

## Digital droplet LAMP as microfluidic App on standard laboratory devices

### Supplementary information

#### Capillary bubble transport

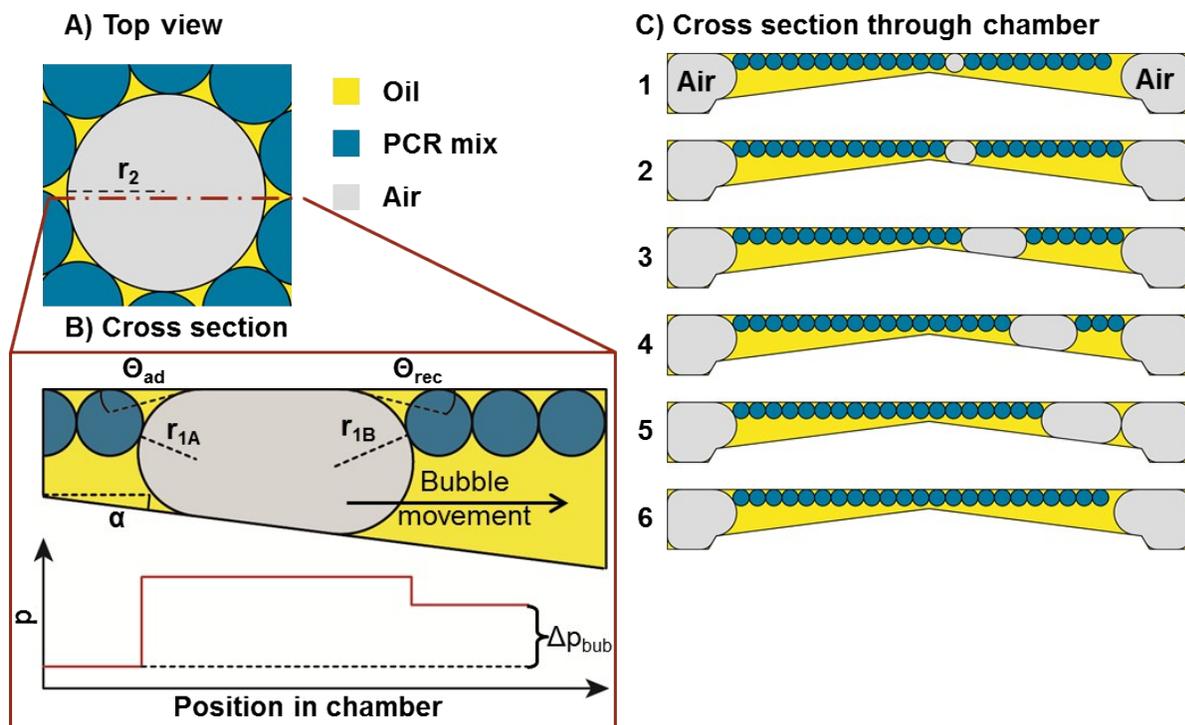


Figure S 1 Schematic of capillary driven bubble removal. A) Top view of an air bubble (grey) amidst PCR droplets (blue) in oil (yellow). From top, the bubble has a circular shape. B) Cross section through the bubble. The bubble is trapped between ceiling and bottom of the chamber. The ceiling and bottom are tilted with respect to each other at an angle  $\alpha$ . The pressure in the oil/air is depicted below. The pressure is constant in the oil, increases sharply at the interface and is static in the air volume again (otherwise there would be air flow to equate the pressure differences). The second pressure jump across the interface is smaller since the radius  $r_{1B}$  is larger than  $r_{1A}$ . C) Cross section through the chamber. Sequence from 1 to 6: an air bubble is forming and transported away to the bubble drain on the right. The emulsion flows around the bubble. Reprinted with permission from Schuler et al. <sup>1</sup>.

#### Production of DropChip

The DropChip was designed using SolidWorks 2015 (Dassault Systemes Deutschland GmbH, Stuttgart, Germany). The design was then milled in Polymethyl methacrylate (Maertin, Freiburg, Germany) by the Hahn-Schickard Lab-on-a-Chip Design & Foundry Service. <sup>2</sup> A PDMS cast was produced using Elastosil 607 and Elastosil 675 (1 : 1, with monomer/crosslinker ratios of 9 : 1 and 1 : 1 respectively) (Wacker, Munich, Germany). This PDMS cast was used for hot embossing in COC (Topas 5013) with a hot press (WMP-30, Wickert, Landau in der Pfalz, Germany). Afterwards, the height of the DropChip was reduced by milling to 1 mm to meet DIN ISO 8037-1 specifications. The DropChip was then sealed with pressure sensitive

adhesive foil (#900 320, HJ Bioanalytik, Erkelenz, Germany) using a custom built lamination tool (PCS 30, Jakob Weiß & Söhne, Sinsheim, Germany).

### LAMP Primers and probe

The sequences for primers and probe from Tanner et al. <sup>3</sup> were used and are shown in the table below. All primers and probe were supplied by Biomers.net GmbH (Ulm, Germany).

Table S 1 Sequence of LAMP primers and probe.

Name	5'→3' Sequence
FIP	CTGCCCCGACGATAGGCTTAATCGTGGTCTGGTGAAGTTCTACGG
BIP	TCCAGTGCACCTGCTGGGTGGGTATTGTTCCGCCAGTAC
F3	GATCACCGATTTACCAACC
B3	CTTTTGAGATCAGCAACGTCAG
LoopF	TGCGCCATGTCCCGCT
LoopB	TGAGTTAACCCACCTGACG
FIP-Q	BHQ2-CTGCCCCGACGATAGGCTTAATCGTGGTCTGGTGAAGTTCTACGG
Fd	ATTAAGCCTATCGTCGGGGCAG-Cy5

### ddPCR

For ddPCR the following primers and probe were used:

Name	5'→3' Sequence
Forward	GGCAATTGCGGCATGTTCTTCC
Reverse	TGTTGCATTTGCAGACGAGCCT
Probe	FAM-ATGCGAACGGCGGCAACGGCAACATGT-BHQ1

Primers and probe were used in 300 nM and 200 nM concentration. Bio-Rad Supermix for probes was used in 1x concentration. The thermal protocol consisted of a hot-start at 95 °C for 10 min and 40 cycles of 30 s at 95 °C and 60 s at 60 °C followed by 98 °C for 10 min. Cycling was conducted using a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad, Munich, Germany). For all other steps manufacturer protocol was followed.

### Measurement of the coefficient of variation (CV) of droplet size and absolute droplet size

A low CV of droplet size is important for digital experiments. Here, the CV was determined by producing droplets of LAMP mix in the DropChip. Then a microscopic image of the droplets was taken. To avoid manual bias the droplet diameter was determined automatically using a Matlab-routine that detected the droplet diameter. Coefficient of variation was 1.4% including inter- and intra-experimental variability (>300 droplets from 4 individual experiments) and <1% considering only inter-experimental variability. The droplet diameter (122 µm) was determined by comparing the droplet diameter to structures of known size.

### Semi-automated image recognition

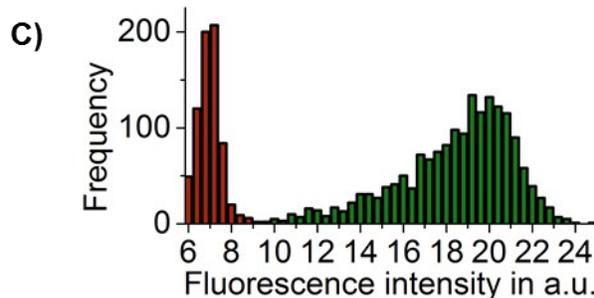
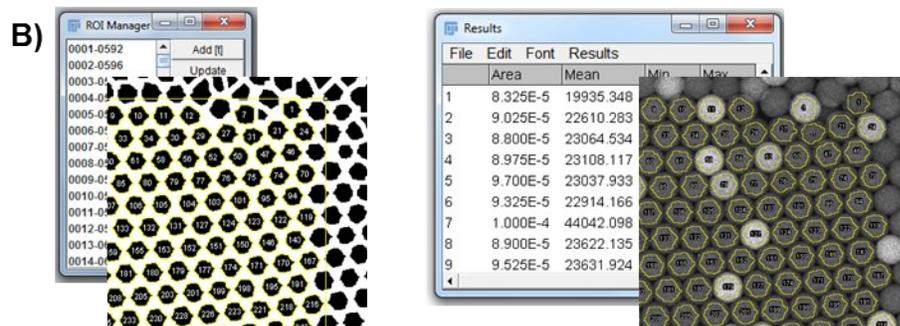
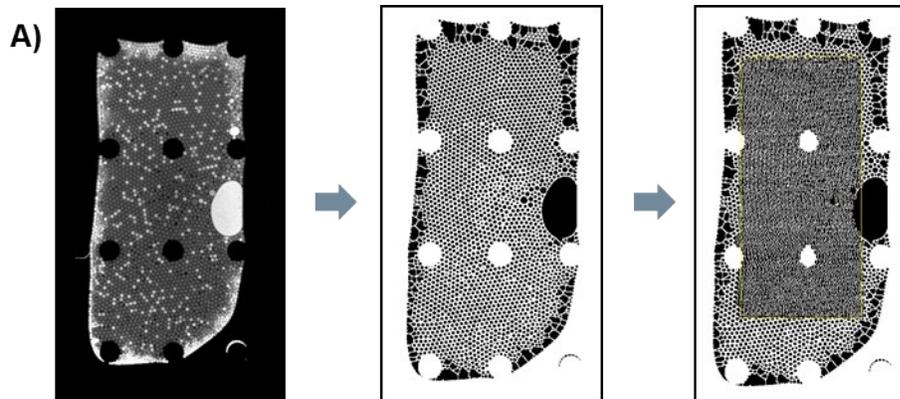


Figure S 2: A) Left: fluorescence image; middle: after “watershed” and “erode” filters were applied; right: after “analyzing particle” filter is applied. B) Left: cropped section of ROIs defined by ImageJ; right: ROIs as overlay on fluorescence image C) Histogram of fluorescence intensities of droplets.

The tiff-file from the fluorescence scanner was opened and the “minimum” filter was applied with a radius of 2 pixels. Then the “threshold” function was used to convert file to only two different intensity values, a suitable threshold was defined manually. The functions “watershed” and “erode” were used to separate the droplets. Afterwards the “analyzing particles” function was used to create regions of interest (ROI). The upper and lower boundary for droplet size were set manually. Regions showing above mentioned edge effects or multiple layers of droplet were not included. Next, droplets that were missed by the algorithm were added manually (typically 0 – 20). Then the grey values for all ROIs were measured and analyzed in Excel.

### Poisson statistics

For calculation of the copy number in the sample base on the numbers of positive and negative droplets the following formula was used:

$$\# \text{ of DNA targets} = -\ln\left(1 - \frac{\# \text{ of positive droplets}}{\# \text{ of droplets}}\right) \cdot \# \text{ of droplets}$$

## References

- 1 F. Schuler, M. Trotter, M. Geltman, F. Schwemmer, S. Wadle, E. Domínguez-Garrido, M. López, C. Cervera-Acedo, P. Santibáñez, F. von Stetten, R. Zengerle and N. Paust, *Lab on a chip*, 2015.
- 2 Daniel Mark, *Hahn-Schickard Lab-on-a-Chip Design + Foundry Service: Customer-specific lab-on-a-chip systems*, available at: [http://www.hahn-schickard.de/fileadmin/media/03\\_Fertigung/01\\_Lab-on-a-Chip\\_Design\\_Foundry\\_Service/2015-05-26\\_FY\\_DINlang\\_LOAC\\_Design\\_\\_\\_Foundry\\_Service\\_-\\_web\\_-\\_en.pdf](http://www.hahn-schickard.de/fileadmin/media/03_Fertigung/01_Lab-on-a-Chip_Design_Foundry_Service/2015-05-26_FY_DINlang_LOAC_Design___Foundry_Service_-_web_-_en.pdf), accessed 11.12.15.
- 3 N. A. Tanner, Y. Zhang and T. C. Evans, *BioTechniques*, 2012, **53**, 81–89.