A High-Throughput Image-Processing Based Analysis of Dynamic Cell Surface Interactions in a Microfluidic Chip

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Abstract:

Immunoaffinity-based lab-on-a-chip technologies offer high-throughput, label-free methods of cell characterization and are attractive for developing low cost point-of-care devices for cellular diagnostics. A biologically functionalized microfluidic channel would allow for detection of cells of particular interest, such as cancer cells as different cells have varying levels of affinity for different antibodies. This project focused on the development of an image-based analysis method combined with a microfluidic platform to identify cells using surface affinities of plasma membranes. A microfluidic channel was designed and constructed with polydimethylsiloxane (PDMS) using soft lithography. The channel was partially functionalized with different coatings including poly-L-lysine (PLL), a positively charged amino acid polymer that electrostatically attracts the negatively charged plasma membrane of cells. It was predicted that the cells would slow down as they traverse the coated region due to the electrostatic attraction. The motion of cell population traversing the channel was captured before and after they reach the PLL-coated region using a high-speed camera. A custom image processing software was developed to analyze and compare average velocity profiles of cells and neutral particles that are similar in size. The developed platform can be used to study the dynamics of immunoaffinity-based cell capture in microfluidic devices.

Experimental Procedure:

Microfluidic Device Design and Fabrication. The microfluidic device was designed in the form of a serpentine channel with an inlet and an outlet at respective ends to maximize the channel length. The serpentine microfluidic channel was 34 μm high, 1.4 mm wide, and 1.9 m long. The length of the channel ensures that cells sediment and reach a steady state on the floor of the channel before they interact with the coated region. The sedimentation process was modeled using Stokes flow [1], assuming a cell density (ϱ_c) of 1.05 g/cm³ and a fluid density (ϱ_f) of 0.996 g/cm³. The calculated sedimentation velocity was 7.6 μm/s. Therefore, the time required for all cells to sediment was approximated to be 5 s, which is much shorter than the time cells spend in the microfluidic channel during our experiments.

The device was fabricated using soft lithography (Figure 1). SU-8 negative photoresist was spun on a 4-inch silicon wafer and was patterned using standard photolithography to create a mold. The fabricated mold was inspected using a Dektak 150 profilometer to ensure consistent channel height across the device.
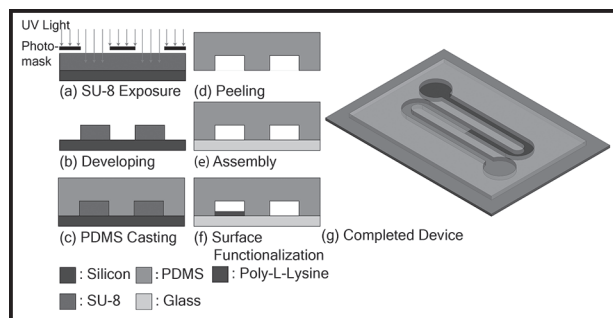

Figure 1: Overview of device fabrication process using soft lithography.

PDMS prepolymer and cross-linker (Sylgard 184) mixed at 10:1 ratio was poured on the SU-8 mold and cured for at least for four hours at 65°C. The cured PDMS was peeled off from the master mold and was bonded to a 1-inch by 3-inch glass slide following surface activation using oxygen plasma. The bonded microfluidic channel
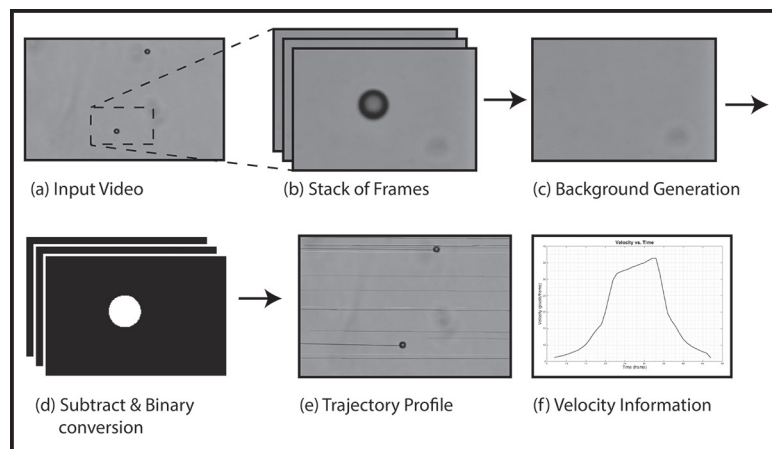
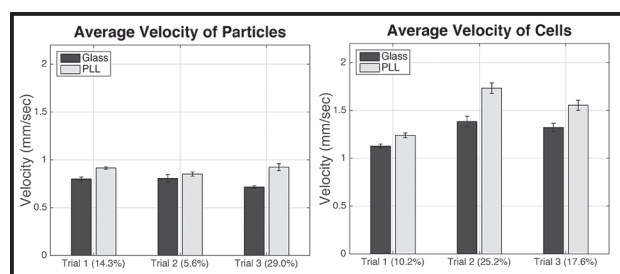Figure 2: Schematic of developed image processing software.

Figure 3: Velocities of particles and cells in the device. Error bars represent 95% confidence intervals of mean velocities. Values next to trial numbers represent percent change in mean velocities.

was then partially coated by flowing a 0.01% PLL solution from the outlet to cover approximately half of the channel. Following a 30 min incubation, the device was flushed thoroughly with de-ionized (DI) water from the inlet.

Data Acquisition and Image Processing. Using a syringe pump, cells and polymer beads (Polybead® Microspheres with 15 μm diameter) suspended in phosphate-buffered saline (PBS) were driven through the microfluidic device from the inlet at a rate of 5 μL/min. The cells used in the experiment were human ovarian cancer (HEY) cells. Confluent cultures of cells were trypsinized and dissociated by gentle pipetting to create single cell suspension in PBS.

A high-speed camera (Vision Research, Phantom v7.3) connected to an inverted optical microscope (Nikon Ti) was used to record the movement of the cells and particles as they flow in the uncoated and the PLL-coated region. The field of view in each video was aligned such that the measurements were taken at identical points along the width of microfluidic channel to account for the parabolic flow profile. The recorded video was analyzed using a cell-tracking program developed using MATLAB for this experiment. The program was designed to; 1) analyze the video frame by frame, 2) create a static background, and 3) perform background subtraction to find the location of

cells and particles in each frame. Using the known frame capture rate and calibrated pixel dimension, actual particle velocities were calculated (Figure 2).

Results:

We measured the average velocities of particles and cells in repeated experiments. Overall, the polymer beads showed a 16.2% increase in mean velocity, whereas the cells showed a 17.7% increase in mean velocity when they entered the PLL-coated region (Figure 3). These results are in conflict with our initial predictions. We hypothesized that the cells should move slower on the PLL-coated region due to the electrostatic attraction between plasma membranes and the PLL layer. Our measurements indicate higher velocities for both cells and particles over PLL-coated surface. Moreover, there is negligible difference between the cells and particles' velocities suggesting an effect independent of electrostatic interactions.

Future Work:

Future experiments will focus on the investigation of various factors that can affect the changes in velocity of moving particles and cells. We will use surface characterization tools to analyze device surface coatings and then optimize our surface functionalization protocols. The image analysis program will be improved to increase the signal-to-noise ratios in cell and particle velocity measurements. Following the analysis of the parameters that mainly affect the motion of cells, functionalization of the channel using antibodies will be studied to study specific cell detection.

Acknowledgements:

References:

[1] R. J. Garde. 2000. Mechanics of Sediment Transportation and Alluvial Stream Problems. 3rd ed. Delhi, India: New Age International.