Electronic Supplementary Material (ESI) for Nanoscale.


# Supporting Information

## Quantitatively Mapping the Interaction of HER2 and EGFR on Cell Membranes with Peptide Probes

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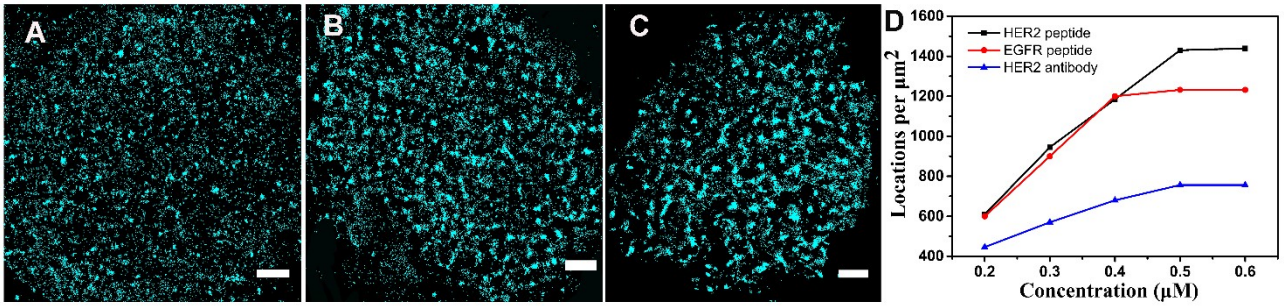


Fig. S1 Determination of the labeling concentration of different probes. (A-C) The reconstructed dSTORM images of HER2 under different labeling concentrations of peptide-probes, 0.2 μM (A), 0.4 μM (B) and 0.5 μM (C), respectively. Scale bars are 2 μm. (D) The plot of localizations per μm$^2$ at different labeling concentrations of HER2 peptide, EGFR peptide and HER2 antibody.

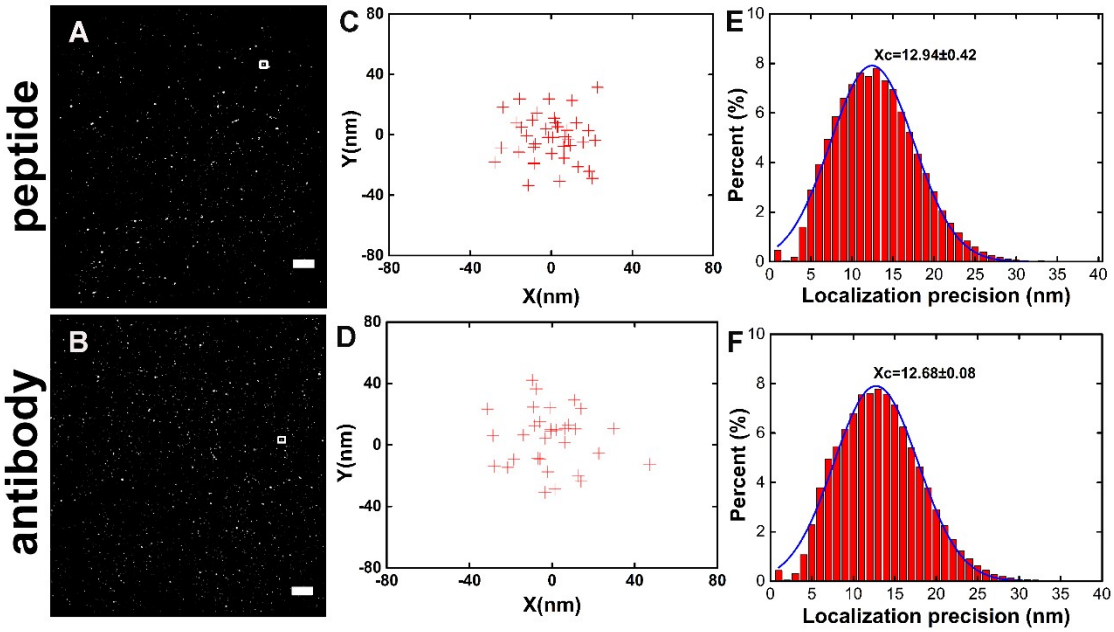


Fig. S2 Measurement of the localization precision of single peptide and antibody probes. (A and B) Representative dSTORM images of Cy3-conjugated peptide (A) and antibody (B) on the empty coverslip. Scale bars are 2 μm. (C and D) The distribution of repeated localizations from a single emitter under the two condition. (E and F) The localization uncertainty of single peptide probe (E) and single antibody probe (F).

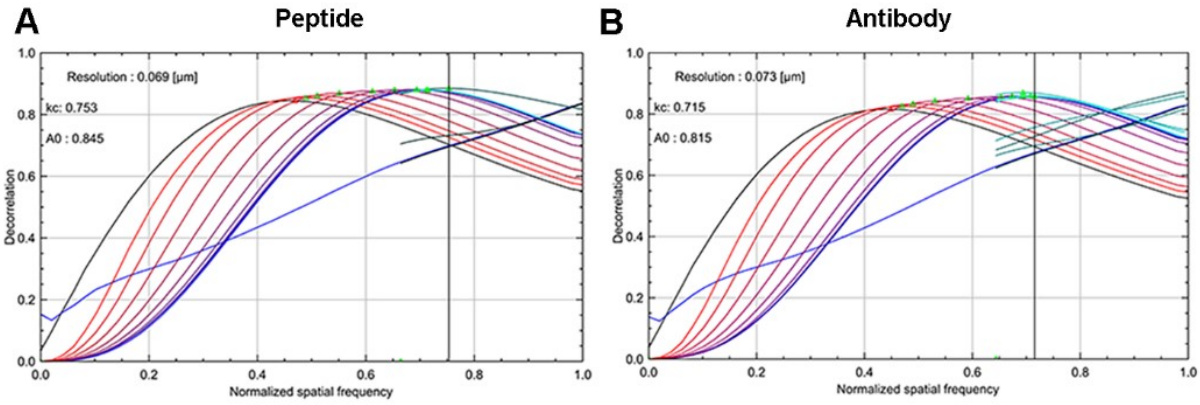


Fig. S3 ImageJ plot of non-filtered decorrelation analysis of single peptide and antibody probe. The resolution for them was 69 nm and 73 nm, respectively.

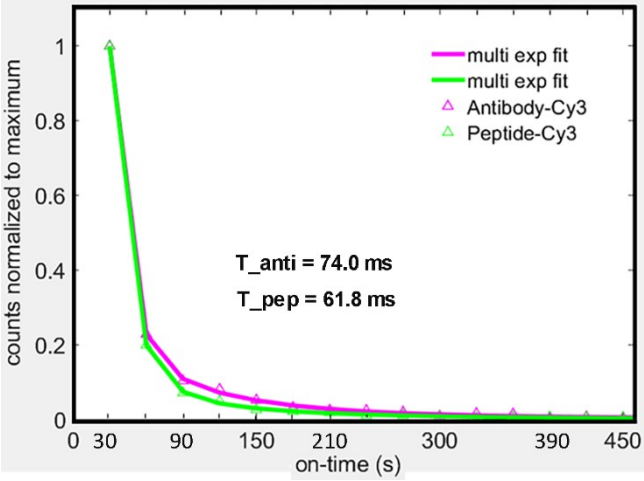


Fig. S4 Distribution of fluorescence-on time of Cy3-conjugated antibody and Cy3-conjugated peptide probe.

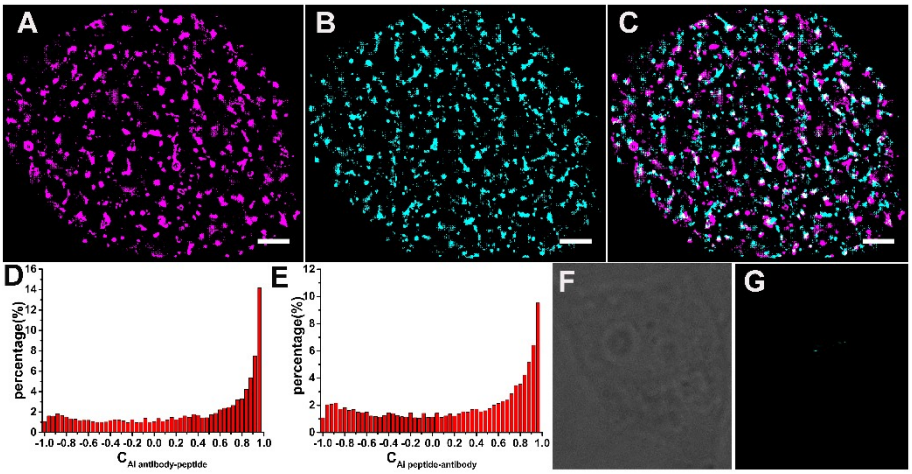


Fig. S5 Detection of the specificity of peptide to HER2. (A-C) Dual-color imaging of HER2 on with antibody-probe (A) and peptide-probe (B) on the same COS-7 cell membrane, and the merged image of the two channels (C). Scale bars are 2 μm. (D-E) The CBC value showed the distributions of the colocalization parameter for peptide and antibody. (F-G) The bright-field image of a MCF-7 cell (F) and corresponding dSTORM image (G) of peptide labeled HER2. Data were from 30 regions with area of 2 μm × 2 μm from 10 cells in 3 independent experiments.

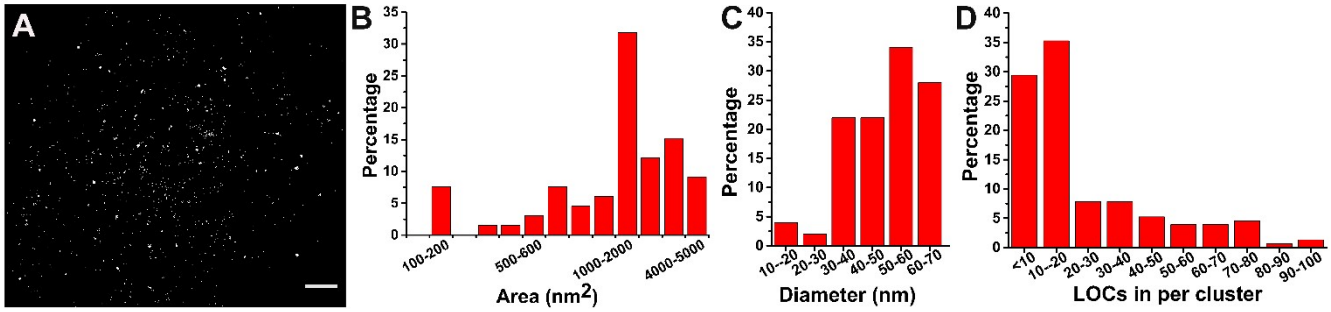


Fig. S6 Characterization of HER2 peptide probes by SR-Tesseler method. (A) dSTORM image of peptide probes on COS-7 cell membrane at a low concentration (0.05 μM). Scale bar is 2 μm. (B-D) Quantitative characterization of single peptide probes. The distribution of area (B), diameter (C), and the number of localizations per molecule (D). Statistics were from 10 cells in five independent experiments. LOCs is the abbreviation for localizations.

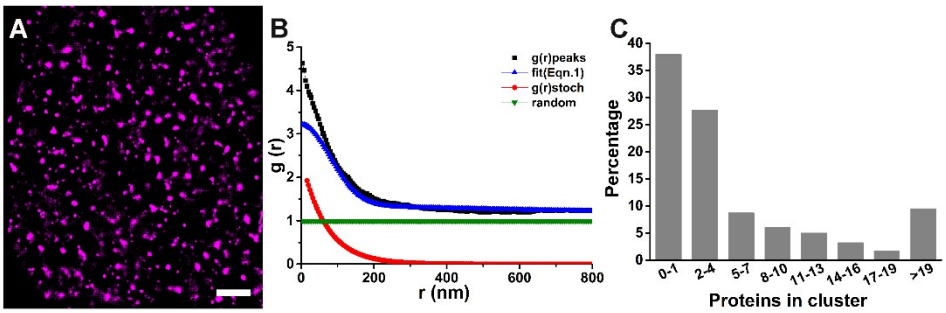


Fig.S7 Clustering analysis of EGFR molecules on COS-7 cell membranes. (A) A representative dSTORM image of EGFR labeled with peptide-probe. Scale bar is 2 μm. (B) The correlation function (g(r) peaks) of EGFR clusters and the corresponding multiple appearances of a single protein (g(r) stoch) extracted from fitting procedure. (C) The number of proteins per cluster derived from pair-correlation functions. Data were obtained from 50 regions with an area of 2 μm × 2 μm from 10 cells in 3 independent experiments.

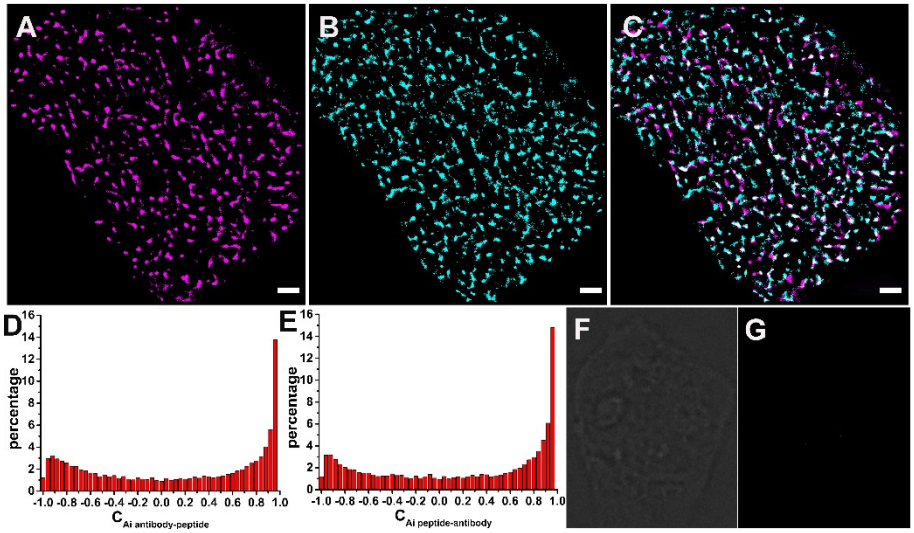


Fig. S8 Detection of the specificity of peptide to EGFR. (A-C) Dual-color imaging of EGFR with antibody-probe (A) and peptide-probe (B) on the same COS-7 cell membrane, and the merged image of the two channels (C). Scale bars are 2 μm. (D-E) The CBC value showed the distributions of the colocalization parameter for peptide and antibody. (F-G) The bright-field image of a MCF-7 cell (F) and corresponding dSTORM image of peptide labeled EGFR (G). Data were from 30 regions with area of 2 μm × 2 μm from 10 cells in 3 independent experiments.

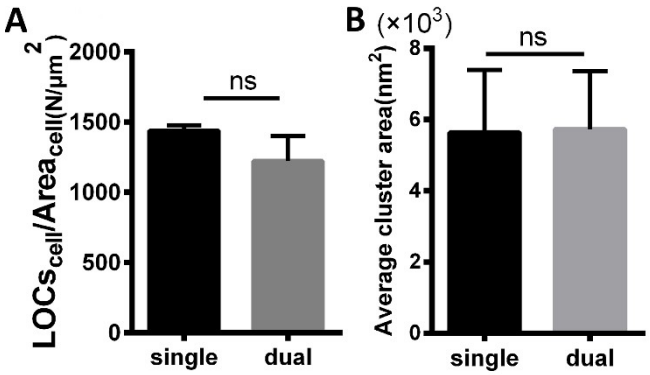


Fig. S9 Analysis of the localization density (A), and cluster area (B) of HER2 in single imaging and dual-color imaging (HER2 and EGFR). The HER2 and EGFR were labeled with the Cy3-peptide and Cy5-peptide, respectively. Data were collected from 10 cells in 3 independent experiments (mean ± SD).

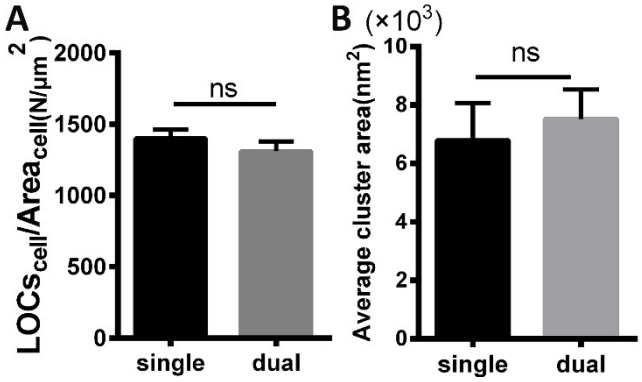


Fig. S10 Analysis of the localization density (A), and cluster area (B) of EGF-stimulated HER2 in single imaging and dual-color imaging (HER2 and EGFR). Data were collected from 10 cells in 3 independent experiments (mean ± SD).

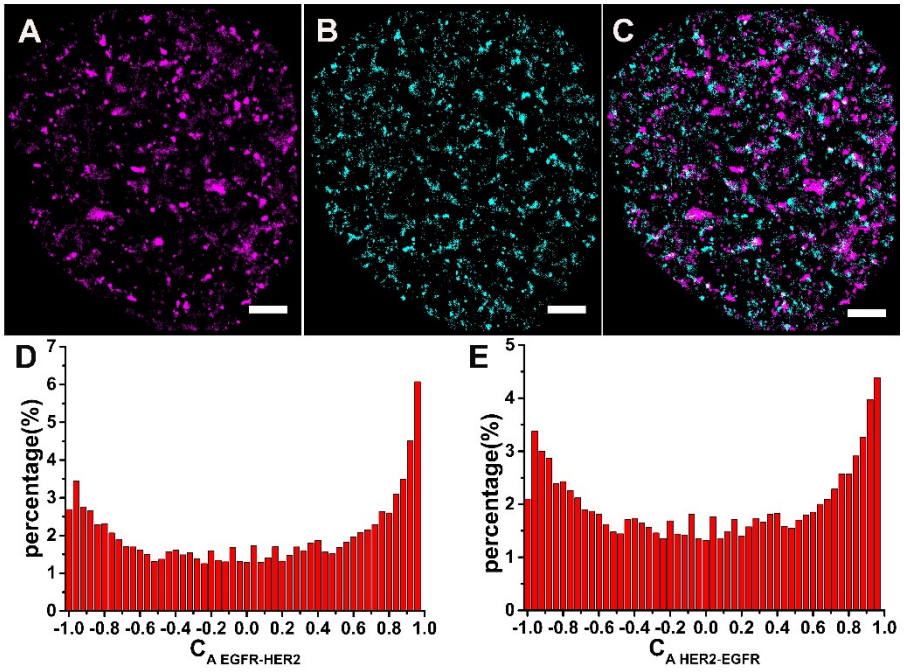


Fig. S11 Dual-color dSTORM imaging of the spatial distribution of HER2 and EGFR on COS-7 cell membrane with antibody-probe. (A-C) Reconstructed dSTORM images of EGFR labeled with Cy5-conjugated antibodies (A), HER2 with Cy3-conjugated antibodies (B), and the merged image of the two channels (C). Scale bars are 2 μm. (D-E) The histograms show the distributions of the colocalization parameter $C_{Ai}$ for EGFR to HER2 (D) and for HER2 to EGFR (E). Data were from 30 regions with area of 2 μm × 2 μm from 10 cells in 3 independent experiments.

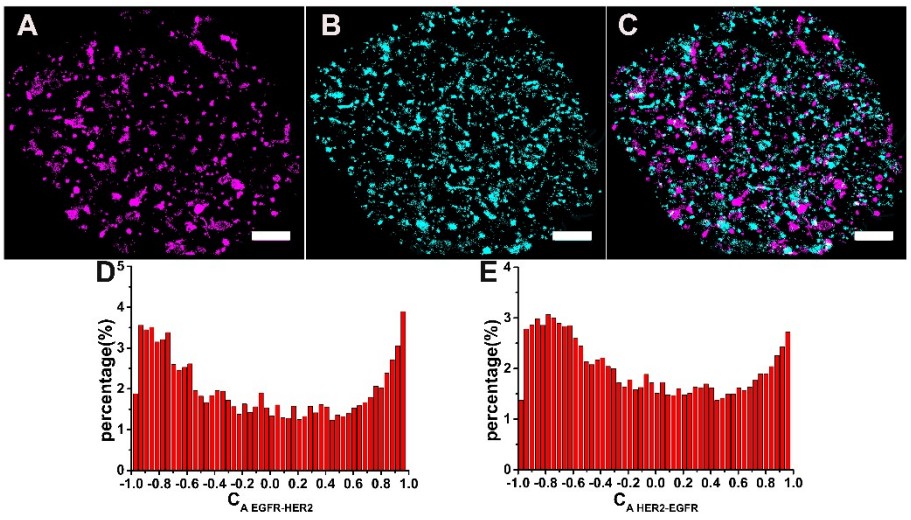


Fig. S12 Dual-color dSTORM imaging of the spatial distribution of EGF-stimulated HER2 and EGFR on COS-7 cell membrane with antibody-probe. (A-C) Reconstructed dSTORM images of EGFR labeled with Cy5-conjugated antibodies (A), HER2 with Cy3-conjugated antibodies (B), and the merged image of the two channels (C). Scale bars are 2 μm. (D-E) The histograms show the distributions of the colocalization parameter $C_{Ai}$ for EGFR to HER2 (D) and for HER2 to EGFR (E). Data were from 30 regions with area of 2 μm × 2 μm from 10 cells in 3 independent experiments.

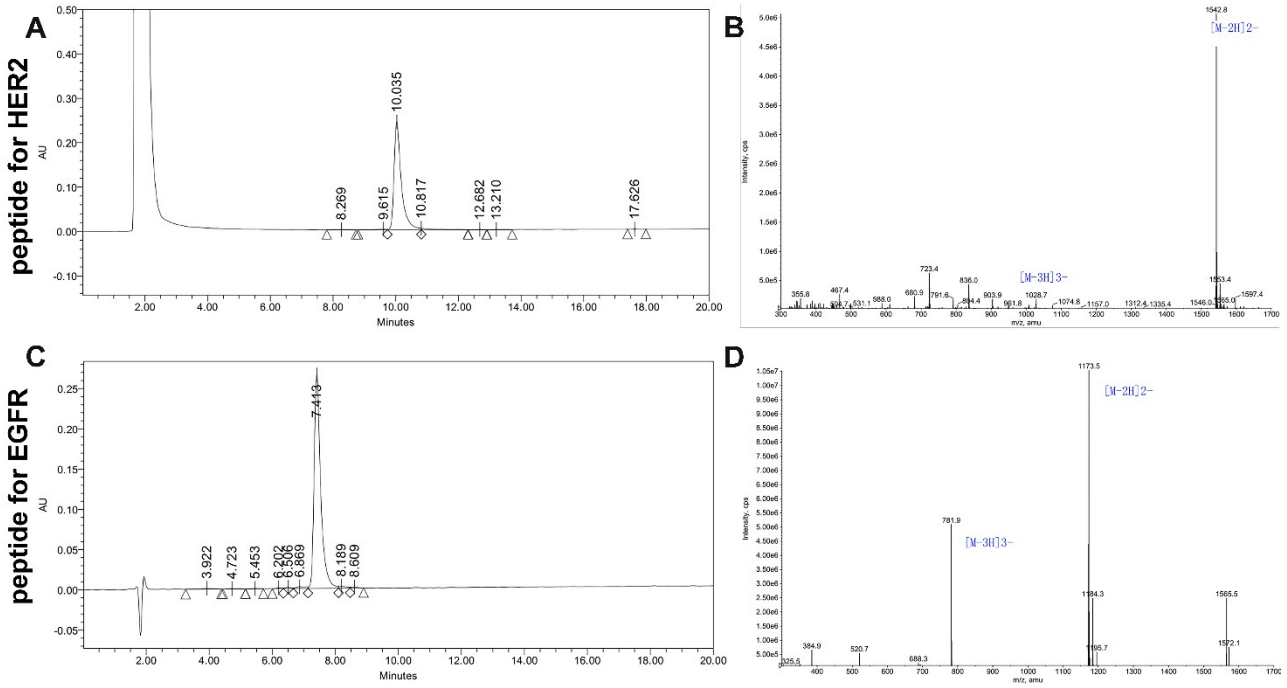


Fig. S13 High performance liquid chromatography spectrum and mass spectrum of peptide probe for HER2 (A and B) and EGFR (C and D), respectively.

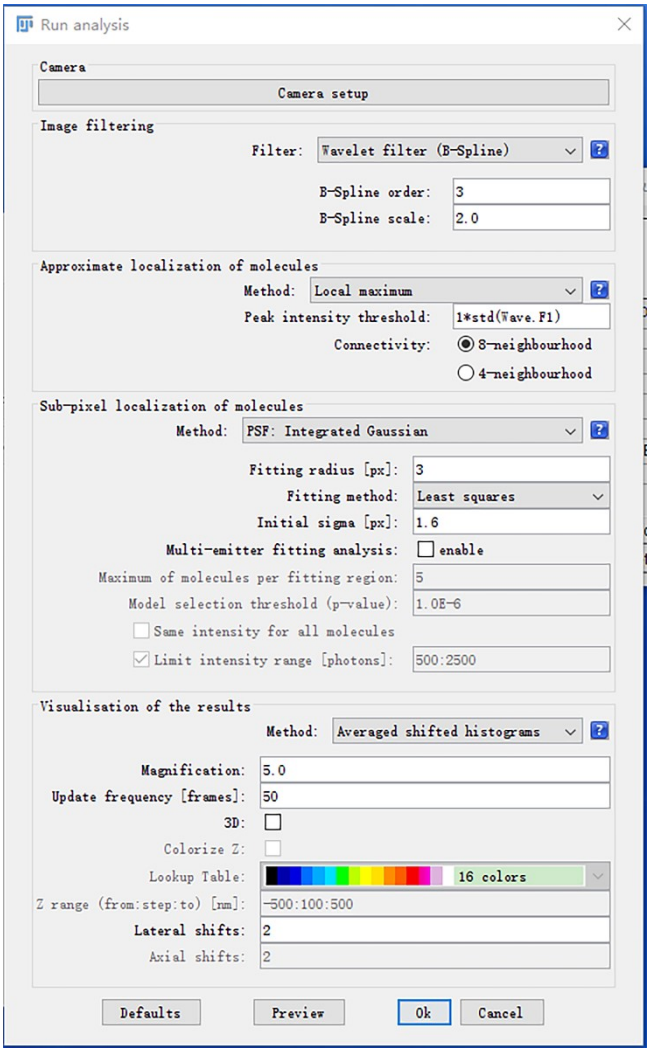


Fig. S14 The parameter setting for our data analysis in ThunderSTORM.