NANO small MICRO

Supporting Information

for *Small,* DOI: 10.1002/smll.201804326

3D Printed Microfluidic Mixers—A Comparative Study on Mixing Unit Performances

*Anton Enders, Ina G. Siller, Katharina Urmann, Michael R. Hoffmann, and Janina Bahnemann**

Supporting Information

3D printed Microfluidic Mixers – a Comparative Study on Mixing Unit Performances

Anton Enders, Ina Siller, Katharina Urmann, Michael R. Hoffmann and Janina Bahnemann*

1. Color intensity measurement and calculation

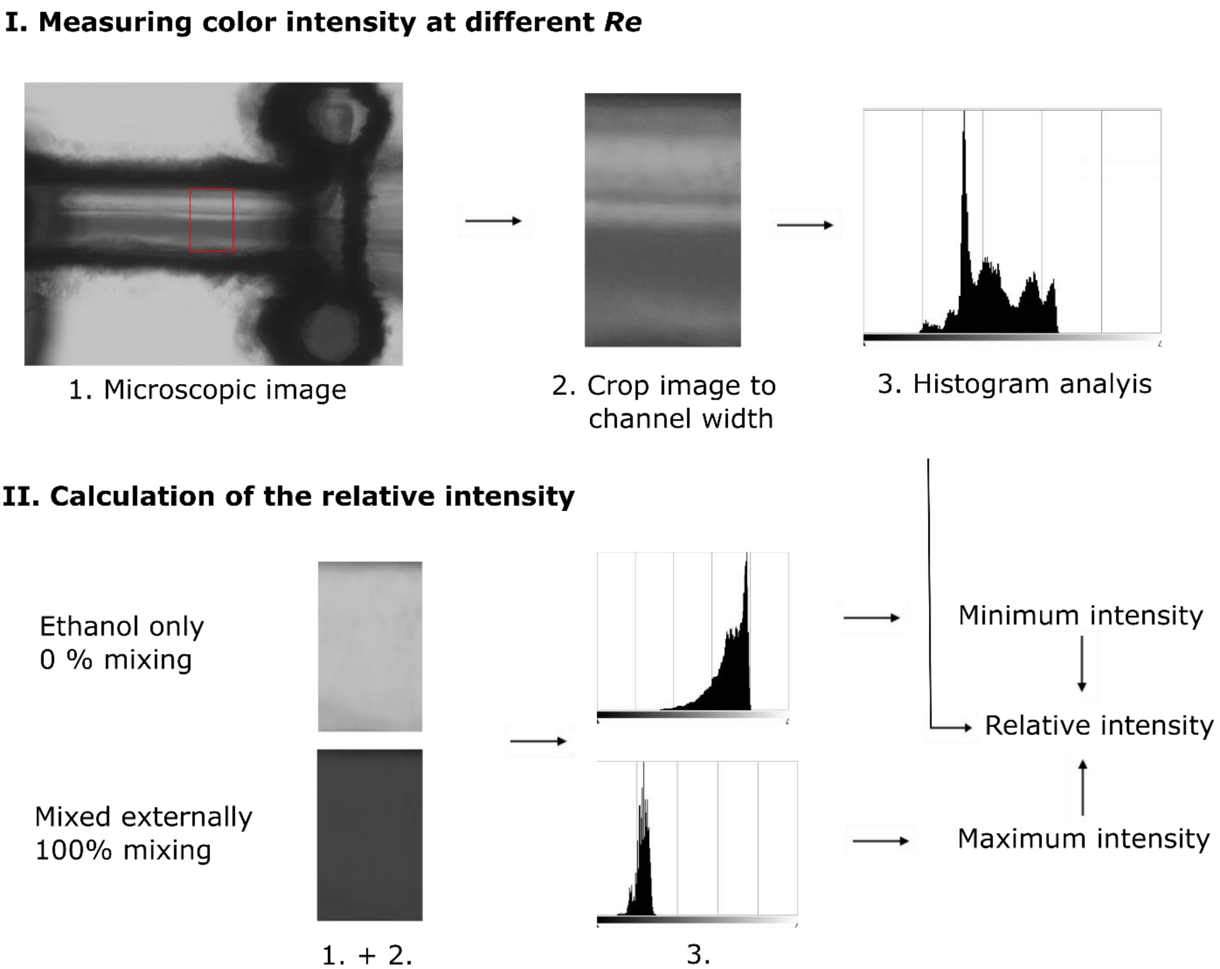

Figure S1: Schematic of the color intensity measurement and calculation of the relative intensity.

Microscope images of different mixer sections were taken to quantify the mixing performance. The images were cropped to the channel width and the mean color intensity was calculated from the histogram using GIMP (GNU Image Manipulation Program 2.8.22, The GIMP-Team). To calculate the relative intensity, the minimum and maximum intensity were determined by pumping only ethanol (minimum intensity) and externally mixed solutions (maximum intensity) through the system and taking microscope images of the same mixer sections. Every flow rate was imaged three times at every measurement location. The flow rates were set in three different orders (descending, ascending and random) to account for possible influence of the previous flow rate before taking the measurement. **Table S1** shows sample values of the HC mixer after the third mixer unit.

Table S1: Determined intensity values for the calculation of the relative intensity $\bar{I}$ at different Re from one measurement location in the HC mixer.

	I_{mean}	$I_{inverted}$	$I_{normalized}$	$\bar{I}$
Ethanol	181,07	73,93	0	0
Re = 37.04	66,73	188,27	114,33	0,99
Re = 27.78	66,37	188,63	114,7	0,99
Re = 18.52	72,43	182,57	108,63	0,94
Re = 9.26	74,27	180,73	106,8	0,92
Re = 3.7	69,93	185,07	111,13	0,96
Externally mixed	65,13	189,87	115,93	1

The mean intensity was determined from three cropped microscope images for every flow rate.

The values were inverted using Equation S1.

$$I_{inverted} = 255 - I_{mean} \quad \text{(S1)}$$

$I_{inverted}$ was normalized using Equation S2 to account for the background of the mixer channels.

$$I_{normalized} = I_{inverted} - I_{inverted,\, ethanol} \quad \text{(S2)}$$

The relative intensity $\bar{I}$ was calculated using Equation S3.

$$\bar{I} = \frac{I_{normalized}}{I_{normalized,\, externally\ mixed}} \quad \text{(S3)}$$

2. Comparison of simulations and experimental data

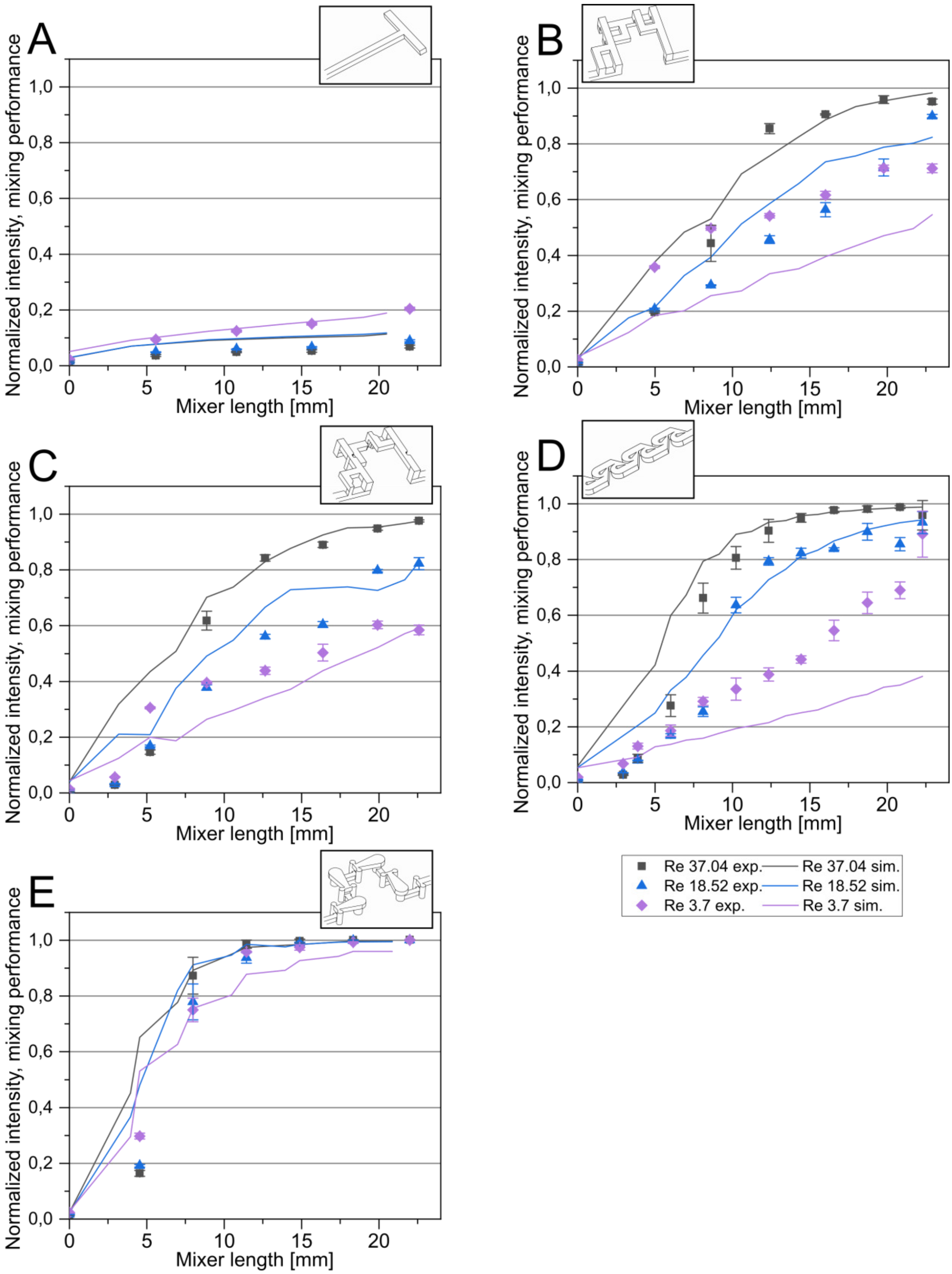

Figure S2: Comparison of simulated mixing performance (lines) and normalized intensity (dots) at different Re. A: T-mixer, B: Caterpillar mixer, C: enhanced Caterpillar mixer, D: Tesla-like mixer, E: HC mixer.

Figure S2 shows additional graphs for the comparison of simulations and experimental results. As discussed in the paper, the simulations and experimental results generally show similar results with greater deviations in the first third of the mixer length. The T-mixer shows very similar normalized intensity and mixing performance at all flow rates. The channels of the T-mixer chip are probably very smooth with no turns or round corners in the mixer itself. The small deviations at higher flow rates might originate from the missing chemical reaction simulation of sodium hydroxide (NaOH) with phenolphthalein, which could lead to a delay between the degree of mixing and the color intensity. The Caterpillar mixer, enhanced Caterpillar mixer, HC mixer and Tesla-like mixer all show better alignment of experimental and simulated results at higher Re with greater deviations at lower Re and at lower mixer lengths. Therefore, data for the Tesla-like mixer was discussed in the paper representatively.

3. Shear stress influence on cell viability and concentration

In additional experiments, CHO-K1 cells were cultivated in CHOMACS CD Medium (Miltenyi Biotec, Bergisch Gladbach, Germany) and pumped through the HC mixer and Tesla-like mixer systems at Re = 9.26. Cell viability was determined using CEDEX AS20 cell counter. The viability of the cells, directly after passing through the mixers (and a control with no mixing), is shown in **Figure S3.**

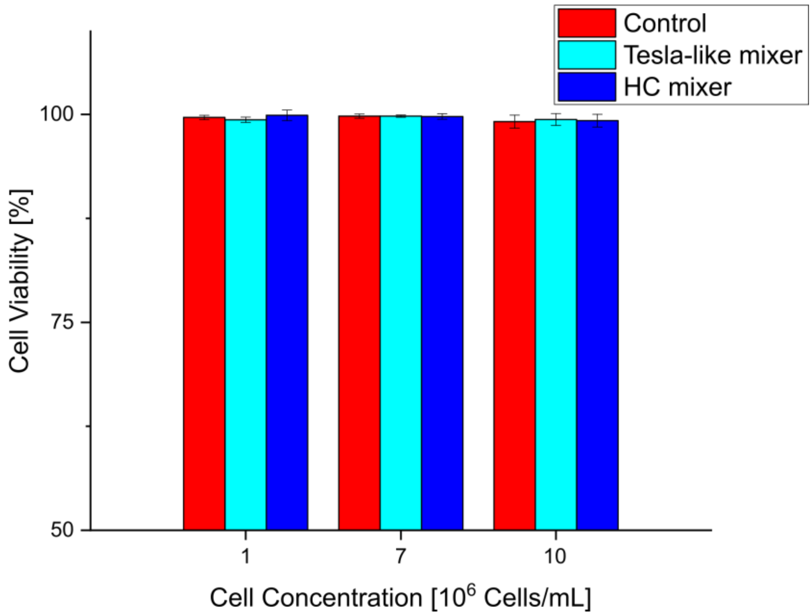

Figure S3: Cell viability after pumping the cells through the Tesla-like mixer (turquoise), HC mixer (blue) and without pumping the cells (Control, red). The cell concentrations tested were $1 \cdot 10^6$, $7 \cdot 10^6$ and $10 \cdot 10^6$ cells $\cdot$ mL^{-1}.

As discussed in the study, the cell viability was not significantly affected by passing the cells through the mixers at any tested concentration (Figure S3).