Supplementary for

A microfabricated electrical differential counter for the selective enumeration of CD4+ T lymphocytes

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S 1. Phase contrast imaging of captured cells

Fig. 1 shows a composite phase contrast image of the entire capture region from a single experiment after cell counting was completed. The largest density of cells was located near the midpoint of the chamber's length (inset 2), while a smaller density was found near the entrance (inset 1). The cells would have had little time to interact with the antibody layer just upon entering the chip compared to the interaction time from reaching the center of the chip. The low capture density near the exit (inset 3) can be explained by the fact that a smaller concentration of cells leading the entire population reached the exit counter first, causing reversal of the flow before higher concentrations could reach the end of the chip. One cause of these leading cells could be that they were traveling at or near the center of the microbore tubing (or the center of the capture chamber's height), where they would travel at approximately twice the velocity of the other cells, which were traveling at the average flow velocity under laminar flow conditions.

S 2. Cell capture purity analysis using fluorescent labels

Fig. 2 depicts the analysis of three areas of a chip's capture capture region as an example of fluorescence analysis for quantification of captured cell purity. The percentage of captured cells being leukocytes was calculated by dividing the number of DAPI-positive cells by the number of cells found in the bright field image. The percentage of captured cells being CD4+ was calculated by dividing the number of Alexa Fluor® 488-positive (CD4+) cells by the number of DAPI-positive cells. The percentage of captured cells being monocytes was calculated by dividing the number of PE-positive (CD14+) cells by the number of Alexa Fluor® 488-positive cells. ImageJ was used to determine the grayscale threshold level which defined the presence of a cell. False coloring was used to illustrate the different fluorescent labels used in the experiments.

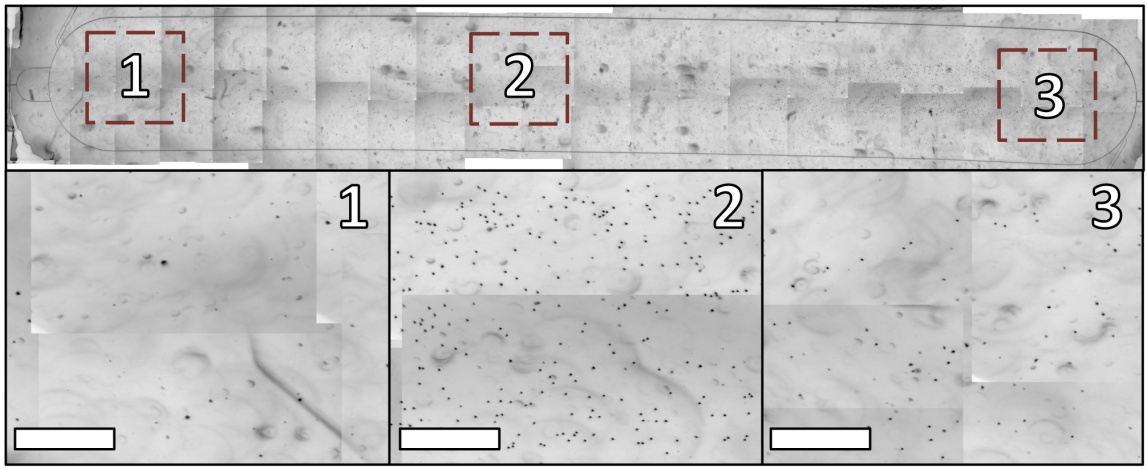

Fig. 2 Composite phase contrast image of entire 34 mm-long capture chamber after a differential counting experiment. The bottom three panels are magnifications of the regions denoted in the main figure. Error bar lengths are 500 µm, and grayscale intensity was inverted to enhance visualization of captured cells (dark spots).

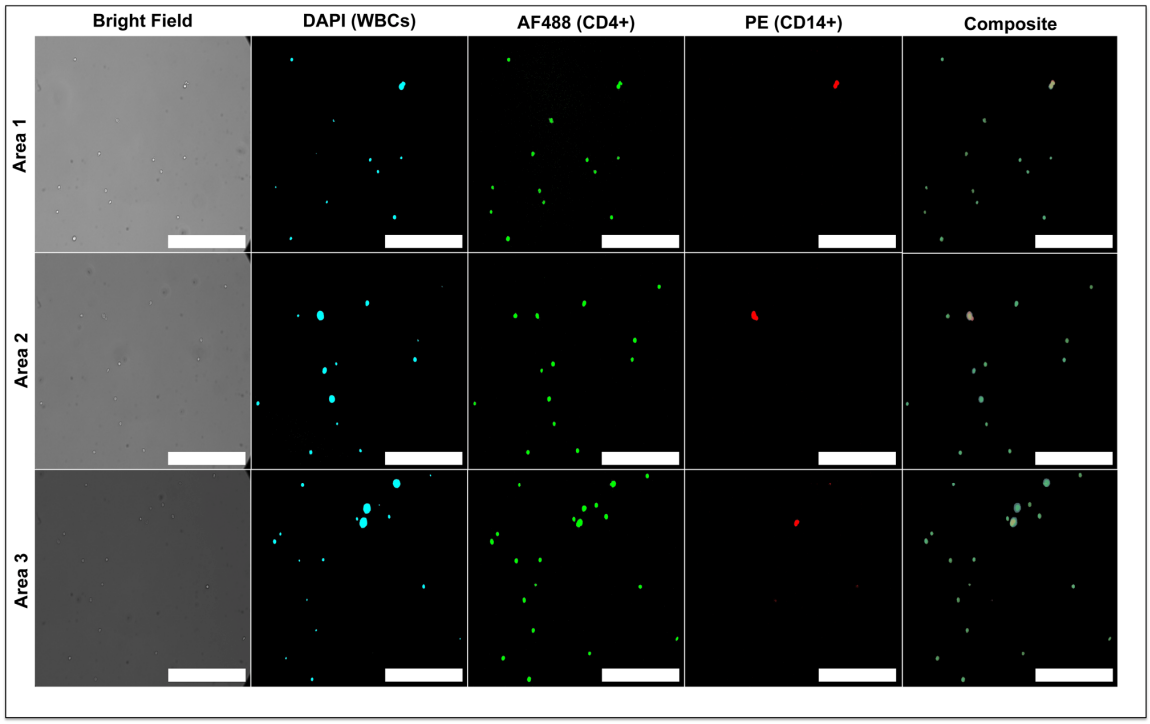

Fig. 2 Example of selective fluorescence analysis of captured cells shown in bright field images for three different areas of the chip's capture region. ImageJ threshold analysis was used to define cells; false coloring was used to discriminate among the three fluorophores used: DAPI (blue; nucleated cells or white blood cells), Alexa Fluor® 488 (green; CD4+; present on helper T cells or monocytes), and PE (red; CD14+; present on monocytes). Scale bars represent 200 µm.