Micropillar-Based Microfluidic Device for Cell Capture and DNA Analysis

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Primary CNF Tools Used: Unaxis, ABM contact aligner, photoresist spinning and baking stations

Abstract:

We present a valveless dual-channel microfluidic device for targeted cell capture, imaging, and on-chip DNA analysis. The two channels are positioned with a perpendicular intersection filled by an array of micropillars. These micropillars are functionalized with DNA aptamers, analogous to antibodies, which are used to bind targeted cells such as cancer cells. These bound cells can then be imaged in place or lysed to extract their DNA for further analysis. Our past work has demonstrated high efficiency DNA extraction using micropillar arrays from varying cell counts (hundreds) down to a single cell [1] as well as an improvement to DNA amplification uniformity as compared to conventional methods. Here, we sought to integrate the various capabilities published in our previous work into a single device. We report the device's ability to perform cancer-cell specific capture with the DOV4 aptamer as well target-specific isothermal amplification [2]. Furthermore, we expanded upon our previous year's DNA amplification work to show an improved amplification uniformity in single cell whole genome amplification as measured by exome sequencing. We report a 33.5% improvement in genome mapping and up to 81.08% genome coverage at 10x read depth [3].

Summary of Research:

The dual-channel device shown in Figure 1 is composed of two perpendicularly intersecting microfluidic channels. One channel is designed for cell loading and surface functionalization of micropillars while the other channel is designed for DNA lysis. The intersection of the two channels, shown in Figure 1 (left panel), contain an array of 50 μm diameter pillars rotated 4° to increase cell-pillar collision rates during cell capture. These capture cells are then lysed towards the direction of a second pillar array region consisting of densely spaced 1.5 μm diameter pillars, shown in Figure 1 (right panel).

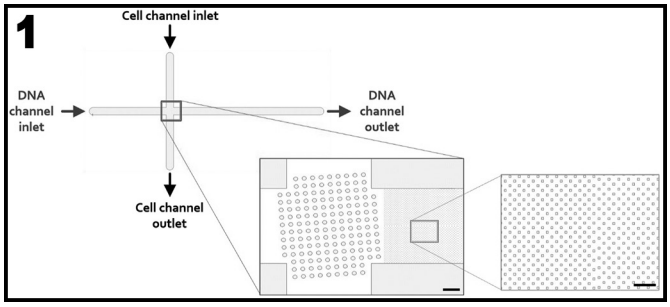

Figure 1: Dual-channel device.

Through functionalizing the 50 μm diameter pillars with DNA aptamer DOV4 which has been reported as a cancer-cell specific aptamer [4], we were able to capture two cancer cell lines tested, HeLa human cervical cancer cell line and CAOV-3 human ovarian cancer cell line. Our positive controls, performed with human non-cancer ovarian cell lines Ect1/E6E7 and End1/E6E7 did not bind to the DOV4 micropillars indicating successful aptamer driven cell capture. Figure 2 then shows our ability to lyse the captured cells, in this case CAOV-3 cells, and stain and image the DNA in-channel.

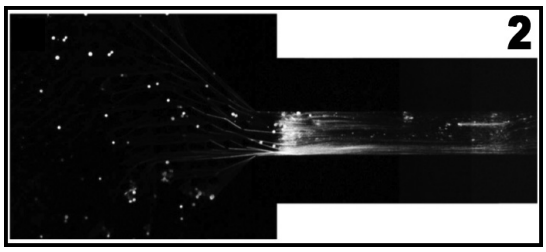

Figure 2: Shows our ability to lyse the captured cells, in this case CAOV-3 cells, and stain and image the DNA in-channel.

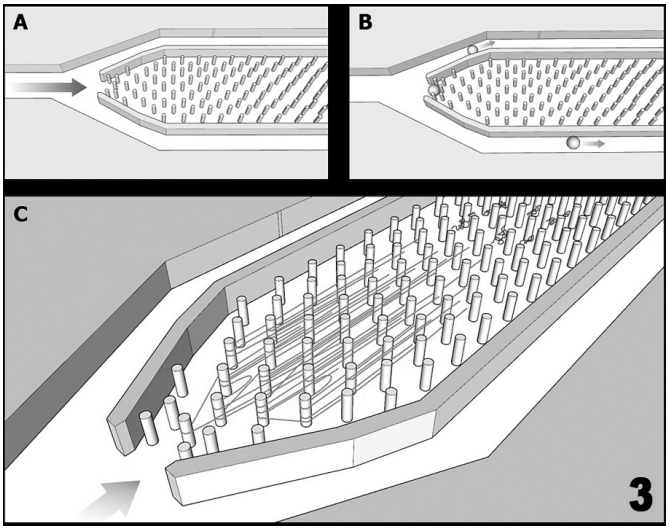

Figure 3: Cartoon of DNA amplification device.

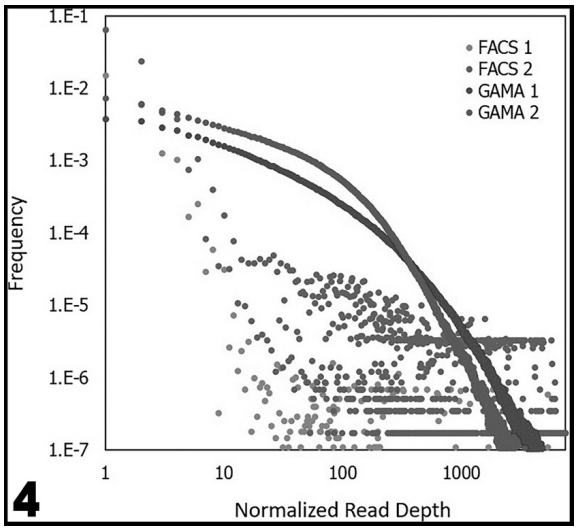

Figure 4: Two on-chip single cell samples.

Our DNA amplification device, as depicted by the cartoon representation in Figure 3(A), contains a micropillar array region enclosed by a barrier. At the front of the barrier is an opening wide enough to fit a single cell. In Figure 3(B), a suspension of human cervical cancer cells (HeLa cells) are loaded into the input single cells were captured at the apex of the micropillar array while excess uncaptured cells flow through to the output ports where they were removed. Upon introducing a cell lysis agent into the channel Figure 3(C), the genonic DNA of entrapped cell become physically entangled on the micropillars immediately downstream from the original position of the cell and this gDNA can be used for in-channel chemistries such as DNA amplification or be directly imaged on-chip after fluorescence DNA staining.

The extracted gDNA can then be isothermally whole genome amplified on-chip using commercially available multiple displacement amplification (MDA) reagents. Because the amplified DNA fragments are below the size threshold necessary to become entangled upon the 2 μm diameter micropillars, they flow through the pillars and can be collected at the output port at the end of the amplification.

To assess amplification bias, we compared our amplified DNA pools to DNA amplified within a 96-well plate from single cell isolated through fluorescence activated cell sorting (FACS). From analyzing the exome sequencing data on more than 20,000 loci across the genome, we determined that we were able to map 98.5% of the reads from our on-chip samples whereas control samples with FACS only reached 65.0% reads mapped.

Shown in Figure 4, the two on-chip single cell samples show a slower decaying distribution of read count as depth increases indicating much more uniform amplification of the genome. Furthermore, we were able to cover 70.35% of the loci with a 1x read depth and 80.8% of the loci with a 1x read depth, comparable to other leading single cell DNA amplification technologies.

References:

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[3] "Single cell on-chip whole genome amplification via micropillar arrays for reduced amplification bias", H. C. Tian, J. J. Benitez, H. G. Craighead, PLOS One, (2018).

[4] "Study of the Molecular Recognition of Aptamers Selected through Ovarian Cancer Cell-SELEX", D. V. Simaeys, D. Lopez-Colon, K. Sefah, R. Sutphen, E. Jimenez, W. Tan, PLOS One, (2010).