Electronic Supplementary Information

Interfacing centrifugal microfluidics with linear-oriented 8-tube strips and multichannel pipettes for increased throughput of digital assays

Yu-Kai Lai[ab], Yu-Ting Kao[b], Jacob Hess[ab], Silvia Calabrese[a], Felix von Stetten[ab] and Nils Paust[ab*]

S1: PCR buffer (1×) preparation

1 fold Supermix dilution in water was used for disperse phase for droplet size characterization. 2× ddPCR Supermix for probes (Bio-Rad Laboratories Inc., Hercules, CA, USA) was diluted to 1:1 (v/v) in distilled water (UltraPure DNase/RNase free, Invitrogen Corp., Carlsbad, CA, USA). Viscosity measurement was carried out by Physica MCR 101 rheometer (Anton Paar, Graz, Austria) at 20 °C. The measured viscosity is 1.653 ±0.003 mPa·s. The density was measured by weighing 1 ml of the buffer in lab tube. The value is ~1000 kg/m^3.

S2: Viscosity of emulsion flow

We noted that multiple factors (viscosity of disperse phase and continuous phase, number of droplet in the channel, droplet morphology, etc.) affect the viscosity of emulsion flow η_{eml}. Since we design identical fluidic condition as the work from Schulz et al. [1], we also expect that the η_{eml} derived from the reference work would cover other factors (e.g. capillary pressure at nozzle). η_{eml} was determined as follows: First, a series of solutions for $\bar{Q}_{spl}$ in the proposed network simulation model (described in the manuscript) using geometry from previous work [1] were solved by sweeping potential η_{eml} from 2 to 18 mPa·s at intervals of 0.1 mPa·s. Secondly, the corresponding η_{eml} was interpolated with the measured $\bar{Q}_{spl}$. The result is shown in Fig. S2. A linear function was applied for an estimation of η_{eml} and therefore η_{eml} was obtained $\sim$10.5 mPa·s in the case $\eta_{spl} = 1.65$ mPa·s.

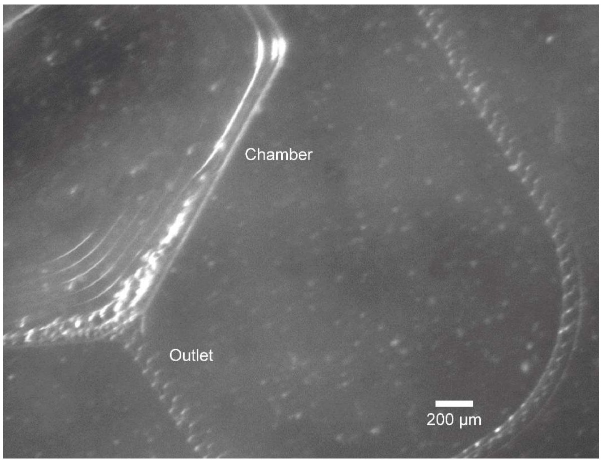

Figure S1 Squeezed droplet in the outlet channel.

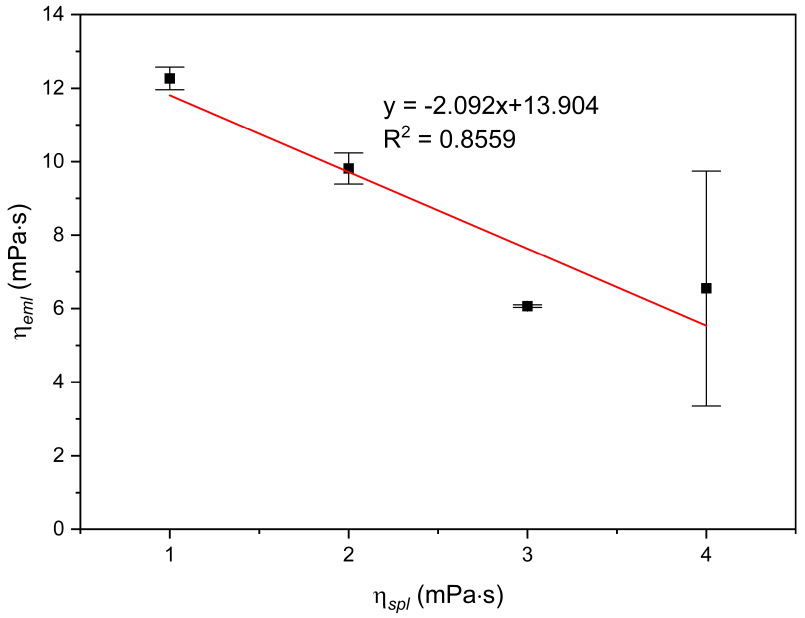

Figure S2 the fitting η_{eml} as a function of η_{spl}.

S3: Design geometry:

Table S1 List of geometry and material parameters used in the microfluidic design.

Abbreviation	Description	Value
d_{oil}	Depth oil supply channel	37 µm
w_{oil}	Width oil supply channel	49 µm
L_{oil}	Length oil supply channel	Varies on DGU case
ρ_{oil}	Density fluorinated oil	1614 Kg·m^{-3}
η_{oil}	Dynamic viscosity fluorinated oil	1.24 mPa·s
d_{spl}	Depth sample supply channel	58 µm
w_{spl}	Width sample supply channel	62 µm
L_{spl}	Length sample supply channel	Varies on DGU case
ρ_{spl}	Density sample	~1000 Kg·m^{-3}
η_{spl}	Dynamic viscosity sample	1.653 ±0.003 mPa·s
d_{nzl}	Depth nozzle	25 µm
w_{onzl}	Width nozzle	40 µm
L_{nzl}	Length nozzle	500 µm
d_{out}	Depth outlet channel	100 µm
w_{out}	Width outlet channel	100 µm
L_{out}	Length outlet channel	Varies on DGU case

Table S2 List of outlet channel length (unit: mm)

Abbreviation	DGU1	DGU2	DGU3	DGU4	DGU5	DGU6	DGU7	DGU8
L_{out}	13.53	13.41	13.59	13.66	13.91	14.13	14.35	14.35

S4: Geometry verification after manufacturing

The key feature for step emulsification are the nozzle dimensions. Hence, we checked the nozzle dimension after CNC milling of the PMMA substrate. The nozzles were measured by confocal microscopy (DUO Vario, Confovis GmbH, Jena, Germany). Here, we averaged the dimension of 8 nozzles from corresponding DGU for each DGU case.

Table S3 Measured nozzle dimensions by profilometer. (unit: µm)

Abbreviation	DGU1	DGU2	DGU3	DGU4	DGU5	DGU6	DGU7	DGU8
d_{nzl}	24.6 ± 0.38	24.6 ± 0.19	24.7 ± 0.24	25.4 ± 0.13	26.0 ± 0.18	26.2 ± 0.19	26.3 ± 0.21	24.6 ± 0.22
w_{nzl}	43.7 ± 0.90	43.0 ± 0.59	42.9 ± 0.84	42.3 ± 1.23	42.7 ± 0.49	42.6 ± 0.92	43.2 ± 0.64	42.4 ± 1.05

The channel dimensions were also measured. The measured dimensions are listed below:

Table S4 Measured channel dimensions by profilometer. (unit: µm)

Abbreviation	DGU1	DGU2	DGU3	DGU4	DGU5	DGU6	DGU7	DGU8
d_{oil}	36.3	36.4	35.7	35.9	36.3	35.9	36.1	35.6
w_{oil}	52.7	53.3	52.0	55.1	53.3	51.2	52.4	53.1
d_{spl}	61.1	61.4	61.6	61.5	60.7	60.3	61.8	59.8
w_{spl}	63.6	63.0	64.5	61.9	62.4	64.5	64.9	61.8
d_{out}	96.7	97.2	96.6	97.3	96.9	96.1	96.0	96.0
w_{out}	100.8	102.1	97.5	105.2	105.4	99.4	99.0	99.9

S5: Description of the droplet diameter evaluation in MATLAB

Stitched bright field images were acquired by the microscope Observer Z1 (Zeiss GmbH, Germany), followed by circle recognition based on build-in Hough algorithm in MATLAB. As shown in the Fig. S3, the droplets are detected and outlines are marked in the image (see Fig. S3 b). Diameter profile of 2000 droplets were extracted for further plotting in Origin Pro 9 (OriginLab Corporation, USA).

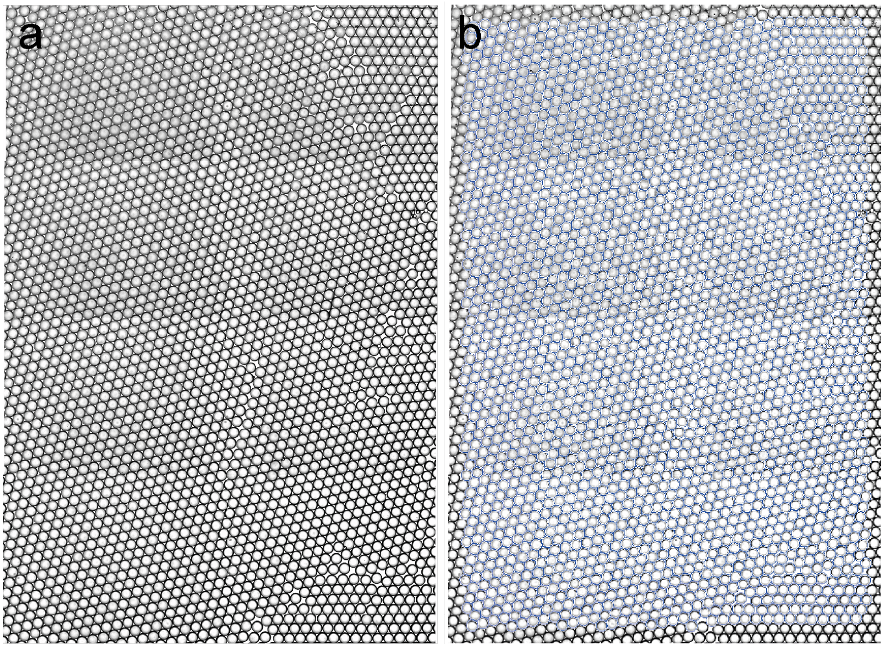

Figure S3 Circle recognition done by customized MATLAB script.

S6: ddPCR result from individual DGU

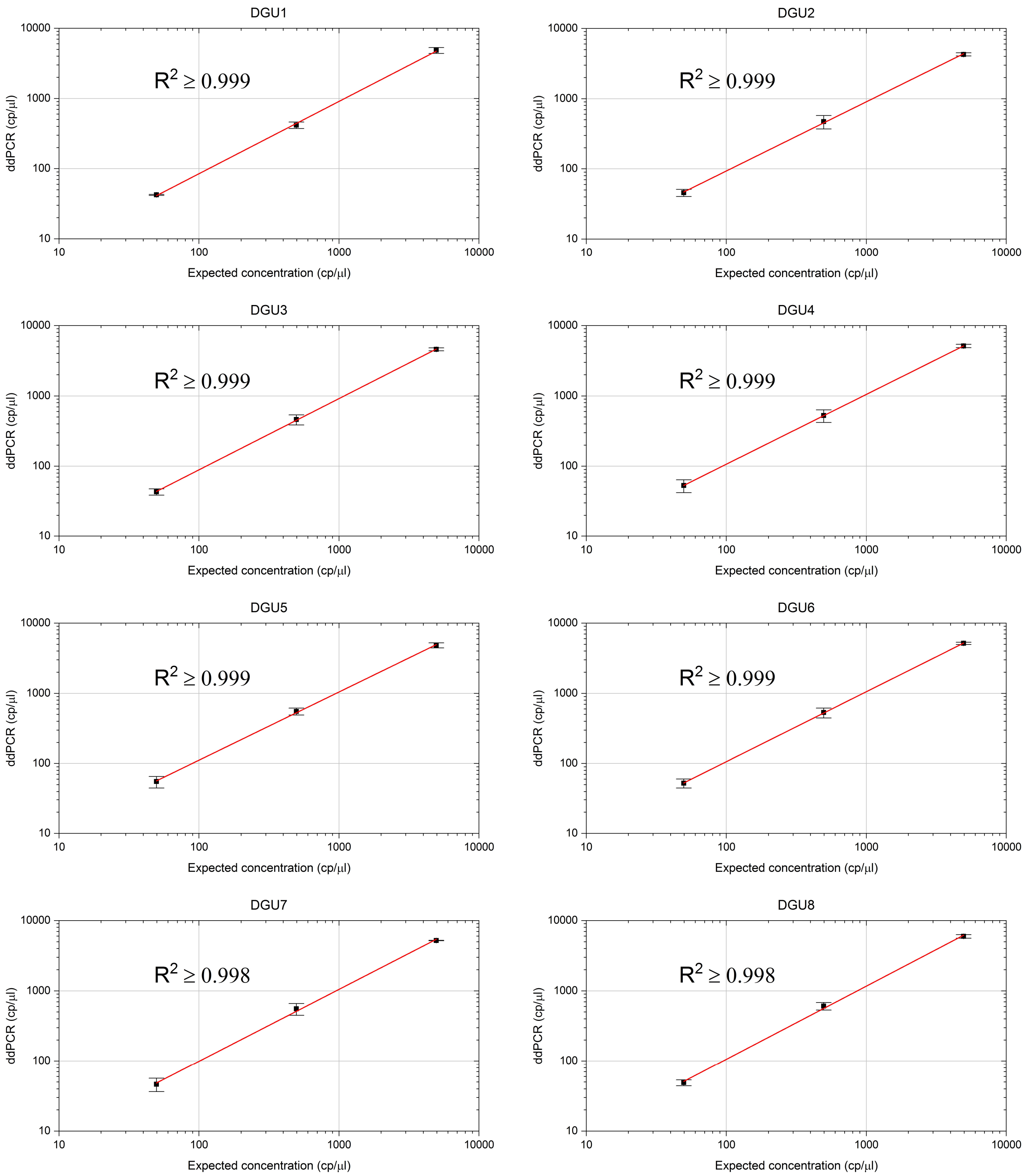

Figure S4 Result of ddPCR from each DGU case. Red lines mark linear regression.

Reference:

1. Martin Schulz, Sophia Probst, Silvia Calabrese, Ana R. Homann, Nadine Borst, Marian Weiss, Felix Von Stetten, Roland Zengerle and Nils Paust, Versatile Tool for Droplet Generation in Standard Reaction Tubes by Centrifugal Step Emulsification, *Molecules*, 2020, **25**, 1914.