Supplementary Material (ESI) for Lab on a Chip

Suplementary Materials

Integration of Semiconductor Quantum Dots into Nano-Bio-Chip Systems for Enumeration of CD4+ T Cell Counts at the Point-of-Need

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Optical Station

This nano-bio-chip (NBC) device was secured firmly beneath an infinity-corrected 10X objective and analyzed with a modified epifluorescent microscope (Olympus, Center Valley, PA) equipped with a 12 bit CCD camera (DVC Co., Austin, TX) and high pressure mercury bulb excitation source, which was evaluated with a universal optical power meter (Melles Griot, Albuquerque, NM). Sample flow was controlled by a variable speed peristaltic pump (FIAlabs, Seattle, WA) with a 500 µL sample loop. The QD 565 images were acquired with a filter cube consisting of a 425 nm downfield excitation filter, a 475 nm long-pass beam splitting dichroic mirror, and a 565 ± 10 nm emission filter. The QD 655 images were obtained via a 420 ± 20 nm excitation filter, a 475 nm long-pass beam splitting dichroic mirror, and a 655 ± 10 nm emission filter. When these two fluorophores were to be visualized concurrently, a 350 ± 25 nm excitation filter, a 475 nm long-pass beam splitting dichroic mirror, and a 520 nm long-pass emission filter were employed. Alexa Fluor 488 was viewed via a standard FITC filter cube: 480 ± 20 nm excitation filter, 505 nm long-pass beam splitting dichroic mirror, and a 535 ± 25 nm emission filter. Alexa Fluor 647 was viewed via a standard cytochrome-5 filter cube. Photomicrographs were captured via DVC software with adjustable gain, offset, and exposure time, and stored for later analysis.

Image Analysis

Raw color photomicrographs were split into their individual Red-Green-Blue (RGB) components as 8-bit grey-scale images to identify the different lymphocyte subsets. This technique allowed a single image collected with the long-pass filter to yield information on two different color channels. Pixel intensity values were then compared to a threshold value of 2 standard deviations above the mean of the background. The resulting image contained pixels having a value of either 0 or 255. Thresholded areas were then subjected to a 'watershed' filter to separate any overlapping lymphocytes and analyzed in terms of size and circularity with these regions defined as an area of interest (AOI). These AOIs were utilized to glean a variety of characteristics from the cells such as mean and maximum intensity, integrated density per pixel, standard deviation, and total count recorded from the original image. In dual staining experiments the geographic location of the red and green cells were also recorded.

To estimate the number of cells on the membrane, the following procedure was used. Representative images of a fraction of the membrane area were recorded and the fractional area therein was used to extrapolate to the total number of cells disposed on the entire membrane. This was accomplished in the following manner. First, the average number of cells per 5 fields of view (FOVs) was calculated. Each image captured by the CCD had dimensions of 1292 pixels x 1030 pixels. Via a stage micrometer the image area was found to correspond to 860 µm x 690 µm. Total available membrane area is 0.74 cm^2 resulting in approximately 125 available FOVs. This correction factor was used to calculate the total number of cells immobilized on membrane.