Supplementary Material (ESI) for Lab on a Chip

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Supporting Information

Device Fabrication

The devices were fabricated in a standard microfabrication facility (Microsystems Technology Laboratories of MIT). The nanofluidic channels and through-holes were defined and etched into a Si wafer using projective photolithography (Nikon Precision Inc., CA) and reactive-ion etching techniques to a depth of 40 nm. Following the through hole etching on silicon wafer in a 80^{o}C, 20% KOH solution and silicon nitride stripping, a 500 nm thermal oxide layer was grown to provide an electrical isolation between the conductive silicon substrate and buffer solution. As for the microchannels and dam structure, a 200 nm a-Si layer was deposited on the Corning Pyrex wafer to work as the hard make for isotropic BOE wet etching. The deep trench channels are first patterned and etched by contact photolithography and reactive-ion etching. Following the hard mask etching, a 6.5 μm wet etching was done in diluted HF solution buffered by Nitric acid. After the first step etching, the shallow dam structure was patterned and etched with the same procedure to a thickness of 5.5 μm. This two step etching, as a result, gives a 12 μm deep microchannel with a shallow dam region of 5.5 μm for bead trapping at the predefined location.

At the end, the device was sealed by bonding the Pyrex wafer on the silicon wafer after alignment. The bonded wafers were then cut into individual devices for experiments in a custom-made clamp. The depths and surface uniformity of the nanochannels and wet-etched microchannels were measured by imaging the cross-section of the nanofilter with scanning electron microscopy (JEOL USA, Inc., Peabody, MA) after anodic bonding.

Protein sample preparation

R-phycoerythrin (R-PE, MW 240 kDa, Invitrogen, Grand Island, NY), Recombinant Green Fluorescent Protein (rGFP, MW 27 kDa, Biosciences, Franklin Lakes, NJ), also written as GFP in this paper, biotinylated anti-GFP (Fitzgerald Industry, Concrod, MA), biotinylated anti-R-PE (BD Biosciences, San Jose, CA) were purchased and used as received without further purification and diluted in the sample buffer (10 mM dibasic sodium phosphate buffer with 0.02% sodium azide) prior to the experiment.

Antibody surface immobilization

325 μl Streptavidin bead (Applied Biosystem, Foster city, CA) at concentration of 30×10^{6} beads/ml concentration was mixed with 800 μl dibasic sodium phosphate buffer with 8 μg anti-R-PE and incubated 2 hrs with constant mixing at room temperature. After the incubation, the beads are isolated by a centrifugal molecular weight cut-off filter (Millipore, Billerica, MA) and resuspend in to 1 ml dibasic sodium phosphate buffer. Same procedure was used with anti-GFP and can be a robust and versatile antibody immobilization method for other biotinylated antibodies. These beads were further diluted by 1000 times before loading into the device.

Fluorescent imaging

All the experiments were imaged with an inverted epi-fluorescence microscope (IX-71, Olympus, Melville, NY) equipped with a thermoelectrically cooled CCD camera (Sensicam QE, Cooke Co., Auburn Hill, MI) and a 100W mercury lamp (Chiu Technical Corp., Kings Park, NY). Fluorescence imaging was visualized through either the TRITC filter set (excitation: 562 nm, emission: 624 nm, Semrock, Rochester, NY) or the FITC filter set (excitation: 482 nm, emission: 536 nm, Semrock, Rochester, NY). Sequences or images were analyzed with image processing software (IPLab 3.6, Scanalytics, BD Bioscience, Rockville, MD).

Immunoassay response measurement

Each of the new devices was first loaded with 1% BSA solution for 30 min to prevent nonspecific binding of samples. After coating, diluted beads labeled with anti-R-PE or anti-GFP were loaded into the channel, and a few beads were controlled to prevent stacking between each other (typically 4-6 beads). After bead loading, sample buffer was introduced and incubated with pressure driven flow similar to flow injection analysis experiments. The fluorescent signal was recorded using computer controlled shutter at various intervals to minimize photobleaching on the samples. The signal was then analyzed by threshold algorism (subtracting background signals) to average only signals from the beads.