

Doubling the resolution of single-molecule localization microscopy with image scanning microscopy

Supplementary information

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1 Figures

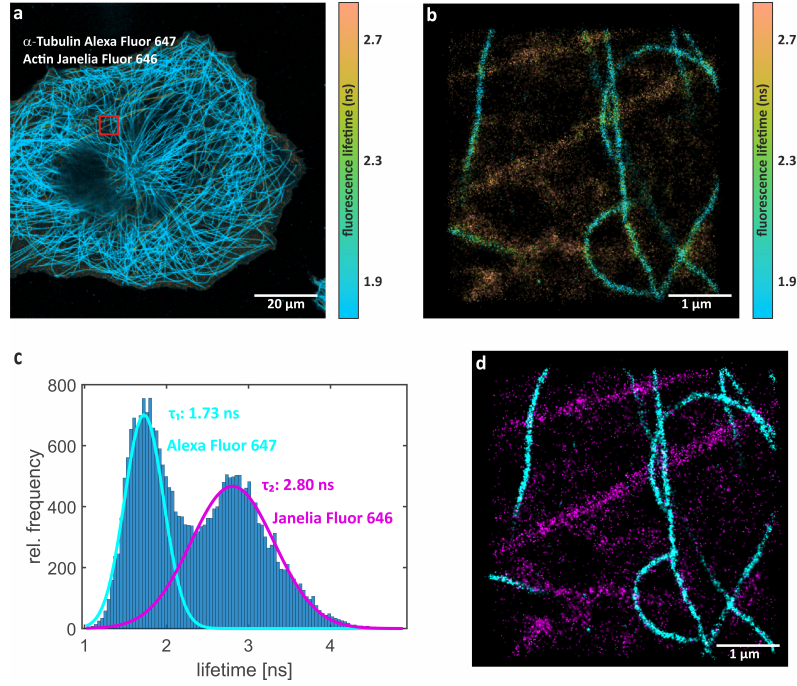


Fig. S1 dSTORM based FL-iSMLM multiplexing. (a) FL-ISM overview of a COS-7 cell where α -tubulin was labeled with Alexa 647 and actin with phalloidin-Janelia Fluor 646 fluorophores. Zoom is the actual $5 \mu\text{m} \times 5 \mu\text{m}$ region of interest of the dSTORM measurement. Colors represent fluorescence lifetime, determined from TCSPC tail fitting. (b) Super-resolved Fourier-reweighted ISM dSTORM reconstruction FLIM image made from $6.5 \cdot 10^4$ images with 10 frame binning. (c) Fitted lifetime histogram based on individual single-molecule localizations for the corresponding region of interest. (d) Dual-color super-resolved image based on the lifetime data. The two lifetime components were separated using lifetime pattern matching classification. Here localizations events are colored cyan if their decay pattern belongs to that of Alexa 647 with a probability of more than 99%. And magenta, if the same is true for the Janelia Fluor 646 pattern.

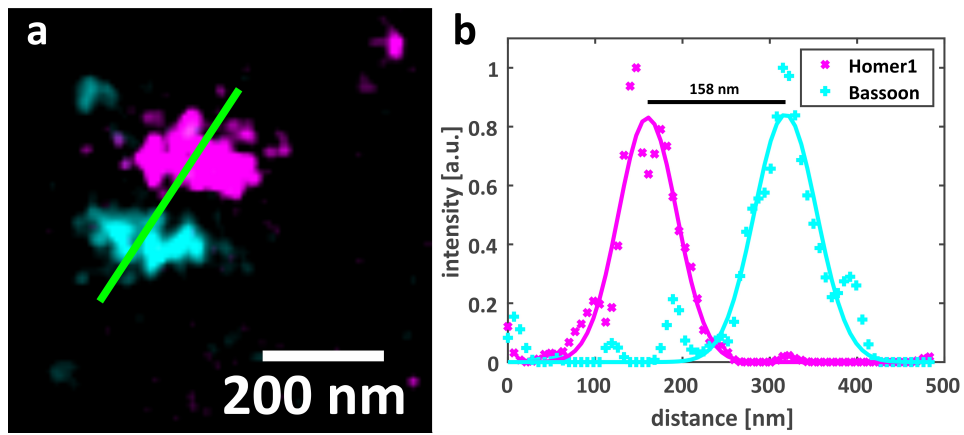


Fig. S2 An example of one synapse with the distance between Bassoon and Homer1 measured across the synaptic cleft. (a) DNA-PAINT iSMLM reconstruction of zoom-in I depicted in Fig. 4(a). Homer1 was labeled with Atto 565 and is depicted in magenta, Bassoon was stained with Cy3b and is shown in cyan (b) Intensity profile along the line shown in green with individual Gaussian fits for the two proteins. Distance was calculated from the absolute difference between the mean values of the respective Gaussian fits.