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Aqueous Two-Phase System-Mediated Antibody Micropatterning Enables Multiplexed Immunostaining of Cell Monolayers and Tissues

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Supporting Information (Figure S1, Figure S2 and an Enumerated Immunofluorescence Protocol)

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John P. Frampton[1], Michael Tsuei[1], Joshua B. White[1], Abin T. Abraham[1] and Shuichi Takayama*[1,2]

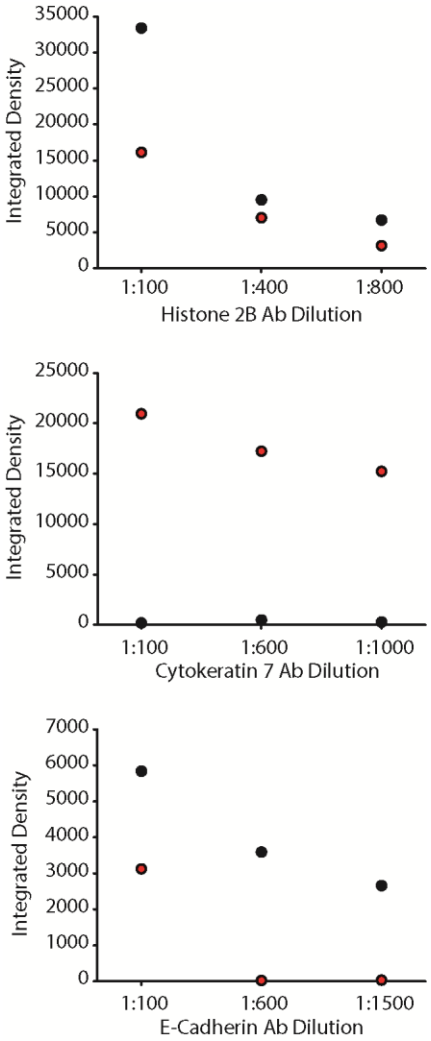

Figure S1- Quantification of antibody concentration versus signal (integrated density) for the images shown in Figure 1 for Histone 2B (top), Cytokeratin 7 (middle) and E-Cadherin (bottom). Black symbols represent data for MCF7 cells and red symbols represent data for HeLa cells.

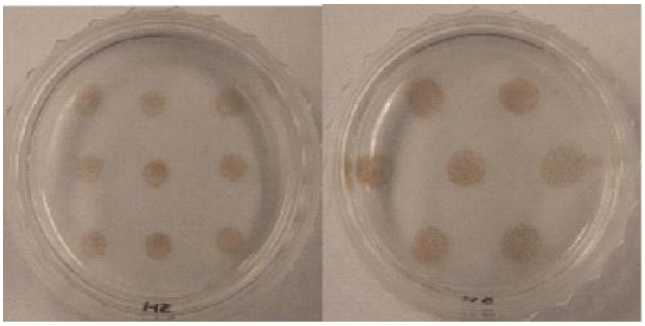

Figure S2- Patterned immunostaining of cell monolayers (35 mm dishes) visualized using a chromogenic developer. The plate on the left shows a 3X3 grid of antibody detection spots generated using a handheld micropipette. The plate on the right shows an arbitrary pattern of 7 antibody detection spots generated using a handheld micropipette.

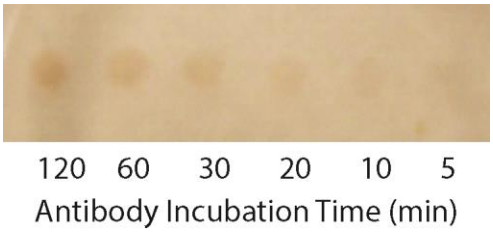

Figure S3- Chromogenic detection of CK7-stained spots with incubation times ranging from 120 to 5 minutes.

Enumerated Immunofluorescence Staining Protocol for Cell Culture Samples

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Legend:

*Hint

⇨ATTENTION

✋REST

Reagents:

DMEM (Life Technologies, Carlsbad, CA)

Penicillin-Streptomycin-Glutamine (PSQ; Life Technologies, Carlsbad, CA)

Fetal Bovine Serum (FBS; Sigma, St. Louis, MO)

Methanol (Fisher Scientific, Hampton, NH)

Phosphate Buffered Saline (PBS; Life Technologies, Carlsbad, CA)

Dextran (DEX T500 10%wt in PBS; Pharmacosmos, Denmark)

Polyethylene Glycol (PEG MW 35,000 g/mol 10%wt in PBS; Sigma, St. Louis, MO)

Bovine Serum Albumin (BSA, 96% purity; Sigma, St. Louis, MO)

10% Normal Goat Serum (Life Technologies, Carlsbad, CA)

Mouse-anti-cytokeratin 7 Antibody (Sigma, St. Louis, MO)

Mouse-anti-CDH1 Antibody (Sigma, St. Louis, MO)

Rabbit-anti-Histone 2B Antibody (Sigma, St. Louis, MO)

Alexa-594-goat-anti-rabbit IgG (Life Technologies, Carlsbad, CA)

Alexa-594-goat-anti-mouse IgG (Life Technologies, Carlsbad, CA)

Cell Seeding:

1. Seed MCF7 and HeLa cells at ~200,000 cells per 35 mm cell culture-treated Petri dish in 2 mL DMEM containing 10% FBS and 1% PSQ. Maintain cultures in a humidified incubator for 24 to 48 hours at 37 °C under 5% CO_2.
2. Microscopically observe the cells. At 70-90% confluence, the cultures are ready for fixation.

   *Hint: Once the cells in the areas you are planning to immunostain reach the appropriate density, the dishes are ready to be fixed. Thus, even non-uniformly seeded samples can be used.

Fixation/Permeabilization:

3. Aspirate the culture medium from each dish and replace it with 2 mL of ice-cold methanol to fix and permeabilize the cells.

   ⇨ATTENTION: Depending on the antigen, different fixation/permeabilization reagents may be required (i.e., 4% paraformaldehyde for fixation and 0.1% Triton X-100 for permeabilization).

4. After 5 minutes of methanol fixation at -20 °C, aspirate the methanol and wash 3 times with PBS.

   ✋REST: The samples can be stored at 4° C before blocking.

Blocking:

5. Aspirate the PBS and apply 10% normal goat serum for 1 hour to block the samples.

Primary Antibody Patterning:

6. After blocking, wash 3 times with PBS.
7. Aspirate the PBS and add 2 mL of 10% PEG containing 0.1% BSA to each dish.
8. Dispense the primary antibody solutions in the 10% DEX by pipette onto the surface of the sample.

   ⇨ATTENTION: Once a dish is spotted, keep the dish on a level surface and do not move the dish suddenly. Failure to do so might impact the fidelity of the patterned DEX droplets.

   *Hint: The antibody solution can be prepared during the blocking step and stored on ice until use.

   ✋REST: Each spotted 35 mm dish should be sealed with parafilm and carefully transported/stored on a level surface at 4° C overnight.

Secondary Antibody Application:

9. Aspirate the PEG and DEX solutions from each dish and quickly proceed to washing 5 times with PBS.

   *Hint: It is recommended to perform three rapid washes, where the PBS is applied and immediately aspirated and two long washes of 5 minutes each.

   ⇨ATTENTION: The initial short washes should be done quickly to avoid the possibility of trace unbound primary antibodies binding to undesired regions of the cell monolayer.

10. Once the PBS is aspirated add the secondary antibodies at the appropriate dilutions in PBS and incubate at room temperature for two hours.

    ⇨ATTENTION: Upon application of fluorescent secondary antibodies, immediately wrap each dish in Aluminum foil or place in a dark area to avoid photobleaching.

Analysis:

11. After two hours, apply at least three washes with PBS.
12. Immediately observe the immunofluorescently stained cells using the appropriate microscope filters.