Exploiting Femtoliter Microwells for the Sensitive Measurement of Protein Adsorption

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Supplemental Information

Measurement of fluorescence intensity

Fluorescent images are analyzed using ImageJ as follows. Each microarray is divided into 6,000 regions, one for each microwell. Each microwell region is measured by 289 pixels, in a 17 x 17 array. Each pixel is assigned an intensity value between 0 and 255. The outer pixels measure background intensity from the top surface surrounding the microwell. The inner pixels measure intensity from the microwell. Typically, pixels in the center have the highest intensity. Since fluorescence originates as essentially a point source, we measure the fluorescence intensity of a microwell as the difference between the pixel with the highest intensity less the pixel with the lowest intensity. This difference normalizes fluorescence variations along the array caused by spatial variations in the excitation beam intensity.

Wells of fluorescence intensity less than 6 are counted as empty.

Histograms of control (blank) arrays show a distribution of intensities between 3 and 6, with 95% of wells with fluorescence intensity below 6, as shown in Figure A. This background fluorescence distribution can overlap with the fluorescence distribution when arrays partially contain adsorbed APSA, as shown in Figure B.

Assuming that the 57% of microwells with fluorescence intensity less than 6 are empty, the background intensity distribution of these wells is calculated by normalizing the data from Figure A; shown as the red line in Figure B. The distribution of filled wells is then obtained by subtracting this background distribution from the total distribution, shown as the green line.

There is some ambiguity for wells with intensity = 5. We choose to minimize false positives by counting all wells with intensity less than 6 as empty. All wells of intensity 6 or greater are counted as filled.

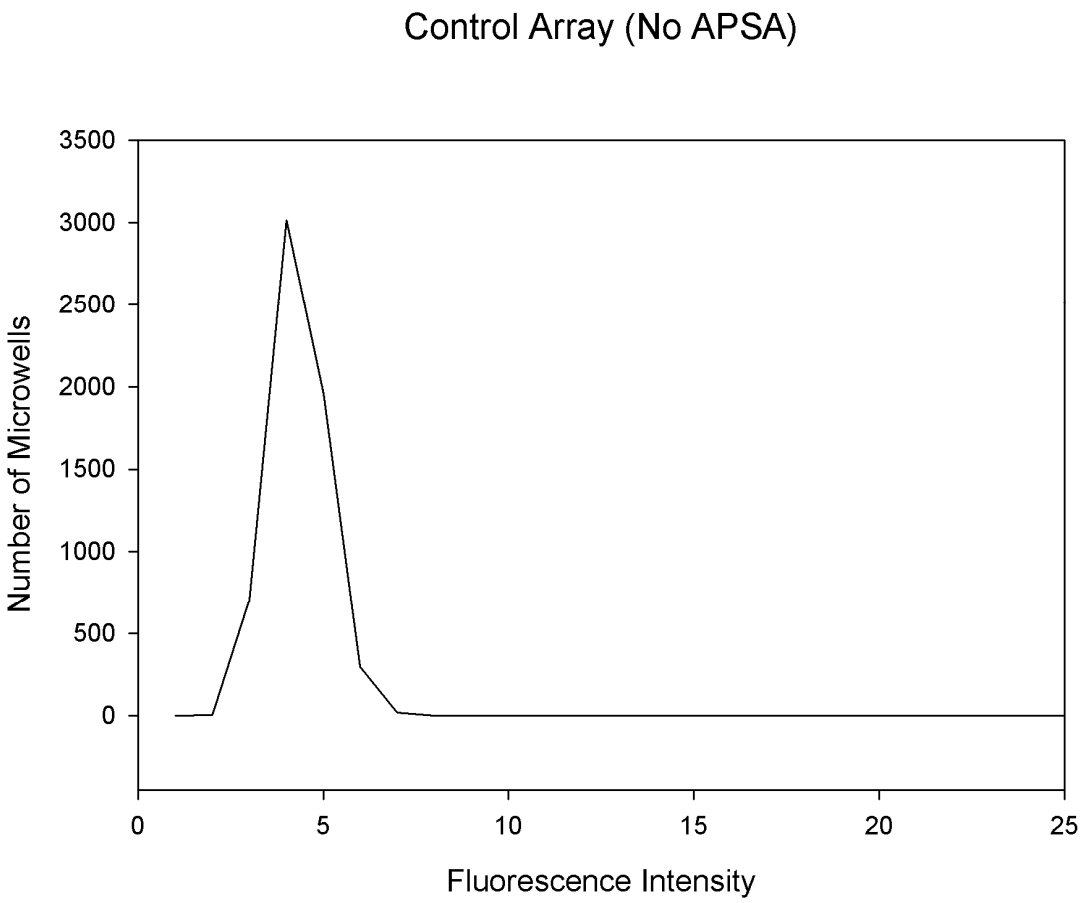

Figure A. Typical histogram of a microwell array with no adsorbed APSA. The peak width = 3 units; 95% of microwells have fluorescence less than 6 units (out of 256).

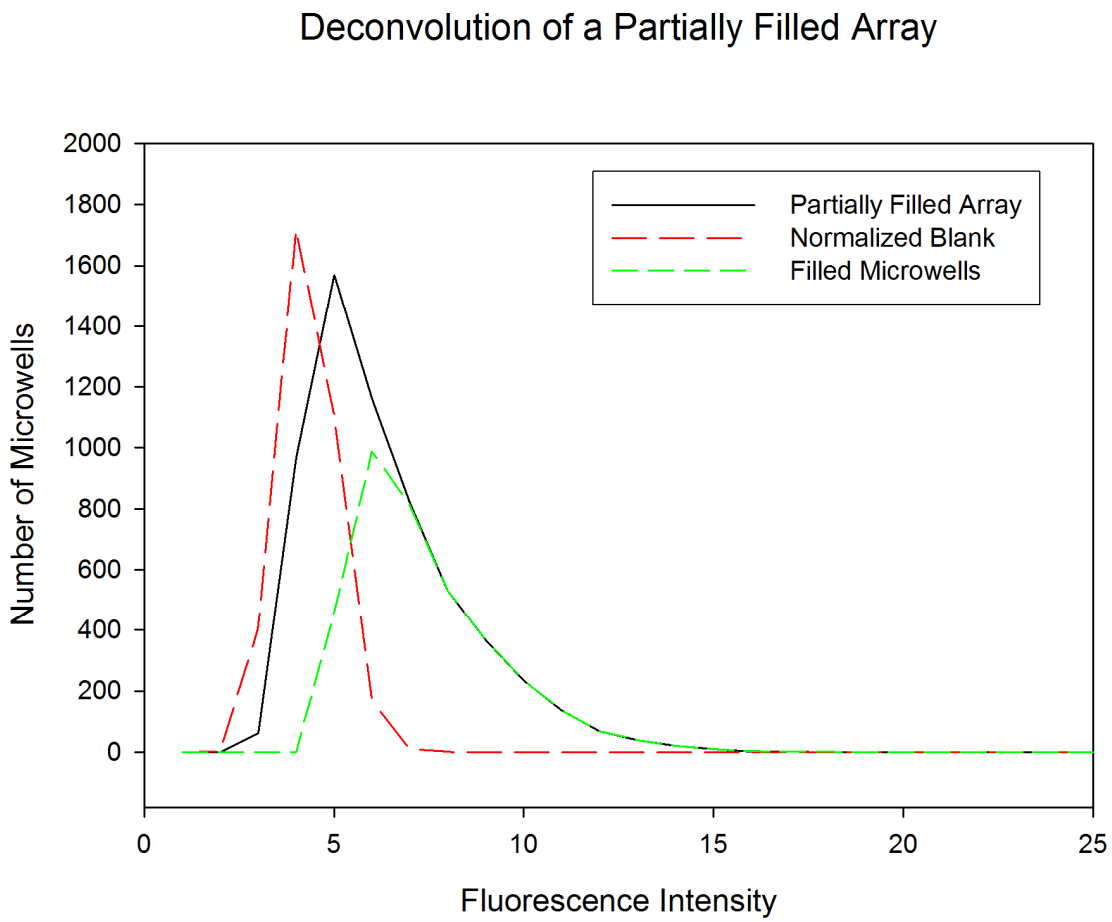

Figure B. Black: Histogram of a partially filled array; 57% of microwells have fluorescence less than 6 units. Red: Normalized blank assumed for 57% of wells. Green: Distribution of fluorescent wells obtained by subtracting the normalized blank.