Supporting Information

Quantitative analysis of multiple breast cancer biomarkers using DNA-PAINT

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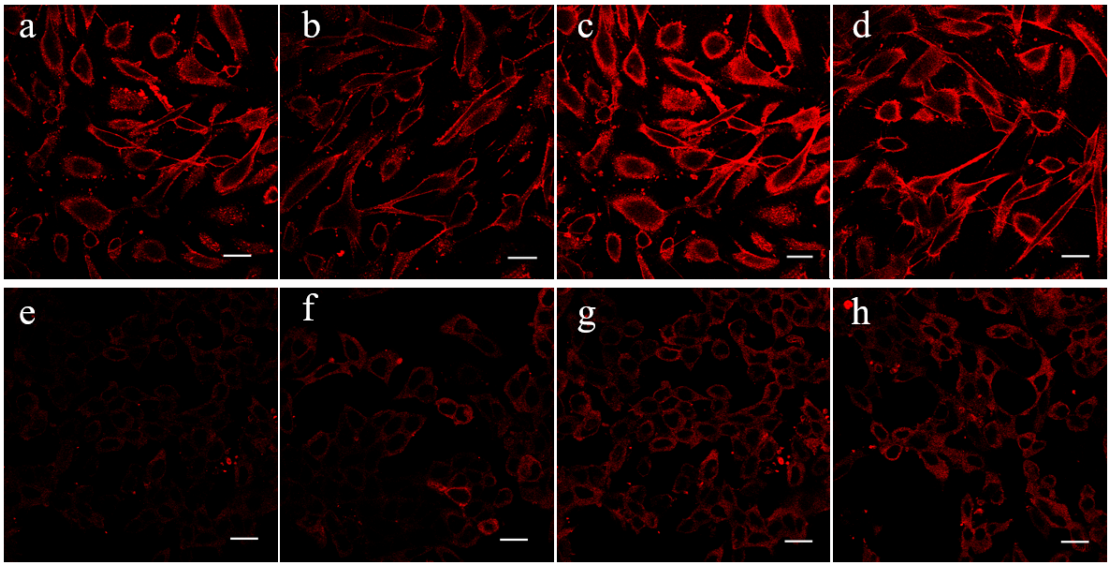

Fig. S1. CLSM images of PD-L1 in MDA-MB-231 cell (a-d) and MCF-7 cell (e-h). Scale bar: 20 μm. The scanning speed of a, b, e and f was 4.0 μs/pixel, and that of c, d, g and h was 8.0 μs/pixel. The other imaging parameters (e.g. laser power, pinhole size, etc) were the same.

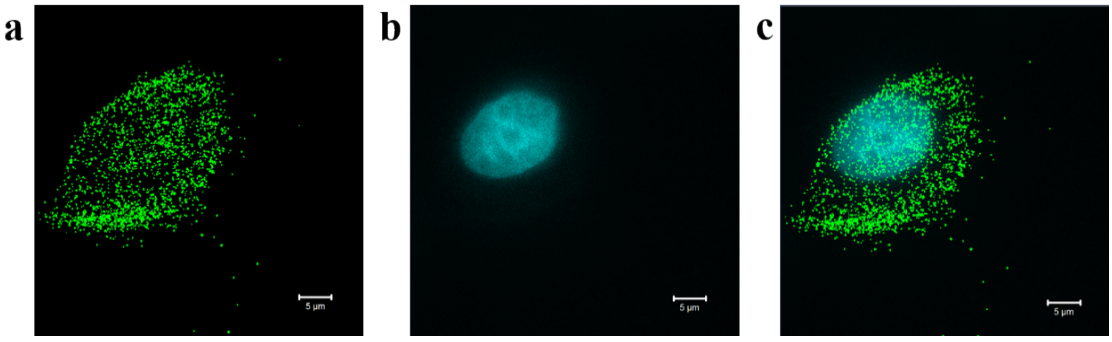

Fig. S2. DNA-PAINT imaging of PD-L1 protein on MCF-7 cell. (a) DNA-PAINT image of PD-L1 protein on the surface of MCF-7 cell. (b) Nuclei staining fluorescence image of MCF-7 cell. (c) The merged image of the first two channels of MCF-7 cell. Scale bar: 5 μm.

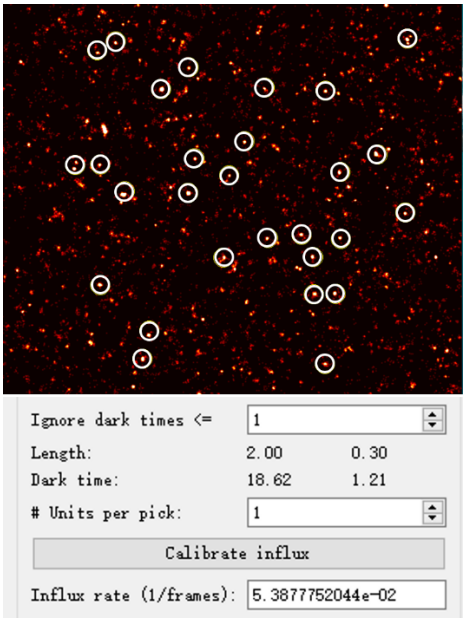

Fig. S3. The influx rate was obtained by calculating the average dark time of multiple binding sites using Picasso software.

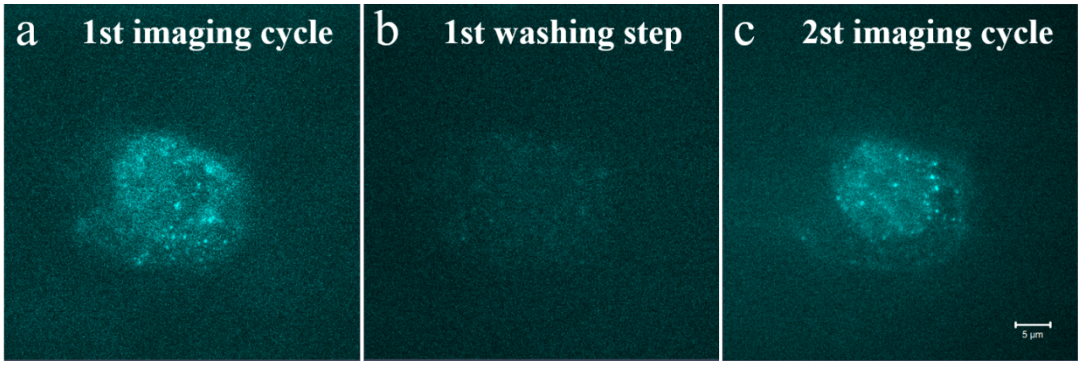

Fig. S4. The wide-field images of PD-L1 and CTLA-4 proteins before and after washing. No obvious fluorescence was detected after washing step, indicating independent imaging between cycles. Scale bar: 5 μm.

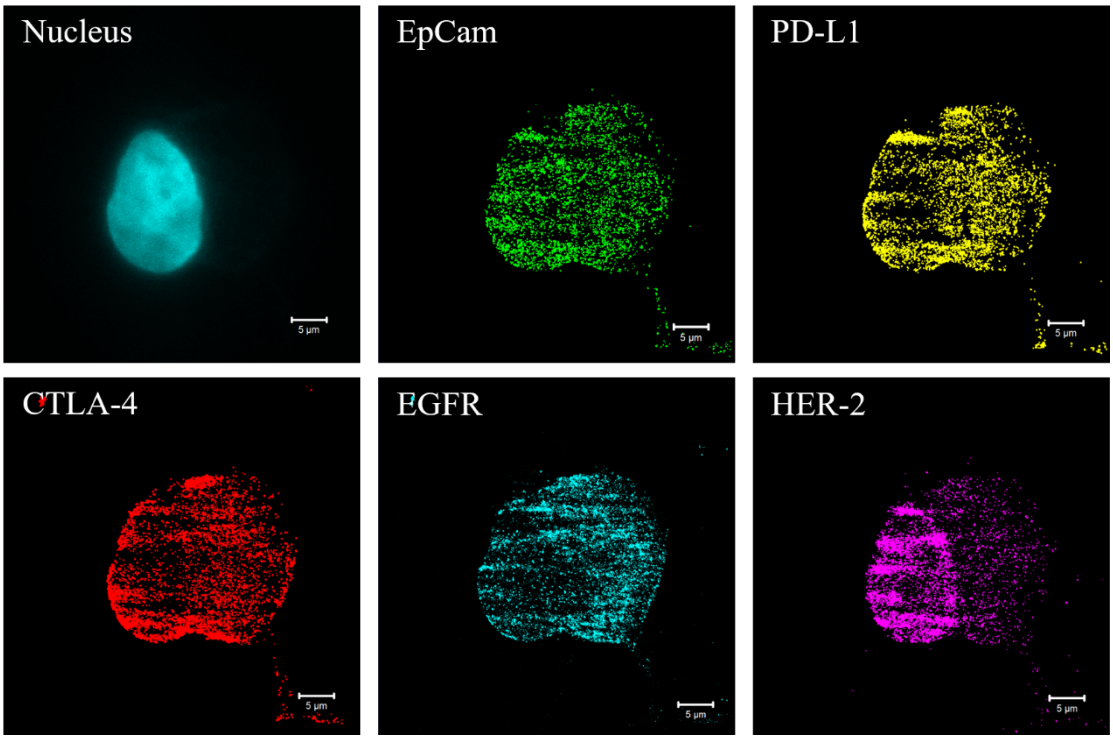

Fig. S5. Exchange-PAINT imaging of MCF-7 cell. The figure shows DNA-PAINT images of the five proteins on the same MCF-7 cell surface. The position of nucleus overlapped well with the proteins, indicating the labeling was successful. Scale bar: 5 μm.

Supplementary Table S1. The sequences of docking and imager strands for individual biomarker.

Identification	Sequences	Identification	Sequences
Docking strands for EpCam	Azide-TTAATTAGGAT-3’	Imager strands for EpCam	5’-CATCCTAATT-Cy5
Docking strands for HER-2	Azide-TTATGAATCTA-3’	Imager strands for HER-2	5’-GTAGATTCAT-Cy5
Docking strands for PD-L1	Azide-TTATCTACATA-3’	Imager strands for PD-L1	5’-TATGTAGATC-Cy5
Docking strands for EGFR	Azide-TTAATTGAGTA-3’	Imager strands for EGFR	5’-GTACTCAATT-Cy5
Docking strands for CTLA-4	Azide-TTTCTTCATTA-3’	Imager strands for CTLA-4	5’-GTAATGAAGA-Cy5