molecules

MDPI

Supplementary Materials

Systematic assessment of freely-diffusing single-molecule fluorescence detection using Brownian motion simulations

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Received: date; Accepted: date; Published: date

Figure Legends

Figure S1. The model for the effective excitation volume is the point-spread function (PSF). The PSF was modeled using PSFLab[5] for a typical 60x water immersion objective with a numerical aperture of 1.2, with a sample mounted on top a 150 μm coverglass and with sample excitation at a wavelength of 532 nm.

Figure S2. The positions of diffusing molecules when they emitted photons that were detected and selected by the burst analysis, with burst analysis parameters m=10, F=6 & a minimal burst size threshold of 40. In the top, central & bottom panels we show the 2D projections at the yz, xz & xy planes when x=0, y=0 & z=0, respectively. Each dot in the scatter plots is an emitted photon. These results are for the simulation of 15 molecules in 425 fL rectangular box (yielding a concentration of 62 pM), where the diffusion coefficient of the molecules was 22.5 $\mu m^2/s$. The colors of the points correspond to the burst number out of the overall number of bursts. In each panel, the 1D projections are shown in histograms.

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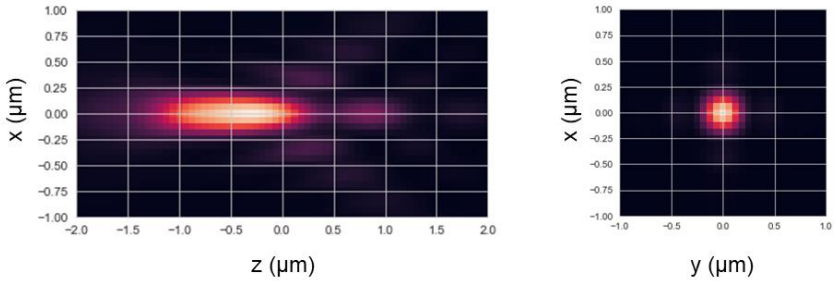

31 **Figure S1.**

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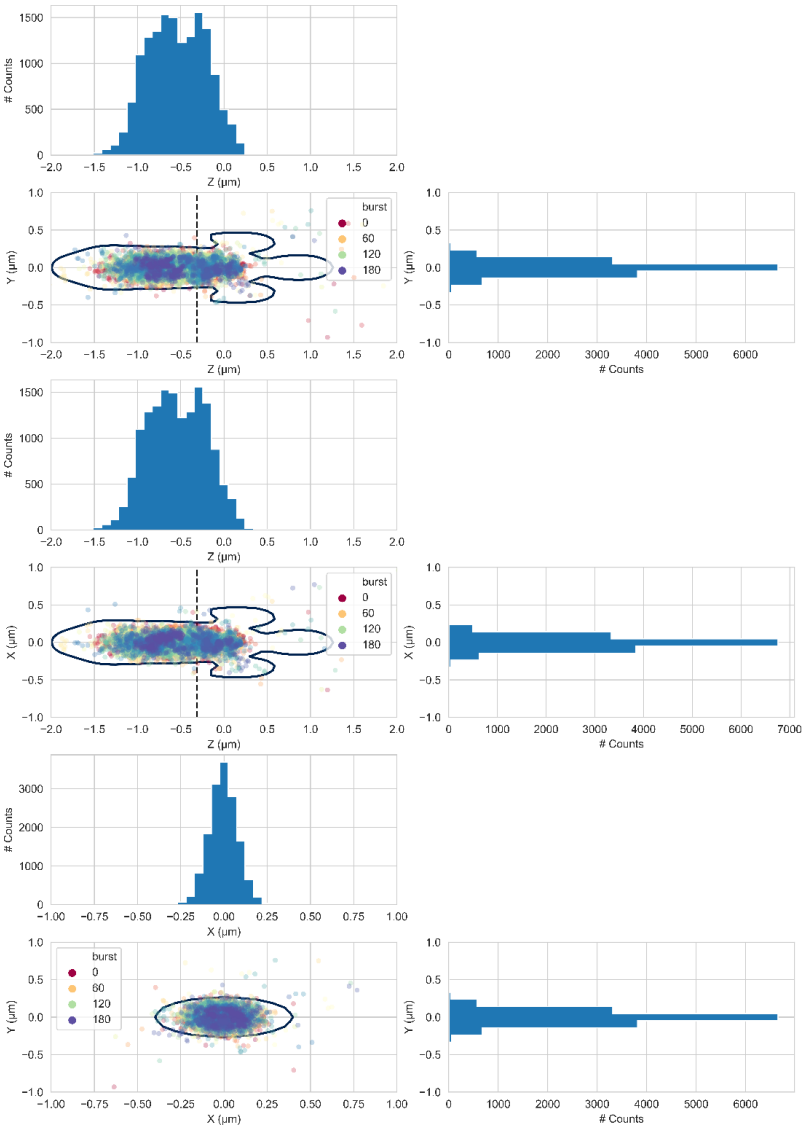

Figure S2.