Supplementary Materials

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

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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 μm²/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.
Figure S1.
Figure S2.