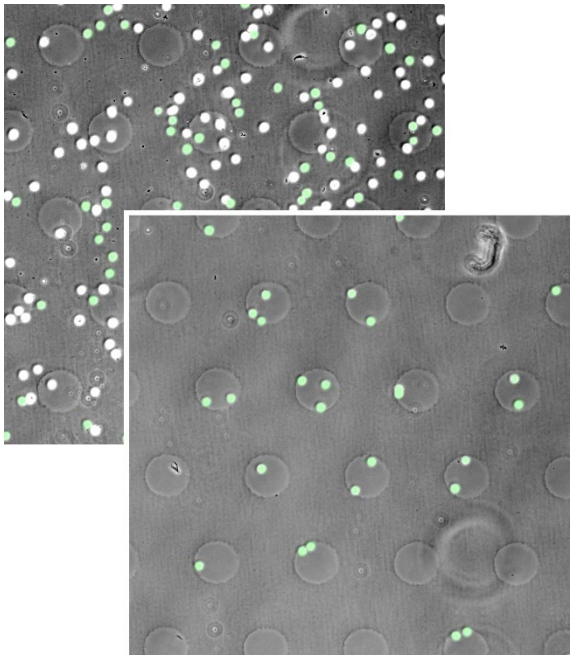
cells in focus ibidi®

Selective and Localized Cell Adhesion using a CD19-Coated Custom µ-Pattern ibiTreat

Immune cells can be identified and separated based on their cluster of differentiation (CD) markers. Cell type-specific CDs, such as the B lymphocyte-specific CD19, can be used to separate a cell population from a heterogenic cell suspension. This Application Note describes a protocol using a CD19 antibody coating on µ-Pattern ibiTreat slides to selectively capture CD19-positive Nalm-6 cells from a mixed population containing CD19-negative Jurkat cells. The separated cells can then be used for downstream immunoanalytical applications.



ibidi Solutions for Micropatterning

- µ-Pattern ibiTreat for Single-Cell Arrays
- µ-Pattern ibiTreat for Multi-Cell Arrays
- µ-Pattern ibiTreat for Line Arrays
- Custom µ-Pattern ibiTreat

Related Documents

- AN 65: Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization (PDF)
- AN 78: Cell Culture and Immunofluorescence Staining in the µ-Slide VI 0.4 µ-Pattern ibiTreat (PDF)
- AN 80: Formation and Long-Term Cultivation of Spheroids in the µ-Slide 8 Well high µ-Pattern ibiTreat (PDF)
- Video: The ibidi µ-Patterning Technology—Achieve Spatially Defined Cell Adhesion with Micropatterning
- Rüdiger, D., *et al.*: Selektive und ortsaufgelöste Zellanbindung mit µ-Pattern ibiTreat, 2023, BIOspektrum, 4, 391

1 Materials

1.1 Reagents and Buffers

- Nalm-6 (DSMZ, ACC 128, B cell precursor leukemia CD19$^+$)
- Jurkat (DSMZ, ACC 282, T cell leukemia CD19$^-$)
- RPMI 1640 Medium (Gibco, 21875034) with 10 % FCS (Gibco, 10270106)
- C19 monoclonal antibody (Invitrogen, 14-0199-82)
- Blocking buffer (1 % BSA + 0.2 % Trition-X-100 in PBS)
- Antibody dilution buffer (1 % BSA + 0.05 % Trition-X-100 in PBS)
- BSA (Sigma Aldrich, A1470-10G)
- Trition-X-100 (Thermo Fisher Scientific, A16046)
- Anti-Mouse-IgG Atto 594 (Sigma Aldrich, 76085)
- Sterile PBS (Gibco, 14190144)
- CellBrite Green Cytoplasmic Membrane Dye (Biotium, 30021)

1.2 Labware

- µ-Slide VI $^{0.4}$ with 100 µm spots and 250 µm pitch (ibidi, Custom µ-Pattern ibiTreat)

2 Procedure

Before starting the experiment, prepare the cells in a standard cell culture flask (e.g., T75). The cells should ideally be sub-confluent and healthy on the day of the experiment.

2.1 Coating of the Channels

- Dilute the CD19 antibody with sterile PBS to prepare a 10 µg/ml working solution.
- Add 30 µl of the CD19 antibody solution per channel.
- Incubate the slide for at least 1 hour at 37 °C.
- Wash five times with sterile PBS.

2.2 CD19 Staining (Optional)

The CD19 staining is used to visualize the coating on the pattern.

- Add 100 µl of blocking buffer per channel.
- Dilute secondary antibody anti-mouse-IgG Atto 594 1:200 with the antibody dilution buffer.
- Add 100 µl of the staining solution per channel.
- Incubate for 2 hours in the dark at room temperature.
- Wash twice with 100 µl of the blocking buffer per channel.
- Add 100 µl PBS per channel for imaging (Nikon TiE with a 10x objective, TexasRed filter).

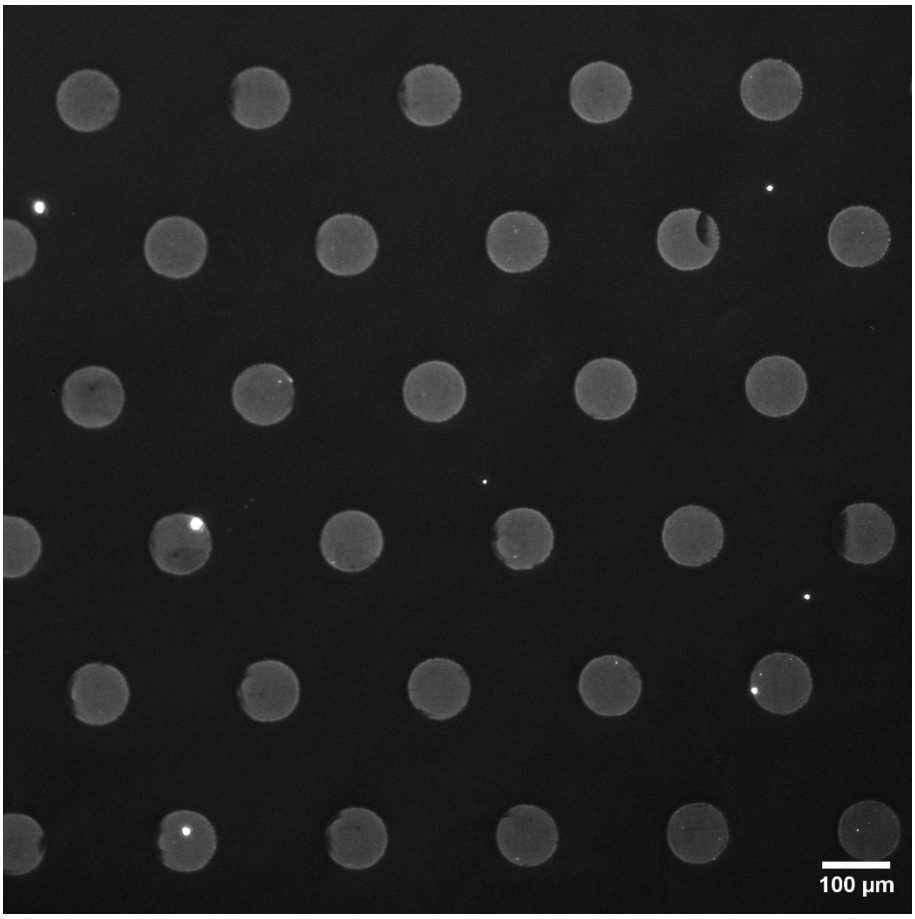


Figure 1: Fluorescence imaging of the CD19 coating. All patterns are uniformly coated with CD19, and the signal shows a clear contrast to the background.

2.3 Cell Labeling and Seeding

Important Note: Before seeding the cells, please read the Instructions of the µ-Slide VI $^{0.4}$. Perform all steps under sterile conditions. It is recommended that the slides and cell culture medium are placed in the incubator one day before seeding the cells to avoid the formation of air bubbles during handling. It is essential to work swiftly during the whole procedure to prevent the cells from drying.

Cell labeling is used to distinguish between the two cell types.

- Harvest Nalm-6 cells and dilute to 1 × 10^6 cells/ml in 2 ml.
- Add 10 µl CellBrite Green to the cell suspension.
- Incubate cells for 20 minutes at 37 °C.
- Centrifuge and resuspend the cells in fresh medium three times
- Count labeled Nalm-6 cells and dilute to 5 × 10^5 cells/ml.
- Harvest Jurkat cells and dilute to 5 × 10^5 cells/ml.
- Mix Nalm-6 and Jurkat cells 1:1 (final concentration of 2.5 × 10^5 cells/ml for each cell type).
- Add 150 µl of the cell mixture per channel.

2.4 Imaging

- Directly after cell seeding, image the slide with a 10x objective, for example.
- Incubate cells for 2 hours at 37 °C.
- Wash cells three times with sterile PBS.
- Add fresh medium to the channels.
- Image the slide again with the same imaging parameters.

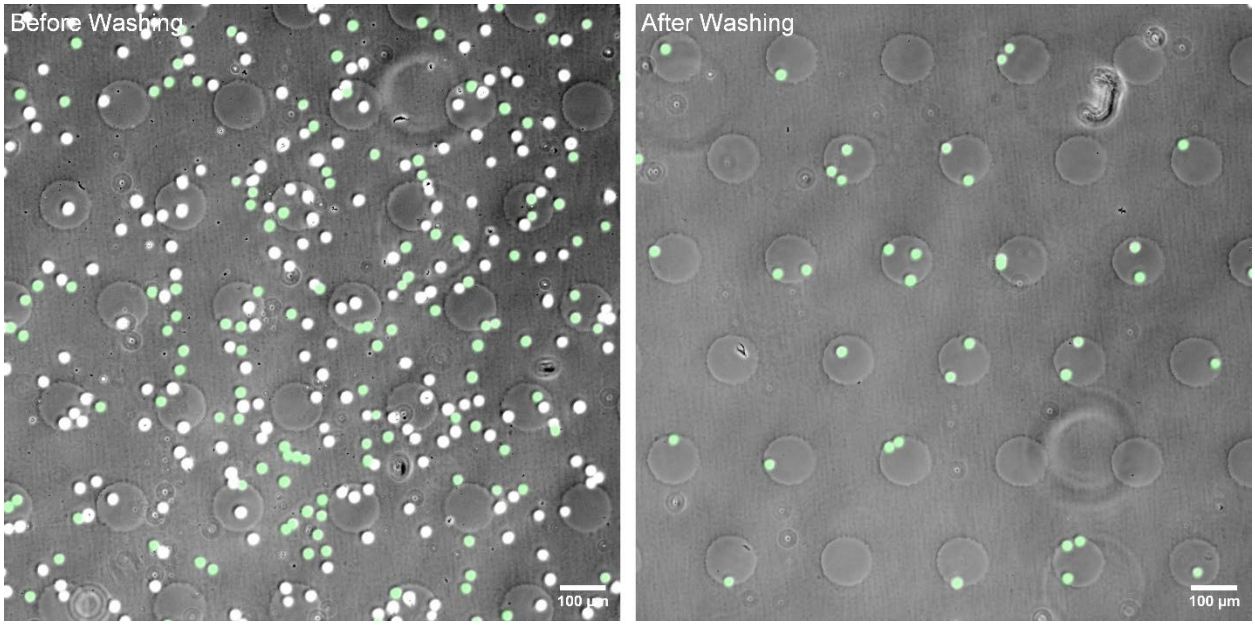


Figure 2: Selective cell binding of CD19-positive Nalm-6 cells. Before washing (left), Nalm-6 cells ($CD19^+$, labeled green with CellBrite Green) and Jurkat cells ($CD19^-$, unlabeled) are evenly distributed in the channel. After a 2-hour incubation and the washing step, all Jurkat cells are removed. Only the Nalm-6 cells can adhere to the CD19 antibody on the pattern (right).

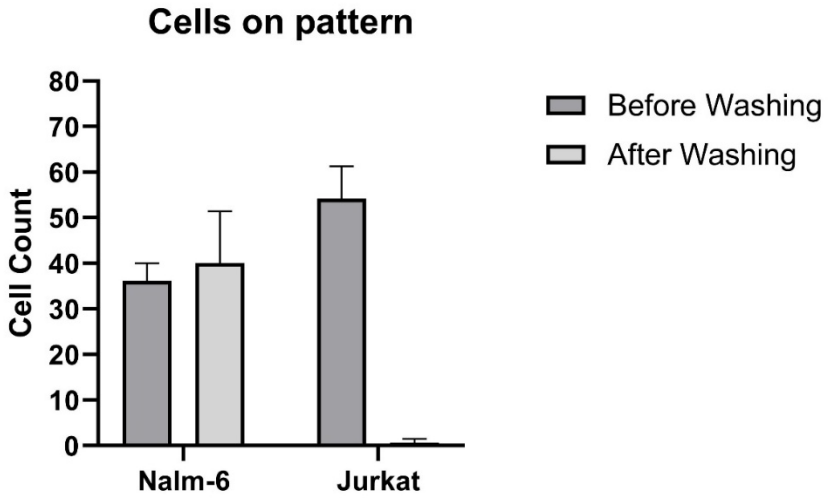


Figure 3: Quantification of cell counts. The quantification of the two cell types shows that before the washing, Nalm-6 cells ($CD19^+$) and Jurkat cells ($CD19^-$) are approximately equally distributed. After the 2-hour incubation and the washing step, almost exclusively Nalm-6 cells adhered to the pattern with CD19 antibody. The numbers are expressed as the mean with the standard deviation (n=6).