**Supporting Information**

# Ion concentration polarization focusing at a millimeter-scale microbead junction: towards higher volumetric throughput

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This supporting information contains information about: 1. Current transients obtained during ICPF experiments at 100 V in the four devices (0.5, 1.0, 1.5, and 2.0 mm-tall channels), 2. ICPF experiments carried out in a single-channel device at 100 V, 3. ICPF experiments carried out in a dual-channel device at 110 V, 4. Calculating the enrichment factor, percent dye leakage and downstream conductance, 5. The .stl files of the design of the dual-channel millimeter-scale

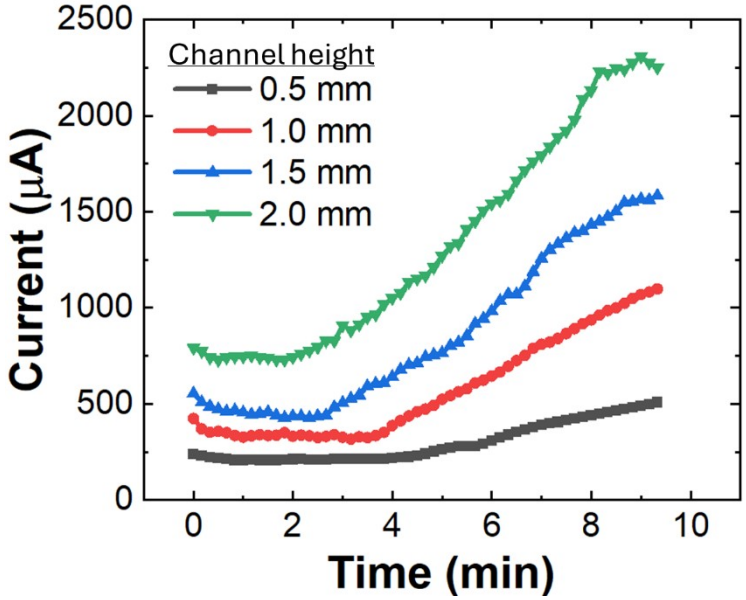


**Figure S1.** Current transients obtained during ICPF experiments in the dual-channel devices, with four distinct channel heights. Background electrolyte: 10.0 mM Tris·$HClO_4$. Applied voltage: 100 V.

device (1.0 and 2.0 mm tall).

**Current transients during ICPF experiments: Figure S1** shows the current transients obtained during the ICPF experiments in 10.0 mM Tris·$HClO_4$, in the four devices used for the scalability study. As expected, the current increases with increasing channel height.

**ICPF in a single-channel millimeter-scale device at 100 V:** The first set of ICPF experiments were carried out in a millimeter-scale device which contained only the main channel (no auxiliary channel as described in the main article) with the microbead beds. The device design is shown in **Figure S2a**. However, we were not able to achieve reproducible stacking of $BODIPY^{2-}$, due to backflow of the fluid into the anodic and cathodic reservoirs. This effect was more severe when 30 µm cation selective beads were used. **Figure S2b** shows a fluorescence micrograph obtained during the ICPF of $BODIPY^{2-}$ in such a single-channel device containing a bed of 200 µm glass beads and a bed of 200 µm DVB-$SO_3^-$ beads. Although the flow rate was set to 30 µL/min, we believe that the flow rate through the channel is lower than that due to backflow.

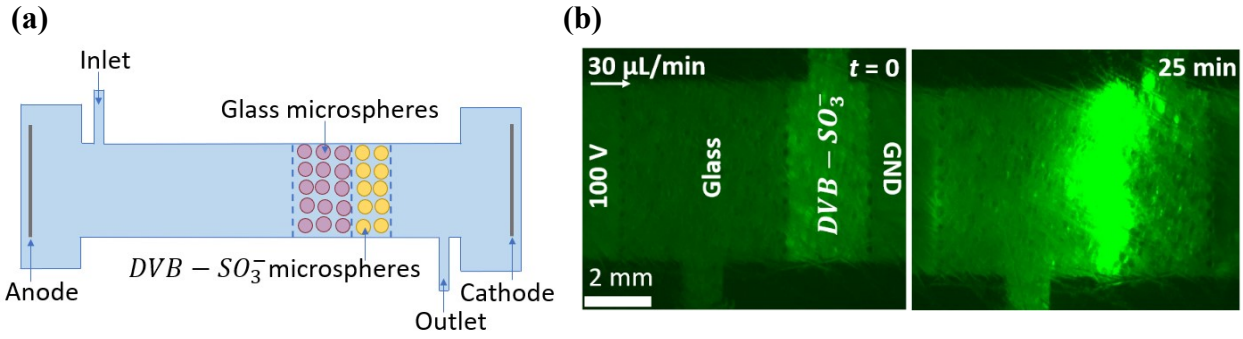


**Figure S2.** (a) Schematic of the single-channel packed microbead-bed device. (b) Fluorescence micrographs showing the focusing of 0.1µM $BODIPY^{2-}$ in a 1.0 mm-tall single-channel device consisting of two bead beds; 200 µm glass and 200 µm DVB-$SO_3^-$. The background electrolyte is 10.0 mM NaCl. Flow rate: 30 µL/min, voltage: 100 V.

**ICPF in a dual-channel millimeter-scale device at 110 V:** In addition to the ICPF experiments

performed at 90 and 100 V as shown in **Figures 3 and 4** in the main article, we performed ICPF

experiments at 110 V as well. **Figure S3a** shows a series of fluorescence micrographs obtained in

one such experiment where 110 V was applied to drive ICP. The device consisted of three bead

beds in the main channel; 200 µm glass, 30 µm DVB-$SO_3^-$, and 200 µm DVB-$SO_3^-$. We observed that the dye slowly starts to focus on the glass bead bed after about 30 s of voltage application. Within 5 minutes, a stable focused plug is formed. However, the fluorescence intensity upstream of the bead bed starts decreasing after ~8 min, due to quenching of the dye (similar to the experiment at 100 V). At 110 V, this effect is more severe, and a significant portion of the focused

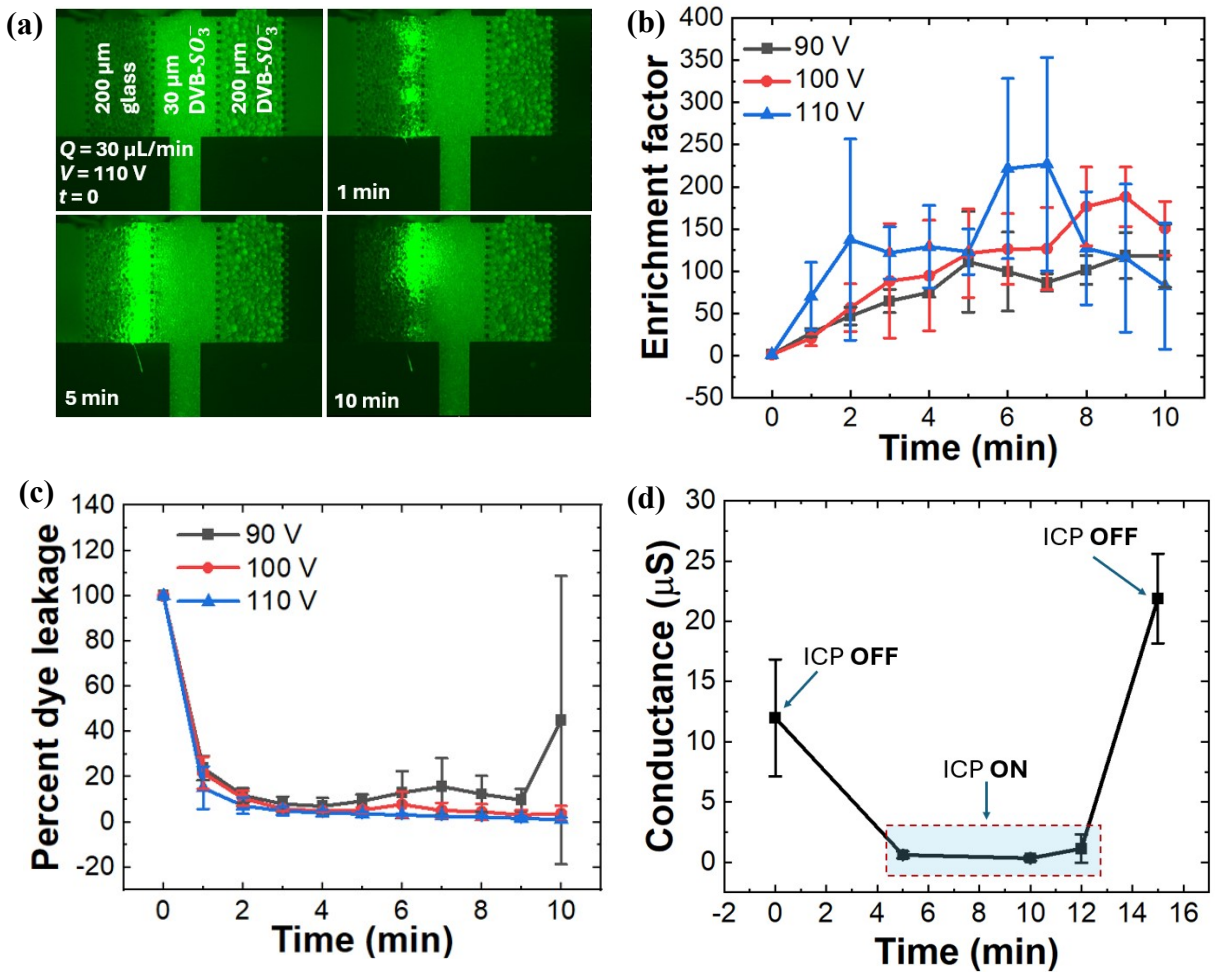


**Figure S3.** (a) Series of fluorescence micrographs showing the focusing of 0.1µM $BODIPY^{2-}$ in 1.0-mm tall channel consisting of three bead beds; 200 µm glass, 30 µm DVB-$SO_3^-$, and 200 µm DVB-$SO_3^-$. The background electrolyte is 10.0 mM NaCl. Flow rate: 30 µL/min, voltage: 110 V. (b) Plot showing the evolution of maximum enrichment factor and (c) percent dye leakage over time, at two distinct voltages. Flow rate: 30 µL/min. (d) Variation of conductance downstream of the bead bed with time before, during and after application of 110 V to drive ICP. 10.0 mM NaCl, 30 µL/min.

plug is also quenched. Because of this (and also due to increased vortex flow at higher voltages), the variation of enrichment factor (**Figure S3b**) with time is the noisiest at 110 V.

**Figure S3c** shows the percent leakage of the model analyte through the cation-selective bead bed. At 100 and 110 V, the percent dye leakage reaches below 10% within 2 min. That is, more than 90% of the analyte is retained upstream of the cation-selective bead bed due to repulsion from the IDZ. **Figure S3d** shows the variation of the conductance downstream of the bead beds before, during, and after the application of 110 V to drive ICP in the system. The downstream conductance reduces to 97% of its initial value after the initiation of ICP. This result indicates that more than 97% of ions in the electrolyte are repelled from the IDZ. After turning off the voltage that drives IDZ, the focused analyte and electrolyte ions "leak" through the bead bed, thus, increasing the current. This conductance is higher than the conductance observed before ICP initiation, since now the local salt concentration downstream is higher than the initial bulk salt concentration.

**Calculating the enrichment factor.** For calculating the maximum enrichment factor, the average fluorescence intensity at a given time point ($I_{final}$) within a square ROI of 200 x 200 μm$^2$ placed on the brightest spot of the enriched plug was considered. All images were processed using NIS Elements software (Nikon, Japan). The enrichment factor was calculated as follows.

$$\text{Enrichment factor} = \frac{I_{final} - I_{background}}{I_{initial} - I_{background}}$$

where $I_{initial}$ is the average fluorescence intensity at t=0, and $I_{background}$ is the background fluorescence intensity (prior to introducing $BODIPY^{2-}$) to the channel.

**Calculating the percent dye leakage:** To calculate the percent dye leakage, the average fluorescence intensity at a given time point within a rectangular ROI running across the entire

channel width (**Figure S4**), approximately 500 µm from the most downstream bead bed was considered. The percent dye leakage was calculated as follows.

$$\text{Dye leakage (\%)} = \frac{I_{final} - I_{background}}{I_{initial} - I_{background}} \text{ x } 100$$

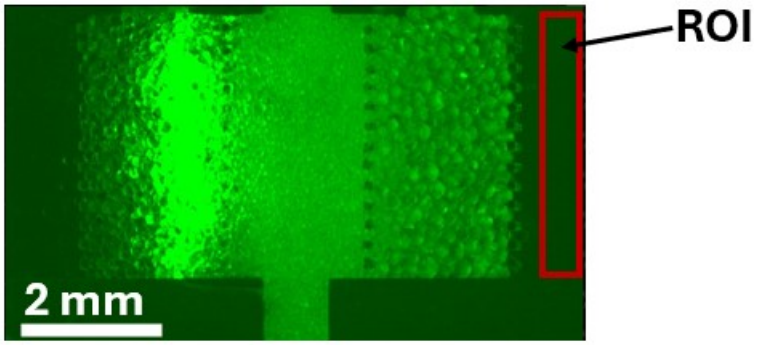


**Figure S4**. A fluorescence micrograph showing the focusing of 0.1µM BODIPY$^{2-}$ in 1.0-mm tall channel consisting of three bead beds; 200 µm glass, 30 µm DVB-$^{SO_3^-}$, and 200 µm DVB-$^{SO_3^-}$. The background electrolyte is 10.0 mM NaCl. Flow rate: 30 µL/min, voltage: 100 V. The average intensity within the ROI shown in red was used to calculate the percent dye leakage.

**Downstream conductance:** The current downstream of the bead bed before and after initiation of ICP was recorded using a custom-made circuit board, by applying a constant voltage of 1.0 V. The recorded current was converted to conductance using the following equation:

$$G = \frac{i}{V}$$

where $G$ is the conductance, $i$ is the current and $V$ is the applied voltage.