Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Extraction of electrokinetically separated analytes with on-demand encapsulation

X.F. van Kooten[a,b], M. Bercovici*[b,c] and G.V. Kaigala*[a]

[a]IBM Research – Zurich, Rüschlikon, Switzerland

[b]Technion – Israel Institute of Technology, Haifa, Israel

[c]The University of Texas at Austin, Austin, Texas, USA

S1. Encapsulation of multiple analyte zones using 'cross-over' geometry

Figure S1 presents a geometry that can be used to encapsulate multiple analyte zones with low dispersion of analyte zones. The lateral flow of the oil phase during encapsulation of a single analyte zone (Fig. 1 of the main text) leads to dispersion of adjacent analyte zones when encapsulating multiple analytes. An example of this can be seen in Figure 4c, where the (initially uniform) analyte band a_2 is dispersed to a parabolic concentration profile during encapsulation of analyte band a_1. The geometry shown in Figure S1 overcomes this issue by providing a 'cross-over' path directly opposite the injection points, preventing lateral displacement of the oil. To prevent loss of passing analyte into the south side channels due to residual hydrostatic pressure, these channels should preferably be filled with an oil phase, which can merge with the oil injected during encapsulation.

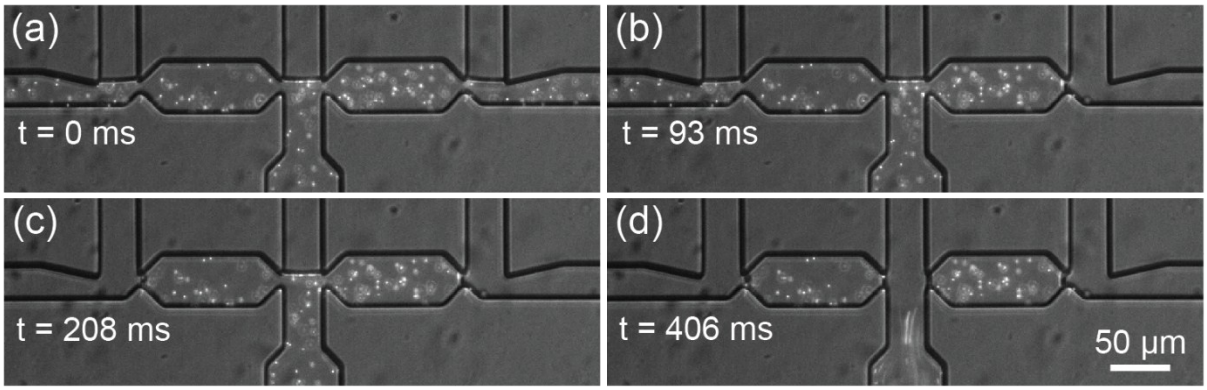

Figure S1. Experimental results showing oil injection in a cross-over geometry for encapsulation of multiple aqueous segments. The addition of a 'south' channel opposite the central side channel enables injection of oil without displacement of the analyte in the encapsulation regions. To prevent loss of the analyte into the south channel during electromigration, this channel can be filled with oil. In the image, the aqueous phase is LE (200 mM bistris, 100 mM HCl, 1% PVP) containing 0.01% red fluorescent beads

(Fluoro-Max 1 µm diameter, Thermo Fisher), and the oil phase is Fluorinert FC-40 containing 2% v/v PFO. The applied pressure was

When using the cross-over geometry to encapsulate multiple segments shown in Figure S1, ejection of analyte droplets from the encapsulation zones can be challenging. Merging of the droplets during extraction may occur when droplets come into contact during ejection. Figure S2 shows how a dedicated ejection channel can be added to each encapsulation region to overcome this issue.

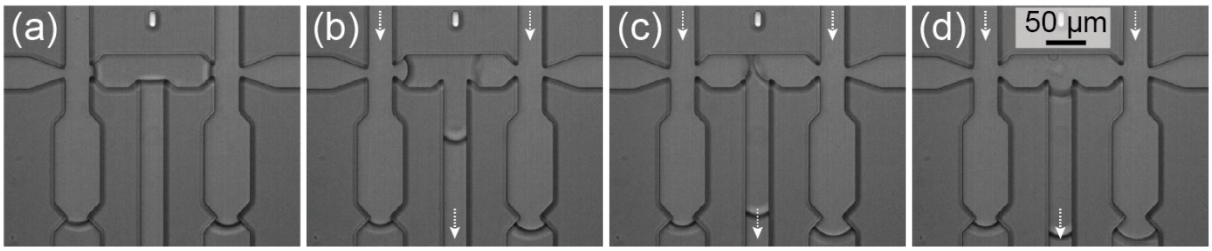

Figure S2. Experimental results showing ejection of an aqueous droplet from the encapsulation region in the center through a dedicated ejection channel in a cross-over geometry. The ejection channel prevents merging of analyte droplets in a multiple-encapsulation geometry. The analyte droplet is ejected by applying an equal pressure (10 mbar) to the left and right north channels, and a vacuum (-30 mbar) to the bottom channel. The pressure difference between either of the north channels and the ejection channel should exceed the Laplace pressure of the second capillary stop to allow the oil interface to enter the encapsulation region. In the image, the aqueous phase is LE (200 mM bistris, 100 mM HCl, 1% PVP) and the oil phase is Fluorinert FC-40 containing 2% v/v PFO.

S2. Experimental setup

Figure S3 presents the experimental setup used to perform on-demand encapsulation. We used a high-voltage power supply (Keithley 2410) connected to a platinum anode and cathode to apply the focusing and separation voltage. We performed the encapsulation using a pressure source (Fluigent MFCS-EZ) connected to two-way valves (Fluigent ESS 2-SWITCH OEM). We achieved fast switching times (~80 ms) by applying a constant pressure to one inlet of the valve and keeping the other at atmospheric pressure. We used a custom Matlab script to control the HV source, pressure source and valves.

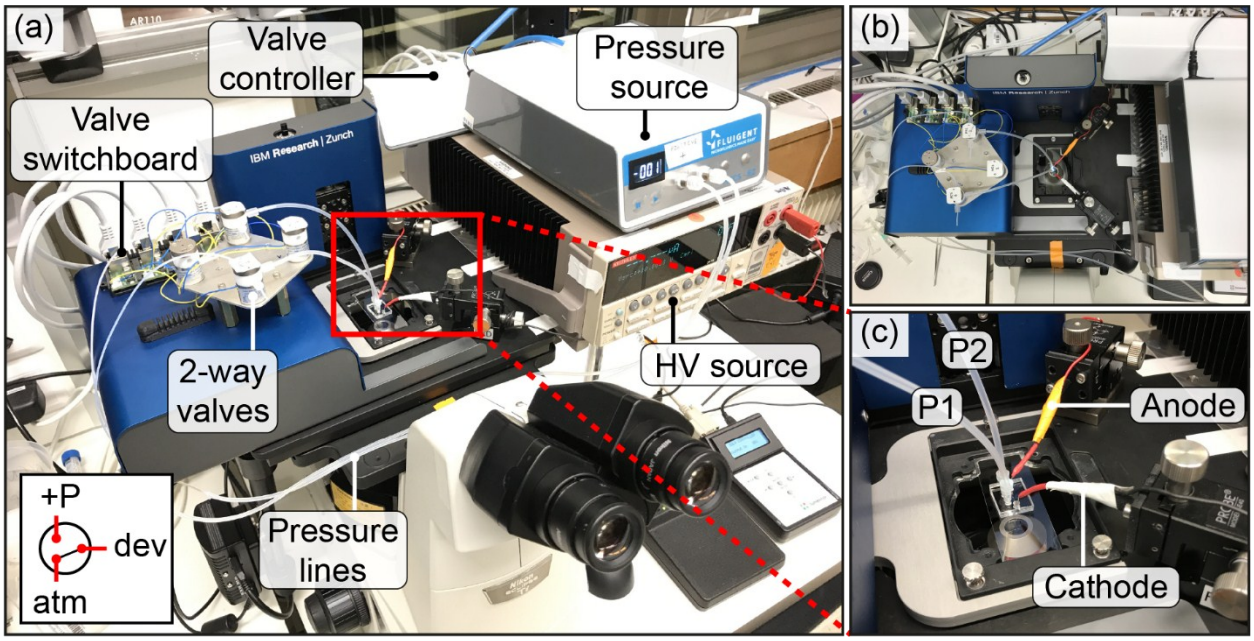

Figure S3. Photograph of the experimental setup on an inverted fluorescence microscope (Nikon Ti-E). The inset in the lower left corner shows the configuration of the two-way valves, with one inlet connected to the positive pressure source (+P), and the other at atmospheric pressure (atm). When triggered, the valves rapidly switch between the atmospheric pressure inlet and the positive pressure inlet. The outlet of the valves is connected to the chip by flexible silicone tubing, and is interfaced with the chip *via* a cut pipette tip to facilitate removal and replacement of the oil in the side reservoirs.

S3. Synchronization of valve triggering

Figure S4 presents a time series in the case where only one of the capillary valves bursts. Despite the imbalanced injection of oil, the second capillary valve (B-C) holds the injected oil interface, preventing it from displacing the focused ITP interface. This behavior relaxes the timing constraints on triggering the capillary valves, as even a large difference in triggering time between the left and right valves does not lead to any dispersion of the interface.

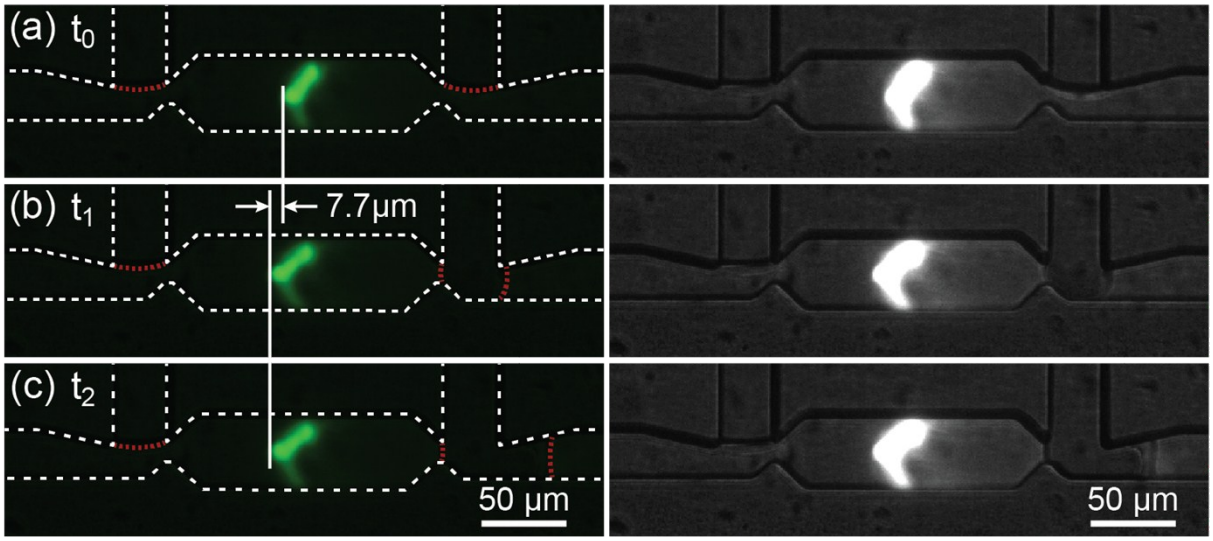

Figure S4. Experimental results showing low analyte displacement in case of a synchronization delay between the two capillary valves. (a) ITP focusing of genomic DNA in an encapsulation device. (b) After 78 ms, the right capillary valve is burst before the left capillary valve, causing the oil to enter the primary channel only on the right side of the encapsulation region. (c) At $t_2 - t_0 = 200\ ms$ the second capillary valve, in the primary channel, prevents the oil from entering the encapsulation region and displacing the analyte. The total measured displacement of the analyte is just 7.7 µm, which is similar to the orifice width on either side of the encapsulation region (7.5 µm). The LE consists of 200 mM bistris, 100 mM HCl and 1% PVP, and the TE is 20 mM bistris and 10 mM tricine. The analyte is genomic DNA from MCF-7 cells, diluted to 350 pg/µL in TE and visualized at the ITP interface by 1× SYBR Green I in the LE, and the oil phase is Fluorinert FC-40 with 10% v/v PFO. The electric field for ITP focusing is 100 V/cm.

S4. Cell culture and extraction of genomic DNA

To extract genomic DNA from cells, we first cultured MCF-7 cells to >80% confluency, and then incubated them in 1 mL trypsin for lysis. After 5 min we stopped the lysis by adding 5 mL complete DMEM medium (DMEM, 1% PenStrep and 10% fetal bovine serum), and centrifuged the suspension. We then used a MiniElute column (Qiagen) according to the manufacturer's instructions to extract genomic DNA, and eluted the DNA in 100 µL. Finally, we incubated the DNA solution at room temperature for 5 min before transferring it to -20 °C for storage.

S5. PCR Amplification

We performed two rounds of PCR: the first to produce amplicon from genomic DNA using β-actin primers, and a second to detect the presence of β-actin amplicon and any genomic DNA, which we tested using BRAF15 primers.

For the first PCR, we added $2\ \mu L$ of $350\ pg/\mu L$ genomic DNA (gDNA) from cultured MCF-7 cells to $5\ \mu L$ 2× Rotor-Gene SYBR Green master mix, $2.6\ \mu L$ RNAse-free water and $0.2\ \mu L$ of $10\ \mu M$ forward and reverse primers per reaction. The primers for β-actin were GGATGCAGAAGGAGATCACT (forward 5'-3') and CGATCCACACGGAGTACTTG (reverse 5'-3').

For the second PCR, we added $5\ \mu L$ 2× Rotor-Gene SYBR Green master mix and $4.6\ \mu L$ RNAse-free water to the vial containing the oil and the analyte droplet. For multiplexed reactions, we adjusted the volumes of master mix and water accordingly. We then centrifuged the vial twice for 10 s at 6000 rpm (2000×g), vigorously mixing the contents by pipetting in between centrifugation steps. We found centrifugation to be critical for breaking the emulsion. After mixing, we split the master mix containing analyte into 9.6 uL reaction volumes and added $0.2\ \mu L$ of $10\ \mu M$ forward and reverse primers to each reaction vial before running the PCR reaction. The primers for BRAF15 were TCATAATGCTTGCTCTGATAGGA (forward 5'-3') and GGCCAAAAATTTAATCAGTGGA (reverse 5'-3').