Supporting information
Experimental
Materials

Optical fiber bundles (1.4 mm-diameter) containing ~50,000- 3.1 micron diameter fibers were obtained from Schott Fiber Optics, Inc. (Southbridge, MA). Lapping films for fiber polishing were purchased from Allied High-Tech Products (Rancho Dominguez, CA). Amine-modified methylstyrene-divinylbenzene polymer microspheres (3.1-μm diameter) were purchased from Bangs Laboratories, Inc. (Carmel, IN). All oligonucleotide probes used in this study were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Europium (III) theonoyltrifluoroacetonate-3H_2O (Eu-dye) was obtained from Acros Organics (Morris Plains, NJ). Sterile water used to reconstitute oligonucleotide probes was purchased from Abbott Laboratories (North Chicago, IL). Tetrahydrofuran (THF), methanol (MeOH), glutaraldehyde (8% aqueous solution), and avidin were purchased from Sigma-Aldrich (St. Louis, MO). Tris-Starting Block, PBS Starting Block Tween-20, and Protein free (PBS) blocking buffers were purchased from Pierce Biotechnology (Rockford, IL). All reagents were used without further purification. Mouse monoclonal capture antibodies (Abs) directed against human IL-8 (clone 6217), recombinant human IL-8, and biotinylated polyclonal anti-human IL-8 antibodies were obtained from R&D Systems, Inc. (Minneapolis, MN).

Microsphere encoding

Every microsphere on the array is encoded with a unique optical barcode comprised of Eu dye incorporated into each microsphere and then decoded using image-processing techniques to allow positional registration of the entire array (Figure 3 A and B (1)). Encoded microspheres were prepared from 50 μL (5 mg) aliquots of a 3.1 μm amine-functionalized microsphere stock. The aliquots were washed in triplicate with 200 μL PBS and were then washed in triplicate with 200 μL THF. A 200 μL solution of 0.5 M Eu-dye in THF was then added, and the microsphere suspension was shaken in the dark for 2 h at room temperature (RT). The reaction vessel was centrifuged, and the microsphere pellet was washed six times with 200 μL MeOH and then washed six times with 300 μL PBS (0.154 M NaCl, 2.7 mM KCl, 10 mM sodium phosphate and 1.7 mM potassium phosphate, pH 7.4). The encoded microspheres were then suspended in 500 μL PBS with 0.01% Tween-20 and stored at 4 °C in the dark.

Preparation of sensors

A 100 μL aliquot (1 mg) of the encoded microsphere suspension was centrifuged, the supernatant was removed, 1 mL of 8% glutaraldehyde in PBS was added, and then the mixture was shaken at RT for 2 h in the dark. The microspheres were then washed three times with 300 μL of PBS. Subsequently, 45.3 μg of IL-8 monoclonal capture antibodies were added to 1 mg of the glutaraldehyde-activated microspheres (encoded 0.5 M Eu-dye, respectively) suspended in 500 μL PBS.

The microcentrifuge tube containing the mixture was covered with aluminum foil and shaken at RT for 4 h. The microspheres were washed once with 300 μL Tris-Starting Block (blocking buffer), and then were suspended in 300 μL blocking buffer. The suspension was shaken at RT for 30 min in the dark, and then washed once with 300 μL

blocking buffer. The microsphere probes were suspended and stored in 100 µL blocking buffer at 4° C, protected from light.

Microarray Fabrication

The ends of the optical fiber bundles were sequentially polished with 30, 15, 6, 3, 1, 0.5, and 0.05 µm lapping films. The fiber bundles were then sonicated in water for 10 s to remove residue on the finished ends. One end of the polished fiber bundle was chemically etched to form microwells as described previously.[33] Etched fibers were thoroughly rinsed with Nanopure water. The etched end of the fiber bundle was blocked with 200 µL Protein free (PBS) blocking buffer for 30 min. Anti-IL-8 (encoded with 0.5 M Eu-dye) microspheres were loaded into the array by pipetting a 0.5 µL aliquot onto the etched end of the fiber, and allowing the solution to dry for 10 min. The end of the fiber containing the microspheres was then blocked a second time with 200 µL Starting Block Tween-20 (PBS) blocking buffer for 30 min at RT. The fiber was incubated in 100 µL of Starting Block Tween-20 (PBS) buffer containing 10 nM of IL-8 protein, for 2 h, and then rinsed with 1mL Starting Block (PBS) Tween-20 buffer. The fiber was subsequently incubated with 100 µL of a solution containing of 3 µg/mL of biotinylated detection antibody (anti-IL-8) for 30 min and then rinsed with 1 mL of Starting Block Tween-20 (PBS) buffer. After incubation in PBS solution containing avidin (20 µg/ml) for 45 min, the fiber was rinsed with 1 mL of PBS. Subsequently, the fiber was incubated in PBS solution containing 10 µM of biotinylated capture DNA probes for 45 min at RT and then rinsed with 1 mL of PBS. The AND gate microarray was then incubated in PBS solution containing 50 µM of target DNA for 1h at RT and then rinsed with 1 mL of PBS.

In order to employ presented array for protein-DNA and single protein detections, two fluorescent-labeled signal probes were designed for the detection step. Fibers were incubated with 1 µM of the mix solution of Cy3 and Cy5 - labeled signal probes in 50 nM of PBS for 30 min at RT. After incubation, the fiber bundle end was washed once using 1 mL of PBS. Finally, the array was imaged using an epi-fluorescence microscope.

Signal processing

A minimum of 30 beads on each of the fiber surfaces were averaged for each measurement. A pre-set signal threshold, show as a line in Figure 2C and D was defined as the average measurement of three negative control fiber arrays (buffer sample including all assay reagents excluding analytes; target DNA and protein for AND gate and IL-8 protein for INHIBIT gate) +3x standard deviation (SD). Any net signal greater than this threshold was considered a positive response (output 1) while any net signal bellow than this threshold was considered a negative response (output 0).

Imaging system

A custom-built epi-fluorescence imaging system was used to acquire all fluorescence images. The system included a mercury light source, excitation and emission filter wheels (Chroma, Rockingham, VT), microscope objectives (Olympus, Center Valley, PA), and a CCD camera (Orca-ER, Hamamatsu). Filter wheels and shutters were computer-controlled and analysis was performed with IPlab software (Scanalytics, Fairfax, VA). The system was equipped with a chuck to immobilize the fiber-optic bundle. After the microspheres were loaded onto the fiber optic bundle, their

locations on the array were registered using their unique optical barcode that consisted of a defined level of Eu-dye (excitation 360 nm/emission 600 nm) (Figure 3A and B (1)). Cy3 fluorescence (excitation 550 nm/ emission 570 nm) and Cy5 (excitation 649nm/ emission 670 nm) fluorescence (Figure 3A (2) and B (3)) was monitored to evaluate the microsphere assay. Fluorescence intensity values were acquired for encoding, background, and signal images using a 200 ms image exposure time and 20 x magnification. The images were analyzed with IPlab software.