Lab on a Chip

ARTICLE

Automated serial dilutions for high-dynamic-range assays enabled by fill-level-coupled valving in centrifugal microfluidics

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Electronic Supplementary Information (ESI)

Details of network simulation on fill-level-coupled TCR actuated valving

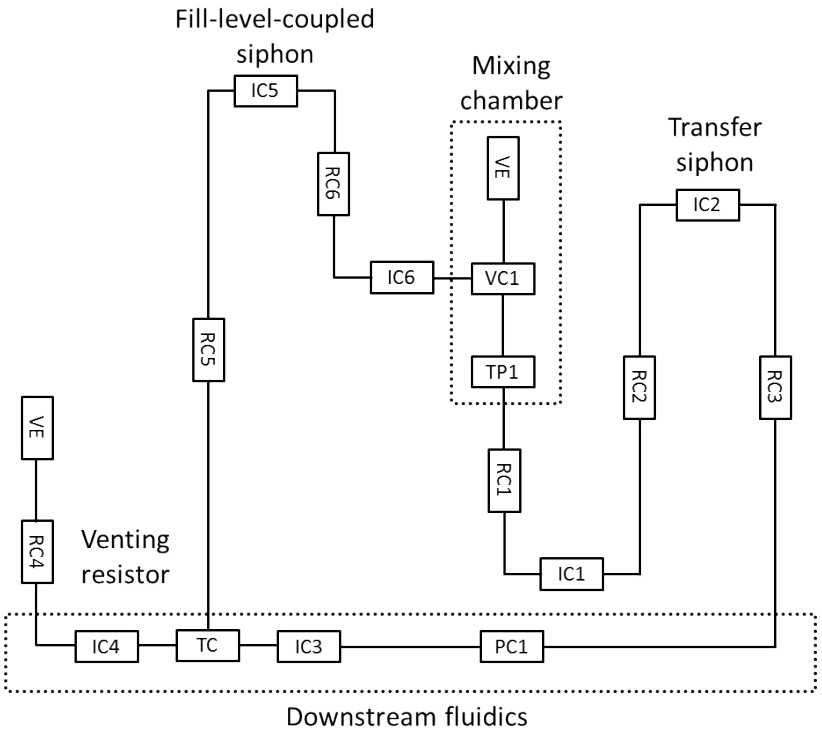


Figure S1. Block model of the performed network simulation, as described by Schwarz et al. [1]. The model contains radial channels (RC), isoradial channels (IC), a pneumatic chamber (PC), a two-port chamber (TP) as bottom part of the mixing chamber, a vented two-port chamber (VC) as top part of the mixing chamber, a T-junction (TC) and two venting elements (VE). The waste channel is neglected in this simulation, due to its minor influence in the first part of the siphon valving process (low pneumatic volume).

Table S1: Definition of all radial and isoradial channels for the network simulation model.

Radial	Width in µm	Depth in µm	Radial inner position in mm	Radial outer position in mm
RC1	300	200	39.9	41.0
RC2	300	200	28.0	41.0
RC3	300	200	28.0	43.2
RC4	40	40	32.2	43.2
RC5	300	200	23.6	41.0
RC6	300	200	23.6	34.0

Isoradial	Width in µm	Depth in µm	Radial position in mm	Isoradial length in mm
IC1	300	200	41.0	3.2
IC2	300	200	28.0	1.2
IC3	300	200	41.0	1.0
IC4	300	200	41.0	1.0
IC5	300	200	23.6	1.2
IC6	300	200	23.0	1.0

Table S2: Definition of all chambers for the network simulation model.

Element	Initial liquid volume in µl	Chamber volume in µl	Radial outer position in mm	Coefficient for height function $\frac{h(V)}{mm} = P_1\left(\frac{V}{\mu l}\right)^{0.5} + P_2\frac{V}{\mu l} + P_3\left(\frac{V}{\mu l}\right)^{1.5} + P_4\left(\frac{V}{\mu l}\right)^2$	Venting channel length in mm	Venting channel width and depth in µm
VC1	5.6	8.5	35.1	P_1 = -0.4059 E-1 P_2 = 0.8879 E-2 P_3 = -0.1904 E-3 P_4 = 0.0333 E-3	262	300 x 200
TP1	45.0	45.0	39.9	P_1 = 0.2852 E-1 P_2 = 0.1278 E-1 P_3 = -0.0343E-2 P_4 = 0.0036 E-2	-	-
PC1	0	132.7	50.7	P_1 = 0.2652 E-1 P_2 = 0.0375 E-1 P_3 = 0.0053E-2 P_4 = 0.0008E-2	-	-

Table S3: Definition of gas and liquid properties.

Parameter	Value	Comment
Gas viscosity	0.019 mPa s	Air
Gas density	1.21 kg/m³	Air
Liquid viscosity	1.30 mPa s	qPCR reaction mix
Liquid density	980.7 kg/m³	qPCR reaction mix
Liquid advancing contact angle	61.6°	qPCR reaction mix on COC 8007
Liquid receding contact angle	9.3°	qPCR reaction mix on COC 8007
Liquid surface tension	50.2 mN/m	qPCR reaction mix
Atmospheric pressure	101300 Pa	-

Table S4: Results of a frequency sweep parameter experimentally study on temperature change rate actuated valving events. The temperature protocol started at room temperature (20°C), slowly (+0.5 K/s) increasing to 50°C and finally fast (-1.7 K/s) decreasing down to 40°C. The subsequent further decrease to room temperature can take place at significantly lower decrease rates and is not considered in this simulation. The simulation results predict a window for fill-level-coupled TCR actuated siphon valving in between of 7 and 15 Hz. For lower frequencies the capillary forces cause undesired valving before TCR actuation, for higher frequencies no valving takes place at all. In all cases the coupled-vent-siphon remains non-valved, as desired.

Frequency [Hz]	Fill level in coupled siphon before/during TCR actuation [%]	Fill level in transfer siphon before/during TCR actuation [%]	Comment
6	63 / -	100 / -	Undesired valving before TCR actuation
7	58 / 76	98 / 100	Fill-level-coupled TCR actuated siphon valving takes place
8	48 / 73	89 / 100	Fill-level-coupled TCR actuated siphon valving takes place
9	39 / 69	83 / 100	Fill-level-coupled TCR actuated siphon valving takes place
10	34 / 67	78 / 100	Fill-level-coupled TCR actuated siphon valving takes place
11	29 / 66	75 / 100	Fill-level-coupled TCR actuated siphon valving takes place
12	26 / 64	73 / 100	Fill-level-coupled TCR actuated siphon valving takes place
13	24 / 63	71 / 100	Fill-level-coupled TCR actuated siphon valving takes place
14	22 / 60	70 / 100	Fill-level-coupled TCR actuated siphon valving takes place
15	21 / 60	68 / 100	Fill-level-coupled TCR actuated siphon valving takes place
16	20 / 58	67 / 97	No valving

Liquid contact angles of different qPCR mastermixes on COC foil

Table S5: Static contact angles, determined on a customized COC 8007 foil (Tekni-Plex Europe NV, Belgium) by optical contact angle measuring and contour analysis systems OCA 25 (DataPhysics Instruments, Germany). Each value represents a mean value of n=6 contact angle determinations, performed on three individually dispensed droplets (left and right contact angle). Mastermix concentration was chosen as suggested by the manufacturer and BSA Molecular Biology Grade (New England BioLabs, US) was added in a concentration of 2 µg/µl. Template DNA, primer, probes and universal reporters were found to not affect static contact angle measurements significantly and thus, have been replaced by water.

qPCR Mix	Manufacturer	Static contact angle [°]
QuantiNova Multiplex PCR Master Mix (4x)	QIAGEN, Netherlands	38.5 ± 4.1
PerfeCTa Multiplex qPCR ToughMix (5x)	Quantabio, US	48.7 ± 5.4
Invitrogen Platinum Taq DNA Polymerase Kit (10x)	Thermo Fisher Scientific, US	66.6 ± 1.6
LightCycler® Multiplex DNA Master (5x)	Roche Diagnostics, Switzerland	45.5 ± 2.7

CAD design of mixing chambers for significant fill level differences between low and high state

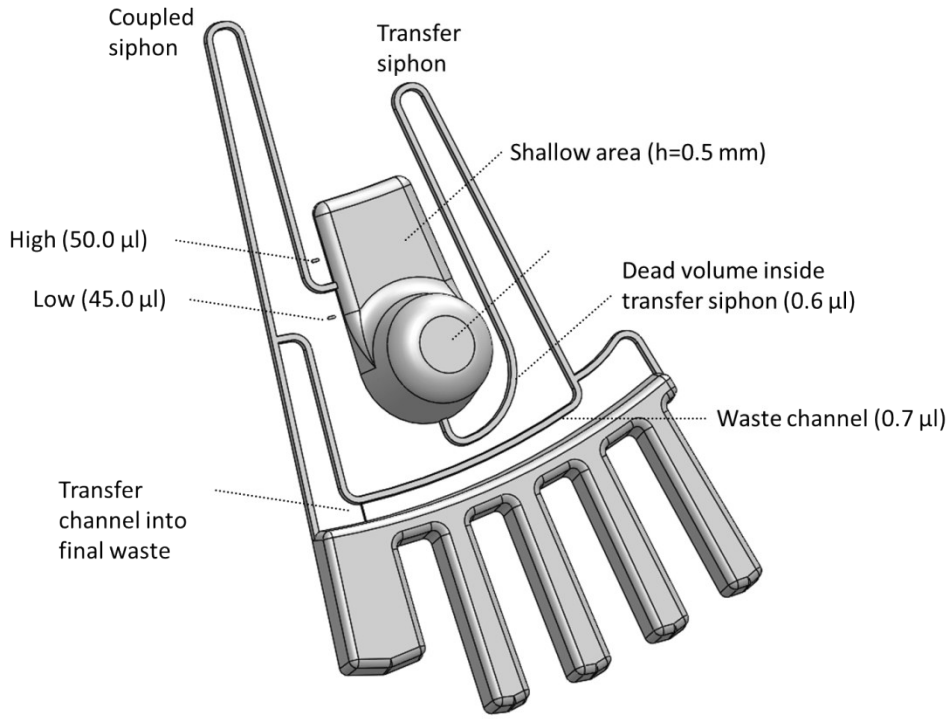
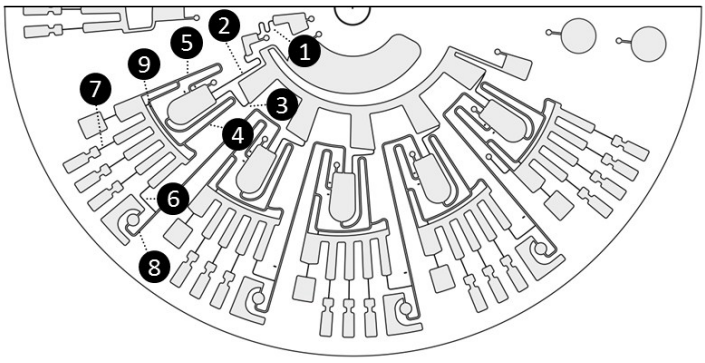


Figure S2. CAD model of the mixing chamber and its connected channels. The deep area enables to host comparably large liquid volumes in a highly integrated microfluidic design. The shallow top area causes significant fill level differences between the *low* and *high* states of the fill-level-coupled valving logic. The transfer siphon cross section of 200 x 300 µm results in a dead volume of 0.6 µl, respectively 1.2 % of the 50.0 µl mixing volume, fulfilling the design guideline $V_{mixing} >> V_{dead}$. The waste channel can host 0.7 µl, consequently the entire transfer siphon dead volume, which is according to the design guideline $V_{waste} \geq V_{dead}$.

Microfluidic design parameters



#	Description	Dimension w x h x l [µm]
1	Sample transfer channel	40 x 40 x 9,000
2	Sample delay channel	40 x 40 x 21,000
3	Buffer transfer channel	40 x 40 x 28,000
4	TCR valving siphon	300 x 200 x 29,000
5	TCR valving flow restriction vent	40 x 40 x 12,000
6	TCR valving blocking vent	300 x 200 x 34,000
7	Dilution transfer channel	40 x 40 x 5,000
8	Inward pumping channel	300 x 200 x 62,000
9	Waste connection channel	40 x 40 x 700

Figure S3: Drawing of the microfluidic design for an automated serial dilution, including all relevant geometrical dimensions.

Workflow of qPCR experiments

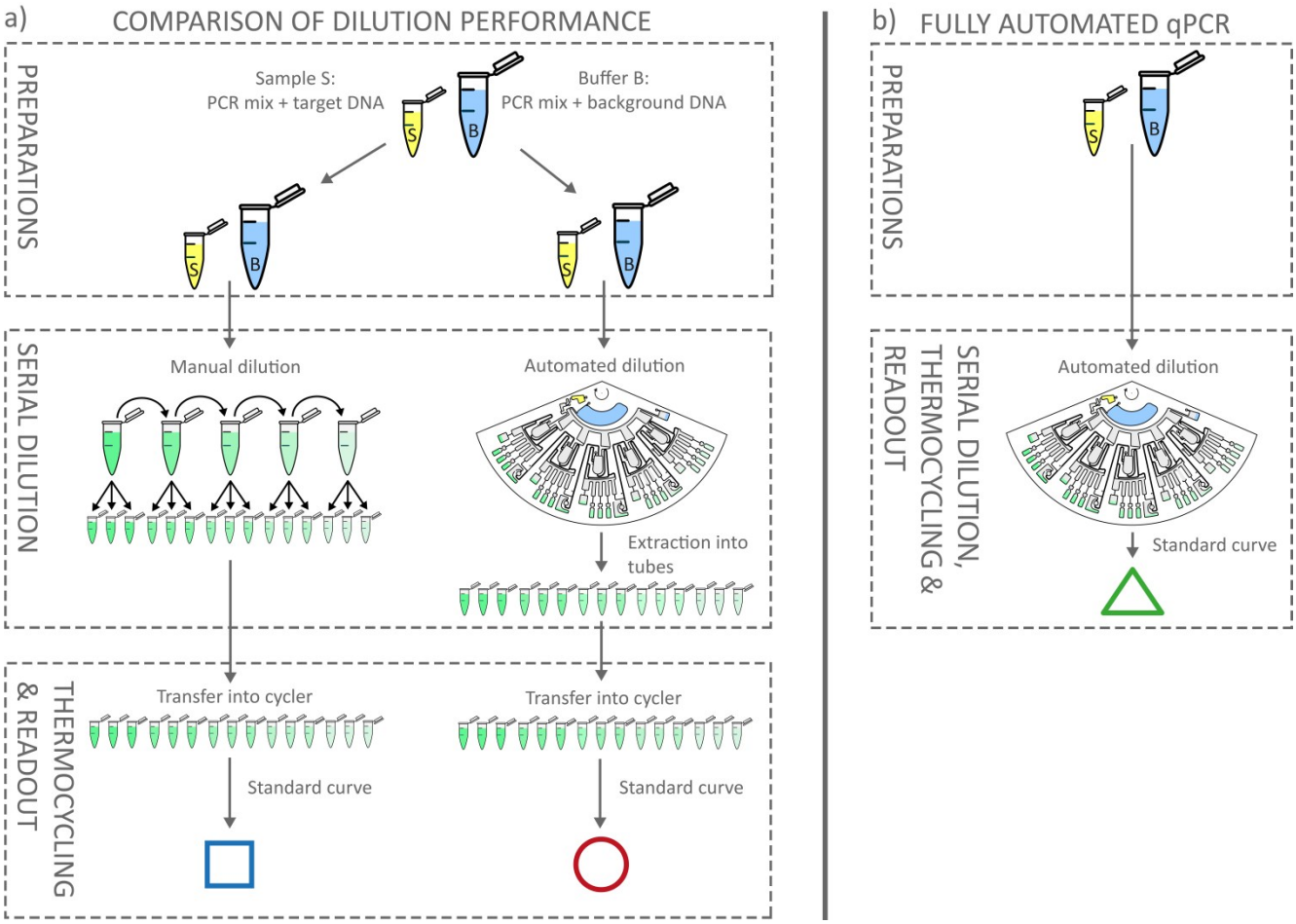


Figure S4. a) Experimental workflow for comparison of manual and automated serial dilutions. Sample and buffer PCR mixes are split into two groups to assure identic conditions for manual and automated serial dilution generation. While manual dilution generation is carried out by conventional pipetting, vortexing and centrifuging, automated dilution generation takes place fully automated in the *LabDisk*. Thermocycling and readout of both serial dilutions is performed simultaneously in the same device (*RotorGene Q* tube cycler). Plotting of standard curves is used to evaluate serial dilution performance. **b)** Experimental workflow for demonstration of a fully automated on-chip qPCR. The ability for integration of downstream analytics is demonstrated. After automated serial dilution generation the microfluidic cartridge remains inside the *LabDisk Player* for qPCR thermocycling and real-time fluorescence readout. Again the result is evaluated by standard curve plotting.

System characterization: Automated LabDisk vs. manual serial dilution performance

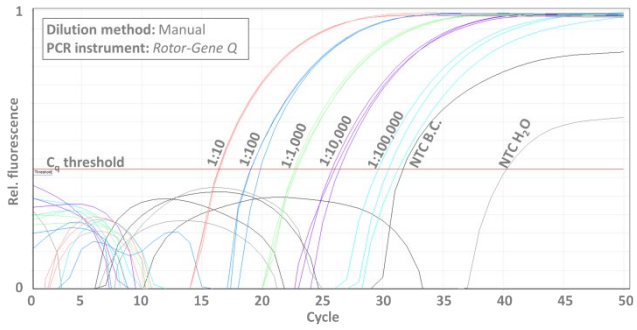


Figure S5: qPCR results Run #1: Serial dilution carried out manually and cycling performed inside a Rotor-Gene Q. The NTC B.C. reaction crossing the threshold at cycle 32 can be explained as unspecific amplification. The NTC H2O reaction crossing the threshold at cycle 40 can be explained as artefact of software curve filtering.

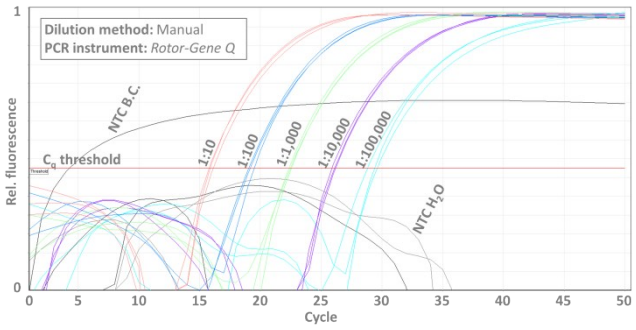


Figure S6: qPCR results Run #2 as a repetition of Run #1. The NTC B.C. reaction crossing the threshold at cycle 11 can be explained as artefact of software curve filtering.

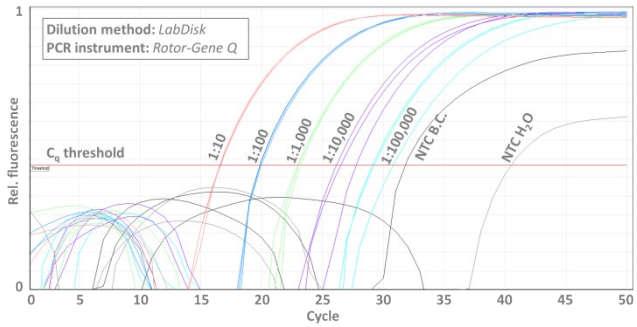


Figure S7: qPCR results Run #3: Serial dilution carried out fully automated inside the LabDisk, then extracted and cycling performed inside Rotor-Gene Q tubes. NTC curves are identical with Run #1 (Same batch of mastermix, same run).

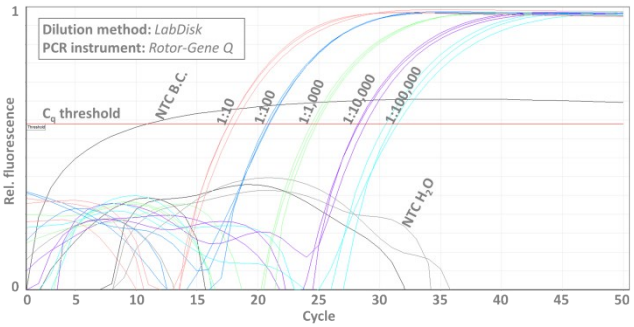


Figure S8: qPCR results Run #4 as a repetition of Run #3. NTC curves are identical with Run #2 (Same batch of mastermix, same run).

Table S6: qPCR result in commercial thermal cycler *Rotor-Gene Q* (manual serial dilution)

Excepted dilution ratio	Expected copies per reaction	Run #	C_q value	Copies per reaction	Copies per reaction AVG ± SD	**Resulting dilution ratio**
1:10	50,000	1	16.5 / 16.4 / 16.4	37713 / 40312 / 39329	39118 ± 1312	**1 : 13**
		2	16.1 / 15.9 / 15.8	42479 / 50725 / 53650	48951 ± 5793	**1 : 10**
1:100	5,000	1	19 / 19.1 / 19.8	6820 / 6711 / 4104	5878 ± 1538	**1 : 85**
		2	19.2 / 19.5 / 19	5250 / 4220 / 5705	5058 ± 761	**1 : 99**
1:1,000	500	1	22.9 / 22.8 / 22.6	536 / 578 / 634	583 ± 49	**1 : 858**
		2	22.5 / 22.3 / 22.5	514 / 587 / 540	547 ± 37	**1 : 914**
1:10,000	50	1	26 / 25.8 / 26.4	68 / 80 / 53	67 ± 14	**1 : 7,463**
		2	26.2 / 25.9 / 26.1	42 / 52 / 44	46 ± 5	**1 : 10,870**
1:100,000	5	1	30.5 / 31.3 / 29.6	4 / 2 / 6	4 ± 2	**1 : 125,000**
		2	28.8 / 29.6 / 29.4	7 / 4 / 5	5.3 ± 1.5	**1 : 93,750**

Table S7: qPCR result in commercial thermal cycler *Rotor-Gene Q* (automated serial dilution)

Excepted dilution ratio	Expected copies per reaction	Run #	C_q value	Copies per reaction	Copies per reaction AVG ± SD	**Resulting dilution ratio**
1:10	50,000	3	16.6 / 16.6 / 16.6	49597 / 48500 / 51209	49769 ± 1363	**1 : 10**
		4	17.6 / 18.1 / 17.6	53939 / 39057 / 53518	48838 ± 8473	**1 : 10**
1:100	5,000	3	20 / 19.8 / 19.9	4723 / 5219 / 5126	5023 ± 264	**1 : 100**
		4	20.9 / 20.7 / 20.9	5923 / 6689 / 5656	6089 ± 536	**1 : 82**
1:1,000	500	3	23 / 23.2 / 23	589 / 500 / 570	553 ± 47	**1 : 904**
		4	24.8 / 24.6 / 24.4	412 / 466 / 553	477 ± 71	**1 : 1,048**
1:10,000	50	3	26.5 / 26.1 / 27.7	51 / 65 / 21	46 ± 22	**1 : 10,949**
		4	28.8 / 28.2 / 28.3	27 / 42 / 40	36 ± 8	**1 : 13,761**
1:100,000	5	3	30.8 / 29.1 / 29.2	3 / 8 / 8	6.3 ± 2.9	**1 : 78,947**
		4	31.5 / 30.5 / 31.1	5 / 9 / 6	6.7 ± 2.1	**1 : 75,000**

Application demonstration: On-disk generation of qPCR standards with leukemia gene target

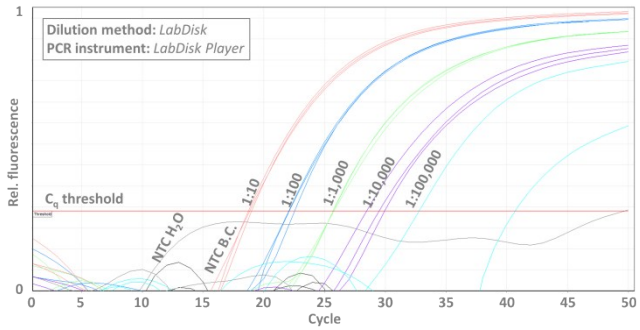


Figure S9: qPCR results Run #5: Both, serial dilution and cycling performed inside the LabDisk and by using LabDisk Player only.

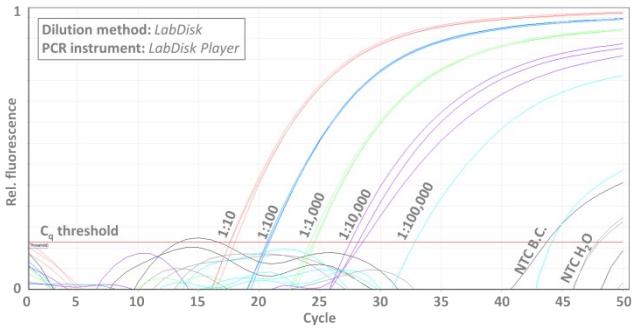


Figure S10: qPCR results Run #6 as a repetition of Run #5. Late threshold crossings of NTCs can be explained as artefact of software curve filtering.

Table S8: qPCR result in *LabDisk Player* (automated serial dilution)

Excepted dilution ratio	Expected copies per reaction	Run #	C_q value	Copies per reaction	Copies per reaction AVG ± SD	**Resulting dilution ratio**
1:10	10,000	5	18.9 / 19 / 18.7	9784 / 8809 / 10486	9693 ± 842	**1 : 10**
		6	17.6 / 18 / 17.6	9534 / 7350 / 9668	8851 ± 1301	**1 : 11**
1:100	1,000	5	22.1 / 22.1 / 22.5	1140 / 1163 / 849	1051 ± 175	**1 : 95**
		6	20.7 / 20.6 / 20.8	1198 / 1287 / 1120	1202 ± 84	**1 : 83**
1:1,000	100	5	25.8 / 25.7 / 25.7	99 / 103 / 105	102 ± 3	**1 : 977**
		6	24.6 / 24.5 / 24.2	91 / 100 / 119	103 ± 14	**1 : 968**
1:10,000	10	5	29.9 / 29.5 / 28.5	6 / 9 / 17	11 ± 6	**1 : 9,375**
		6	28.5 / 28.1 / 27.5	7 / 9 / 13	10 ± 3	**1 : 10,345**
1:100,000	1	5	neg / neg / 32.8	0 / 0 / 1	n/a	**n/a**
		6	neg / 32.8 / neg	0 / 1 / 0	n/a	**n/a**

Sequences for *ETV6-RUNX1* Mediator Probe PCR assay

Table S9: Oligonucleotide and target sequences (C3 = C3-Spacer; Q2 = BHQ-2; Underlined: Primer binding positions; Underlined and bold: Mediator probe binding position).

Oligonucleotide / target	Description	Sequence (5'-3')
Fw primer	Forward primer	CGGGTAGGAGAGGAAAACAG
Rv primer	Reverse primer	TGCCCATGTTTCAAATTCAT
MP	Mediator probe	tctgggctctacgaccCAAATGGCCCAGCAACACCTGCCTC-C3
UR	Universal Reporter	Q2-GACCGGCCAAGACGCGCCGGT(dC-Atto647N)TGTTggtcgtagagcccagaACGA-C3
Target	*ETV6-RUNX1*	TGAGGGCAATTGGAGGCTTCTGCTTGGATGAGGCTAAATCCCTAATGGCTTGGTTAATGAGC CGCTGGGATGGAGTAGTTAATGAGCCTCAGAAATGTTAAGAAACAAATGTCCTACGTCCAGC TTACAAGGAGAGTCACATCAGAATCAAGGCTAAGCGAAAACATTTAAAATAAAAGGTTTATG AGCATGCTAATGGCCCTGTCCTGAAGTTTCCAGCAGCTAATTAATGACCAGACACAGCATAA AGAAGCTTTGTCTCAGATTCAGGCCTGTAATTCCATCCTGTGAGCGGTAACGCCATGCAGCCT TCACCTTAGGAGCGGGTAGGAGAGGAAAACAGGATTATGGTATTG**GAGGCAGGTGTTGCT GGGCCATTTGG**ACAGAGGAAACCACTCCCAGATTCTCTTTCATTTATTATAAGAAGCCCAAAT TTGCTTACTTAAGGGAAGAAACCAGTGGAAACCAGTGGGAAAAATATCTACAAGTCCCCTGC TGATAAGAGAGTGAGATGAATTTGAAACATGGGCACCACATCCATGTTCTTGTAGGCCTGGT TTGTGGTCCAGGGCCAATTGTAAGCTGGGATCACAAGACAGACACTTCTCCAGCAAGCAGAC AACAAGGGAAGGTCTGAACTTAAAGCTGCC

Frequency- and temperature protocol for automated serial dilution and qPCR on *LabDisk*

Table S10: *LabDisk Player* temperature- and frequency protocol (RT = Room temperature).

Step	Description	Frequency [Hz]	Acceleration or Deceleration [Hz/s]	Hold time [s]	Temperature [°C]
		Automated serial dilution – initial phase			
1	Reagent loading	0	0	n.a.	RT
2	Buffer aliquoting	80	30	0	RT
3	Buffer and sample transfer	80	0	360	RT
		Automated serial dilution – iterative phase (5 cycles)			
4	Mixing by shake-mode	10 / 30	30	80 (Alterations: 20)	50
5	TCR actuated siphon valving	7	10	5	RT (Ramp: ≥ - 1K/s)
6	Centrifugo-pneumatic aliquoting	20	30	5	RT
7	Metering and pneumatic loading	80	30	25	RT
8	Inward pumping	15	10	1	RT
		Automated qPCR (50 cycles)			
9	Denaturing	40	10	8 (1st cycle: 300)	93
10	Annealing + Extension	40	0	30	62
11	Readout	0	10	1	62

Stroboscopic imaging: Filling dynamics of waste channel

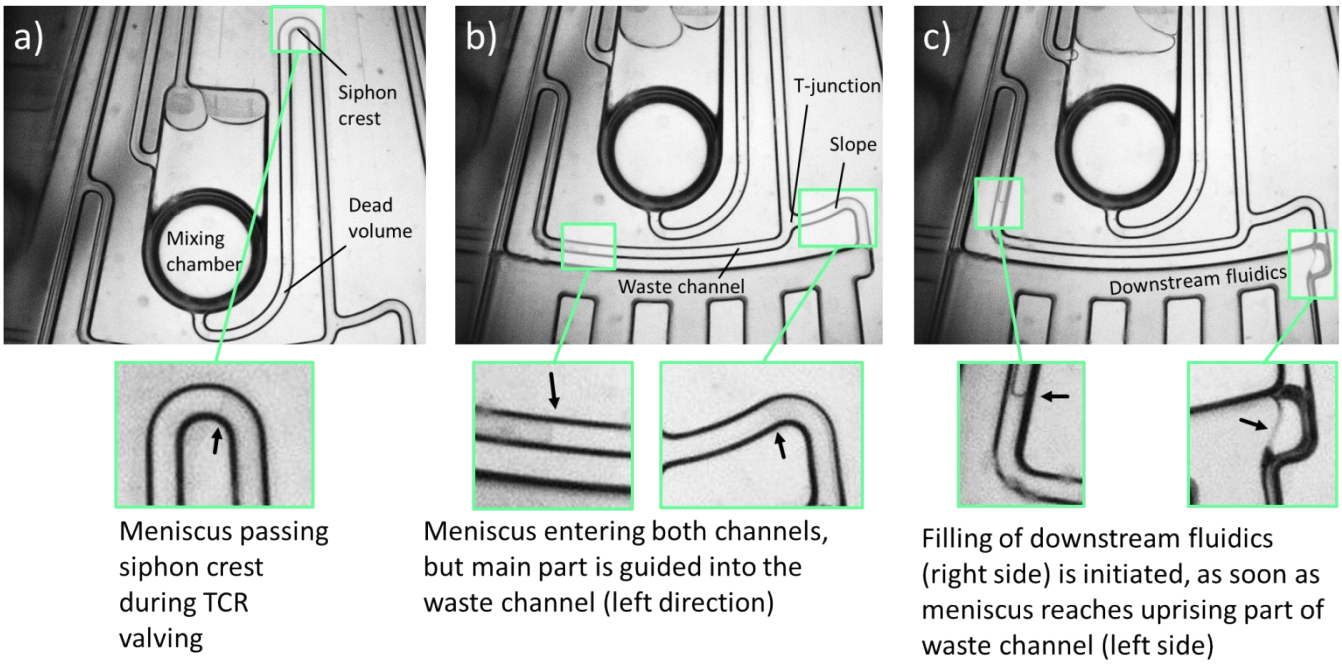


Figure S11: Meniscus tracking of the waste channel filling dynamics during fill-level-coupled TCR valving. **a)** During TCR induced underpressure the meniscus is raised across the siphon crest. **b)** As intended, the majority of poorly mixed dead volume is routed into the waste channel (left side of T-junction). **c)** As soon as the meniscus reaches the uprising part of the waste channel, the centrifugal pressure is causing a counter-pressure, overcoming the counter-pressure in the slope and resulting in a routing of liquid towards the downstream fluidics (right side of the T-junction).

Stroboscopic imaging: Metering accuracy and precision

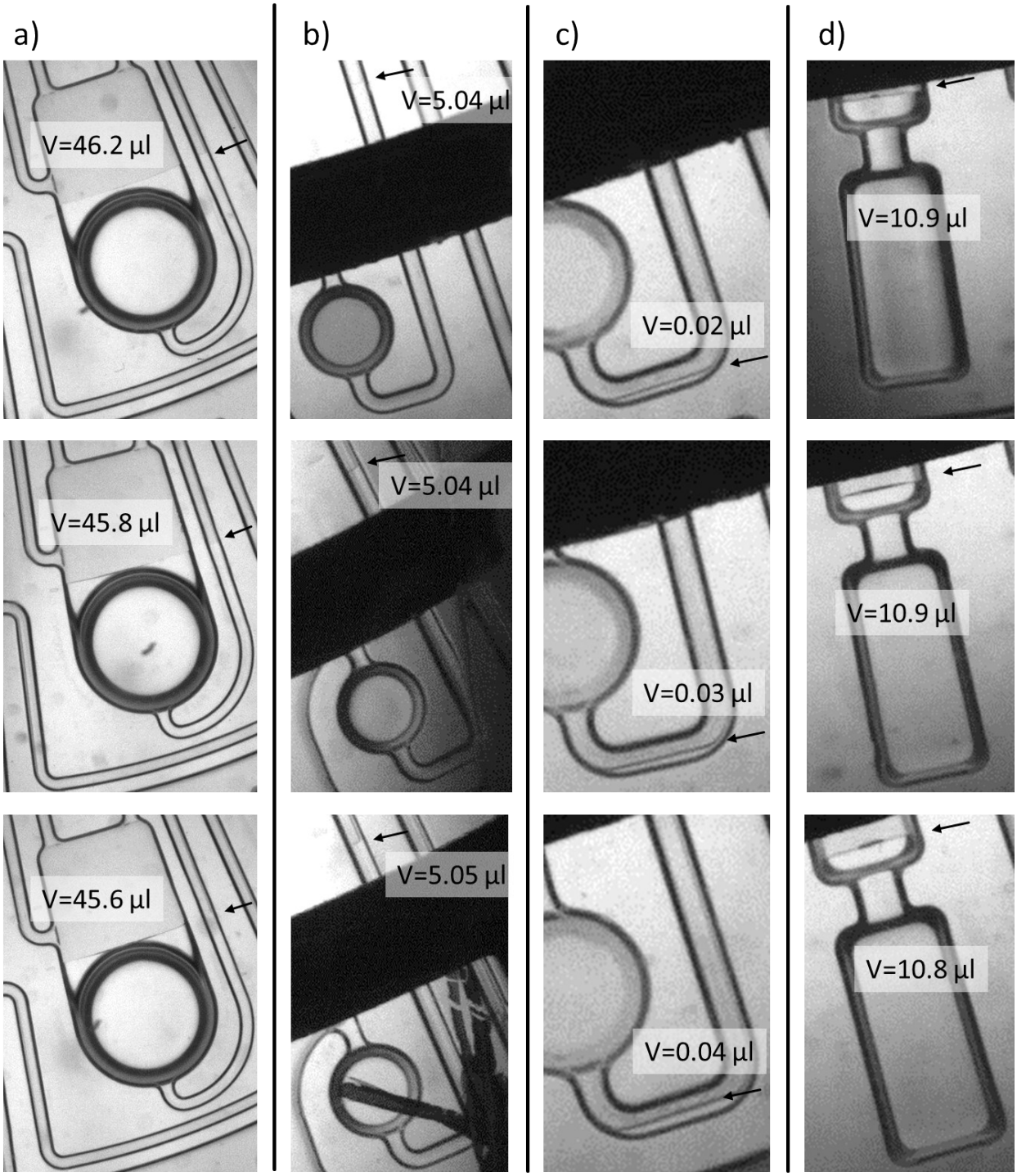


Figure S12: Images for determination of volume accuracies. **a)** Metered buffer volume *V_buffer* in a mixing chamber. **b)** Metered transfer volume *V_transfer.metering* in a pneumatic inward pumping structure. **c)** Residual liquid *V_transfer.residual* after inward pumping. **d)** Metered qPCR reaction replicate volumes.

Table S11: Results of metering accuracy and reproducibility determination by optical fill level evaluation. For each parameter *n = 3* individual measurements in three different, randomly chosen chambers were performed.

Value	Description	Nominal [µl]	Measured #1 [µl]	Measured #2 [µl]	Measured #3 [µl]	AVG±SD [µl]
a) V_buffer	Metered qPCR buffer (Containing 0.6 µl dead volume inside transfer siphon)	45.6	46.2	45.8	45.6	45.9 ± 0.3
b) V_transfer.metered	Metered transfer volume in pneumatic inward pumping structure	5.0	5.04	5.04	5.05	5.04 ± 0.01
c) V_transfer.residual	Residual liquid after inward pumping	0.0	0.02	0.03	0.04	0.03 ± 0.01
V_transfer	Delta of metered and residual transfer volume	5.0	-	-	-	5.01 ± 0.02
d) V_reaction	Volume of qPCR reaction replicates	10.0	10.9	10.8	10.8	10.8 ± 0.1

References

1 I. Schwarz, S. Zehnle, T. Hutzenlaub, R. Zengerle and N. Paust, *Lab on a chip*, 2016, **16**, 1873–1885.