaces; streptavidin and biotinylated CF[®] Dye conjugates, and CF[®] Dye nucleotides. Many cellular stains validated for super-resolution imaging and probes for apoptosis, endocytosis, and other cellular processes are also available (see page 4).

CF® Dyes Validated for Super-Resolution Microscopy

Dye	Abs/Em (nm)	STORM	STED	SIM	2-Photon	TIRF	Other Applications	Features
CF®405S	411/431			•				<ul style="list-style-type: none"> Brighter than Alexa Fluor® 405
CF®405M	416/452		•	•	•			<ul style="list-style-type: none"> More photostable than Pacific Blue™ Excellent choice for SIM imaging
CF®488A	490/516	•	•	•	•	•	DNA-PAINT	<ul style="list-style-type: none"> Less non-specific binding than Alexa Fluor® 488
CF®505	505/519	•						<ul style="list-style-type: none"> Identical to ATTO 488
CF®535ST	535/569	•						<ul style="list-style-type: none"> Orange dye designed specifically for STORM imaging
CF®555	554/568	• ¹		•				<ul style="list-style-type: none"> Brighter and more photostable than Cy®3 Less non-specific binding than Alexa Fluor® 555
CF®568	562/584	• ¹	•	•		•		<ul style="list-style-type: none"> Yields much brighter conjugates vs. Alexa Fluor® 568 Outperforms Cy®3b in STORM Pairs well with Biotium's CF®647 and CF®680 in multi-color STORM
CF®583R	585/609	• ¹						<ul style="list-style-type: none"> One of two top-performing dyes specifically designed for STORM with green laser (also see CF®597R)
CF®594	593/615		•		•			<ul style="list-style-type: none"> Significantly brighter than Alexa Fluor® 594 and Texas Red® Extremely photostable
CF®597R	597/619	• ¹						<ul style="list-style-type: none"> Deep-red fluorescent dye designed for STORM Top-performing dye specifically designed for STORM with green laser (also see CF®583R)
CF®633	629/650					•	FIONA, gSHRImp, SMT	<ul style="list-style-type: none"> Significantly brighter than similar far-red dyes Far more photostable than Alexa Fluor® 647
CF®640R	642/663		•	•	•	•	FLImP	<ul style="list-style-type: none"> Offers improved brightness and photostability over ATTO 647N and spectrally similar dyes
CF®647	652/668	• ¹						<ul style="list-style-type: none"> Spectrally similar to Cy®5 and Alexa Fluor® 647 Pairs well with CF®568 for multi-color STORM The best far-red dye for demixing-based multi-color (d)STORM imaging when paired with CF®680
CF®660R	662/682					•	SMLM, DNA-PAINT	<ul style="list-style-type: none"> Much brighter than Alexa Fluor® 660 The most photostable 660 nm dye Validated for use with DNA-PAINT SMLM
CF®660C	667/685	• ¹					MINFLUX	<ul style="list-style-type: none"> Much brighter and more photostable than Alexa Fluor® 660 Ideal for long high-intensity 3D (d)STORM image acquisitions with minimal photobleaching
CF®680	681/698	• ¹					Dual-color 3D SMLM, MINFLUX	<ul style="list-style-type: none"> The brightest among spectrally similar 680 nm dyes Pairs well with CF®568 for multi-color STORM The best near-IR dye for demixing-based multi-color (d)STORM imaging when paired with CF®647
CF®680R	680/701	• ¹	•		•		Single-molecule spectroscopy, SMT	<ul style="list-style-type: none"> The most photostable 680 nm dye Suitable for labeling nucleic acids and small biomolecules
CF®750	755/779	•						<ul style="list-style-type: none"> Exceptionally bright and photostable near-IR dye Patented pegylated dye for superior performance

¹ Dye was validated in multi-color STORM experiments.

A bullet indicates that the respective dye has been published and/or validated for the application. See our [current list of references](#) validating CF® Dyes for SRM and other applications. For more information regarding SRM validation data, or questions about the compatibility of CF® Dyes with expansion microscopy protocols contact techsupport@biotium.com.

FLImP: Fluorophore localization imaging with photobleaching; SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastic optical reconstruction microscopy; TIRF: Total internal reflection fluorescence microscopy; FIONA: Fluorescence imaging with one-nanometer accuracy; SMT: Single-molecule tracking; SMLM: Single-molecule localization microscopy.

Membrane and Organelle Stains for Super-Resolution

Biotium offers a wide selection of novel and classic cellular stains that have been validated for SRM applications. This includes stains for labeling nuclei, cell membranes, lysosomes, extracellular vesicles, and more. See the table below for a list of cellular stains and validated SRM applications that Biotium offers.

Cellular Stains for Super Resolution

- **Membrane Dyes for STORM:** MemBrite® Fix-ST Dyes were developed specifically for STORM
- **EV Dyes for STORM:** ExoBrite® STORM Dyes designed for labeling isolated or bead-bound EVs for STORM
- **Organelle Stains:** View table below for SRM- validated stains for nuclei, lysosomes, microtubules & more

Cellular Stains Validated for Super Resolution

Localization	Catalog Number	Product	Abs/Em (nm)	STORM	STED	SIM	2-Photon*	TIRF
Nucleus	40046, 40047	Hoechst 33342	352/458 (with DNA)			•	•	•
	40011, 40009, 40043	DAPI	358/461 (with DNA)			•	•	•
	40090	Oxazole Yellow Homodimer (YOYO®-1)	491/509 (with DNA)	•				
	40081	NucSpot® Live 488	503/518			•		
	40082	NucSpot® Live 650	655/681	•	•	•		
Membrane / Cell Surface	30092	MemBrite® Fix 405/430	405/430			•		
	30093	MemBrite® Fix 488/515	488/515		•		•	•
	30095	MemBrite® Fix 568/580	568/580	•		•		•
	30096	MemBrite® Fix 594/615	594/615				•	
	30097	MemBrite® Fix 640/660	640/660			•		•
	30099	MemBrite® Fix 680/700	680/700	•	•		•	
	30101	MemBrite® Fix-ST 650/665	650/665	•				
	30102	MemBrite® Fix-ST 667/685	667/685	•				
	30103	MemBrite® Fix-ST 681/698	681/698	•				
	30104	MemBrite® Fix-ST 755/777	755/777	•				
	30090	CellBrite® Fix 488	480/513			•		
	30088	CellBrite® Fix 555	542/570			•		
	30107	CellBrite® Steady 550	562/579	•				
	30108	CellBrite® Steady 650	656/676	•				
	30109	CellBrite® Steady 685	686/708	•				
	30023	CellBrite® Red	644/665			•	•	•
	70020, 70022	SynaptoGreen™ C4 (FM®1-43)	480/598				•	
Cytoplasm	30050	ViaFluor® CFSE	495/515				•	
Microtubules	70063	ViaFluor® 647	650/675		•	•		
Lysosomes	70067	LysoView™ 488	506/532			•		
	70059	LysoView™ 650	650/675		•	•		
Lipid Droplets	70065	LipidSpot™ 488	427/585			•		
EVs & Exosomes	30115	ExoBrite™ STORM CF®505	505/519	•				
	30116	ExoBrite™ STORM CF®583R	583/609	•				
	30117	ExoBrite™ STORM CF®647	652/668	•				
	30118	ExoBrite™ STORM CF®680	681/698	•				

*We also offer a wide selection of ion indicator and cell tracing dyes for use in 2-Photon Microscopy.

A bullet indicates that the respective dye has been published and/or validated for the application. For questions regarding the validation of our cellular stains for SRM, please contact techsupport@biotium.com.

SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastic optical reconstruction microscopy; TIRF: Total internal reflection fluorescence microscopy.

CY Dye is a registered trademark of Cytiva; Alexa Fluor and Texas Red are registered trademarks, and Prolong and Pacific Blue are trademarks of Thermo Fisher Scientific.