Supporting Information

Image-based single-cell isolation in high-density seeded cells using photoactivatable surfaces

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Supporting Methods

Cell proliferation on photoactivatable PEG-lipid surfaces after light-induced anchoring

BaF3-EGFP cells were cultured on glass-bottom dishes to evaluate their proliferation. Cells were seeded on photoactivatable PEG-lipid–modified glass surfaces either before or after irradiation at 405 nm (10 J/cm²), a dose sufficient for cell anchoring, to examine whether light exposure and surface anchoring affect cell proliferation. In both cases, after a 10-min incubation period to allow anchoring, non-anchored cells were removed by gentle washing. For comparison, approximately the same number of cells as those anchored on the photoactivatable PEG-lipid–modified surfaces were seeded on a BSA-coated glass surface, which served as a naive biocompatible surface. Cells were cultured in 200 μL of RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 ng/mL IL-3. Cell numbers were quantified for each dish immediately before culture initiation and at days 1, 2, and 3 of culture using the TrackMate plugin in Fiji (version 2.14.0/1.54f, ImageJ distribution) on EGFP fluorescence images covering the entire culture area.[1] The fold increase in cell number at each time point relative to the initial count was then calculated. All samples exhibited comparable proliferation rates, indicating that neither light exposure nor surface anchoring significantly affected cell proliferation (Fig. S1).

Supporting Figures

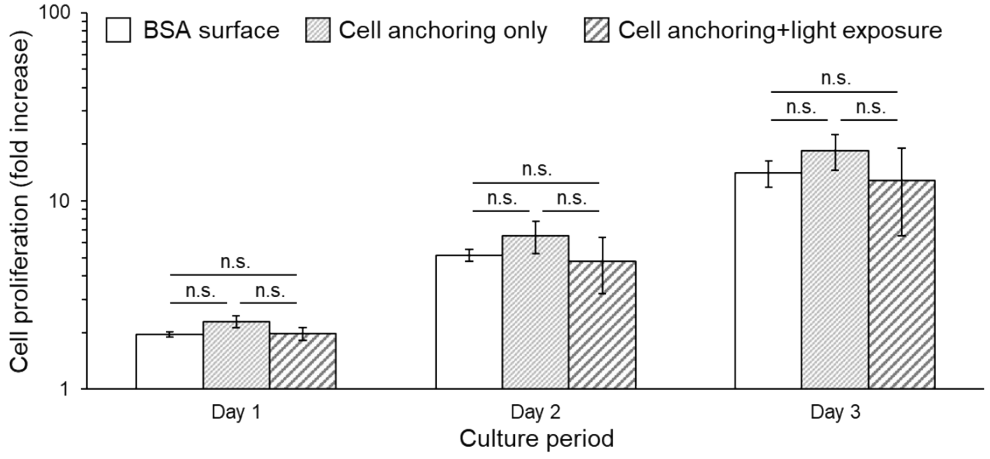

Figure S1. Effects of light exposure and surface anchoring on cell proliferation on photoactivatable PEG-lipid–modified surfaces. Fold increase in cell number over 3 days of culture (n = 3). Bars indicate mean ± SD. n.s., not significant ($p > 0.05$, Student's t-test).

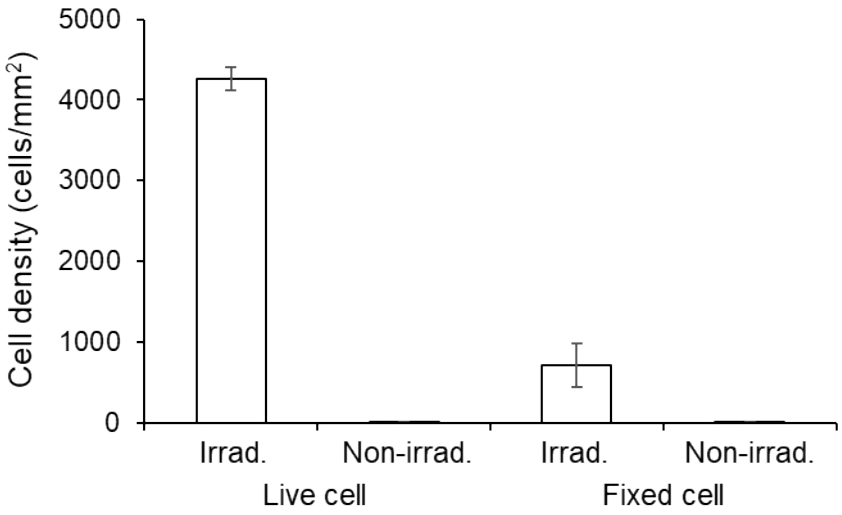

Figure S2. Comparison of capture efficiency between live and paraformaldehyde-fixed cells. The number of captured cells was quantified on the photoactivatable surface using either live cells or cells fixed with 4% paraformaldehyde in PBS prior to seeding. Data represent mean ± s.d. from three independent experiments (n = 3).

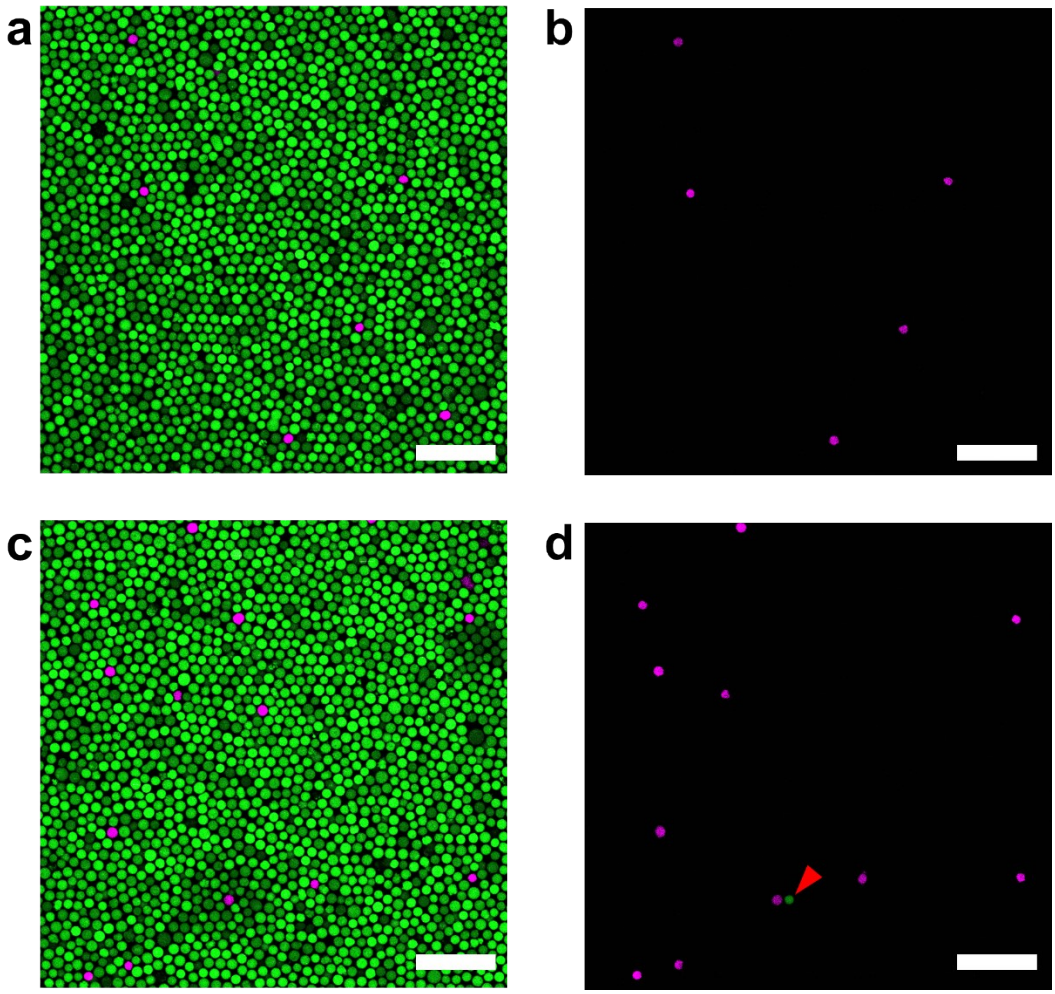

Figure S3. Additional independent experiments of light-induced selective anchoring of single cells. (a,c) Fluorescence images of BaF3 cells stained with green fluorescence and red fluorescence (displayed as magenta in pseudo-color) before selective light irradiation. (b,d) Fluorescence images of the corresponding fields of view after selective light irradiation of red fluorescence–stained cells followed by rinsing. An adjacent off-target cell is co-captured, as indicated by a red arrow in Fig. S3d. Scale bar, 100 μm.

Supporting Movies

Movie S1. Light-induced selective single-cell isolation

This movie shows a screen capture of the control interface of the confocal laser scanning microscope (TCS SP8, Leica Microsystems GmbH, Wetzlar, Germany). Bright-field and Calcein-AM fluorescence images (detected at 506–522 nm) were acquired using a 20× objective lens under 488 nm laser excitation. The time displayed at the upper-left corner represented the actual elapsed time during the experiment. Using the microscope's bleach

mode function, a bleach point was set on a Calcein-AM–fluorescent PBMC drifting within a microchannel coated with photoactivatable PEG-lipid **1.** At 142 s on the time display, 405 nm laser irradiation (10 ms exposure) was applied to photoactivate the PEG-lipid for capturing the single target cell. At 358 s, buffer flow was initiated to wash away non-target cells.

Additional References

1. J.-Y. Tinevez, N. Perry, J. Schindelin, G. M. Hoopes, G. D. Reynolds, E. Laplantine, S. Y. Bednarek, S. L. Shorte and K. W. Eliceiri, *Methods* 2017, **115**, 80–90.