

Supplementary Information

Fluorescein in Tris-HCl buffer at pH 9 in gradient device – determination of optimal speed

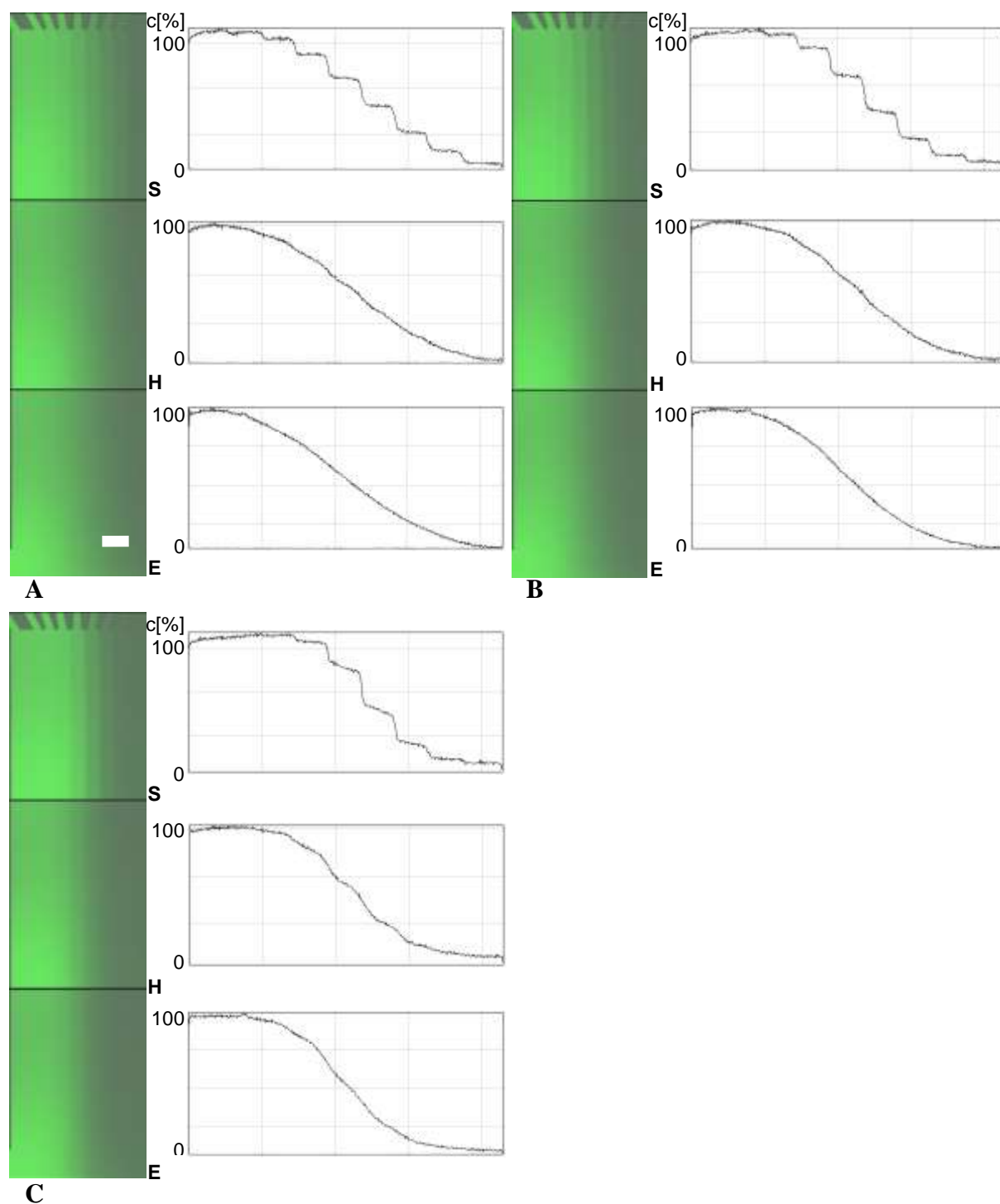


Figure 1, A-C: Fluorescence intensity images with respective fluorescence intensity profiles horizontally across the microchamber of Tris-HCl pH 9 with fluorophore fluorescein (left inlet) and without fluorescein (right inlet) at varying pump speeds. Per speed fluorescence

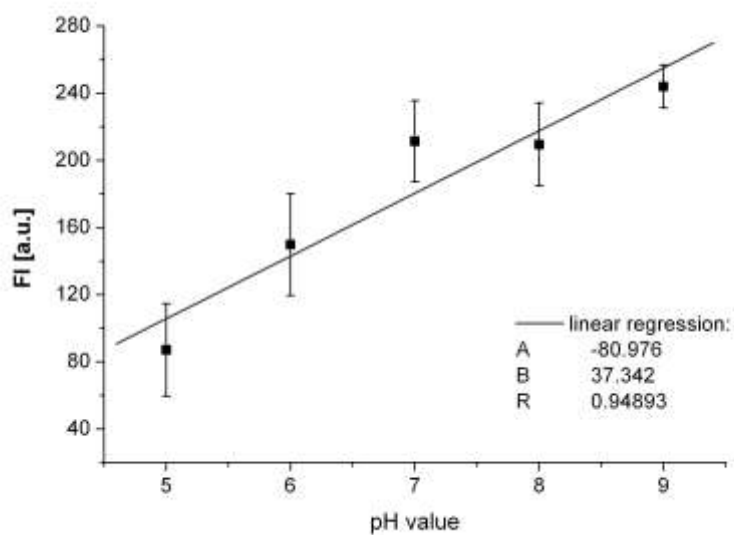
intensity images were taken at the start (S), in the middle (H) and at the end (E) of the microchamber. A: 50 $\mu\text{L}/\text{min}$, B: 100 $\mu\text{L}/\text{min}$ and C: 200 $\mu\text{L}/\text{min}$. Concentration c of fluorescein in [%], scale bar 200 μm applies to A-C.

By definition laminar flow is an essential requirement for microfluidic devices creating linear gradients [21, 22, 26]. Under laminar flow conditions present within the gradient μFL device, mixing of differently concentrated solutes occurs via diffusion, which can be described by FICK's laws. Diffusion, i.e. the lateral movement (perpendicular to the flow direction) of solutes, as well as the gradient profiles of the solutes at all points along the microchamber (parallel to the flow direction) are determined by the flow speed. To optimise conditions for formation of a stable linear gradient along the microchamber, the effect of flow speed on concentration gradient formation was first investigated using fluorescein solutions.

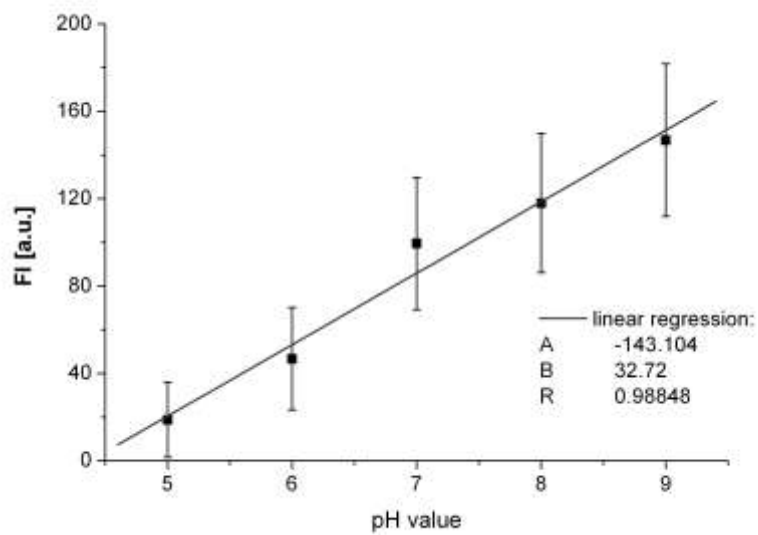
Fluorescence images and profiles shown in **Figure 11** depict concentration gradients of fluorescein since its fluorescence intensity is linearly dependent on the solution concentration within the low concentration range. The fluorescein solution in Tris-HCl buffer at pH 9 was delivered into the left inlet whereas the buffer alone was delivered into the right inlet. The gradients formed at flow speeds of 50, 100 and 200 $\mu\text{L}/\text{min}$ are shown in Figure 11, A, B and C, respectively. Both fluorescence images and their corresponding fluorescence intensity profiles are illustrated for three different positions along the microchamber (S – starting point, H – middle of chamber and E – end of microchamber; see also Figure 10). At position S, 9 serpentine channels from the mixer merge into the microchamber. A step concentration profile of fluorescein was formed at the beginning of the microchamber (Figure 11, A). At position H, this step profile disappears for the flow rates of 50 to 100 $\mu\text{L}/\text{min}$, and a smooth gradient is formed. This smooth, linear gradient persists until position E at these flow rates.

Since lower flow rates could create undesired pulsing effects on mixing, a pump speed between 50 and 100 $\mu\text{L}/\text{min}$ was chosen as optimal.

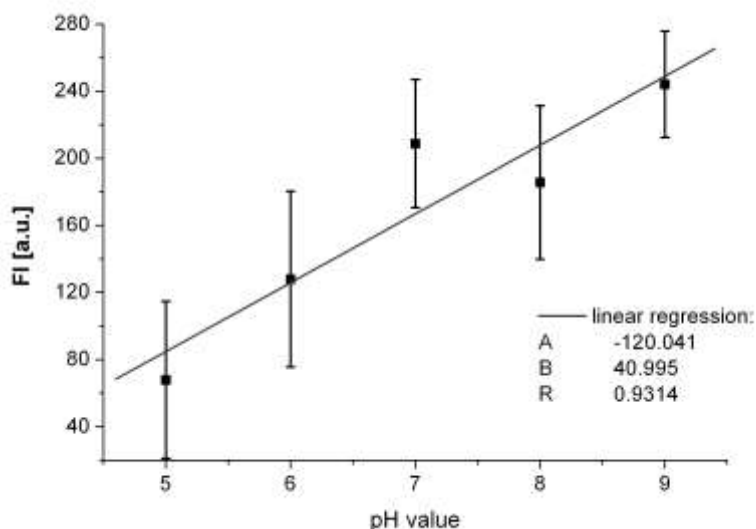
Interrelation between fluorescence intensity and solution pH value



A



B



C

Figure 2, A-C: Fluorescence intensity (FI) in dependence on pH value of the solution: BCECF in washing solution (A), BCECF in polyethylene imine solution (B) and SNARF-5F in heparin solution (C).

Capillaries of precise thickness and geometry were filled with mixtures of pH sensitive fluorophores and respective washing or polyelectrolyte solutions to evaluate the interrelation between fluorescence intensity and solution pH between 5 and 9. Using fluorescence microscopy a linear relation between FI and the resp. solutions' pH value was determined. The linear interrelationship was prerequisite for following characterization of the pH gradients inside the μ FL microchamber.

Flow rate determination

The optimized pump speed of 100 μ L/ min for optimal pH gradients was used to a) calculate the flow rate and b) to measure the flow rate by weighing.

a) Calculation of flow rate

The volume flow rate was calculated to be $F_R = 2.5277 \times 10^{-8} \text{ m}^3/\text{min}$. The measured height of the channels was $52 \text{ }\mu\text{m}$ and the width was $900 \text{ }\mu\text{m}$. Therefore, the cross sectional area of the microchamber was determined to be $A = 4.7 \times 10^{-9} \text{ m}^2$. The linear flow velocity was calculated using the following equation (1) resulting in a value of $v_{AK} = 0.09 \text{ m/s}$.

$$v_{AK} = \frac{F_R}{A} = 5.378 \frac{\text{m}}{\text{min}} = 0.09 \frac{\text{m}}{\text{s}} \quad (1)$$

b) Calibration of flow rate by weighing

The flow rate was calibrated by weighing the fluid that was transported through the microfluidic device within a certain period of time (5 to 10 repetitions for mean value and standard deviation). The cross sectional area calculated above ($A = 4.7 \times 10^{-9} \text{ m}^2$) was used.

The volume flow rate was measured to be $F_R = 26.23 \text{ }\mu\text{L}/\text{min} \pm 0.22 \text{ }\mu\text{L}/\text{min}$. The linear flow velocity was determined using the following equation (2).

$$v_{AK} = \frac{F_R}{A} = 5.581 \frac{\text{m}}{\text{min}} \pm 0.047 \frac{\text{m}}{\text{min}} = 0.093 \frac{\text{m}}{\text{s}} \pm 0.008 \frac{\text{m}}{\text{s}} \quad (2)$$

This resulted in a value of $v_{AK} = 0.093 \text{ m/s} \pm 0.008 \text{ m/s}$. The calibrated and calculated flow rates are consistent with each other.