Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information to

Highly flexible and accurate serial picoinjection in droplets by combined pressure and flow rate control

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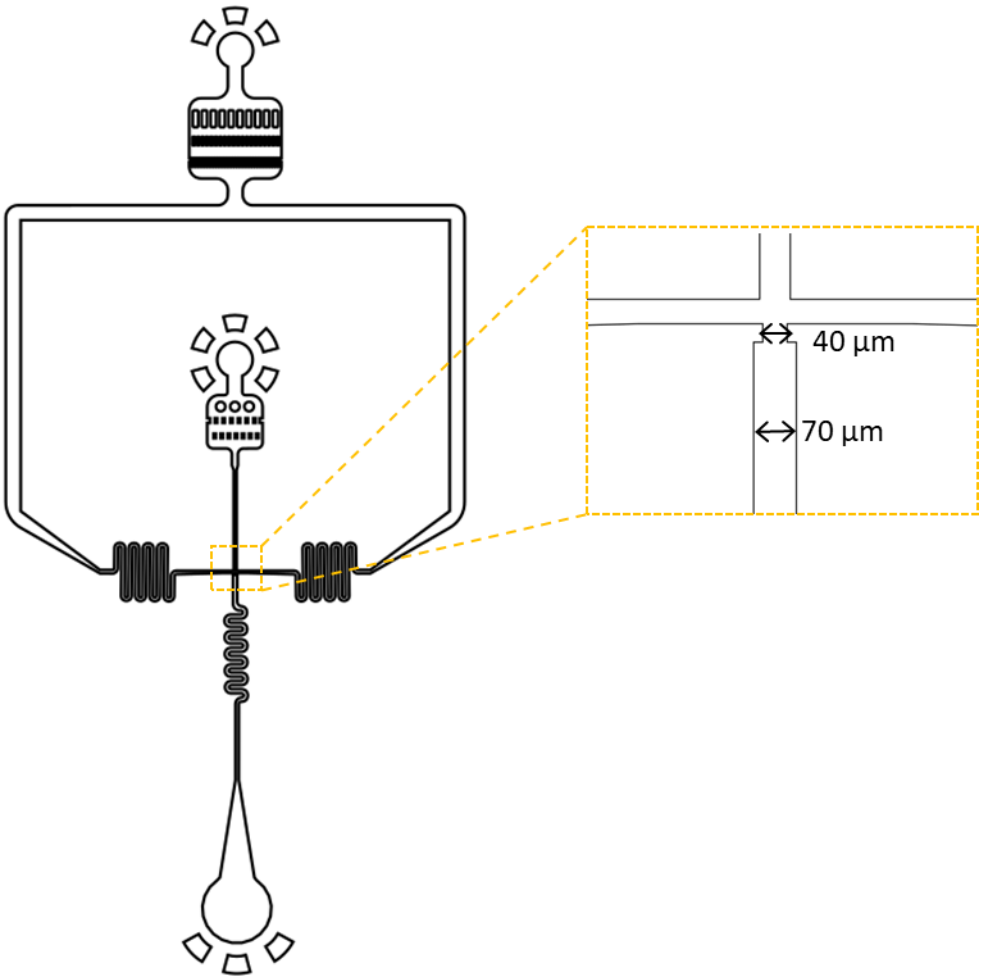

Fig. S1: Flow focusing design

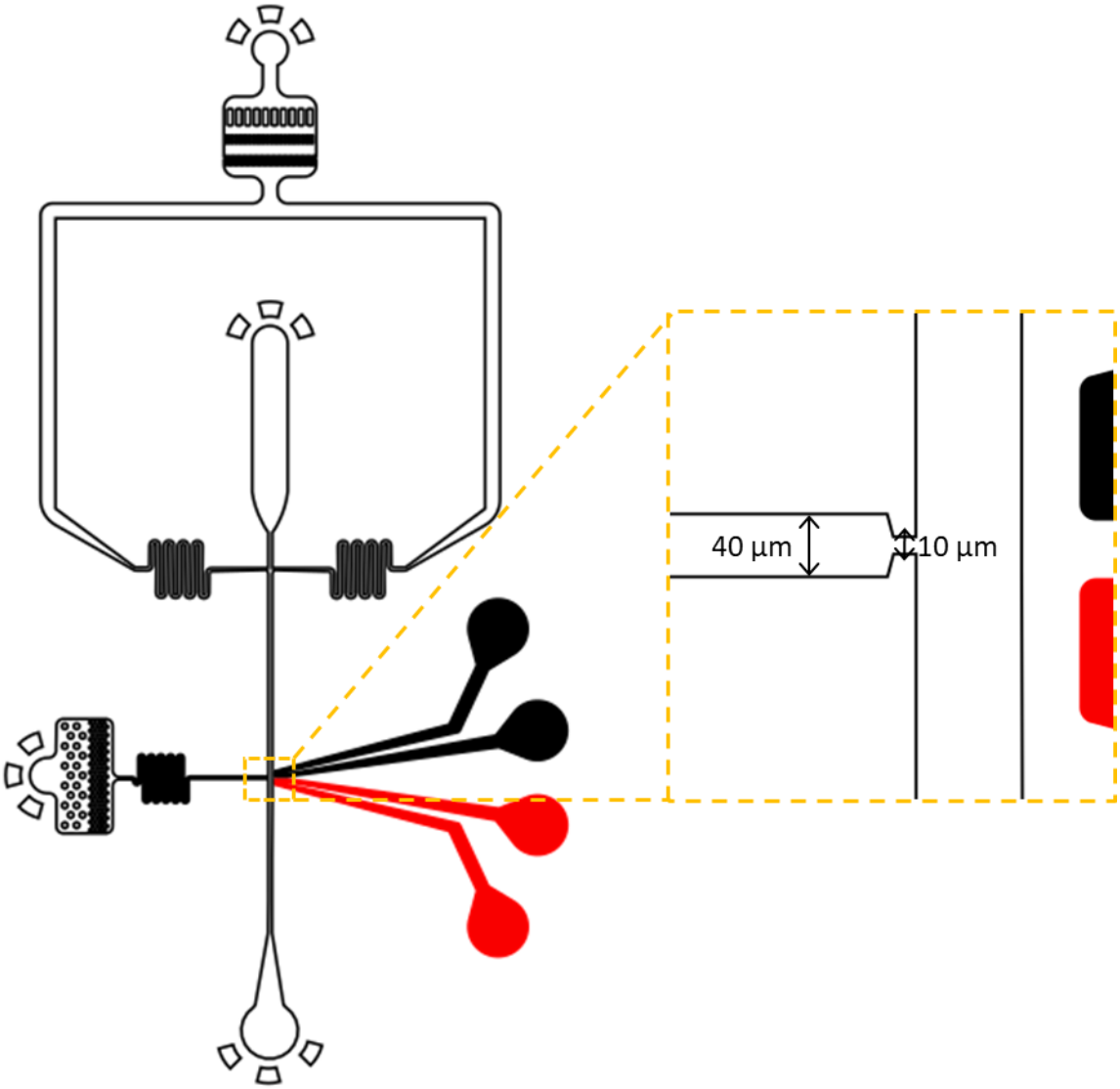

Fig. S2: Design of a picoinjection chip with 1 injector. The picoinjector channel of 40 µm width narrows to an orifice of 10 µm width to enable setting of different equilibrium pressures as defined in the main text (P_{min}, P_{mid}, P_{max}). The black electrode is connected to ground, and the voltage signal is applied to the red electrode.

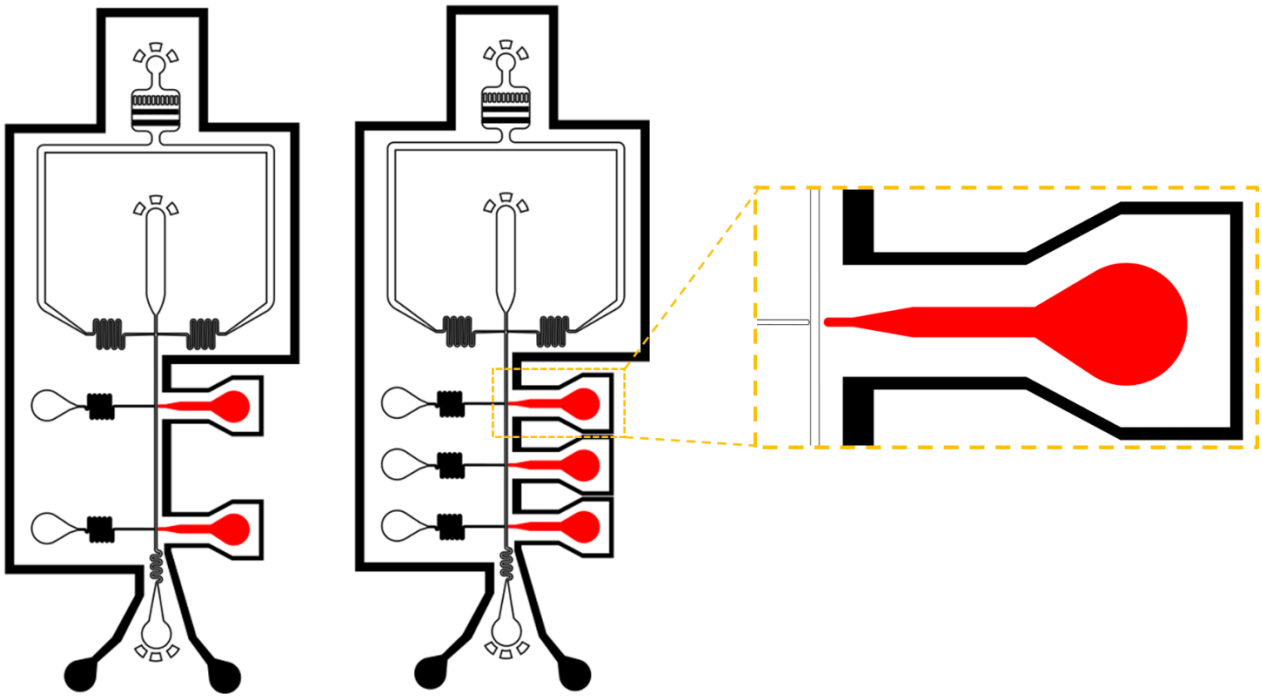

Fig. S3: Design of picoinjection chip with 2 or 3 injectors. The black electrode is connected to ground, and the voltage signal is applied to the red electrodes. The electrode design was based on Sciambi et al.[32]

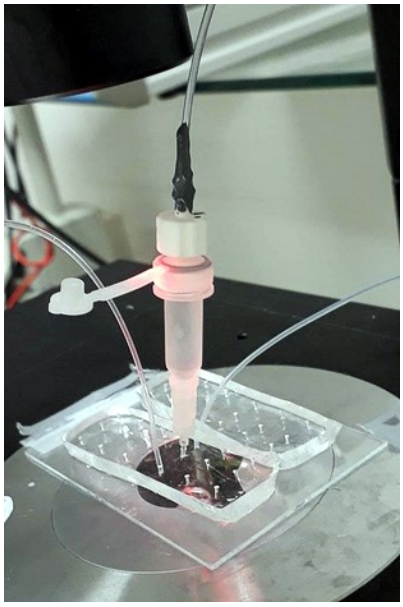

Fig. S4: Cell loading in the microfluidic chip was achieved by filling a microfluidic reservoir (based on 'microfluidic reservoirs for PDMS chip (XXS)' from Darwin microfluidics) with cells at a concentration of 2.5 x 10^6 cells/mL. The microfluidic reservoir was on one side glued to microfluidic tubing that was connected to the pressure pumps and flow sensors, and on the other side plugged into the PDMS microfluidic chip using a short needle.

Table S1: Flow rate settings to determine the linear model for the equilibrium pressure in case of a single injector.

Calibrate P_{eq} for 1 injector	
Q_{oil} (µL/min)	**$Q_{droplets}$ (µL/min)**
8	1.6
9	1.8
10	2
11	2.2
12	2.4
13	2.6

Table S2: Pressure and flow rate settings to determine the linear models for the equilibrium pressure in case of 2 serial injectors.

Calibrate 2 serial injectors	
Setting Injector 1	**Setting Injector 2**
P_{eq}	0.5 µL/min
P_{eq}	1 µL/min
P_{eq}	1.5 µL/min
P_{eq}	2 µL/min
P_{eq}	2.5 µL/min
0.5 µL/min	P_{eq}
1 µL/min	P_{eq}
1.5 µL/min	P_{eq}
2 µL/min	P_{eq}
2.5 µL/min	P_{eq}
P_{eq}	P_{eq}

Table S3: Pressure and flow rate settings to determine the linear models for the equilibrium pressure in case of 3 serial injectors. To perform full calibration, 36 measurement points were defined per injector.

Calibrate injector 1				**Calibrate injector 2**				**Calibrate injector 3**		
Setting injector 1	Setting injector 2	Setting injector 3		Setting injector 1	Setting injector 2	Setting injector 3		Setting injector 1	Setting injector 2	Setting injector 3
P_{eq}	1 µL/min	P_{eq}		1 µL/min	P_{eq}	P_{eq}		1 µL/min	P_{eq}	P_{eq}
P_{eq}	1.5 µL/min	2.5 µL/min		1.5 µL/min	P_{eq}	2.5 µL/min		1.5 µL/min	2.5 µL/min	P_{eq}
P_{eq}	1 µL/min	2 µL/min		1 µL/min	P_{eq}	2 µL/min		1 µL/min	2 µL/min	P_{eq}
P_{eq}	2.5 µL/min	0.5 µL/min		2.5 µL/min	P_{eq}	0.5 µL/min		2.5 µL/min	0.5 µL/min	P_{eq}
P_{eq}	1.5 µL/min	1.5 µL/min		1.5 µL/min	P_{eq}	1.5 µL/min		1.5 µL/min	1.5 µL/min	P_{eq}
P_{eq}	P_{eq}	1.5 µL/min		P_{eq}	P_{eq}	1.5 µL/min		P_{eq}	1.5 µL/min	P_{eq}
P_{eq}	P_{eq}	2 µL/min		P_{eq}	P_{eq}	2 µL/min		P_{eq}	2 µL/min	P_{eq}
P_{eq}	1.5 µL/min	0.5 µL/min		1.5 µL/min	P_{eq}	0.5 µL/min		1.5 µL/min	0.5 µL/min	P_{eq}
P_{eq}	2.5 µL/min	P_{eq}		2.5 µL/min	P_{eq}	P_{eq}		2.5 µL/min	P_{eq}	P_{eq}
P_{eq}	1 µL/min	1.5 µL/min		1 µL/min	P_{eq}	1.5 µL/min		1 µL/min	1.5 µL/min	P_{eq}
P_{eq}	P_{eq}	0.5 µL/min		P_{eq}	P_{eq}	0.5 µL/min		P_{eq}	0.5 µL/min	P_{eq}
P_{eq}	0.5 µL/min	P_{eq}		0.5 µL/min	P_{eq}	P_{eq}		0.5 µL/min	P_{eq}	P_{eq}
P_{eq}	1.5 µL/min	P_{eq}		1.5 µL/min	P_{eq}	P_{eq}		1.5 µL/min	P_{eq}	P_{eq}
P_{eq}	0.5 µL/min	2 µL/min		0.5 µL/min	P_{eq}	2 µL/min		0.5 µL/min	2 µL/min	P_{eq}
P_{eq}	0.5 µL/min	2.5 µL/min		0.5 µL/min	P_{eq}	2.5 µL/min		0.5 µL/min	2.5 µL/min	P_{eq}
P_{eq}	2 µL/min	1 µL/min		2 µL/min	P_{eq}	1 µL/min		2 µL/min	1 µL/min	P_{eq}
P_{eq}	2 µL/min	1.5 µL/min		2 µL/min	P_{eq}	1.5 µL/min		2 µL/min	1.5 µL/min	P_{eq}
P_{eq}	2.5 µL/min	2.5 µL/min		2.5 µL/min	P_{eq}	2.5 µL/min		2.5 µL/min	2.5 µL/min	P_{eq}
P_{eq}	P_{eq}	P_{eq}		P_{eq}	P_{eq}	P_{eq}		P_{eq}	P_{eq}	P_{eq}
P_{eq}	P_{eq}	2.5 µL/min		P_{eq}	P_{eq}	2.5 µL/min		P_{eq}	2.5 µL/min	P_{eq}
P_{eq}	2 µL/min	0.5 µL/min		2 µL/min	P_{eq}	0.5 µL/min		2 µL/min	0.5 µL/min	P_{eq}
P_{eq}	1 µL/min	0.5 µL/min		1 µL/min	P_{eq}	0.5 µL/min		1 µL/min	0.5 µL/min	P_{eq}
P_{eq}	1.5 µL/min	2 µL/min		1.5 µL/min	P_{eq}	2 µL/min		1.5 µL/min	2 µL/min	P_{eq}
P_{eq}	0.5 µL/min	1.5 µL/min		0.5 µL/min	P_{eq}	1.5 µL/min		0.5 µL/min	1.5 µL/min	P_{eq}
P_{eq}	2.5 µL/min	2 µL/min		2.5 µL/min	P_{eq}	2 µL/min		2.5 µL/min	2 µL/min	P_{eq}
P_{eq}	P_{eq}	1 µL/min		P_{eq}	P_{eq}	1 µL/min		P_{eq}	1 µL/min	P_{eq}
P_{eq}	2 µL/min	2.5 µL/min		2 µL/min	P_{eq}	2.5 µL/min		2 µL/min	2.5 µL/min	P_{eq}
P_{eq}	0.5 µL/min	0.5 µL/min		0.5 µL/min	P_{eq}	0.5 µL/min		0.5 µL/min	0.5 µL/min	P_{eq}
P_{eq}	0.5 µL/min	1 µL/min		0.5 µL/min	P_{eq}	1 µL/min		0.5 µL/min	1 µL/min	P_{eq}
P_{eq}	1 µL/min	2.5 µL/min		1 µL/min	P_{eq}	2.5 µL/min		1 µL/min	2.5 µL/min	P_{eq}
P_{eq}	2 µL/min	P_{eq}		2 µL/min	P_{eq}	P_{eq}		2 µL/min	P_{eq}	P_{eq}
P_{eq}	2 µL/min	2 µL/min		2 µL/min	P_{eq}	2 µL/min		2 µL/min	2 µL/min	P_{eq}
P_{eq}	2.5 µL/min	1.5 µL/min		2.5 µL/min	P_{eq}	1.5 µL/min		2.5 µL/min	1.5 µL/min	P_{eq}
P_{eq}	2.5 µL/min	1 µL/min		2.5 µL/min	P_{eq}	1 µL/min		2.5 µL/min	1 µL/min	P_{eq}
P_{eq}	1.5 µL/min	1 µL/min		1.5 µL/min	P_{eq}	1 µL/min		1.5 µL/min	1 µL/min	P_{eq}
P_{eq}	1 µL/min	1 µL/min		1 µL/min	P_{eq}	1 µL/min		1 µL/min	1 µL/min	P_{eq}

Table S4: Pressure and flow rate settings to determine the linear models for the equilibrium pressure in case of 3 serial injectors. The number of settings was reduced to 7, to allow for faster calibration.

Calibrate 3 serial injectors		
Setting Injector 1	**Setting Injector 2**	**Setting injector 3**
P_{eq}	P_{eq}	P_{eq}
P_{eq}	P_{eq}	2.5 µL/min
P_{eq}	2.5 µL/min	P_{eq}
2.5 µL/min	P_{eq}	P_{eq}
2.5 µL/min	P_{eq}	2.5 µL/min
P_{eq}	2.5 µL/min	2.5 µL/min
2.5 µL/min	2.5 µL/min	P_{eq}

Table S5: Tested conditions to measure the response time when switching picoinjector settings.

From	To		From	To
0.5 µL/min	1 µL/min		0.5 µL/min	P_{eq}
1 µL/min	1.5 µL/min		1 µL/min	P_{eq}
1.5 µL/min	2 µL/min		1.5 µL/min	P_{eq}
2 µL/min	2.5 µL/min		2 µL/min	P_{eq}
2.5 µL/min	2 µL/min		2.5 µL/min	P_{eq}
2 µL/min	1.5 µL/min			
1.5 µL/min	1 µL/min			
1 µL/min	0.5 µL/min			
0.5 µL/min	1.5 µL/min			
1.5 µL/min	2.5 µL/min		**From**	**To**
2.5 µL/min	1 µL/min		P_{eq}	0.5 µL/min
1 µL/min	2 µL/min		P_{eq}	1 µL/min
2 µL/min	0.5 µL/min		P_{eq}	1.5 µL/min
0.5 µL/min	2.5 µL/min		P_{eq}	2 µL/min
2.5 µL/min	1.5 µL/min		P_{eq}	2.5 µL/min
1.5 µL/min	0.5 µL/min			
0.5 µL/min	2 µL/min			
2 µL/min	1 µL/min			
1 µL/min	2.5 µL/min			
2.5 µL/min	0.5 µL/min			

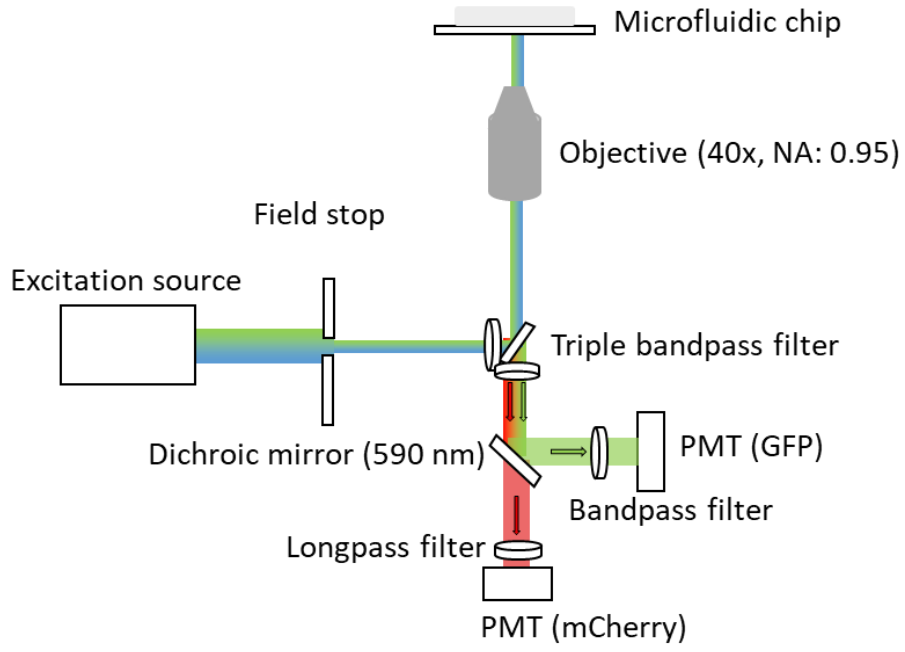

Fig. S5: Detailed microscope set up for dual PMT detection at GFP and mCherry wavelengths. The light from a fluorescent light source (pE-300 white SB, CoolLED, Andover, United Kingdom) passes through a field stop to narrow the light to a spot with the same size as the droplets. Using a triple bandpass filter (69401, Chroma Technology Corporation, Bellows Falls, VT, USA), both blue and green light excite the sample. Upon passing of a fluorescent droplet, green and red light is emitted. A dichroic mirror transmits light above 590 nm through a longpass filter towards the PMT for mCherry detection, and reflects the light below 590 nm through a bandpass filter towards the PMT for GFP detection.

Movie S1: Active picoinjection using flow rate control. The injector flow rate was set at 1.5 µL/min and the electrodes were actuated. The injector liquid bulges out of the injector channel into the main channel, where it merges with passing droplets.

Movie S2: Active picoinjection using flow rate control with a too high flow rate of 3 µL/min. The picoinjector forms droplets at a faster rate than the passing droplets, resulting in faulty injection.

Movie S3: Inactive picoinjection using pressure control. The equilibrium pressure of the injector was set and electrodes were deactivated, resulting in a stable interface between the injector liquid and the main channel.

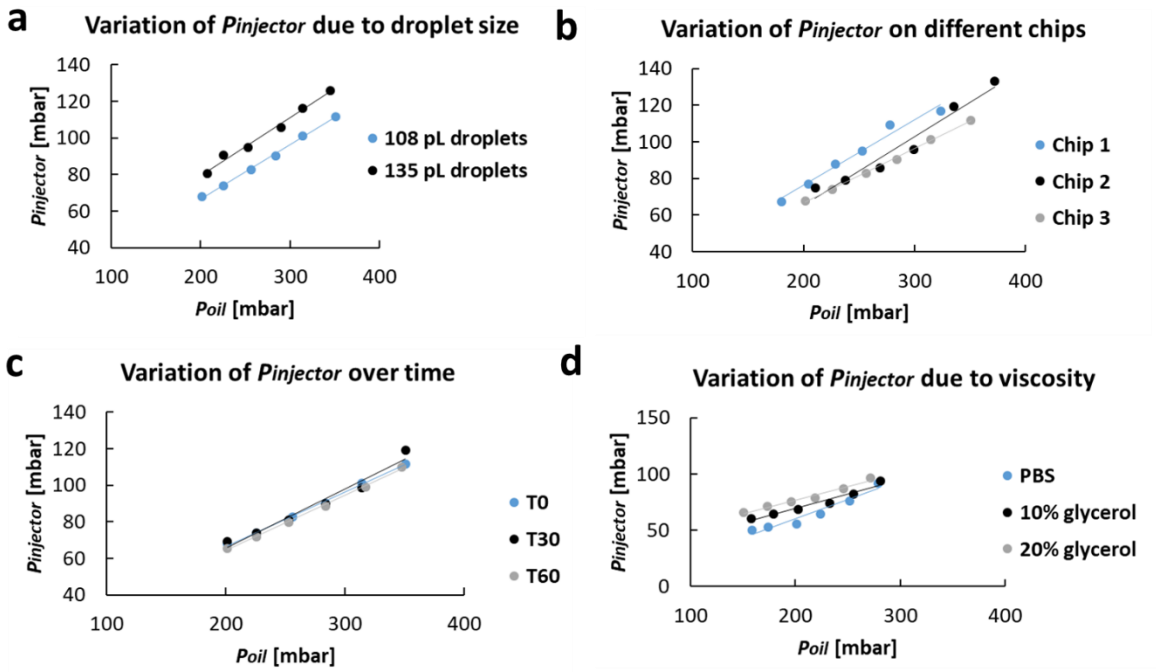

Fig. S6: (a) The equilibrium pressure of the injector depended on droplet size. Measurements were performed on the same chip. Analysis of covariance showed that the slopes of the 2 models were not significantly different whereas the intercepts did differ significantly. (b) The equilibrium pressure of an injector varied between different chips. Measurements were performed with droplets of the same size. Analysis of covariance showed that the slopes of the models were not significantly different, while there was a significant difference between the intercepts of the models obtained for different chips. (c) The equilibrium pressure of an injector remained stable over 60 min. Analysis of covariance showed that there were no significant differences between the slopes and intercepts of the models obtained at different time points. (d) The equilibrium pressure of an injector varied for injector liquids with different viscosity. Glycerol was dissolved as 10 or 20% (V/V) in PBS to increase the viscosity of the injector liquid. Analysis of covariance showed that the slopes of the models were not significantly different, while there was a significant difference between the intercepts of the models obtained for different viscosities.

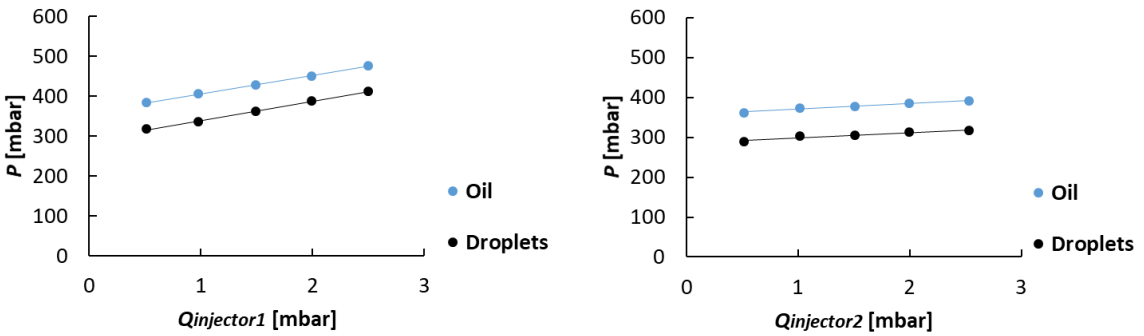

Fig. S7: The pressure required to reach a fixed flow rate of the oil and droplets was influenced by the injector flow rate in a system with 2 picoinjectors.

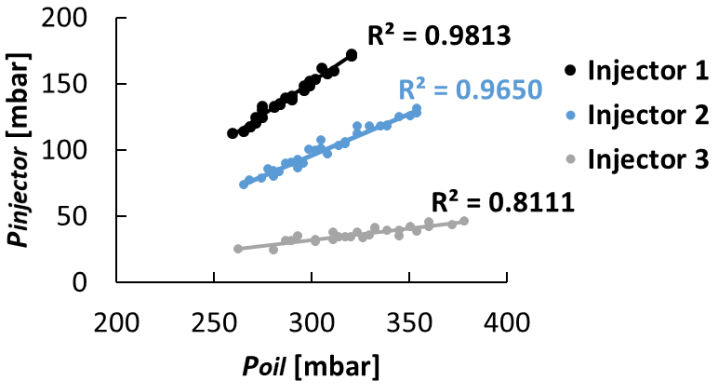

Fig. S8: Results of pressure calibration for a system with 3 injectors in which the third injector was located too close to the outlet, leading to equilibrium pressures below 50 mbar. This resulted in a lower coefficient of determination and consequently less robust picoinjector control.

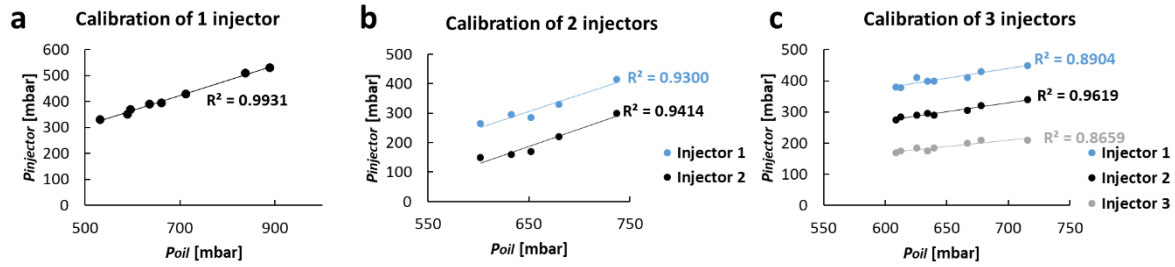

Fig. S9: In a system with smaller dimensions and droplets of around 4 pL, linear relationships between the pressure of the injector and the pressure of the oil were also found for 1(a), 2(b) and 3(c) injectors. Here, the data was obtained differently than described in the manuscript. More specifically, data from different microfluidic chips were combined in which all injectors were set at equilibrium pressure.

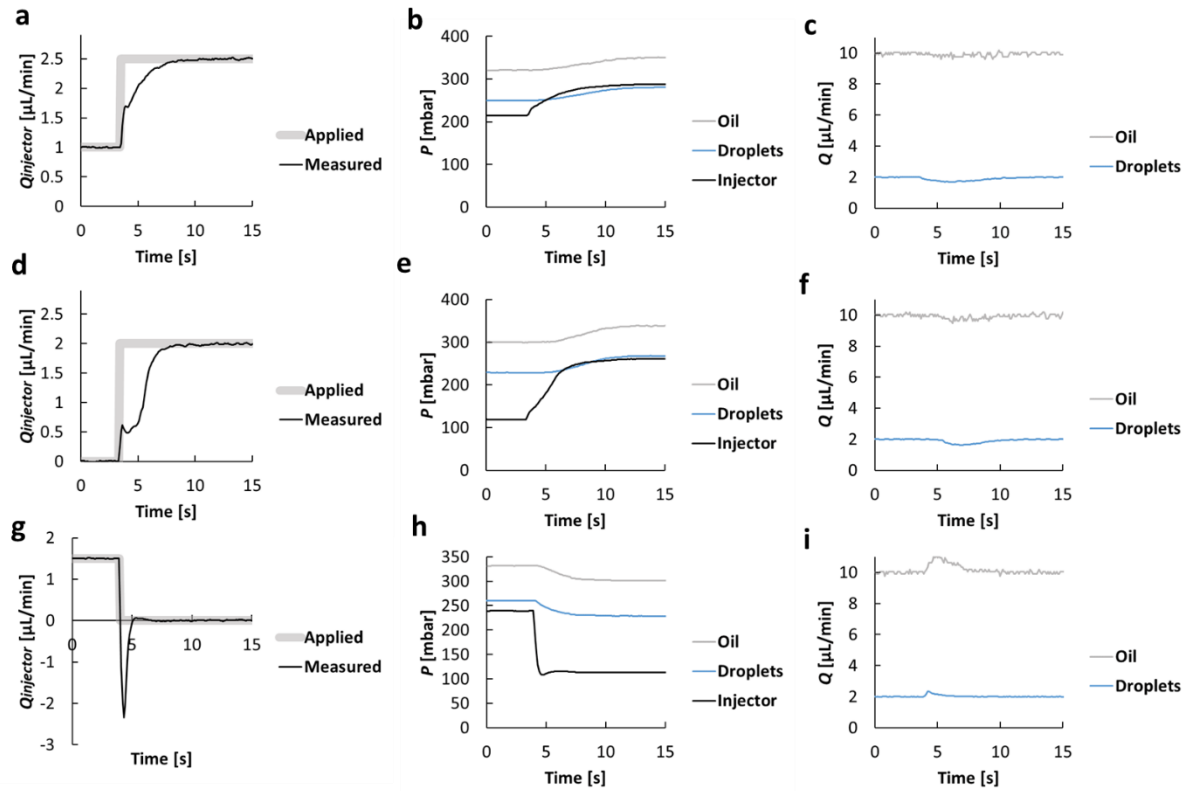

Fig. S10: Stabilization time when changing picoinjector settings. (a),(b),(c) Example data when switching from one injector flow rate to another. (d),(e),(f) Example data when switching from equilibrium pressure to flow rate control. (g),(h),(i) Example data when switching from flow rate control to equilibrium pressure. (a),(d),(g) Applied and measured injector flow rates. (b),(e),(h) Pressure data of the oil, droplets and injector liquid. (c),(f),(i) Flow rate data of the oil and droplets. The stabilization time was defined as the time between (i) the moment at which the injector pressure and flow rate deviated more than 1 mbar and 0.05 µL/min respectively when compared to the initial pressure and flow rate, and (ii) the moment at which the injector pressure and flow rate did not deviate more than 1 mbar and 0.05 µL/min respectively when compared to the final pressure and flow rate.

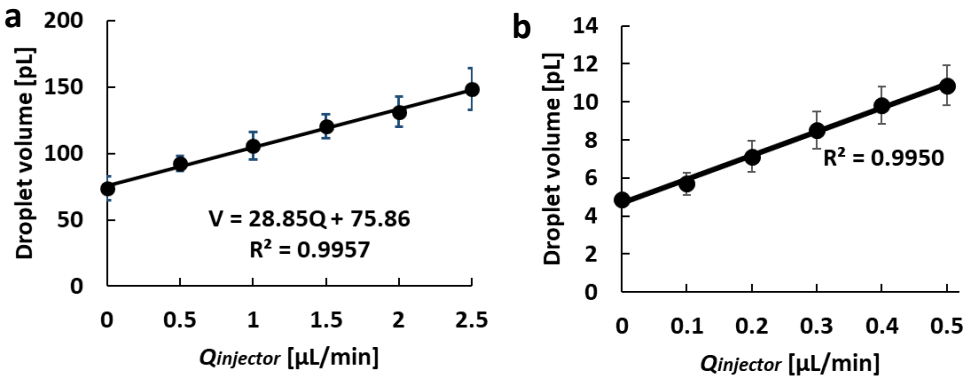

Fig. S11: (a) Increase in droplet volume by injecting PBS with 20% (V/V) glycerol. Droplets remained highly monodisperse (CV < 3.3%) after injection. (b) Increase in droplet volume by injecting PBS into PBS droplets of about 5 pL. Droplets remained highly monodisperse (CV < 3.8%) after injection.

Table S6: Conditions for picoinjection with time-invariant settings using 3 serial injectors.

Picoinjection with time-invariant settings using 3 serial injectors				
	Setting injector 1	**Setting injector 2**	**Setting injector 3**	**Total flow rate**
1	*P eq*	*P eq*	2 µL/min	2 µL/min
2	*P eq*	2 µL/min	*P eq*	2 µL/min
3	2 µL/min	*P eq*	*P eq*	2 µL/min
5	*P eq*	1 µL/min	1 µL/min	2 µL/min
4	1 µL/min	*P eq*	1 µL/min	2 µL/min
6	1 µL/min	1 µL/min	*P eq*	2 µL/min
7	0.5 µL/min	*P eq*	1.5 µL/min	2 µL/min
8	0.5 µL/min	1.5 µL/min	*P eq*	2 µL/min
9	0.5 µL/min	0.5 µL/min	1 µL/min	2 µL/min
10	0.5 µL/min	1 µL/min	0.5 µL/min	2 µL/min
11	*P eq*	0.5 µL/min	1.5 µL/min	2 µL/min
12	1.5 µL/min	0.5 µL/min	*P eq*	2 µL/min
13	1 µL/min	0.5 µL/min	0.5 µL/min	2 µL/min
14	*P eq*	1.5 µL/min	0.5 µL/min	2 µL/min
15	1.5 µL/min	*P eq*	0.5 µL/min	2 µL/min

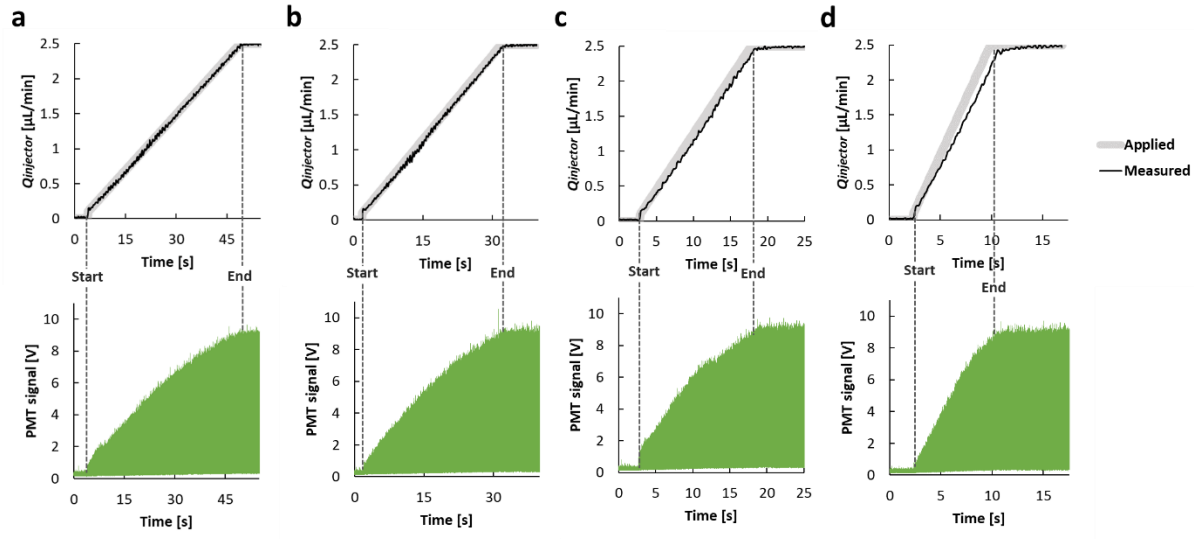

Fig. S12: Measured and applied injector flow rate and accompanying PMT signals for time-variant picoinjector settings. Flow rates were gradually increased from zero (equilibrium pressure) to 2.5 µL/min within a time frame of (a) 45 s, (b) 30 s, (c) 15s, (d) 7.5 s. For a time period of 45 or 30 s, the measured flow rate followed the applied flow rate and the increase in the PMT signal corresponded to the increase in the applied flow rate. For a time period of 15 s or 7.5 s, the measured flow rate lagged behind the applied flow rate, and the increase in the PMT signal corresponded to the measured flow rate.

Table S7: Conditions for picoinjection with time-invariant settings using 2 serial injectors, used for the detergent concentration screening application.

	Calibration by time-invariant picoinjection	
	Setting injector 1	**Setting injector 2**
1	P_{eq}	1.5 µL/min
2	0.5 µL/min	1 µL/min
3	1 µL/min	0.5 µL/min
4	1.5 µL/min	P_{eq}

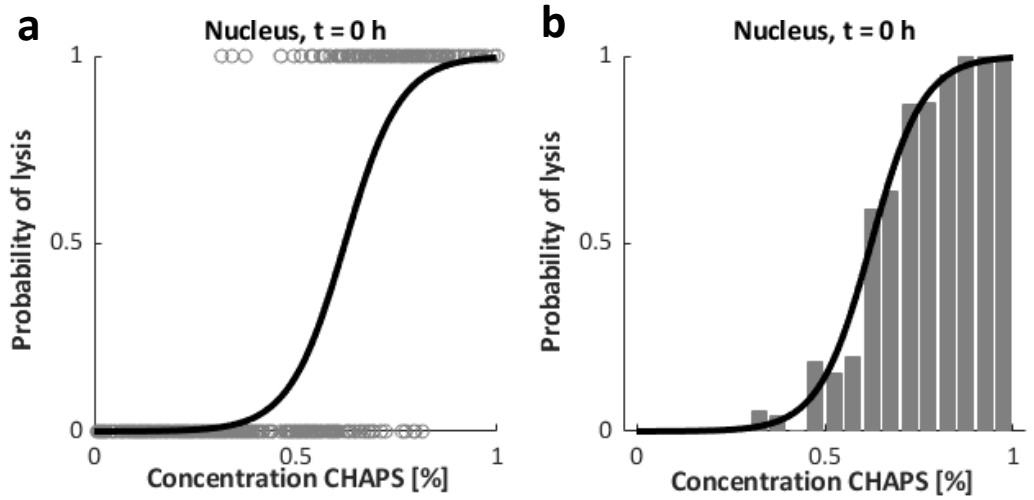

Fig. S13: Logistic plots of the condition of nuclear lysis after an incubation time of 0 h, to give an example of the fitted experimental data. (a) Logistic regression line in combination with scatter plot of each single-cell measurement. For each cell, nuclear lysis was labeled with 0 or 1. (b) For visualization purposes only, the logistic regression line was plotted together with a bar graph containing the single-cell data subdivided in arbitrarily chosen intervals with a width of 0.05% CHAPS. Here, the percentage of lysed cells was calculated per interval based on the 0/1 labeled data.

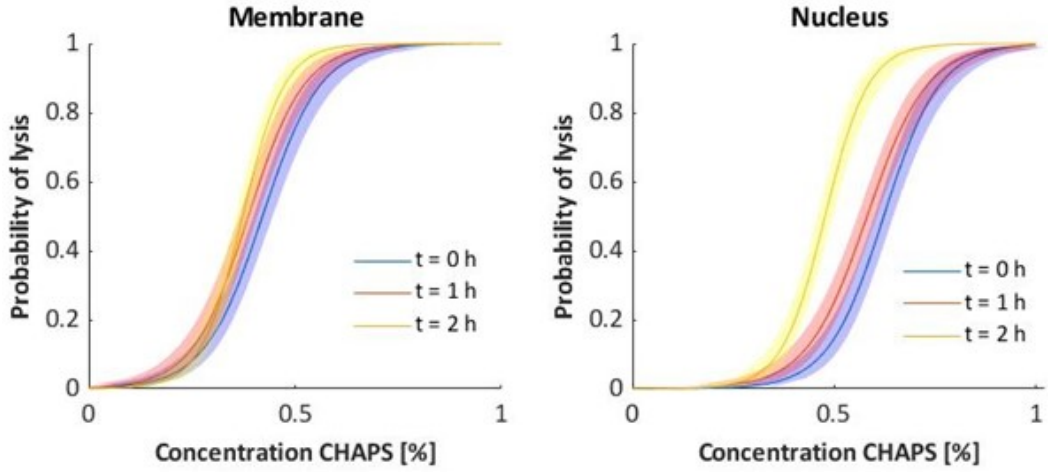

Fig. S14: Logistic regression plots with asymmetrical 95% confidence intervals.

Table S8: Summary of multiple logistic regression of probability of membrane and nuclear lysis in relationship to incubation time and CHAPS concentration.

Membrane lysis				
Parameter estimates				
Term	**Estimate**	**Standard error**	**ChiSquare**	**P value**
Intercept	-6.82	0.39	293.29	<.0001
Concentration CHAPS [%]	16.21	0.89	332.94	<.0001
Time [h]	0.47	0.13	13.43	0.0002
(Concentration CHAPS [%]-0.31828)*(Time [h]-1.00199)	2.48	1.05	5388	0.0182
Whole model test				
Model	**-LogLikelihood**	**DF**	**ChiSquare**	**P value**
Difference	691.83	3	1383.66	<.0001
Full	355.16			
Reduced	1046.99			
Unit odds ratio				
Term	**Odds ratio**	**Lower 95%**	**Upper 95%**	**Reciprocal**
Concentration chaps [%]	11014980	1930051	62863492	9.08e-8
Time [h]	1.60	1.24	2.05	0.63
Nuclear lysis				
Parameter estimates				
Term	**Estimate**	**Standard error**	**ChiSquare**	**P value**
Intercept	-9.51	0.56	288.00	<.0001
Concentration CHAPS [%]	15.04	0.85	312.67	<.0001
Time [h]	1.08	0.14	62.46	<.0001
Whole model test				
Model	**-LogLikelihood**	**DF**	**ChiSquare**	**P value**
Difference	612.54	2	1225.076	<.0001
Full	347.08			
Reduced	960.61			
Unit Odds ratio				
Term	**Odds ratio**	**Lower 95%**	**Upper 95%**	**Reciprocal**
Concentration chaps [%]	3394036	640962	17972153	2.95e-7
Time [h]	2.94	2.25	3.83	0.34