Imaging Diffusion in Living Cells Using Time-Correlated Single-Photon Counting

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SUPPORTING INFORMATION

A. Convergence of correlation functions

Figure S-1: Comparison of (a) autocorrelation and (b) crosscorrelation of dT\textsubscript{20}-TMR in PBS with 50\% (v/v) glycerol recorded with different dwell times (25 ms to 10 s) and analyzed using the photon-by-photon algorithm. The data clearly shows that the bias of the correlation function observed at short measurement times (0.025 – 0.1 s) is decreased at longer measurement times (0.5 – 10 s). Additionally, a tendency to shorter diffusion times in autocorrelation analysis for short dwell times occurs in (a) (black dots and black fit).
B. Cell images

**Figure S-2:** Images of two adherent cells incubated with dT$_{20}$-ATTO488. Images (a)-(c) and (h)-(j) were imaged with an EMCCD camera (iXon, Andor, USA) and show a transmission image of the cells before ((a) and (h)) and after the scan ((b) and (i)) as well as a fluorescence image ((c) and (j)); a cutout region of each cell (red square in (a) and (h)) was scanned using DIFIM, scan images are shown in (e) and (k) (16 x 16 µm$^2$, 100 nm pixel size, 100 ms dwell time) together with fluorescence lifetime images ((f) and (l)) and diffusion time images ((g) and (m)).
C. Influence of pixel sampling time on quality of diffusion images

**Figure S-3:** Living cell incubated with dT20-ATTO488 and imaged with (a) 25 ms and (b) 100 ms dwell time (four images shown are fluorescence intensity, fluorescence lifetime, and diffusion time and cross-correlation amplitude). Additionally, a transmission image recorded with an EMCCD camera (c) before and (d) after the scan is shown.
D. Comparison of autocorrelation and cross-correlation in DIFIM images

**Figure S-4:** Comparison of autocorrelation and crosscorrelation analysis using the photon-by-photon algorithm. 16 x 16 μm² fluorescence intensity image (a) recorded with 100 nm pixel resolution and 100 ms dwell time; (b) fluorescence lifetime image; (c) DIFIM image and (d) correlation amplitude image generated with crosscorrelation; (e) DIFIM image and (f) correlation amplitude image generated with autocorrelation. The data demonstrates that autocorrelation shows a tendency to shorter diffusion times although contrast in both images is similar.