Electronic supplementary information (ESI): High dynamic range digital assay enabled by dual volume centrifugal step emulsification

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S1: DETAILED OVERVIEW OF THE CARTRIDGE

An overview of the fluidic cartridge design is displayed in Figure S1, geometry parameters are listed in Table S1.

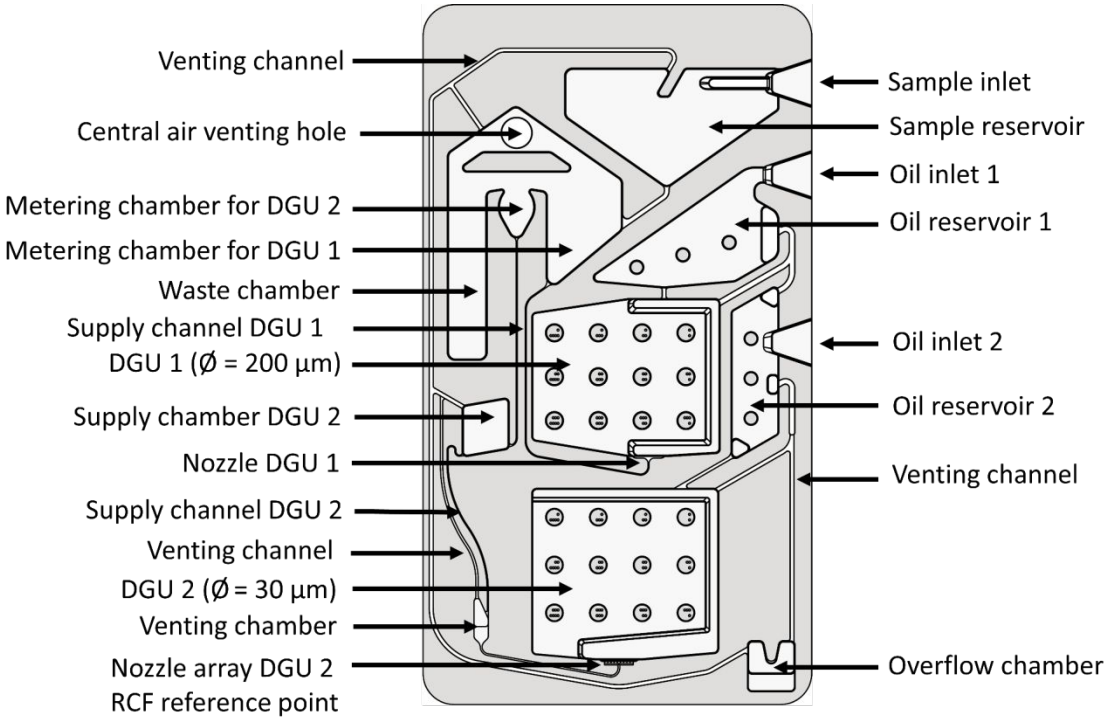

Figure S1. Overview of the microfluidic cartridge design with all channels and chambers marked.

Table S1. List of geometry parameters of all chambers and channels used in the microfluidic design as listed in Fig. S1.

Description	Cross section w·d (µm²)
Supply channel DGU 1	75·70
Supply channel DGU 2	70·70
Nozzle DGU 1	75·70
Nozzle array DGU 2	10 x 11·11
Venting channel	250·200
Description	**Volume (µl)**
Sample reservoir	34
Oil reservoir 1	15
Oil reservoir 2	4
Metering chamber for DGU 1	10.5
Metering chamber for DGU 2	2.5
Waste chamber	23
Supply chamber DGU 2	5
Overflow chamber	3
Venting chamber	0.2

S2: CALCULATION OF THE FLOW REGIMES DURING DROPLET FORMATION

The Reynolds numbers for determination of the flow status in DGU 1 and DGU 2 were calculated as shown below (see eq S1 and eq S2). Used parameters and results are displayed in Tab. S2.

$$\mathrm{Re}_1 = \frac{\rho \cdot \nu_1 \cdot l_1}{\eta} \tag{S1}$$

$$\mathrm{Re}_2 = \frac{\rho \cdot \nu_2 \cdot l_2}{\eta} \tag{S2}$$

with

$$\nu_1 = \frac{Q_1}{A_1} \tag{S3}$$

$$\nu_2 = \frac{Q_2}{A_2} \tag{S4}$$

Table S2. Used parameters and results for calculation of the Reynolds number for both DGUs.

Symbol	Description	Value
ν_1	Disperse phase velocity for DGU 1	$1.12 \cdot 10^{-1}$ m·s^{-1}
ν_2	Disperse phase velocity for DGU 2	$1.23 \cdot 10^{-2}$ m·s^{-1}
Q_1	Volume flow in DGU 1 derived by stroboscopic imaging	$6.44 \cdot 10^{-11}$ m^3·s
Q_2	Volume flow in DGU 2 derived by stroboscopic imaging	$1.35 \cdot 10^{-11}$ m^3·s
ρ	Disperse phase density	998.203 kg·m^3
η	Disperse phase dynamic viscosity	3.1 mPa·s
A_1	Nozzle cross section DGU 1	$5.25 \cdot 10^{-9}$ m^2
A_2	Nozzle cross section DGU 2	$1.21 \cdot 10^{-10}$ m^2
l_1	Depth of nozzle in DGU 1	70 µm
l_2	Depth of nozzle in DGU 2	11 µm
Re_1	Reynolds number DGU 1	0.28
Re_2	Reynolds number DGU 2	0.4

S3: ddLAMP PRIMER SEQUENCES

The following sequences were used for the ddLAMP assay (see Table S3).

Table S3. Primer and probe sequences used in the ddLAMP assay.

Short name	Sequence
FIP	AAGGCTTTCTACGTGGAACTGCCTGTGGGATAGGAGGGGT
BIP	GGTGGATACCGTTGATGATGGGAGTCTGTGGCATAGGTGTG
F3	TGTTTACAACACAAGCGACG
B3	CGCTAAGATGTTTACCGTGA
LB	AGTGCCATGTAACGGCGAT

S4: EXTENDED DATA SET FOR THE GENERATION OF FIG. 4.

Raw data of the biological evaluation using a ddLAMP reaction is displayed below in Table S4.

Table S4. Raw data of the biological evaluation. The values for the measured concentrations represent the mean concentration of three independent runs. The final concentration represents the mean concentration of the two signals for DGU1 and DGU2. Given errors represent the standard deviation.

Concentration step	Measured concentration DGU 1 ($cp \cdot ml^{-1}$)	Measured concentration DGU 2 ($cp \cdot ml^{-1}$)	Final concentration ($cp \cdot ml^{-1}$)
NC	0	0	0
C1	$4.05 \cdot 10^2 \pm 3.49 \cdot 10^1$	-	$4.05 \cdot 10^2 \pm 3.49 \cdot 10^1$
C2	$4.74 \cdot 10^3 \pm 5.56 \cdot 10^2$	-	$4.74 \cdot 10^3 \pm 5.56 \cdot 10^2$
C3	$4.93 \cdot 10^4 \pm 5.74 \cdot 10^3$	$8.05 \cdot 10^4 \pm 7.16 \cdot 10^3$	$6.49 \cdot 10^4 \pm 2.21 \cdot 10^4$
C4	$6.24 \cdot 10^5 \pm 3.21 \cdot 10^4$	$7.34 \cdot 10^5 \pm 1.54 \cdot 10^4$	$6.79 \cdot 10^5 \pm 3.90 \cdot 10^4$
C5	-	$3.77 \cdot 10^6 \pm 1.70 \cdot 10^5$	$3.77 \cdot 10^6 \pm 1.70 \cdot 10^5$
C6	-	$6.46 \cdot 10^7 \pm 1.75 \cdot 10^7$	$6.46 \cdot 10^7 \pm 1.75 \cdot 10^7$

S5: PROCESSING DEVICE FOR PARALLELIZED PROCESSING OF 96 CARTRIDGES

A device for the parallelized processing of 96 cartridges is currently under development by Biofluidix Gmbh. It incorporates in a single device the operations: centrifugal actuation of 96 cartridges, isothermal amplification and fluorescence readout. A picture of the device and preliminary results are depicted in Fig. S2 and S3.

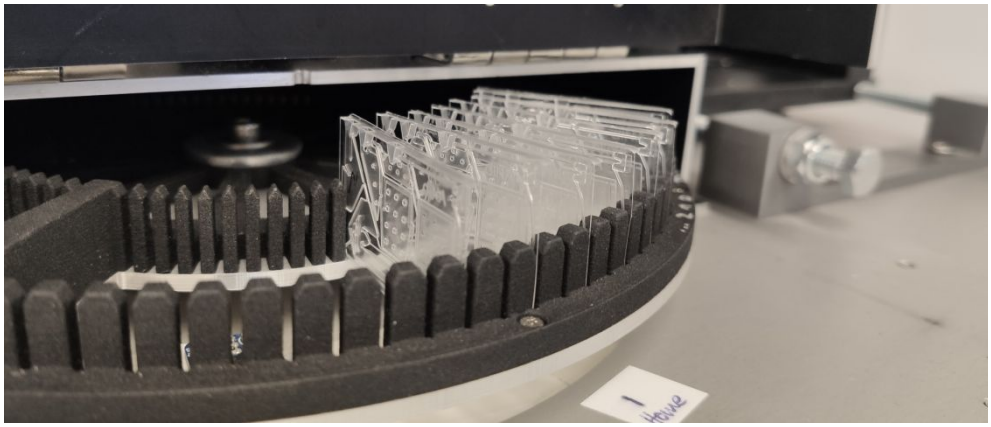

Figure S2. Detailed view on the rotor for parallelized processing of 96 cartridges in a novel processing device which is currently under development. In the picture the rotor is equipped with seven cartridges. The reagents are supplied from top by manual pipetting. Sample and reagent supply, droplet generation, incubation and readout works well in the vertical orientation.

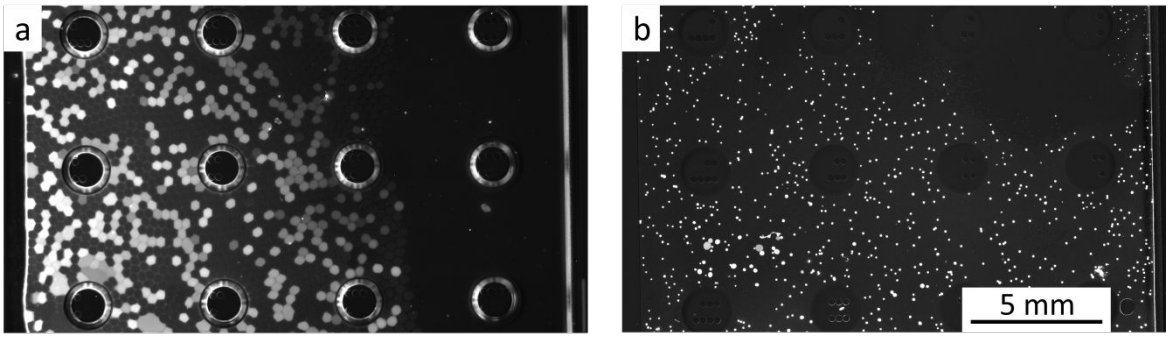

Figure S3. Preliminary results generated in the novel processing device for parallelized processing of 96 cartridges which is currently under development. a) Fluorescent image of the droplet area in DGU 1 (large droplets). b) Fluorescent image of the droplet area in DGU 2 (small droplets). White droplets represent positive signals.