Photobleaching effect

Photobleaching is a destructive process in which the fluorophore loses its ability to emit light under prolonged exposure to excitation. The rate of photobleaching depends on a number of factors including illumination intensity and wavelength, exposure time and so on. According to Gui and Ren [1], when fluorescent dyes of a concentration C_0 is exposed under an excitation light intensity I, the remained dye concentration C after an exposure time t can be expressed as

$$C / C_0 = e^{-kIt}$$

where k is a coefficient considering dye photobleaching speed. In this study, we carried out experiments to determine this coefficient, k. In brief, a 200 μM fluorescein dye solution was loaded into in a channel and it was exposed under the same illumination intensity as in our microfluidic temperature gradient focusing (TGF) experiments. The captured fluorescent image intensities were converted into the corresponding dye concentrations, and a relationship was obtained as $C / C_0 = e^{-0.042t}$, which is shown in Figure 1s below. The results clearly show that there are 92% fluorescein dyes remaining in the solution after 2 min, while only 56% dyes remaining after 14min. This indicates that the concentration (e.g., 2500-fold) of sample solutes converted from image intensity is actually only the concentration of remaining solute dyes, and its value is usually underestimated compared to the actual concentration of solute dyes after time elapsed (e.g., 14 min).

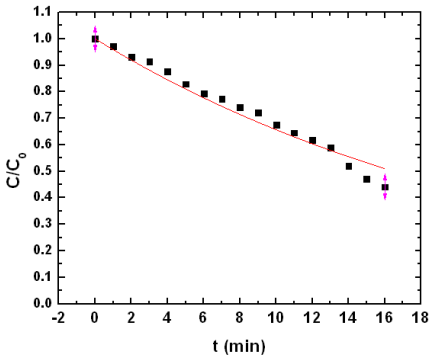

Fig1s Photobleaching effect of fluorescein dyes in temperature gradient focusing (TGF) experiments

Ref 1 L. Gui; C. L. Ren; *Applied Physics Letters* 2008, 92, 02410

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Temporal sequential concentration contours under a combined AC and DC electric field with two square-shaped reservoirs of 16 mm×16 mm in dimensions

Figure 2s shows the temporal sequential experimental images for sample concentration increasing with time, from which increase and tightening of sample concentrations with time can be clearly demonstrated. Also, it shows a slow shift of the sample focusing zone from the narrow channel region towards the conjunction due to the backpressure effect. In the experiment, the homogenous solution containing dilute 0.05 μM Fluorescein-Na dissolved in a 180 mM Tris-borate buffer is loaded into the PDMS microchannel with two square-shaped reservoirs of 16 mm × 16 mm. Once a combined 400 sin(2πft) V AC and 450 V DC field is imposed to the two reservoirs with the left-hand side grounded, the sample solutes are gradually focused at a stagnant point in the narrow channel region. Meanwhile, the backpressure is induced and also increases gradually because EOF pumps the buffer flow from the right-hand side reservoir to the left-hand side reservoir to cause the liquid level change in the two reservoirs. Such induced backpressure then pushes the sample focusing zone towards the conjunction region. Within 14 min elapsed, the focused sample solutes reach a concentration about 125 μM (converted through using the calibration curve from the captured fluorescent intensity); which is about 2500 times the initial concentration of the Fluorescein-Na solution.

Fig. 2s Temporal sequential microscopic fluorescence images for sample solute concentration experiment using microfluidic Joule heating induced temperature gradient focusing technique. In the experiment, a solution containing 0.05μM Fluorescein-Na solutes dissolved in a 180 mM Tris-borate buffer is loaded into a 10mm-long PDMS microchannel with two square-shaped reservoirs of 16 mm × 16 mm. Once a combined 400 sin(2πft) V AC and 450 V DC field is imposed to the two reservoirs with the left-hand side grounded, the sample solutes are gradually focused at a stagnant point in the narrow channel region.

Measurement of solution pH

In our Joule heating induced temperature gradient focusing (TGF) experiment, the fluorescence intensity can be affected by possible change of solution pH due to Faradaic interactions. To address this issue, we did the following test to measure the solution pH values before and after TFG experiment. First, prior to TGF experiment the pH of the solution (i.e., 0.05μM Fluorescein-Na solute dissolved in a 180 mM Tris-borate buffer) was measured by pH paper indicator. After the TGF experiment, we also measured the pH value of the solutions in two reservoirs. In TGF experiment configuration, the left-hand side reservoir was connected with the negative platinum electrode. The right-hand side reservoir was connected with the positive platinum electrode. Based on the following figure, it shows that there was no noticeable change in the solution pH values before and after TFG experiment. The measured pH values before and after TGF experiment were all about 8~9. This is because in the experiment the platinum wires used as electrodes have a diameter of 0.5 mm. The area of the electrodes is very small compared

to the area of reservoirs. Though there are Faradaic interactions around the electrodes, the amount of reaction substances is small. Hence, the possible effect on fluorescence intensity due to pH shift in the TGF experiment can be negligible.

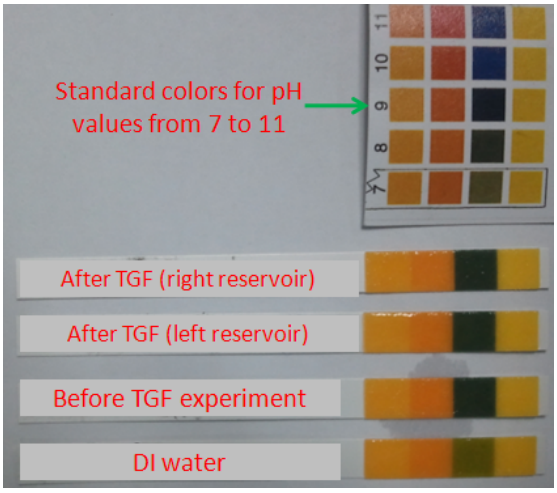

Fig 3s Measured pH values for the sample solution before and after TGF experiment