Electronic Supporting Information

Calibration curve – conversion from intensity to concentration and number of moles

In order to characterize the actual amount of sample accumulated by ITP, we have constructed calibration curves relating the fluorescence signal (arbitrary units) to known concentration (molar units), for both the glass and paper channels. Fig. S1 presents these calibration curves. We used an initial dye concentration of 10 nM, and expected roughly a 1,000-fold increase in concentration and hence based the calibration curve on concentrations of 1, 5, 10, 15 and 20 μM DyLight650. For each concentration, we filled a channel (either glass or paper) with a uniform concentration of the dye and imaged it at different locations along the channel (9 stations for glass, 3 stations for paper). The background corrected intensities in the images, at all locations, were then averaged to obtain the mean intensity corresponding to the given concentration. We fitted 1st and 2nd order trend line to the data in glass and paper respectively, and treated them as the calibration curves. To estimate the number of moles in a given region, we integrated the concentration in the plane and multiplied it by the porosity of the paper and the thickness of the paper channel. We evaluated the porosity of the paper by comparing the weight of a dry paper to the weight of the same paper filled with water. Using the relation[1],

$$\varepsilon = \frac{w_{wet} - w_{dry}}{AL\rho_{water}} \ , \qquad \text{(S1)}$$

we obtained a value of 0.65 +/- 0.08, based on 8 repeats.

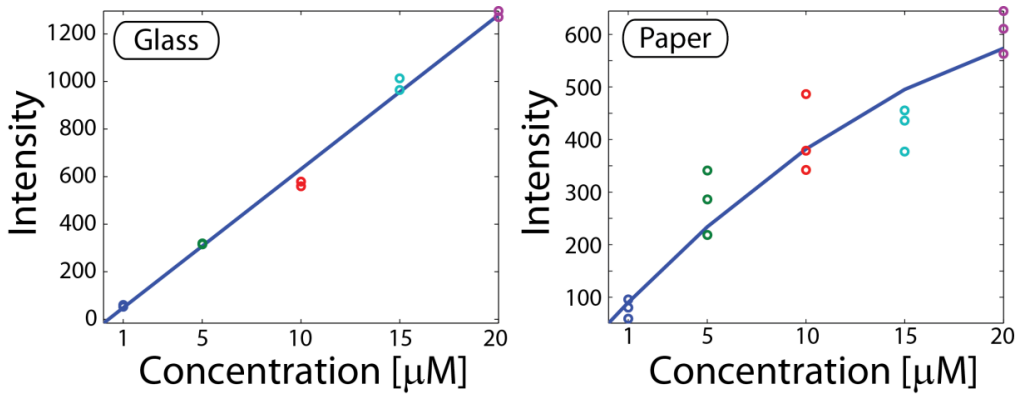

Figure S1. *Calibration curves for glass and paper, relating known concentrations of DyLight650 to measured intensity. In our ITP experiments, we used an initial dye concentration of 10 nM, and expected roughly a 1,000-fold increase in concentration. Hence, we constructed the calibration curve based on concentrations in the range 1 to 20 μM. For each concentration, we filled a channel (either glass or paper) with a uniform concentration of the dye and imaged it at different locations along the channel (nine stations for glass, three stations for paper). The background corrected intensities in the images, at all locations, were then averaged to obtain the mean intensity corresponding to the given concentration. Two and three repeats of the experiment, for each concentration, were conducted in both glass and paper respectively. The solid curves represent 1st and 2nd order trend line to the data in glass and paper respectively, which we utilize as calibration curves in this work.*

Finite sample isotachophoresis injection on μPAD

As described in section 4.2, one of our hypotheses for the reduced efficiency of paper compared to glass is adsorption of the dye to the paper. In order to test this, we conducted a finite-sample ITP experiment on our μPAD. Measuring the total amount of sample as it electromigrates along the channel allows determining whether any significant sample loss occurs.

Finite-sample isotachophoresis assay

We used 100 mM HCl, 200 mM Bistris, and 1% 1.3 MDa poly(vinylpyrrolidone) (PVP) as the LE solution. Our analyte solution contained 1 μM of the fluorescent dye DyLight650 (NHS Ester, Thermo Fisher Scientific, Waltham, MA), and as our TE solution we used 10 mM Tricine, 20 mM Bistris, and 1% PVP.

We began the experiments in our fabricated μPAD by adding 150 μL of LE to the right reservoir, and relied on capillary action for filling the channel with LE solution. After ~7 min, when the LE front was approximately 5 mm away from the designed wax barrier, we injected 1 μL of analyte solution, which filled the area between the wax barrier and the LE front by capillary action. We then located the chip on the microscope, placed the electrodes in each of the reservoirs, and added another 150 μL of LE to the right reservoir. Finally, we filled the left reservoir with 300 μL of TE solution, and applied 200 V across the channel to initiate ITP. The focused sample was imaged at seven stations, located at 0.75, 1, 1.25, 1.5, 1.75, 2 and 2.25 cm from the TE reservoir (stations are printed as 1-7 on the paper-chip in Fig. 4). At each station, the images were background corrected (background was taken in the LE solution, before ITP plug arrives).

Finite-sample isotachophoresis experimental results and discussion

Fig. S2 presents experimental results of the total amount of sample focused at the ITP interface along the channel, for a finite-volume injection. The total amount of sample was calculated as described in section 4.1. The injection of 1 μL of 1 μM Dylight to the channel, results in a theoretical amount of 10^{-12} mol. Experimental results show a similar initial amount, with no observable decrease in sample over time (in fact, a slight increase is observed which we attribute to the accuracy of intensity to concentration conversion). Thus we conclude that there is no significant adsorption of dye to the paper device.

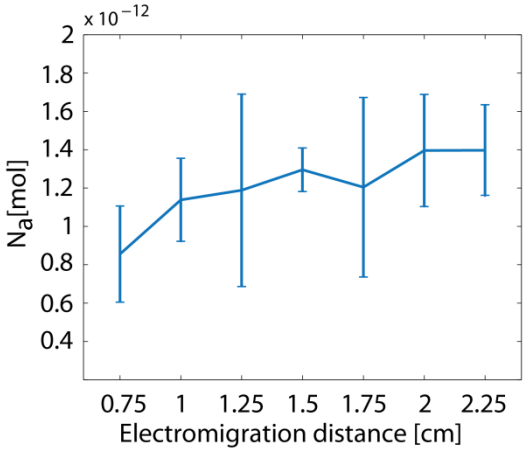

Figure S2. *Experimental results for finite-sample isotachophoresis injection showing the amount of sample focused at the interface along the channel. After a transition period, required for the injected sample to reach the interface, results show a slight increase (but no decrease) in signal over time. Each data point represents the average of three repeats, with range bars representing 95% confidence on the mean (+/- 3 standard deviations). LE is 100 mM HCl and 200 mM BisTris; TE is 10 mM Tricine and 20 mM BisTris. For both buffers we used 1% of PVP.*

References

1. S. Zeng, C.-H. Chen, J. C. Mikkelsen Jr., and J. G. Santiago, *Sens. Actuators B Chem.*, 2001, **79**, 107–114.