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


Electronic Supporting Information

# AMPLIFICATION-FREE DETECTION OF DNA IN A PAPER-BASED MICROFLUIDIC DEVICE USING ELECTROOSMOTICALLY BALANCED ISOTACHOPHORESIS

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**Calibration–intensity to concentration**

In order to characterize the actual amount of sample accumulated by ITP on paper, we have constructed a calibration curve relating the fluorescence signal (arbitrary units) to known concentration (molar units). Fig. S1 presents the calibration curve. In our ITP experiments, we used an initial dye concentration of 10 pM, and expected roughly a 10,000-fold increase in concentration and hence based the calibration curve on concentrations of 1, 10, 100 and 500 nM DyLight650. We filled the reservoir with 150 µL of each concentration, waited 5 min until the paper channel was filled with a uniform concentration of the dye by capillary action, and imaged it. The background corrected intensities in the channels (background images were taken before the filling the channels with dye) were then averaged to obtain the mean intensity corresponding to the given concentration. We fitted a linear trend line to the data, and used it as the calibration curve.

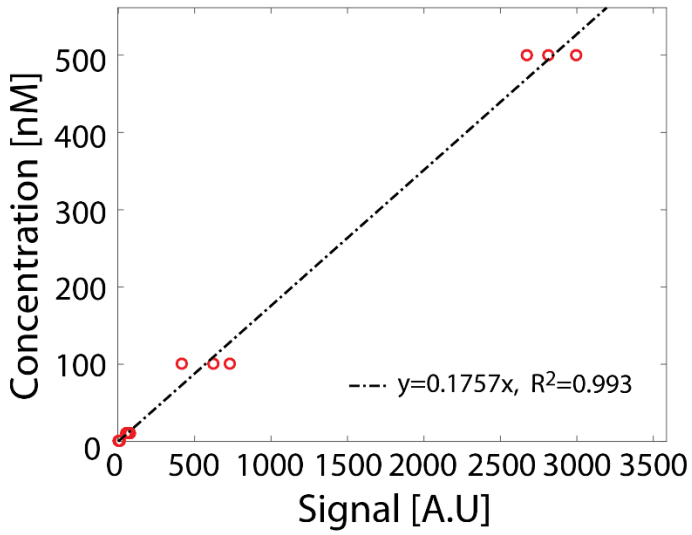


***Figure S1.*** *Calibration curve for the nitrocellulose paper, relating known concentrations of DyLight650 to measured intensity. In our ITP experiments, we used an initial dye concentration of 10 pM, and expected roughly a 10,000-fold increase in concentration. Hence, we constructed the calibration curve based on concentrations in the range 1 to 500 nM (three repeats each). Each marker represents an individual experiment, and the dashed curve represents the linear trend line fit to the data, which we utilize as calibration curves in this work.*

**Calibration – concentration to number of moles**

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. We cut cm-sized pieces of nitrocellulose, blocked them with 3% BSA, let them dry for 1 hour at 37˚C, filled them with DI by capillary flow for 30 min in a closed bottle, cut with scissors the wet areas and weighted them. After 1 hour drying at 37˚C, we weighted them again. Using the relation[1],

$$\varepsilon = \frac{w_{wet} - w_{dry}}{Ah\rho_{water}}, \tag{S1}$$

where $A$ and $h$ are respectively the area and the thickness of the paper, we obtained a porosity value of 0.75 +/- 0.03, based on six repeats.

To estimate the number of moles in an imaged ITP interface, we summed up all concentrations in the plane that are greater than 10% of the maximum width-average, and multiplied it by the porosity of the paper and the volume of one pixel in the image.

$$N = \varepsilon \cdot V_{pixel} \cdot \sum C_{i,j} = \left( \frac{w_{wet} - w_{dry}}{Ah\rho_{water}} \right) \cdot \left( A_{pixel} \cdot h \right) \cdot m \sum I_{i,j} = \left( \frac{w_{wet} - w_{dry}}{A\rho_{water}} \right) \cdot A_{pixel} \cdot m \sum I_{i,j} \ , \quad \text{(S2)}$$

where $A_{pixel}$ is the area of one pixel, $m$ is the slope of the calibration curve, and $I_{i,j}$ is the intensity of the pixel (i,j). Importantly, when using the expression for porosity, the number of moles does not explicitly depend on the thickness of the paper.

## References

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