Supplementary Information for

Biomolecular motor-driven molecular sorter

Experimental

Reagents

80 mM BRB80 was mainly used for a buffer solution, adjusted to pH 6.8 with potassium. 10 μM of Taxol was added into all buffer solutions to prevent microtubule depolymerization. Also, an anti-bleach solution was made by mixing BRB80 buffer with 0.047 mg/ml casein, 0.08 mg/ml catalase, 0.1 mg/ml glucose oxidase, 10 μM glucose, and 10 μM DTT to prevent the photo-bleaching of fluorescently-labeled molecules (all from Sigma-Aldrich). Analytes were prepared by mixing an anti-bleach solution with 2 nM of tetramethylrhodamine (TMR)-labeled streptavidin, 1 mM of ATP, and 0.45 mg/ml of kinesin. Addition of kinesin molecules into the analyte is believed to maintain the surface density of kinesin on nanotracks by reducing the gradient of kinesin concentrations between the bulk analyte solution and the surface.[1]

Kinesin and microtubule preparation

For most experiments we used a bacterially expressed kinesin motor, NKHK560cys. This motor consists of the head and neck domain of *Neurospara crassa* kinesin (amino acids 1-433) and stalk of *Homo sapiens* kinesin (residues 430 to 560) and a reactive cysteine at C-terminal end.[2] Kinesin was expressed and purified as described previously.[3, 4] Tubulin was purified from cow brain by three cycles of microtubule polymerization and depolymerization followed by phosphocellulose ion exchange chromatography, and fluorescently-labelled tubulin (TMR-tubilin) was prepared by reacting polymerized microtubules with a 20 folds excess of tetramethylrhodamine (Molecular Probes) at room temperature for 30 minutes. Labeled tubulin was purified from this mixture by repeated depolymeriztion and polymerization. For all experiments, microtubules were polymerized by incubating 2 mg/ml tubulin, 1mM GTP and 4 mM $MgCl_2$ in BRB80 buffer at 37 °C for 20 minutes. Microtubules were stabilized by the addition of 10 μM taxol. To make biotinylated microtubules, 1 μL of 10 mM of biotin was mixed with 200 μL of polymerized microtubules and then this mixture was incubated about 30 min. To quench free biotin bindings, 2 μL of 1 M of glycine was added to the mixture, followed by additional 10 min incubation. Finally, biotinylated microtubules were purified by repeatedly running a high speed centrifuge process consisting of discarding the supernatant and resuspending the pellet. All motility assays were carried out in BRB80 buffer at room temperature.

Nano-/micro-fabrication

Several nano-/micro-fabrication techniques were developed to build the device. The technique consists of creating nanotracks on glass substrates through direct nanoimprinting of a cyclized perfluoropolymer called CYTOP™, and a polymer inking

method for bonding CYTOP^{TM} nanotracks with the microfluidic channels. In the microfluidic part, the standard microfabrication procedure was used to etch 20 μm deep and 50–100 μm wide microchannels on glass substrate (the top side, including the collector) (see Fig. S1). In parallel, CYTOP^{TM} nanotracks on glass coverslips (the bottom side) were fabricated via nanoimprint lithography (NIL). The mold used for NIL has nanoscale grating structure with 700nm in period and 50% of duty cycle made by interference photolithography and dry etching. Such dimension allows us to create 350nm-wide track-arrays separated by 350 nm-wide CYTOP^{TM} barriers. Since CYTOP^{TM} (Asashi, Japan), a type of Teflon, has a good adhesion to glass substrates but low differential protein binding compared to glass. The imprinting temperature and pressure was 150 °C and 600 psi, respectively. After mold separation, SF_6 reactive ion etching (RIE) was applied to remove CYTOP^{TM} residuals in the nanoscale trenches and expose glass surface (see Fig. S1 step B1–B3). The more detailed process of nanotracks and its guiding efficiency of microtubule are found in our previous study.[5]

To integrate microfluidic channels with CYTOP^{TM} nanotracks, a polymer transfer bonding technique was used to bond these two parts. As illustrated in Fig. S1, a 500nm thick SU-8 layer was first spun on an oxygen-plasma treated PDMS (polydimethylsiloxane) stamp and, afterward, transferred on the microfluidic chip. Because the surface energy of PDMS stamp is relatively low and SU-8 layer is thin enough, it is possible to selectively transfer SU-8 on top of the microfluidic chip when these two pieces were put into physical contact. After baked at 80 ºC for 2 minutes, the SU-8 topped microfluidic chip was then bonded onto the CYTOP^{TM} grating chip at 75 ºC at 300 psi for 2 minutes. After cooling, the bonded chip was cured by flood UV exposure and hard-baked at 95 ºC for 5 minutes. Since the glass transition temperature (T_g) of CYTOP^{TM} (T_g=110 ºC) is higher than the T_g of SU-8 (T_g=75 ºC), this low temperature polymer transfer bonding was capable of sealing the microfluidic channels and nanotracks by leaving CYTOP^{TM} nanotracks intact. Finally, glass pipettes that were connected on the device by using UV-glue and epoxy served as external reservoirs for loading or draining of analyte solutions and biomolecular motor proteins. A similar microfabrication technique is also found in our previous study.[1]

Experimental procedure

Initially, each 200 μL of BRB80 buffer solution was injected into all reservoirs except ‘waste’ reservoir and then vacuum pressure of ~50 kPa was applied to the ‘waste’ reservoir for 20 min using a hand vacuum pump with gauge (S94224, Fisher Scientific) to clean the channels. To help kinesin adsorb better on channel surfaces and prevent nonspecific binding of target molecules, all channels were flushed with 200 μL of 0.14 mg/ml casein by repeating the previous injection procedure with the same pressure, followed by allowing it to incubate for 5 min. And then, to adsorb kinesin on the nanotracks at a higher density, 100 μL of 0.45 mg/ml of kinesin was injected into all reservoirs except the microtubule outlet reservoir and then vacuum pressure of ~25 kPa was applied to the outlet reservoir for 20 min, followed by 5 min incubation.

Subsequently, 200 μL of an analyte solution was injected into the analyte reservoir in the absence of additional, external pressure. The analyte required about 5 min to arrive at the intersection of the device because the hydrostatic pressure of the reservoir (~100 Pa) produced about 100 μm/s flow speed, which was determined when a microfluidic channel network was designed and fabricated. Finally, functionalized, unlabeled microtubules were injected into the microtubule inlet reservoir and then observations were made using an inverted epifluorescence microscope (Axiovert 200, Carl Zeiss Microimaging, New York, USA) with a 40x oil immersion objective to obtain fluorescent images via a digital CCD camera (Orca ER II, Hamamatsu, Japan). After experiments, quantifications were conducted using Image J.

Figure

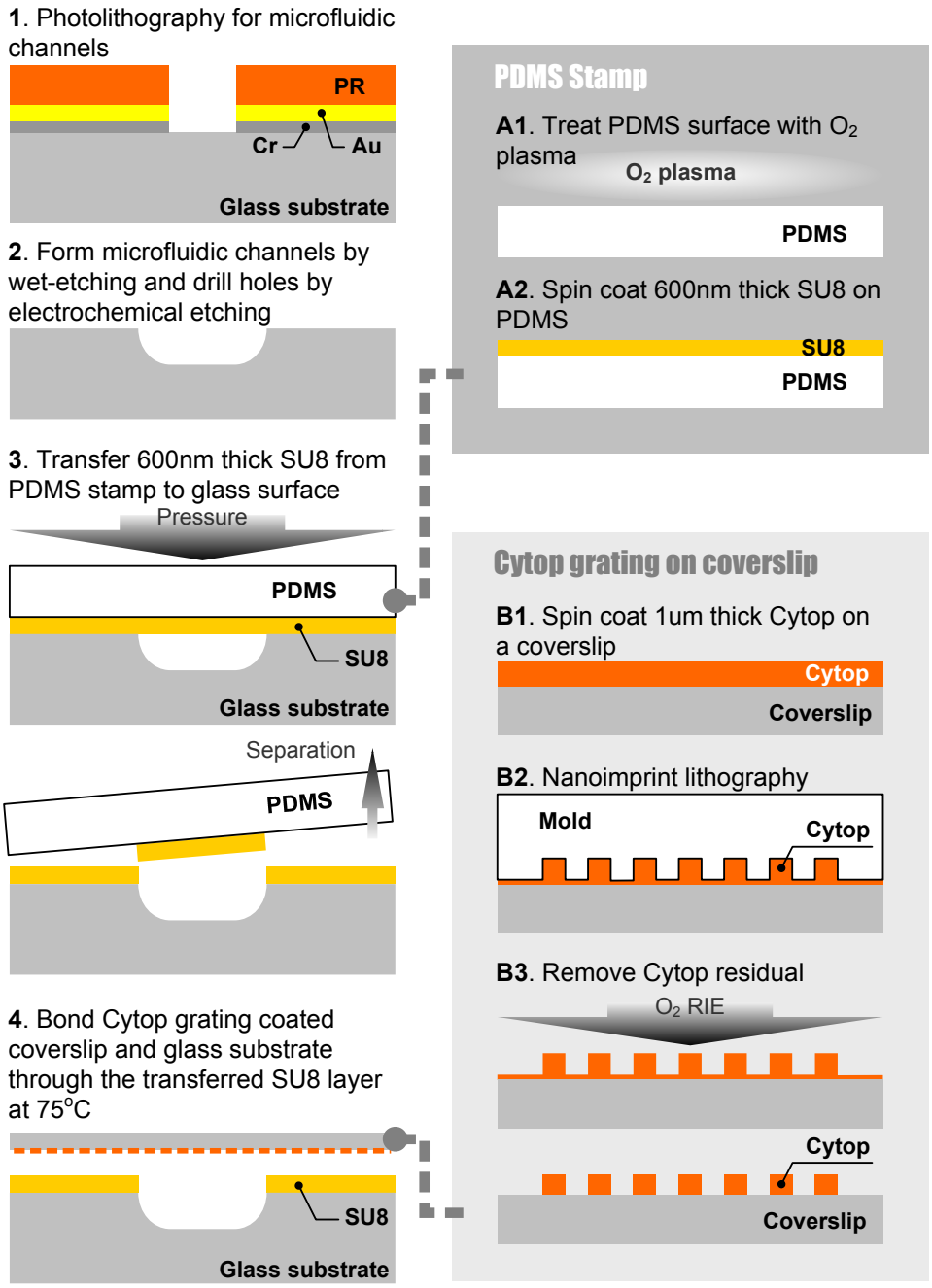

Figure S1. Step 1 and 2 illustrate the procedure of the microchannel fabrication. As the top substrate, microfluidic channels are HF-etched and electrochemically drilled to access the microchannels. In step 3, SU-8 thin film is selectively transferred to the top of microfluidic substrate and served as an adhesive bonding with glass coverslips. This process is detailed in step A1 and A2. As the bottom substrate, CYTOPTM nanotracks

on a glass coverslip are made via nanoimprinting lithography (step B1–B3). Lastly, the top and bottom substrates are bonded as illustrated in step 4.

Reference

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