Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental Information

The photopatterned barcode capture antigen locations were validated by probing with primary and secondary antibodies.

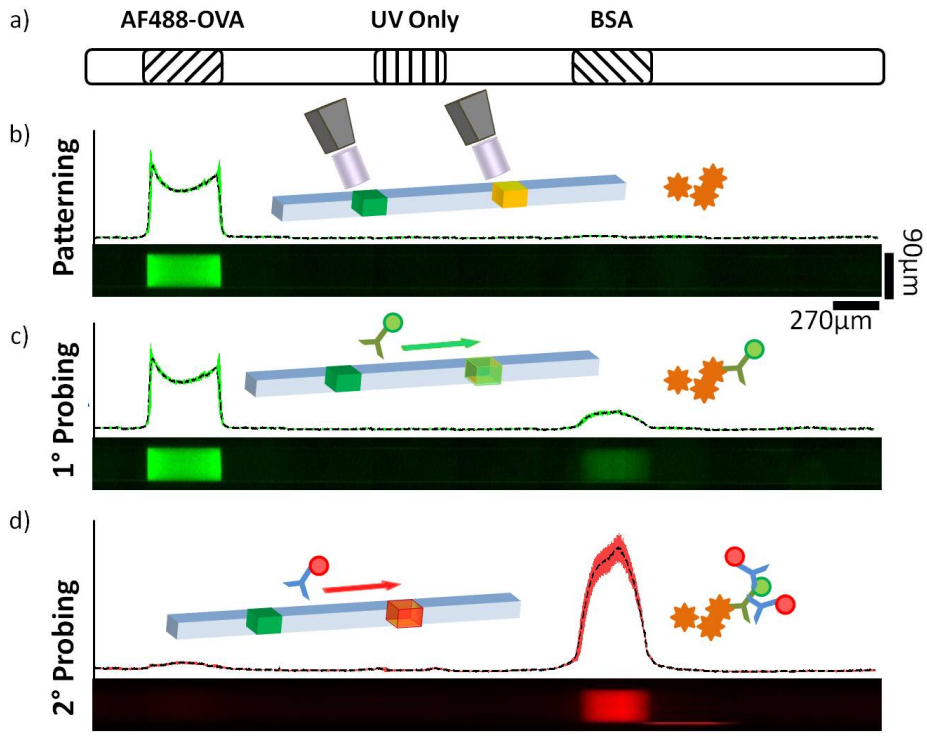

Figure S1. **Validation of protein barcode patterning**. a) Schematic of capture antigen barcode layout. As a negative control, a barcode region exposed to UV illumination with no proteins present is employed. b) Micrograph and line scan of the barcode channel after protein patterning. c) Micrograph and line scan after loading of fluorescently labeled primary antibody. d) Micrograph and line scan after loading of fluorescently labeled secondary antibody.

Increased UV dose decreases fluorescence signal from AlexaFluor-labeled proteins

To scrutinize the impact of UV illumination on the fluorescence signal from AlexaFluor-labeled proteins, individual wells of standard microwell plate were filled with 60uL of BSA-AF488 solution. The microwell plate was a black, flat bottomed, polystyrene 96well plates from Corning. The solution was then illuminated using the Hamamatsu UV source for 0s, 1s, 5s, 10s, 20s, 40s and 60s using the 20% power setting. Each time point was tested in triplicate. The UV exposure durations were selected based on the durations employed in the barcode assay. Fluorescence signal from each microwell containing a 1uM solution of BSA-AF488 was measured using a Tecan Infinite M200 Pro microplate reader before and after UV exposure, as shown in Figure S2.

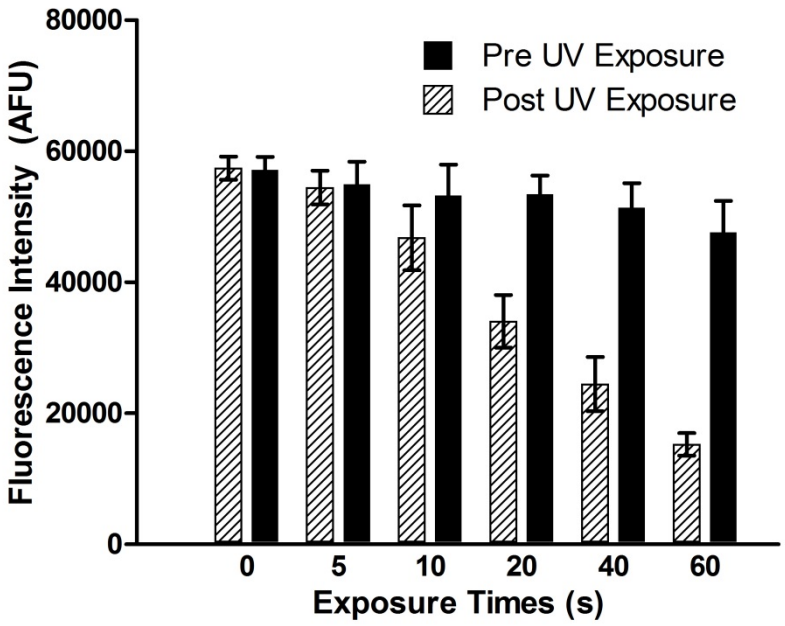

Figure S2. **UV photobleaching of fluorescently labeled proteins**. Microplate reader collected fluorescence signal from AF488-labelled BSA before and after UV illumination shows notable fluorescence signal decrease with increasing UV dose.

While the effective UV dose applied to microwell plate is different than that applied to the microdevice during UV photopatterning, the data support the working conclusion that UV dose can impact fluorescence of the AlexaFluor dyes used here. During photopatterning of the barcode assay, the light guide is placed in proximity to the microchannel (<1mm displacement). For the microwell plate experiment, physical limitations locate the light guide ~9mm from the well floor. Thus, the UV dose applied to the microwells is less than the dose applied to the barcode microchannels. To increase the UV dose in the microwell system, we increased the UV illumination duration to 40s and 60s, yielding increased UV dose and concomitant decrease in AlexaFluor488 signal, as observed in the barcode assay.