

## **Supplementary material**

### **Droplet-based *in situ* compartmentalization of chemically separated components after isoelectric focusing in a Slipchip**

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#### **Material compatibility**

PMMA is a transparent, low cost thermoplastic polymer with good biocompatibility. PMMA is generally not transparent to UV light, but the PMMA sheets used to fabricate the device have a light transmission ratio of 50% at 275nm, and 80% at 290nm, meaning that they can be used for the fluorescence observation with DAPI filters (Figure S1). After coating with Parylene C and Duxcoat, the light transmission was reduced to 70% at 290nm – see Figure S2. The micro machined channel has almost the same optical properties as the raw PMMA. The optical transmission was 83.9% after machining, increasing to 96.7% after reflow with chloroform.

FC-40 oil is a perfluorinated, colourless, biocompatible and thermostable oil widely used in droplet microfluidics. Material compatibility tests were performed in acid (pH = 1) and basic (pH = 13) environments. After overnight (16 hours) immersion of PMMA in acid/basic buffer, no change in material properties was observed.

After chip fabrication and surface treatment, it was observed that the surface contact angle increased from 62.3° to 114.2° (Figure S3). Rhodamine fluorescence dye was used to test whether there is leakage in between the plates. 100mM Rhodamine was added to IEF buffer and injected into the channels at various flow rates. Leakage was observed only when the flow rate was increased to 165(±12)µL/min.

#### **HPMC to prevent pH gradient compression**

pH gradient compression is a phenomenon whereby the pH gradient is compressed into the central part of the separation channel. Compression can occur because the electrolyte diffuses into the sample and the electrolyte/buffer boundary moves towards the centre of channel. pH gradient compression is a common problem in microchip IEF [1, 2], but increasing the viscosity of electrolyte was reported to prevent this gradient compression problem [1, 3, 4]. Such an approach was adopted in this work.

Hydroxypropyl methylcellulose (HPMC) at different concentrations was added to increase the viscosity of the electrolyte. In an ideal case, the theoretical distance of the focused pI 4.0 and pI 8.1 peaks should be 2.9 cm in our system. Without HPMC in the electrolyte, the pI 4.0 and pI 8.1 peaks were measured to be 1.3 cm apart, 44.8% of the theoretical distance (Figure S6a). With 1% (w/v) HPMC added to the electrolyte, the distance increased to 1.8cm, 62.1% of the theoretical number (Figure S6b). At 3% (w/v), the distance of the peaks was 2.8cm, 96.6% of the theoretical (Figure S6c). No improvement was observed with further increase in HPMC concentration.

#### **Current calibration**

IEF Slipchips with three different channel dimensions were prepared. The width and length of channels were fixed at 250 $\mu\text{m}$  and 5cm, while the depth varied from 175 $\mu\text{m}$  to 250 $\mu\text{m}$  and 275 $\mu\text{m}$ . Three electric field strengths, 100V/cm, 150V/cm, and 200V/cm were used and the current in the channel was measured. Figure S5 shows a histogram of peak current for the different experimental conditions. As expected the larger channel and higher field strength gives the highest current. The average resistivity was calculated at 3.51 ( $\Omega\cdot\text{m}$ ), and the relative standard deviation of the peak current was smaller than 2.9% in all of the experiments.

### **IEF focusing in channel**

The focusing accuracy of the fluorescence pI markers was calibrated by scanning the whole channel. One scan was performed every minute for the first 5 minutes and once every 5 minutes thereafter. Figure S6 shows that at the beginning the fluorescence intensity was uniformly distributed along the channel; over the following 30 minutes, the pI markers gradually moved and focused to their pIs along the pH gradient. At the beginning of the separation, the current was at its peak value (Figure S7), falling during the focusing to a stable value after 30 min.

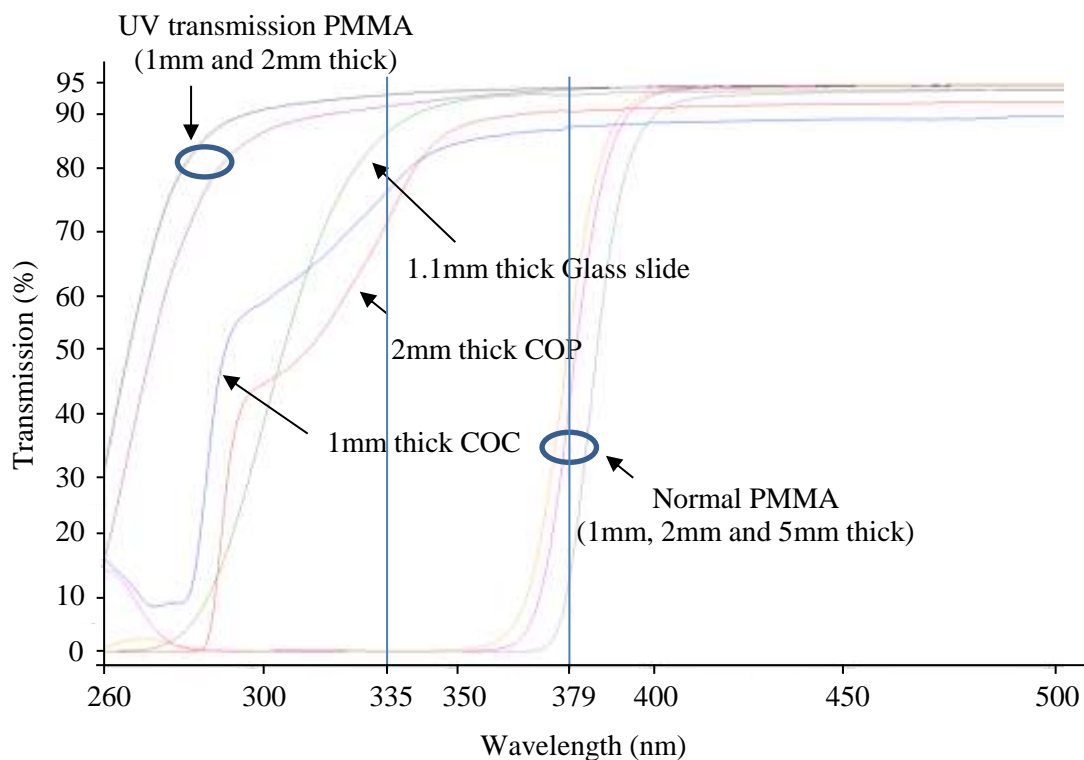


Figure S1. Light transmission for normal PMMA, COC, COP, glass slide and UV transparent PMMA.

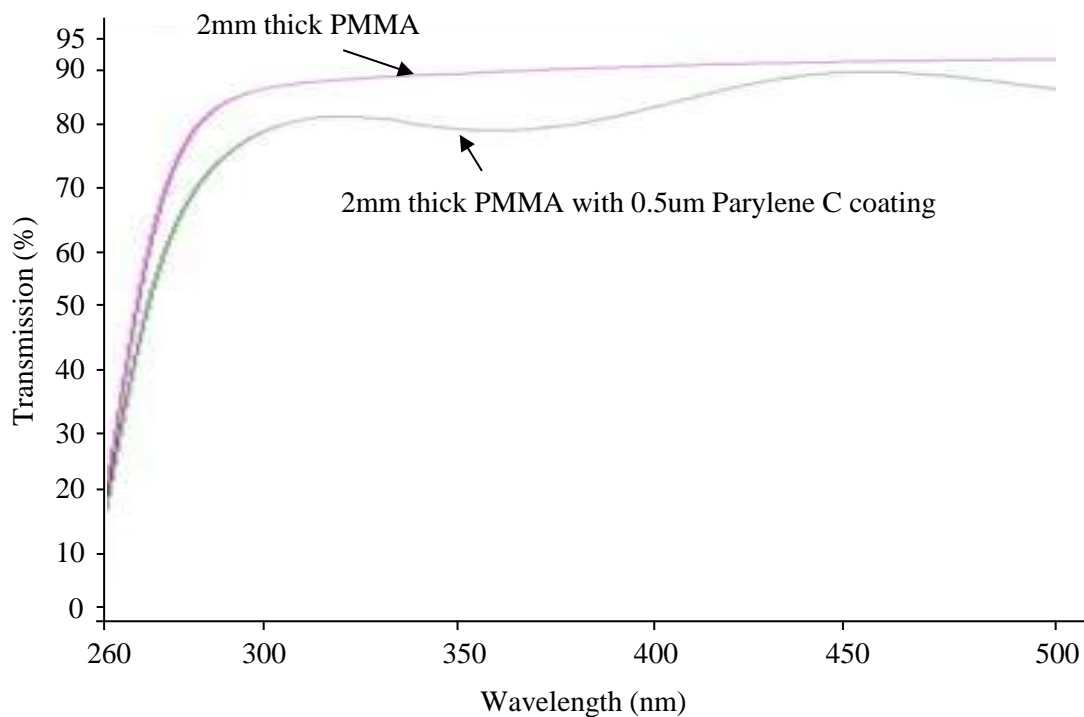


Figure S2. Light transmission for 2mm thick UV transparent PMMA with and without a 500nm thick Parylene coating.

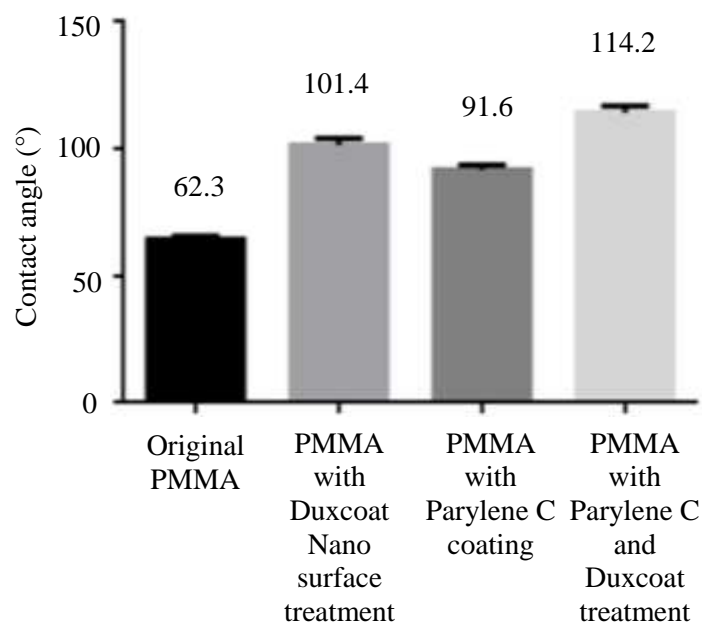


Figure S3. Surface contact angles of the PMMA device after different surface treatment. (A) Original PMMA; (B) PMMA with Duxcoat Nano coating; (C) PMMA with 500nm thick Parylene C coating; and (D) PMMA device with Duxcoat Nano coating on top of a 500nm thick Parylene C coating.

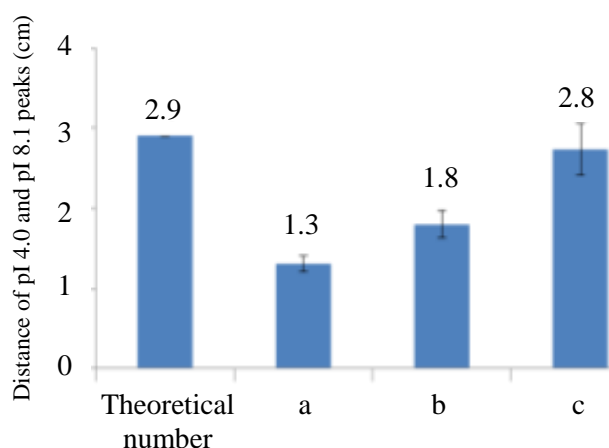


Figure S4. Calibration of HPMC to prevent pH gradient compression in slipchip. After IEF, the theoretical distance of the focused pI 4.0 and pI 8.1 peaks was 2.9 cm. (a) Without HPMC in the electrolyte, the pI 4.0 and pI 8.1 peak was 1.3 cm; 44.8% of the theoretical distance. (b) With 1% (w/v) HPMC added to the electrolyte, the distance of the pI 4.0 peak and pI 8.1 peak was 1.8cm, 62.1% of the theoretical. (c) With 3% (w/v) HPMC added in the electrolyte, the distance of the peaks was 2.8cm, 96.6% of the theoretical. The channel dimension was 250 $\mu$ m wide, 250 $\mu$ m deep and 5cm long; the field strength was 100V/cm, and the focusing time was 30 minutes.

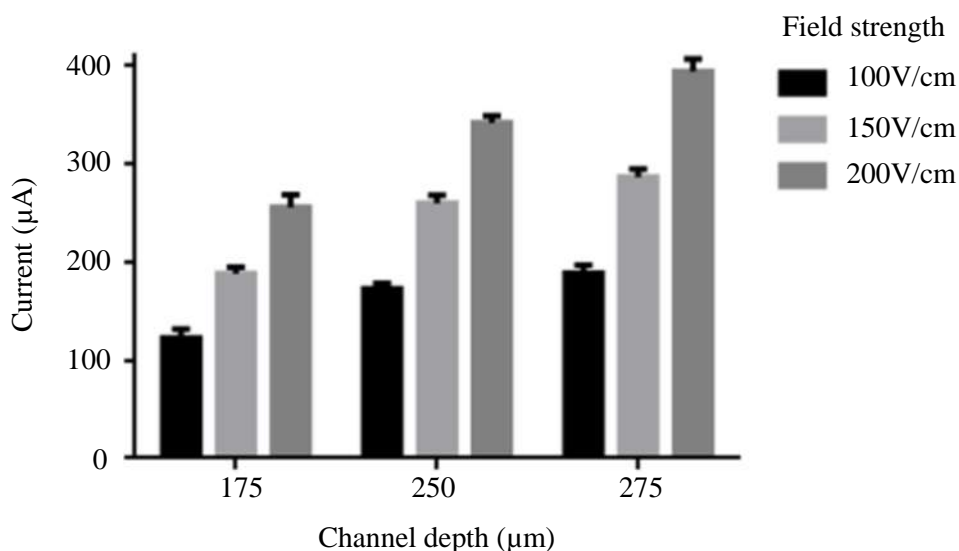


Figure S5 Current calibration in the channel of 5cm length, and 300μm in width for different depths. The electric field strength was varied from 100V/cm to 200V/cm

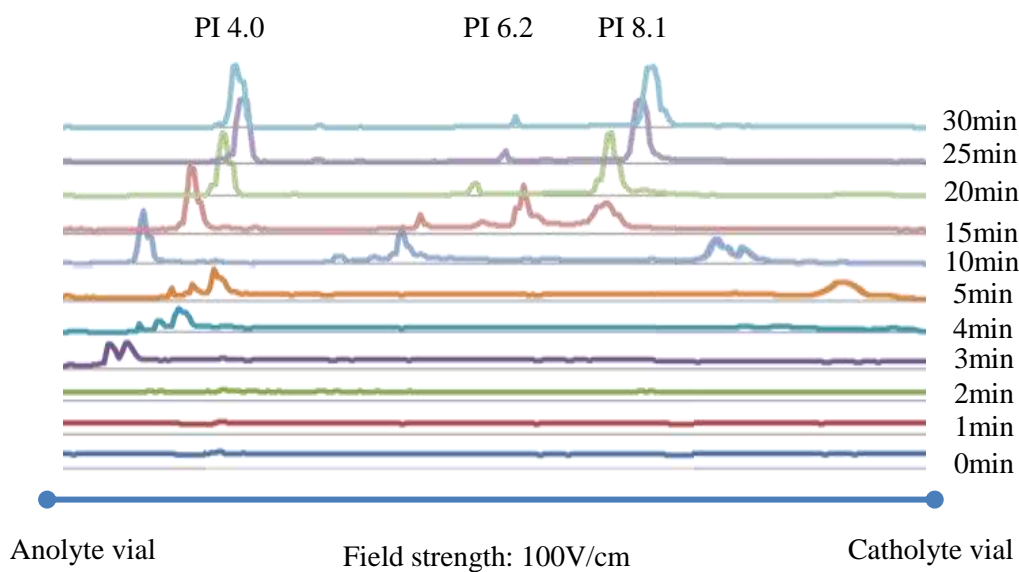


Figure S6. Time traces of fluorescence intensity along the separation channel. Three fluorescence pI markers (pI 4.0, 6.2 and 8.1) were used. The channel dimension was 250μm wide, 250μm deep and 5cm long; the field strength was 100V/cm.

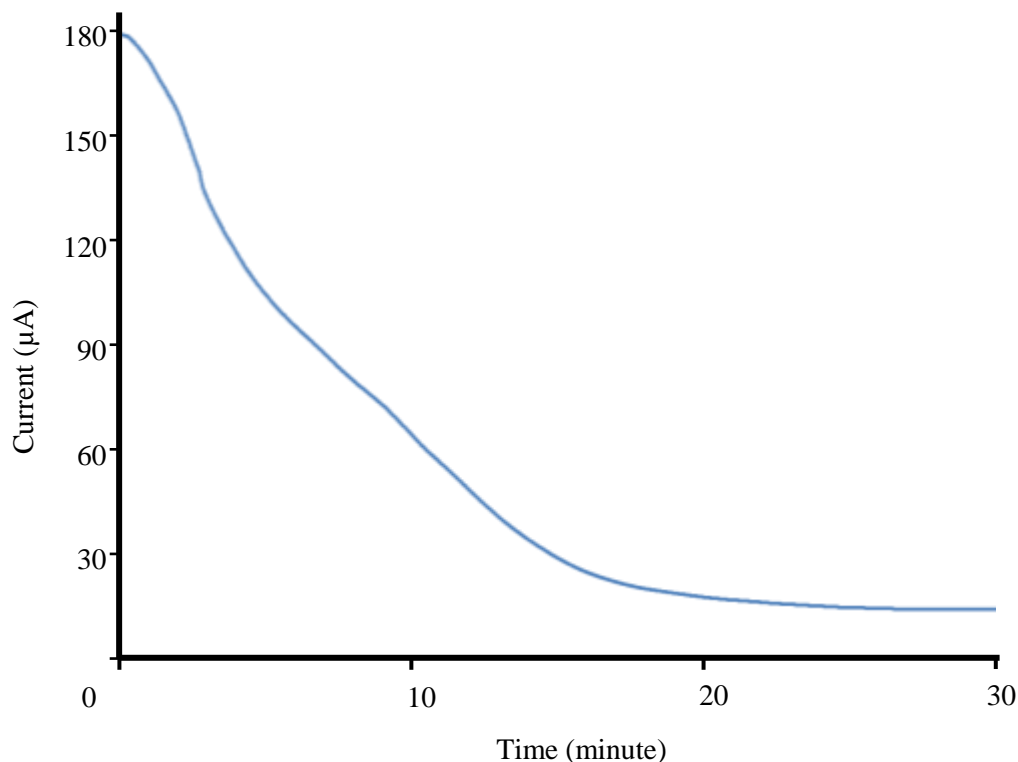


Figure S7. Current-time curve for IEF separation with an electric field of 100V/cm. The channel dimension was 250µm wide, 250µm deep and 5cm long.

### References

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