Supporting Information

Open-tubular Nanoelectrochromatography (OT-NEC): Gel-free Separation of Single Stranded DNAs (ssDNAs) in Thermoplastic Nanochannels

Charuni A. Amarasekara,[1,2] Uditha S. Athapattu,[1,2] Chathurika Rathnayaka,[1,2] Junseo Choi,[2,3] Sunggook Park,[2,3] and Steven A. Soper[1,2,3,4,5,6, *]

[1]Department of Chemistry, The University of Kansas, Lawrence, KS 66045

[2]Center of Biomodular Multiscale Systems for Precision Medicine

[3]Department of Mechanical Engineering, Louisiana State University, Baton Rouge, LA 70803

[4]Department of Mechanical Engineering, The University of Kansas, Lawrence, KS 66045

[5]Bioengineering Program, The University of Kansas, Lawrence, KS 66045

[6]KU Cancer Center, University of Kansas Medical Center, Kansas City, KS 66160

Table S1. Conditions used to imprint nanochannel devices for two different PMMA substrates using the Nanonex 2500 NIL instrument.

Material	Tg /ºC	Imprinting temperature (ºC)	Pressure (psi)	Time (s)	Depth of channel (nm)
IM-PMMA	105	130	300	300	112 nm
NIM-PMMA	122	140	300	300	115 nm

Table S2. Bonding conditions used to assemble nanochannel devices imprinted for two different PMMA substrates with a COC 8007 cover plate. The devices were made via NIL using the Nanonex 2500 machine. Both cover plate and substrate were O_2 plasma treated prior to bonding.

Substrate-coverplate	Bonding conditions (°C, psi, min)
IM-PMMA-COC	**70, 110, 15**
NIM-PMMA-COC	**70, 100, 15**

Detection system for the nanoelectrochromatography. All fluorescence imaging was done using a Nikon TE2000 inverted microscope (Nikon, Minato-ku, Tokyo, Japan), which was equipped with a 100x oil immersion objective (NA 1.3). A Keplerian beam expander (L1, L2) was used to expand the Gaussian laser beam to completely backfill the microscope objective. The lower intensity wings were knocked out using an iris to ensure uniform laser intensity within the microscope's field-of-view. Kohler epillumination was used to focus the laser beam to the back of a microscope objective to allow complete irradiation of the active area of the CCD camera (L4). The excitation beam was passed through a 532 nm laser line filter (F1) and dichroic filter (DF). The emission signal was collected by the microscope objective, passed though the dichroic filter, spectrally selected using a long pass filter (F2) and band pass filter (F3), and imaged onto an iXon 897 EMCCD camera (Andor Technologies Ltd, Belfast, United Kingdom), which was controlled by Metamorph software (see Figure S1).

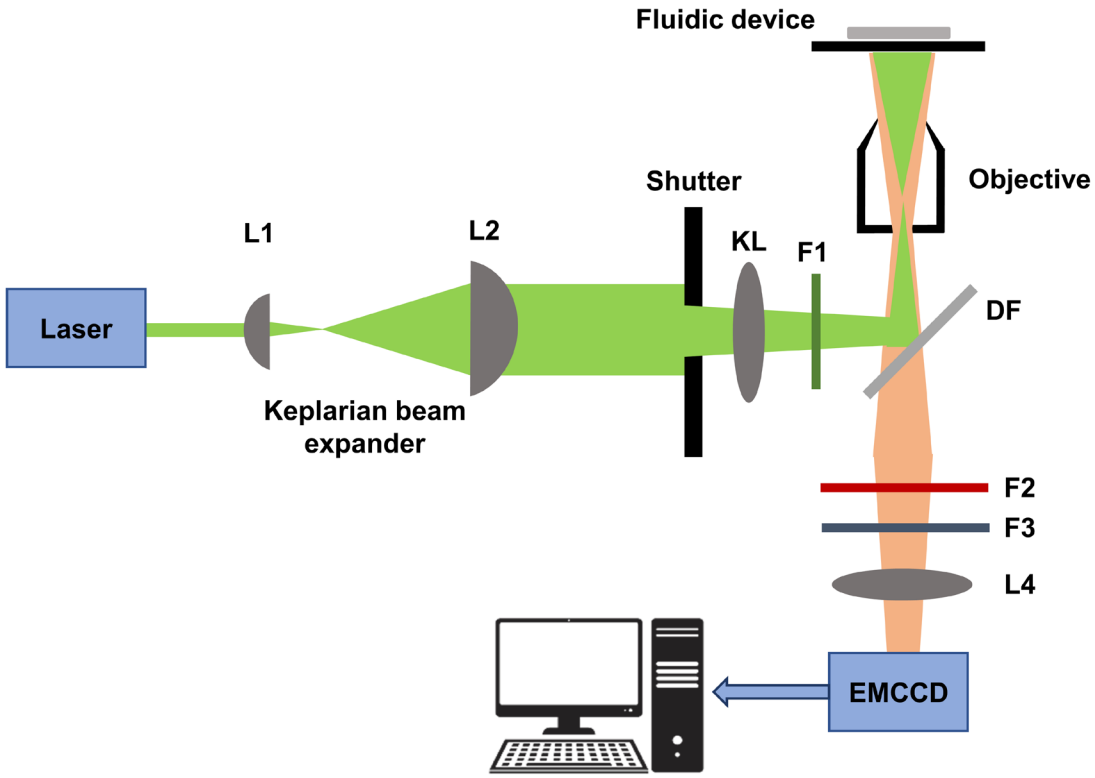

Figure S1. The optical setup of the fluorescence imaging system. The Gaussian beam from the laser (Nd:YAG; λ_{ex} = 532 nm; P = 0.01-5 W; 2.2 mm beam diameter) was expanded 10x using a Keplerian beam expander and the wings knocked out with an iris that ensured uniform laser intensity across the field-of view and also to completely backfill the objective (OBJ). The beam was focused into a 100x oil immersion objective.

Data analysis. ImageJ software[56] was used to analyze the fluorescence intensity generated by the ATTO 532-labeled ssDNAs as they migrated through the plastic nanochannels. Videos were collected using the imaging microscope's EMCCD camera and imported into ImageJ software. The time for the dye front to move between two detection windows placed at the entrance and exit of nanochannel was determined. The data was then exported into Origin 8.5, where it was smoothed and the time of the fluorescence front reaching the entrance (peak 1) and the exit (peak 2) evaluated. The time difference (Δt) between peaks 1 and 2 was taken as the migration time (see Figure S2) for each ssDNA.

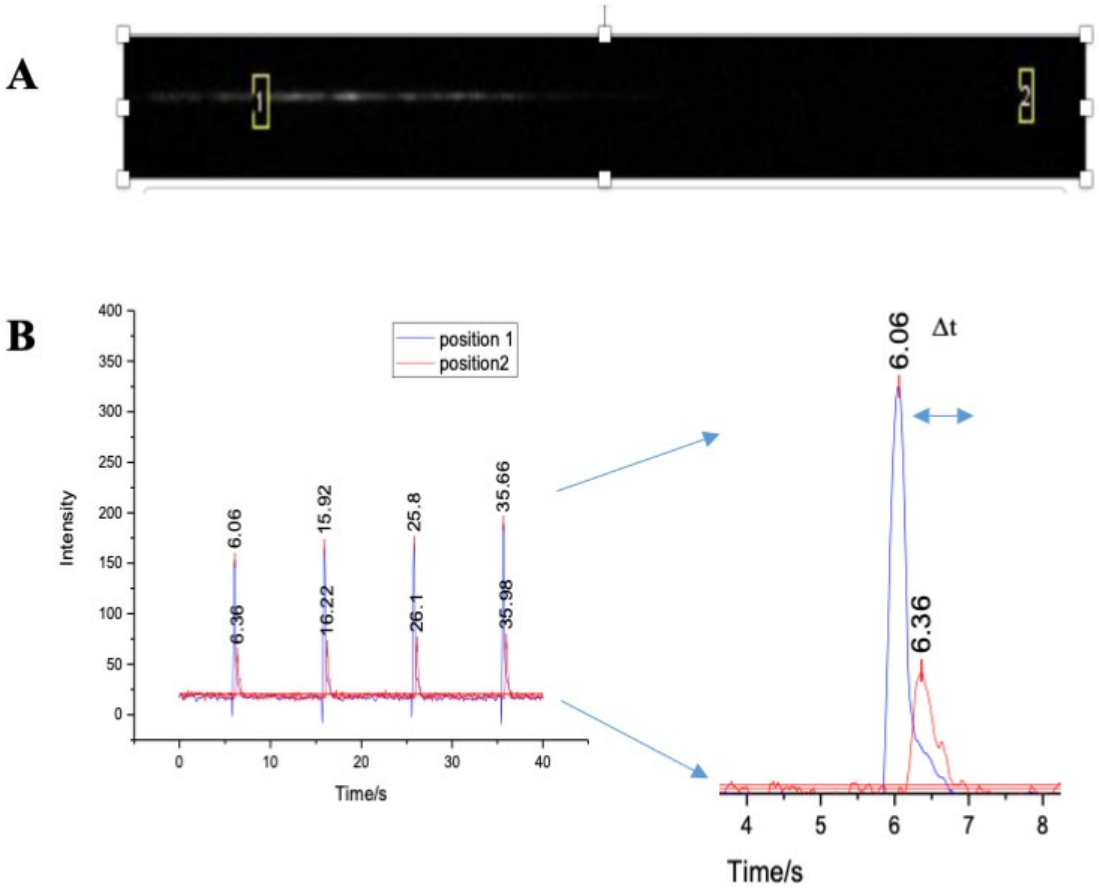

Figure S2. Representation of how the nanochannel electrochromatography and data analysis were performed. **A**) A picture of a fluorescently labeled ssDNA migrating through a thermoplastic nanochannel. The yellow boxes indicate Positions (Pos) 1 and 2. **B)** The fluorescence intensity profile of one injection event was enlarged at Pos1 and 2.

The time difference was used to calculate the effective mobility using equation (S1);

$$\mu_{eff} = (/L/\Delta tV) \qquad \text{(S1)}$$

where *l* is the distance between two detection windows, *L* is the total nanochannel length, and *V* is the applied voltage (Figure S3).

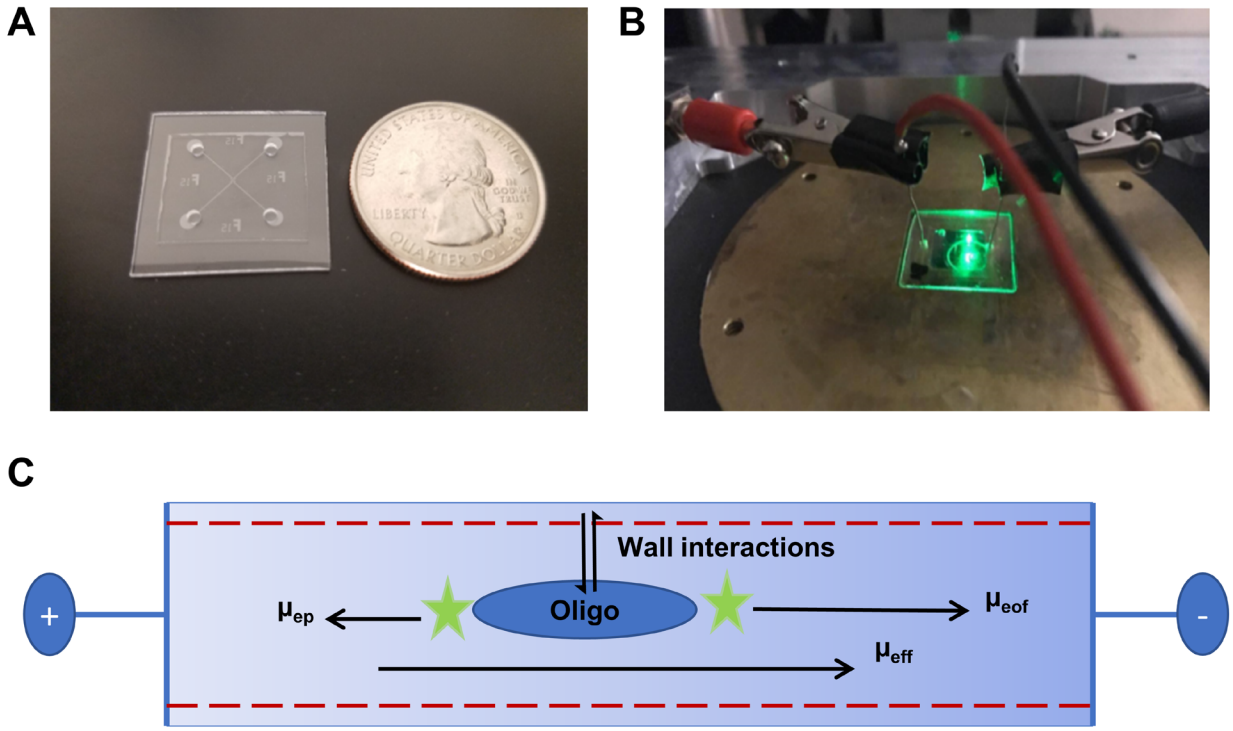

Figure S3. A) Image of a PMMA/COC nanochannel device (100 nm x 100 nm x 107 μm in depth, width, and length, respectively). **B**) Experimental setup for nanoelectrochromatography experiment where anode (red) is at the sample input reservoir and the cathode as the waste reservoir. **C**) Schematic of a nanochannel when an external electric field was applied. Electroosmotic flow (μ_{eof}) was from anode to cathode while the electrophoretic mobility (μ_{ep}) of negatively charged dye-labeled ssDNA was toward the anode. In addition to μ_{ep} and μ_{eof}, the ssDNA can interact with the wall of the channel giving rise to μ_{eff}.