

Evaluation of Slowfade Diamond as a buffer for STORM microscopy: supplement

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Fluorophore	Antibody type	Reference
Dylight 488	Horse anti-mouse	Vectorlab DI-2488
Alexa Fluor 488	Goat anti-mouse	Thermofisher A-11029
CF 488	Chicken anti-mouse	Sigma SAB4600029
Alexa Fluor 514	Goat anti-mouse	Thermofisher A31555
Alexa Fluor 532	Goat anti-mouse	Thermofisher A-11002
CF 532	Goat anti-mouse	Biotium 20365
Alexa Fluor 546	Goat anti-mouse	Thermofisher A-11030
Dylight 549	Horse anti-mouse	Vectorlab DI-2549
Atto 550	Goat anti-mouse	Sigma 43394
Alexa Fluor 555	Donkey anti-mouse	Thermofisher A-31570
Alexa Fluor 555	Goat anti-mouse Fab'2	Thermofisher A-21425
CF 555	Rabbit anti-mouse	Biotium 20235
Cy3	Goat anti-rat(*)	Thermofisher A-10522
CF 568	Goat anti-mouse	Biotium 20101
Alexa Fluor 568	Goat anti-mouse	Thermofisher A-11031
Dylight 594	Horse anti-mouse	Vectorlab DI-2594
Alexa Fluor 594	Goat anti-mouse	Thermofisher A-21135
CF 647	Goat anti-mouse	Sigma SAb4600182
Alexa Fluor 647	Goat anti-mouse	Thermofisher A-21236
Dylight 649	Horse anti-mouse	Vectorlab DI-2649
CF 660C	Goat anti-mouse	Biotium 20050
CF 680	Goat anti-mouse	Biotium 20065
Alexa Fluor 700	Goat anti-mouse	Thermofisher A-21036
CF 750	Goat anti-mouse	Biotium 20463
Dylight 755	Donkey anti-rat(*)	Thermofisher SA510031

Table S1. List of secondary antibodies used

All antibodies were used at a concentration of 1:500

(*):tested with rat anti α -tubulin (abcam ab6160)

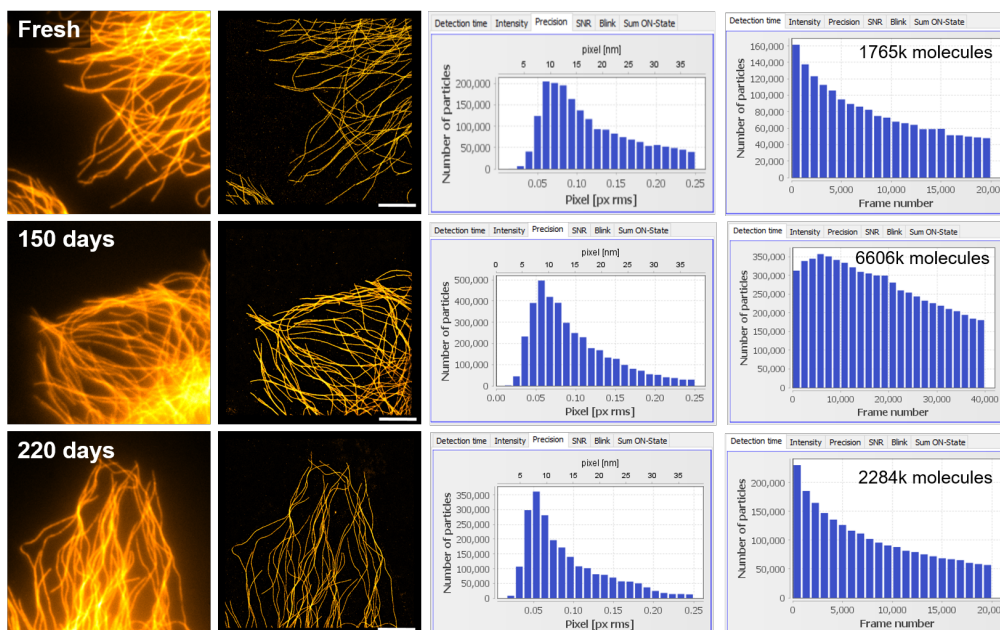


Fig. S1. Further quantification of Alexa Fluor 555 blinking in Slowfade Diamond. From top to bottom: sample freshly mounted, sample mounted ≈ 150 days before, and ≈ 220 days before. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

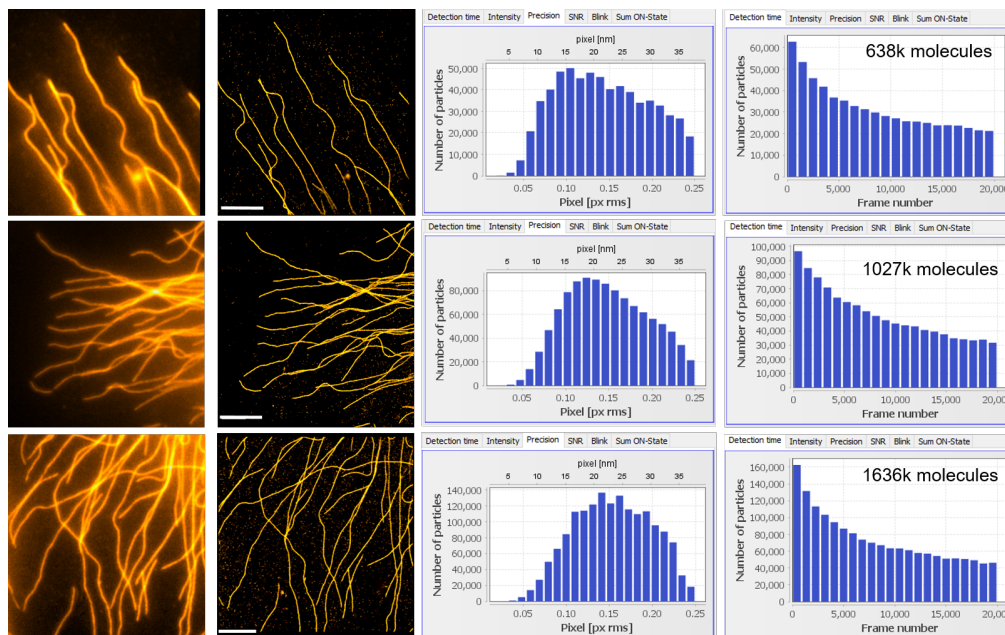


Fig. S2. Further quantification of Alexa Fluor 532 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

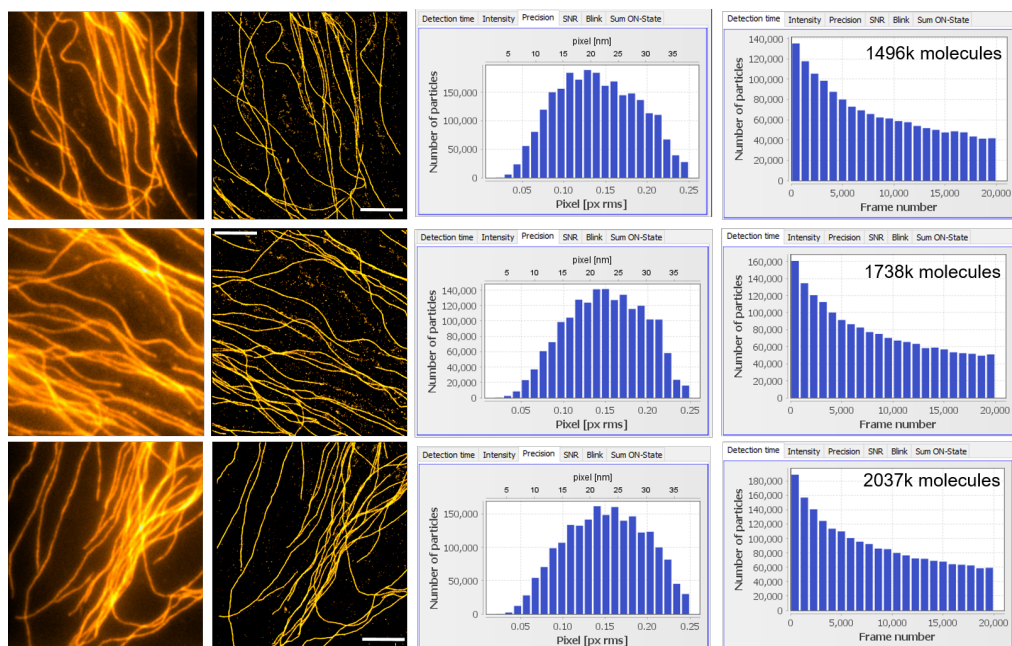


Fig. S3. Further quantification of CF 532 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

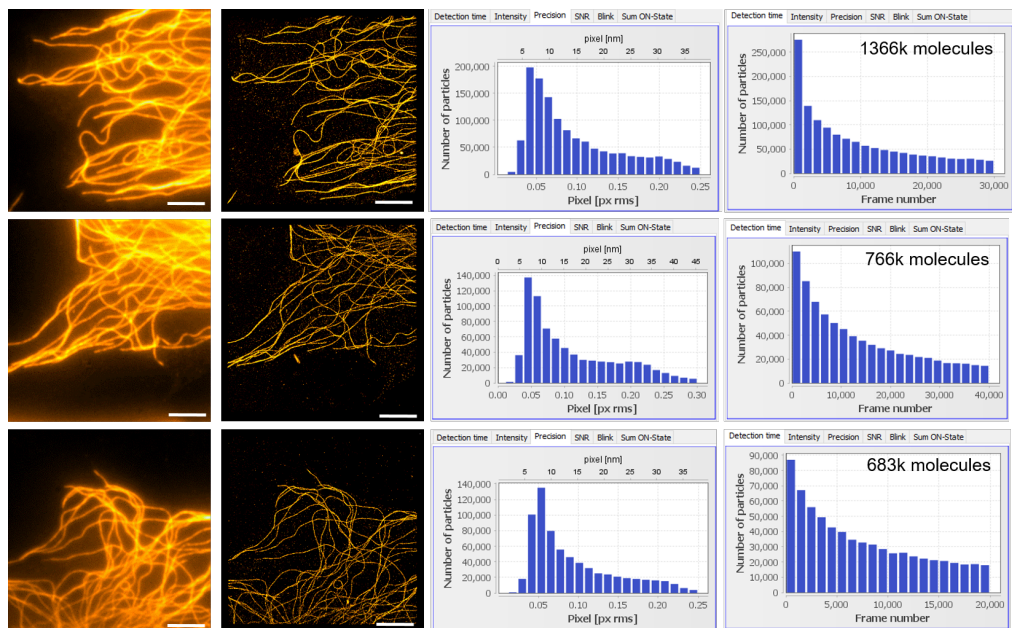


Fig. S4. Further quantification of Dylight 549 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

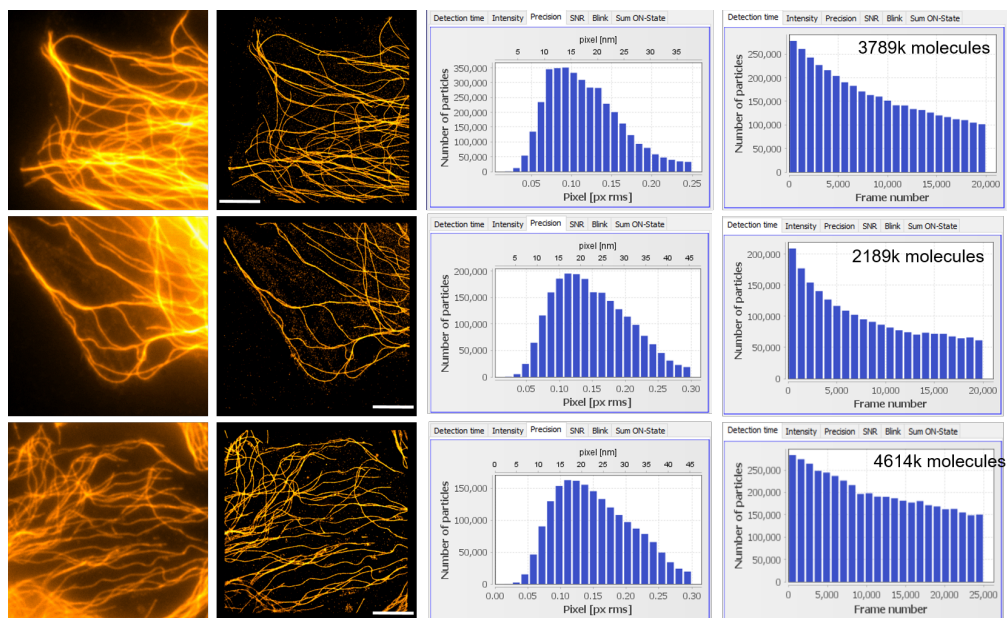


Fig. S5. Further quantification of Atto 550 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

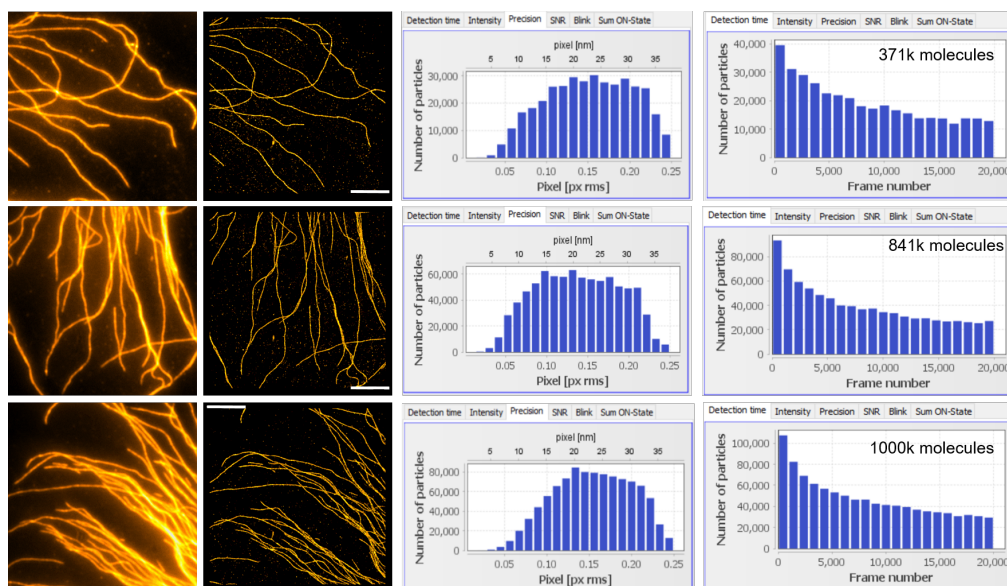


Fig. S6. Further quantification of CF 568 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

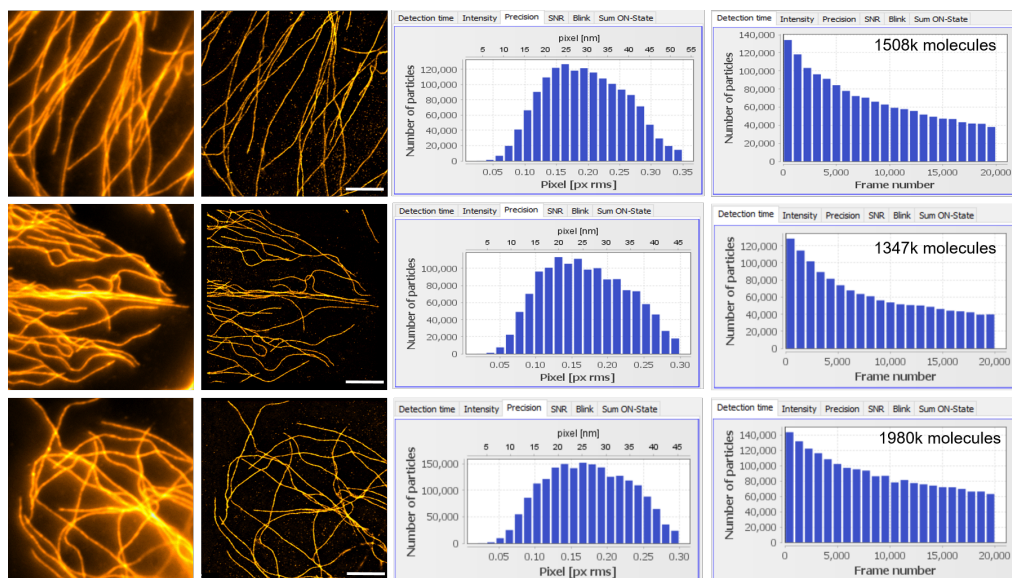


Fig. S7. Further quantification of Alexa Fluor 594 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

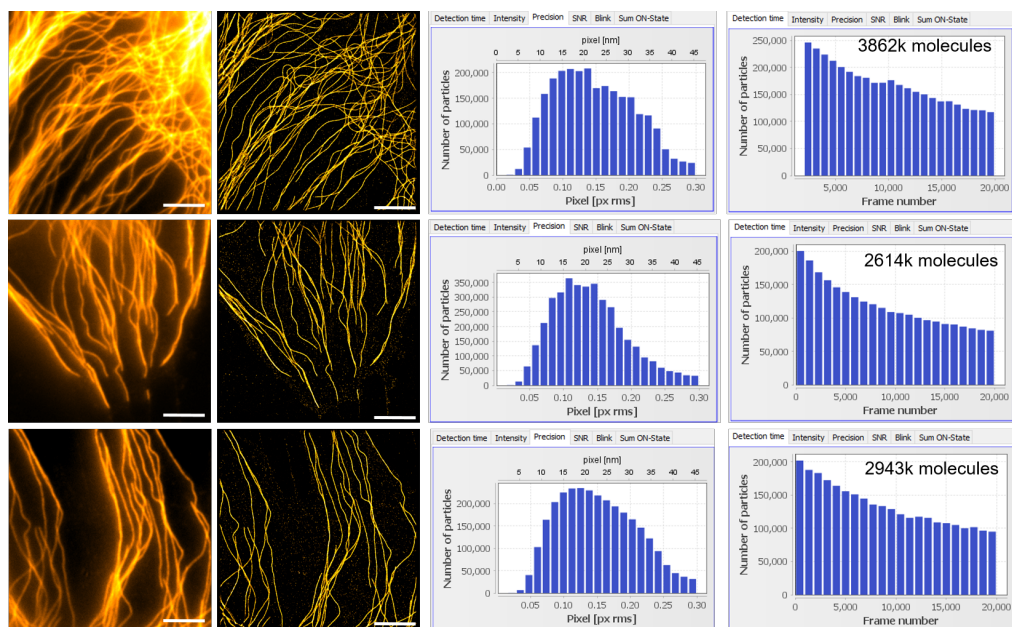


Fig. S8. Further quantification of Dylight 594 blinking in Slowfade Diamond. From left to right: widefield image, STORM image, Localization precision histograms, and number of detected particles per 1000 frames. Scalebars = $5\mu m$

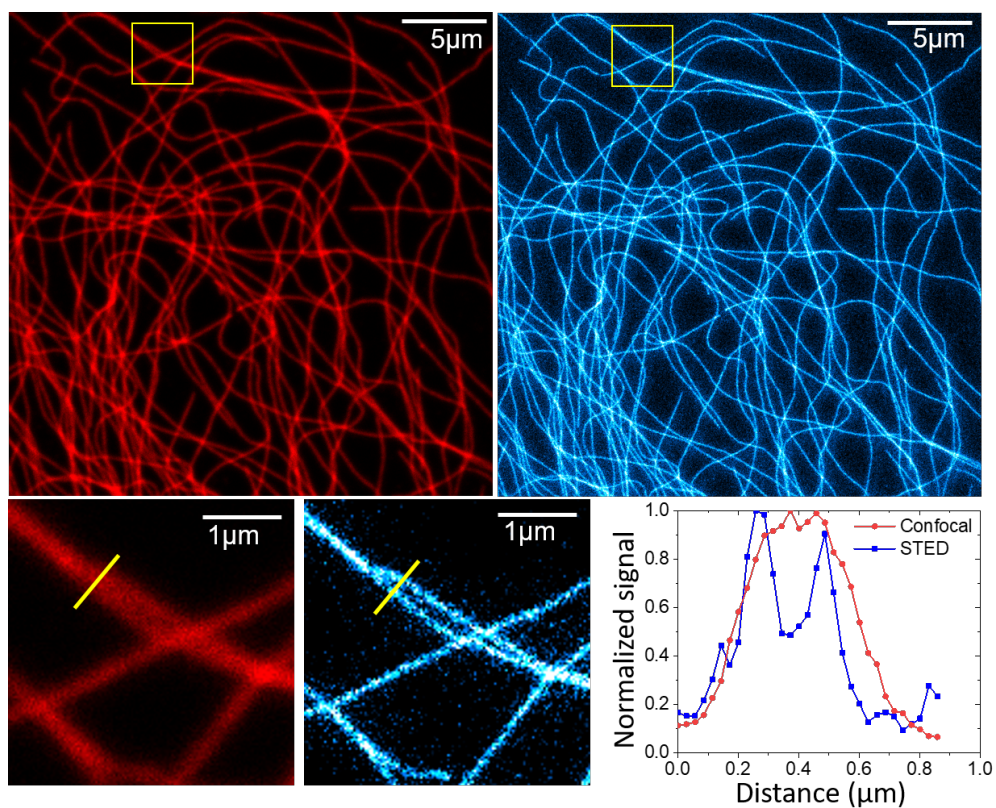


Fig. S9. Confocal and STED imaging of Alexa-555 in Slowfade Diamond. Top row: Confocal image (left) and STED image (right). Bottom row: Zoom to the region highlighted in the yellow boxes, and line profile taken through two neighboring microtubule. This imaging was taken on a sample previously used for STORM imaging. Method: STED imaging was performed on a Leica TCS SP8 STED using a 100X 1.4 NA objective. Excitation was provided by a white-light laser set to 530 nm, and depletion was done using 660 nm laser. Detection was done using HyD detectors, with a pinhole of 1AU (160 μm), and using time-gating of 6 ns. The pixel size was set to 27 nm, and the images displayed are the average of a z-stack of 10 images taken every 250 nm