

Literature Digest

CF[®] Dyes for STORM Super-Resolution Microscopy

Biotium scientists have engineered CF[®] Dyes with exceptional brightness and photoswitching properties that are ideal for super-resolution imaging by STORM. In this digest we have selected three publications that demonstrate the superior performance of CF[®] Dyes over other commercially available probes for STORM-based applications.

Novel Chemical Modifications to Fluorescent Dyes for Improved Super-Resolution Imaging by STORM ... p. 2

Wang, et al. *Angewandte Chemie*, e202113612 (2021).

CF[®]647 and CF[®]680 Dye Pair Demonstrates Optimal Performance for Demixing-Based Multicolor STORM ... p. 3

Lehmann, et al. *Journal of Biophotonics*, 9(1-2), 161–170 (2016).

CF[®]660C Ideal for Optimized High-Throughput Super-Resolution STORM Imaging ... p. 4

Diekmann, et al. *Nature Methods*, 909–912 (2020).

[Download our full publication list](#) for more validated applications using CF[®] Dyes.

Novel Chemical Modifications to Fluorescent Dyes for Improved Super-Resolution Imaging by STORM

Wang, B., Xiong, M., Susanto, J., Li, X., Leung, W. Y., & Xu, K. (2021). [Transforming Rhodamine Dyes for \(d\)STORM Super-Resolution Microscopy via 1,3-Disubstituted Imidazolium Substitution](https://doi.org/10.1002/ange.202113612). Angewandte Chemie. <https://doi.org/10.1002/ange.202113612>

Summary

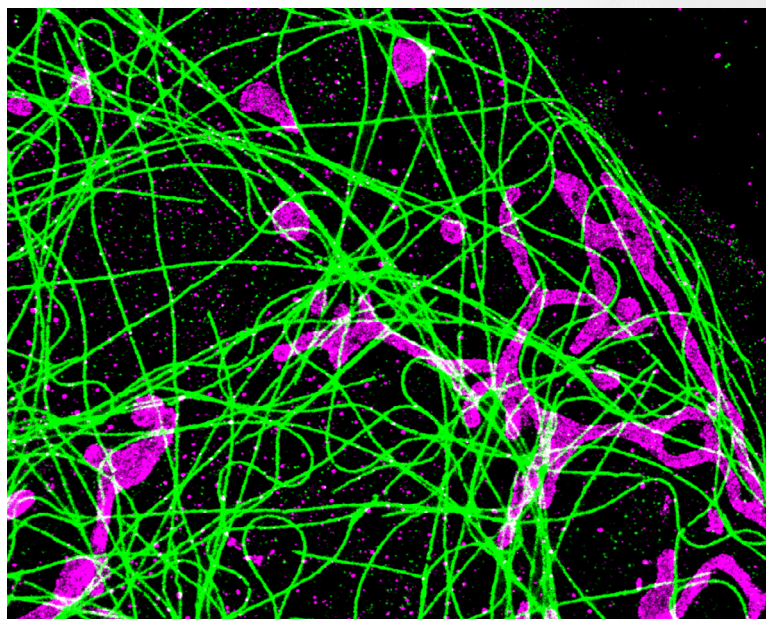
Though there are several red-excitable dyes that are suitable for (d)STORM, generating comparable dyes in other color channels for high quality multi-color STORM remains a challenge. Scientists at Biotium proposed a strategy to improve the photoswitching behavior of rhodamine dyes, leading to two green-excitable dyes that expand the color selection for multicolor STORM imaging. Wang et al. validated CF®583R and CF®597R, two new rhodamine-based dyes developed by Biotium chemists. The dye structures were modified with a positively charged 1,3-disubstituted imidazolium substitution to stabilize the radical dark state and improve photoswitching performance. These green-excited dyes for (d)STORM generated similar results to the ideal, red-excited AF647 and CF®647 in the same imaging buffer. They also showed superior photoswitching and imaging properties compared to other commonly used green-excited dyes, such as AF532, Cy®3B, Atto 565, and AF568. These new CF® Dyes will be essential for expanding the color palette for multicolor (d)STORM and future super-resolution microscopy research.

How CF® Dyes were used

CF®583R and CF®597R were developed to address issues with commonly used green-excited dyes for (d)STORM. The permanent positive charge on the 1,3-disubstitution imidazolium in these rhodamine-based dyes sensitizes the dyes for better photoswitching and imaging results.

Takeaways

- In collaboration with Biotium, the authors validated two novel green-excited dyes, CF®583R and CF®597R, for optimized for (d)STORM imaging.
- Other green-excited dyes are not as compatible with multicolor STORM imaging due to poor photoswitching and production of low-quality images.
- CF®583R and CF®597R demonstrated significantly improved photoswitching properties and were ideal for use with red-excited dyes for multicolor STORM imaging.



Two-color (d)STORM imaging of a fixed COS-7 cell: microtubules were immunolabeled with CF®647 conjugate, and mitochondria were immunolabeled with CF®583R conjugate. Image courtesy of Bowen Wang, Michael Xiong, and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

Recommended CF® Dyes

Dye	Ex/Em (nm)
CF®583R	585/609
CF®597R	597/619

CF®647 and CF®680 Dye Pair Demonstrates Optimal Performance for Demixing-Based Multicolor STORM

Lehmann, M., Lichtner, G., Klenz, H., & Schmoranzner, J. (2016). [Novel organic dyes for multicolor localization-based super-resolution microscopy](https://doi.org/10.1002/jbio.201500119). Journal of biophotonics, 9(1-2), 161–170. <https://doi.org/10.1002/jbio.201500119>

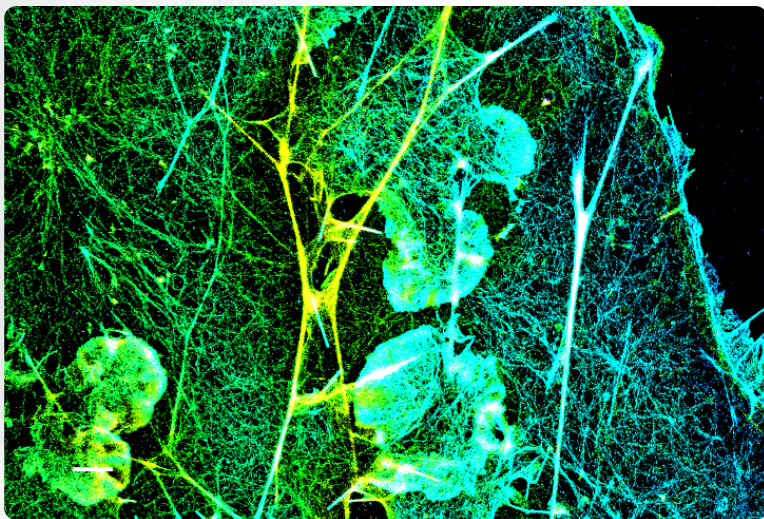
Summary

There are many constraints that limit the use of multicolor single-molecule localization techniques such as STORM, including color channel crosstalk, chromatic alignment errors, and limited availability of bright probes that can resolve distinct molecular species on a nanoscale. To find organic dyes optimal for (d)STORM, Lehmann et al. tested the ability of 28 commercially available dyes to super-resolve a known nanostructure.

Eight of the tested dyes produced high-quality (d)STORM [(direct) stochastic optical reconstruction microscopy] imaging in different spectral regimes. From these dyes, the authors identified CF®647 and CF®680 as an optimal pair for demixing-based multicolor (d)STORM imaging. In addition, the authors found the dye pair could be combined with CF®568 to successfully perform 3-color (d)STORM of cellular nanostructures with low crosstalk. The authors concluded that their three-dye set, CF®647, CF®680, and CF®568, will be useful for further high precision, multicolor SMLM experiments.

How CF® Dyes were used

To optimize precise multicolor (d)STORM methods, the authors tested 28 commercially available dyes, including CF®543, CF®555, CF®568, CF®647, CF®680, and CF®680R, for their photoswitching and low crosstalk properties for multicolor (d)STORM.



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 standard tris-based (d)STORM imaging buffer containing 100 mM cysteamine and an oxygen scavenger. Image courtesy of Professor Ke Xu, College of Chemistry, University of California, Berkeley.

Takeaways

- CF®647 and CF®680 functioned optimally together for demixing-based multicolor (d)STORM imaging.
- CF®647 and CF®680 were combined with separately excited CF®568 to produce high-quality 3-color (d)STORM images.
- Other CF® Dyes, including CF®543, CF®555 and CF®680R, also yielded good quality (d)STORM images.

Recommended CF® Dyes

Dye	Ex/Em (nm)
CF®543	543/563
CF®555	554/568
CF®568	562/584
CF®647	652/668
CF®680	681/698
CF®680R	680/701

CF®660C Ideal for Optimized High-Throughput Super-Resolution STORM Imaging

Diekmann, R., Kahnwald, M., Schoenit, A. et al. [Optimizing imaging speed and excitation intensity for single-molecule localization microscopy](https://doi.org/10.1038/s41592-020-0918-5). Nat Methods 17, 909–912 (2020). <https://doi.org/10.1038/s41592-020-0918-5>

Summary

There is a demand for methods that expedite large volume data acquisition for high-throughput single-molecule localization techniques such as (d)STORM. Faster imaging speeds require high excitation laser intensities to stimulate fluorophore blinking kinetics. However, there is no clear consensus on how increased laser power impacts (d)STORM image quality. Diekmann et al. investigated how excitation intensity influences image quality for a variety of fluorophores.

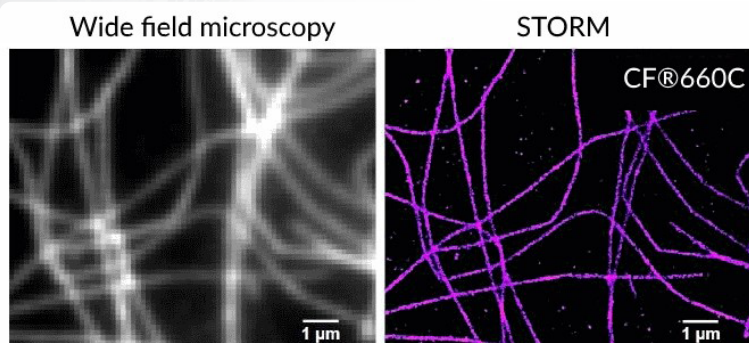
The authors reported the effect of imaging speeds when using AF647, CF®680, CF®660C, Dy-634, and mMaple. CF®660C was much less prone to photobleaching when imaged under high laser intensity than the other tested dyes, allowing higher labeling efficiency under fast imaging conditions. Other similar dyes, such as AF647, exhibited more photobleaching, as well as a decreasing effective labeling efficiency under the same high laser intensity conditions. The authors concluded that CF®660C is ideal for long high-intensity image acquisitions, allowing efficient capture of three-dimensional (d)STORM images in whole cells with minimal photobleaching.

How CF® Dyes were used

Optimization of (d)STORM was conducted using varying buffers, lasers powers, and fluorophores. The authors tested fluorophores CF®680, CF®660C, AF647, Dy-634, and mMaple for correlations in imaging speeds and image quality using low and high-intensity laser excitation.

Takeaways

- High-throughput STORM imaging requires a trade-off between image speed and quality.
- Fast, high-intensity imaging directly impacts data quality due to dye bleaching.
- The minimal photobleaching demonstrated by CF®660C under high-power laser conditions allows efficient capture of three-dimensional (d)STORM images in whole cells.



Comparison of microtubule imaging using conventional wide-field microscopy (left) with STORM (right) using CF®660C dye conjugate. Images courtesy of Sam Kenny and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

Recommended CF® Dyes

Dye	Ex/Em (nm)
CF®660C	667/685
CF®680	681/698

Interested in Learning More About CF® Dyes for Super-Resolution Microscopy?

Check out these educational pages, product selection guides, and other resources available on our website.

Learn More

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[View Biotium's full catalog of probes validated for super-resolution microscopy.](#)

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[CF® Dye Phalloidin & Other Bioconjugates](#)

[Reactive CF® Dyes](#)

Product Selection Guides

[CF® Dye Quick Reference Guide](#)

[CF® Dye Brochure](#)

[Super-Resolution Microscopy Flyer](#)

Other Highlighted Citations & References

[Fast and multiplexed super-resolution imaging with DNA-PAINT-ERS](#)

[A pathologist's guide for using expansion microscopy for nanoscale imaging of clinical specimens](#)

[Multi-color 3D super-resolution microscopy of cellular organelles and protein complexes](#)

[Super-resolution microscopy through thick biological samples](#)

[Integrated imaging of cells in 3-dimensional culture reveals new nuclear structures](#)

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