Multicolor 3D Super-Resolution Imaging by Quantum Dot STORM

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Supporting Information

Excitation and Emission Spectra.

Measurements of emission and excitation spectra of the QDs used in this research are shown in figure S1. The excitation spectrum was acquired by measuring the emission at the nominal wavelength of the QD. The emission spectra of the QDs were measured while excited using 488nm light. The excitation spectrum of the 705nm QD shows a peak at 355nm. Measurement of emission using 355nm excitation light, indicate that two sizes of QDs were in the solution, with average wavelengths of 680nm and 704nm. However when excited with 488nm the QDs with average wavelength of 680nm dominated the emission.

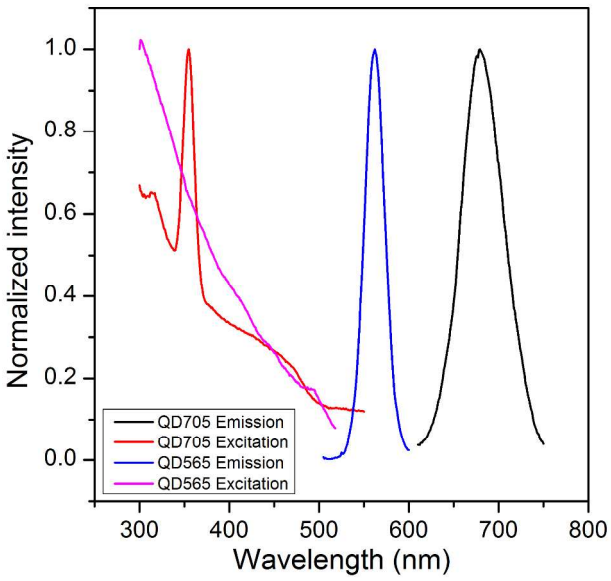

Figure S1. Excitation and Emission spectra of antibody conjugated quantum dots.

Comparison of QD 565, QD 705 and Alexa 647.

In Figure S2, we show a STORM image of microtubules labeled with Alexa 647. The image is comparable to the images taken with QDs. The measured microtubule FWHM is 37nm. Figure S2(d)-(f) show histograms of the measured photon counts per fluorophore for 565nm QDs, 705nm QDs, and Alexa 647 demonstrating that QD blueing can generate more photons than the Alexa dyes. The number of detected photons per localization for Alexa Fluor 647 has been reported to be greater than 3000 photons in MEA buffer in.[1] The collection of efficiency of our optical system is likely lower due to the deformable mirror and additional optics in our system.

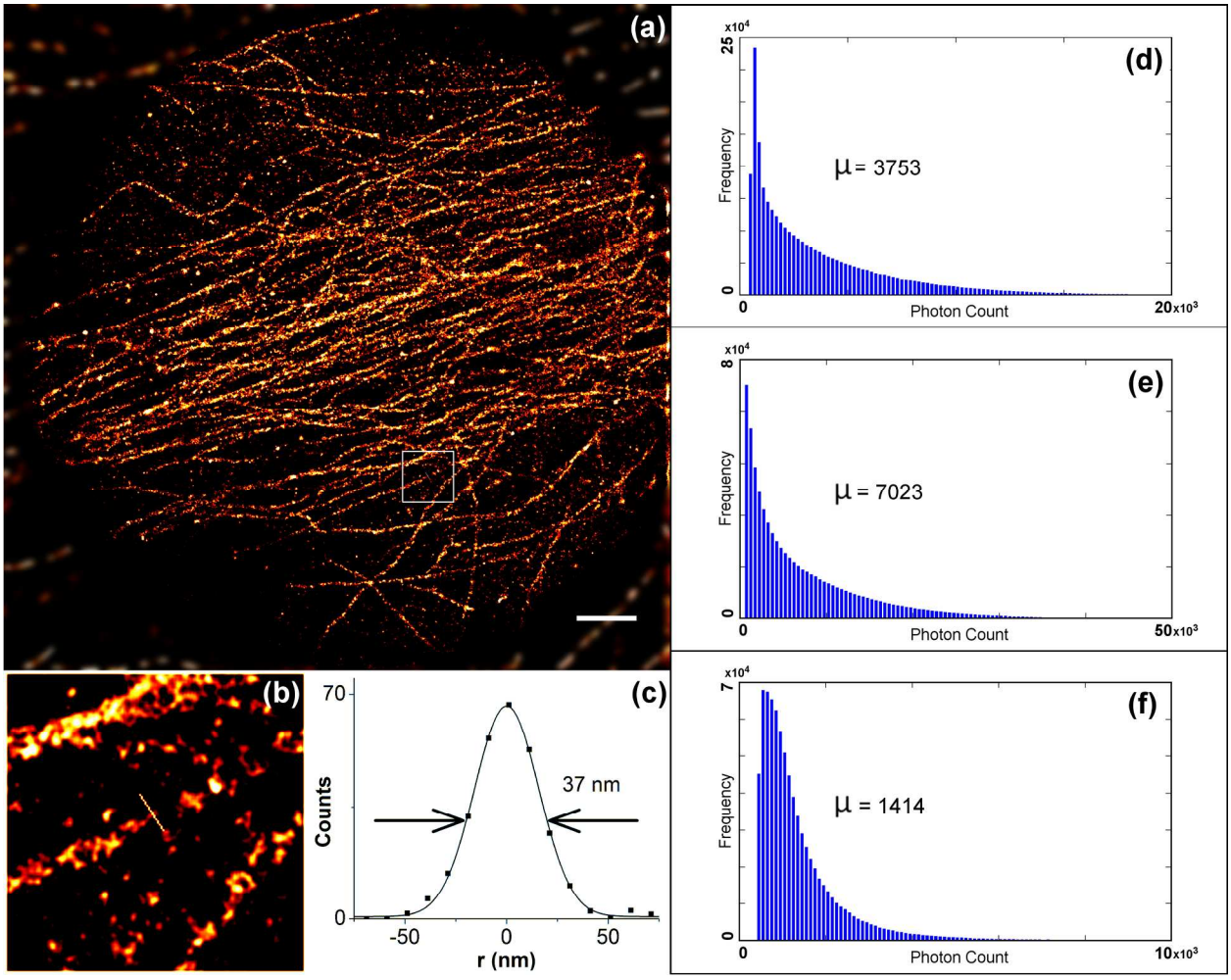

Figure S2. (a) STORM image of microtubules labeled with Alexa 647 in HepG2 cells. Scale bar is 2μm. (b) Magnified image of boxed region in (a). (c) Cross-section of the microtubule as indicated by the yellow line in (b). FWHM is 37nm. (d)-(f) Histogram of photon counts per localization for QD565, QD705 and Alexa647 respectively as calculated from the images Figure 5(a), 5(b) and S2(a). μ is the average photon number per localization.

Calculation of resolution

We measured the resolution of our localization from the fitting parameters by calculating the standard deviation of the uncertainty in the fit to a Gaussian for several single QDs in single color STORM images shown in figure S3 (a) and (b). This method has also been used by Hoyer

et al.[2] to find the resolution. The resolution of our imaging was found to be 24nm laterally and 37nm axially.

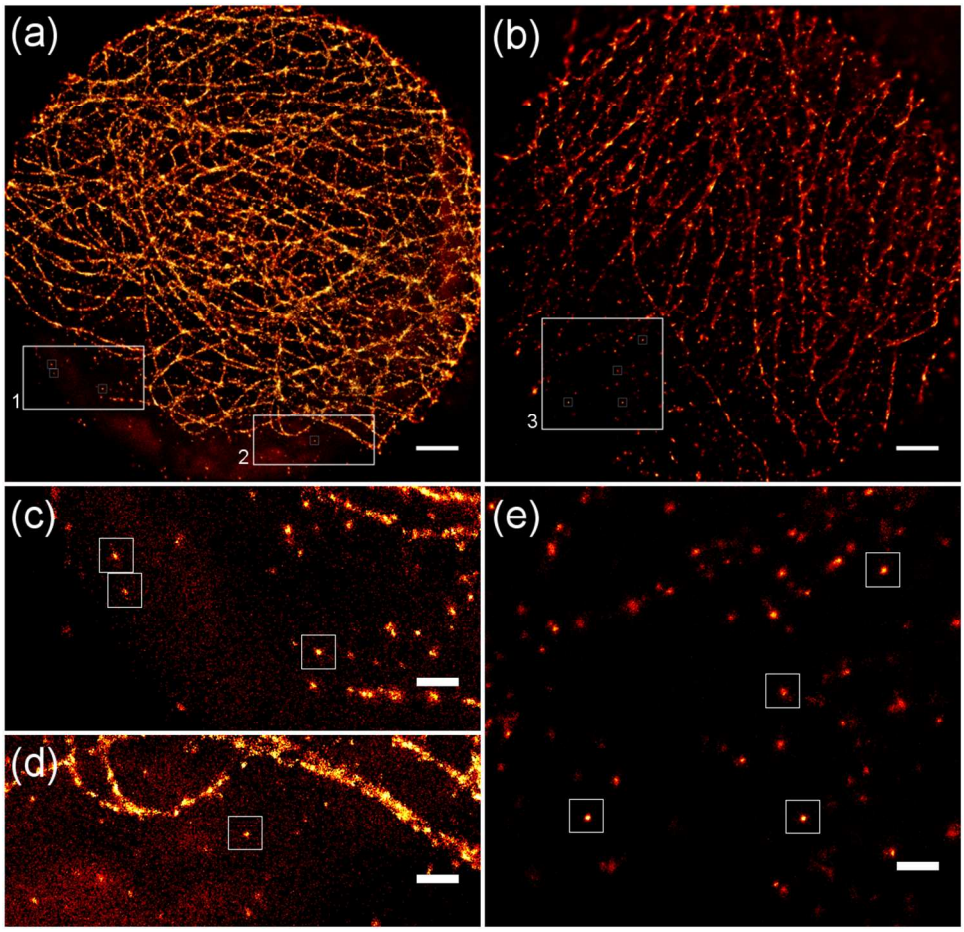

Figure S3. Shows a few single QDs used for the calculation of resolution. (a) and (b) show the samples stained with 565nm QD and 705nm QD respectively. The scale bars are 2 μm. (c-e) are the magnified images of 1-3. Scale bars are 500nm.

Chromatic Calibration

200 nm TetraSpec™ fluorescent microspheres (Invitrogen) were diluted 1:100 to get a distribution of about 20 beads in an area of $380\mu m^2$ (excitation spot diameter 22μm). The solution was dried on a No. 1.5 cover slip and mounted on a microscope slide using glycerol. The microspheres' emission wavelengths include 515 nm and 680 nm, allowing both channels to

pass through the blueing filters. Both channels were excited using the 488nm laser, and the emission intensity of the channels was equalized by a neutral density (ND10) filter in the Green channel. To increase the signal to noise ratio, each location was imaged 100 times (at 33 frames per second) and averaged. 121 locations on the slide (on an 11x11 grid) were imaged resulting in 1,928 microsphere localizations. In each image the microspheres were localized by Gaussian fitting. The difference in red channel and green channel location as a function of microsphere location (green channel) was then fit to a 2^{nd} order polynomial as shown in Figure S4(a). For calibration of 3D imaging, astigmatism (0.8 radians RMS) was applied to the wavefront and the same procedure was repeated on 2,968 microsphere localizations, Figure S4(b). The chromatic correction is demonstrated on microtubules double labeled with 565nm and 705nm QDs, Figure S4(c-e).

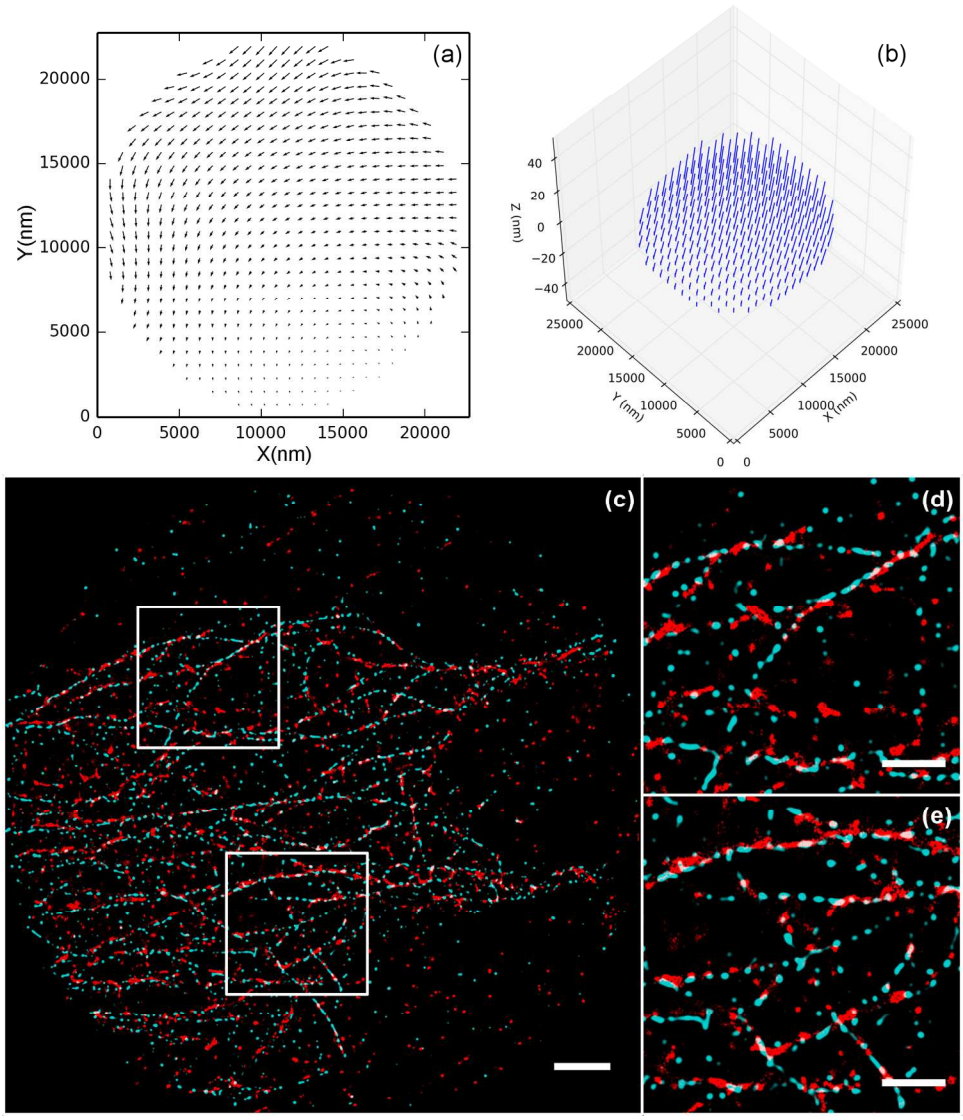

Figure S4. Chromatic correction of two-color STORM. (a) Two-dimensional chromatic correction calculated from a multicolor image of tetraspeck beads. (b) Three-dimensional chromatic correction. (c) Corrected images of microtubules labeled with 565nm QDs (blue) and 705nm QDs (green). Scale bar is 2μm. (d) Image of upper boxed region in (c). (e) Image of lower boxed region in (c). Scale bars in (c) and (e) are 1μm.

Calculation of Microtubule Width

The microtubule width was calculated by doing Gaussian fits to the microtubule cross-section across a section of the microtubule length. A selection of microtubule segments is shown in Figure S5. First the microtubule image was binarized and skeletonized. Then the skeleton was fit to a line so the microtubule image could be rotated to the horizontal. A 10nm (1 pixel) Gaussian blur was applied to the rotated image and a Gaussian fit was performed in the vertical direction at each pixel (every 10nm) along the horizontal direction. The fit parameters were then averaged. The Full Width Half Maximum of the microtubule is measured to be 35nm, 51nm, 46nm, and 45nm for labeling with Alexa488, Alexa647, 565nm QDs, and 705nm QDs respectively.

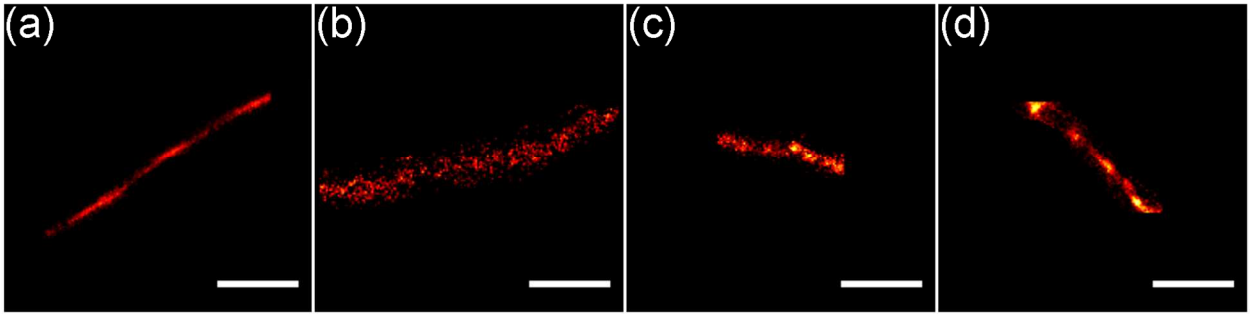

Figure S5. A Gallery of microtubule image sections labeled with (a) Alexa 488, (b) Alexa 647, (c) 565nm QDs, and (d) 705nm QDs. Scale bars are 500nm.

Mitochondrial Labeling with Alexa 488

For comparison with the 705nm QD labeling, we show images of Mitochondria in HepG2 cells labeled using the same primary antibody, MTC02, and Alexa 488 below in Figure S6.

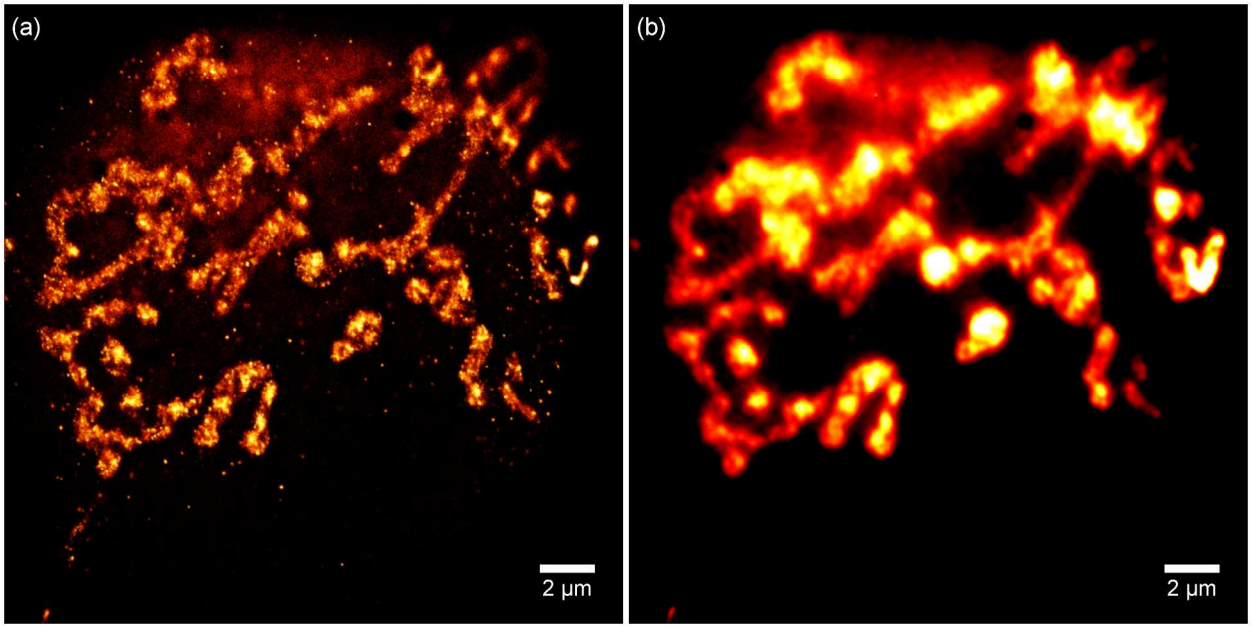

Figure S6. Mitochondria in HepG2. labeled with Alexa488. The primary is MTC02, the same as the quantum dot labeling in the main text. (a) STORM image. (b) Widefield image.

Efficiency of Blueing

To measure the percentage of QDs that undergo blueing, a layer of 565nm IgG conjugated QDs were dried on a glass coverslip and mounted in a 20% glycerol / 80% deionized water solution. The QDs were imaged at 50 frames per second under continuous exposure to 2.6kW/cm^2 of 488nm excitation. A measurement consisting of 4 cycles of blueing followed by a measurement at the original emission wavelength was performed. 5000 frames were measured with a 504nm/12nm filter followed by 500 frames measured with a 580nm/60nm filter to detect remaining QDs. These measurements demonstrate that 95% of the QDs are blued in 5000 frames. The rate of blueing may be somewhat slower in cells as compared to directly exposed to the buffer.

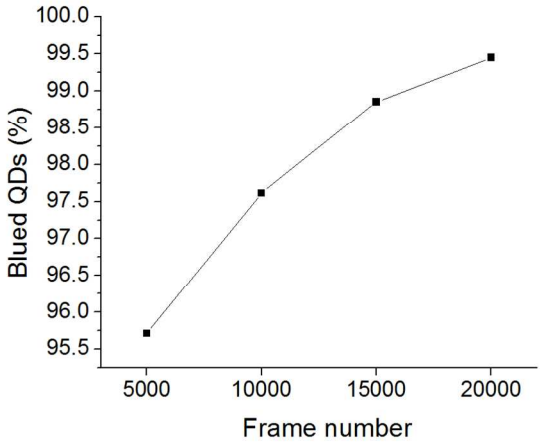

Figure S7. Percentage of blued QDs with emission between 550nm and 610nm (emission measured with a 580/60 nm emission filter) *vs.* the number of frames measured at 50fps. The QDs were continuously exposed to 2.6kW/cm^2 of 488nm excitation.

REFERENCES

1. Dempsey, G. T.; Vaughan, J. C.; Chen, K. H.; Bates, M.; Zhuang, X. Evaluation of Fluorophores for Optimal Performance in Localization-Based Super-Resolution Imaging. *Nat. Methods* **2011,** *8*, 1027-1036.

2. Hoyer, P.; Staudt, T.; Engelhardt, J.; Hell, S. W. Quantum Dot Blueing and Blinking Enables Fluorescence Nanoscopy. *Nano Lett* **2010,** *11*, 245-250.