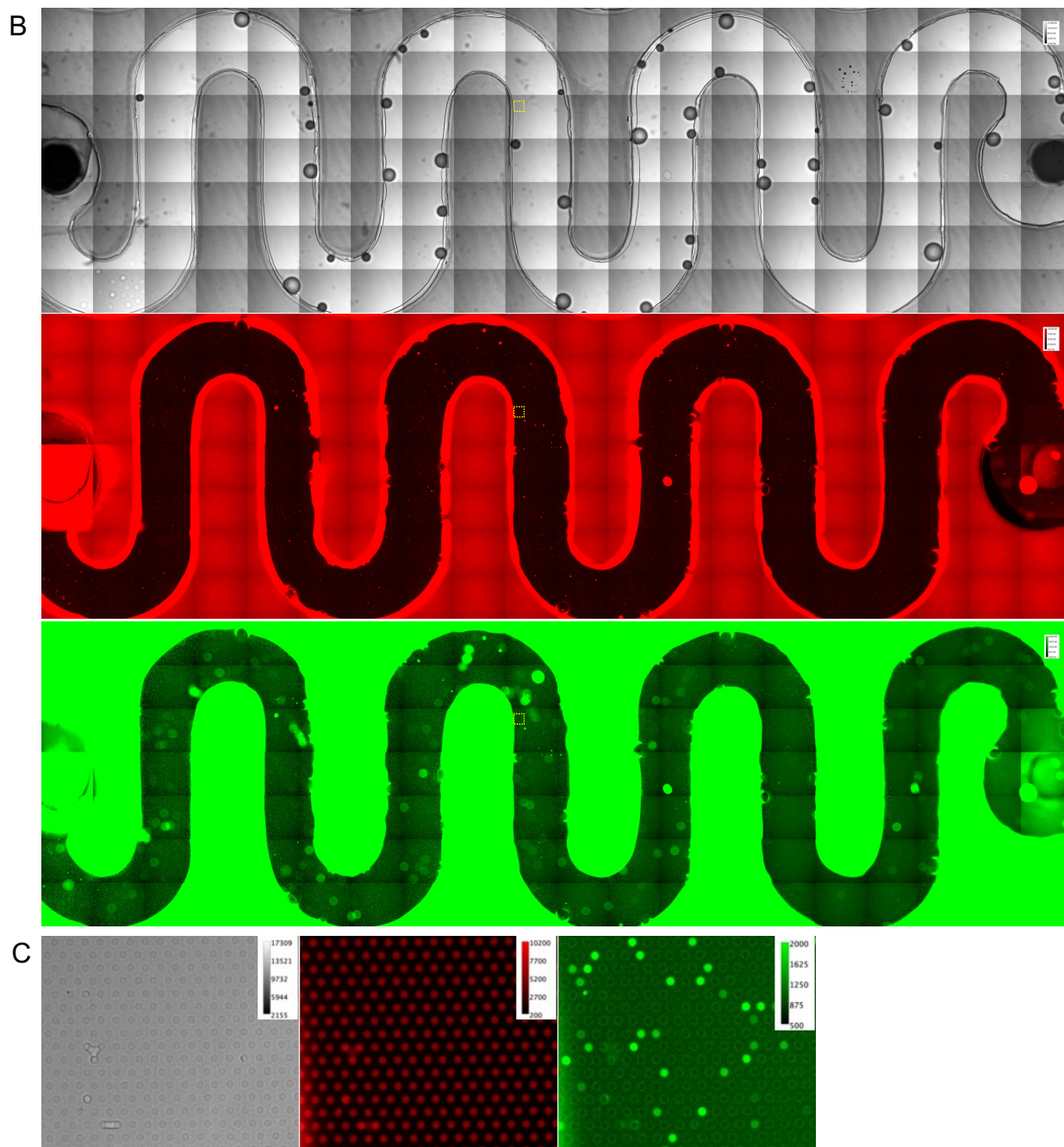
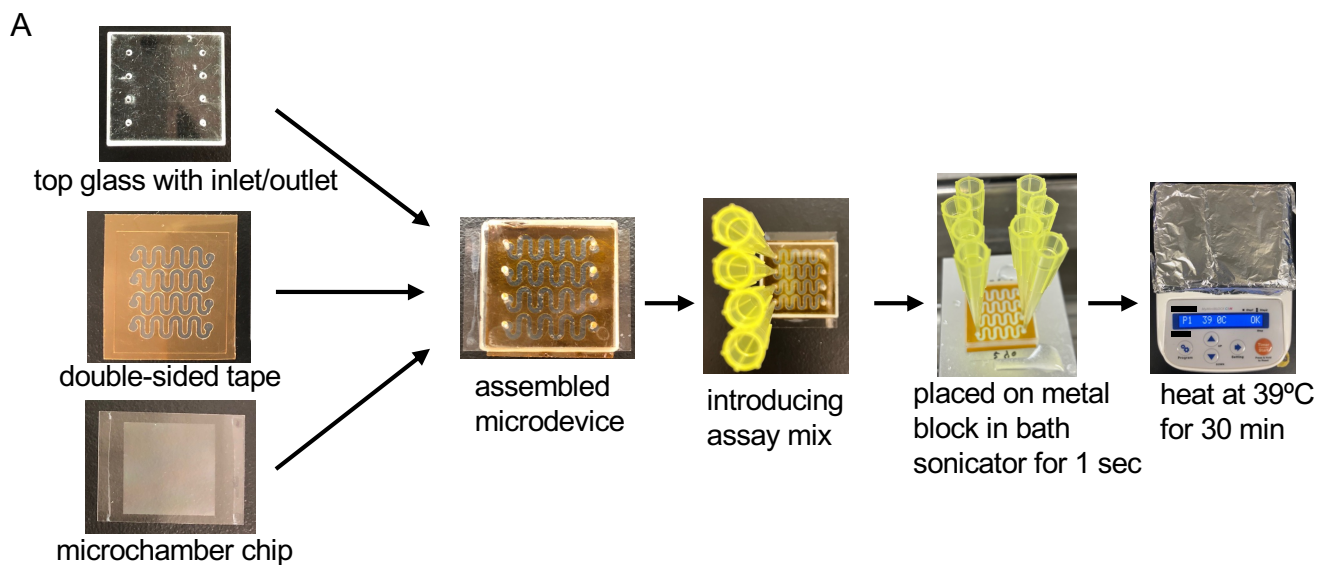
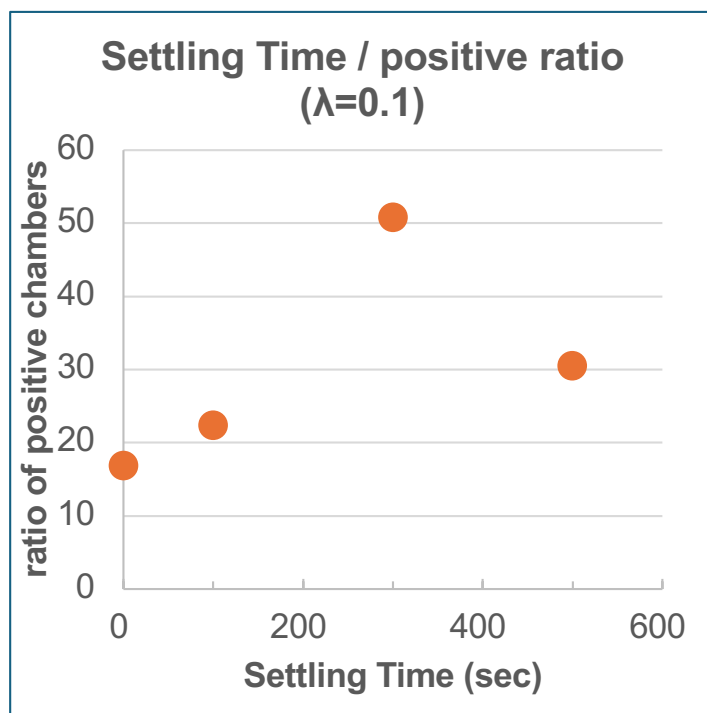
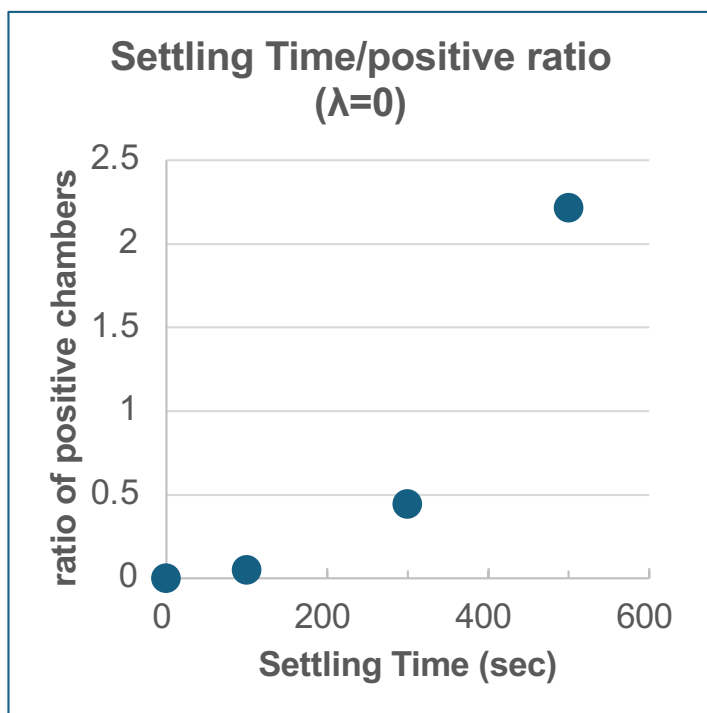




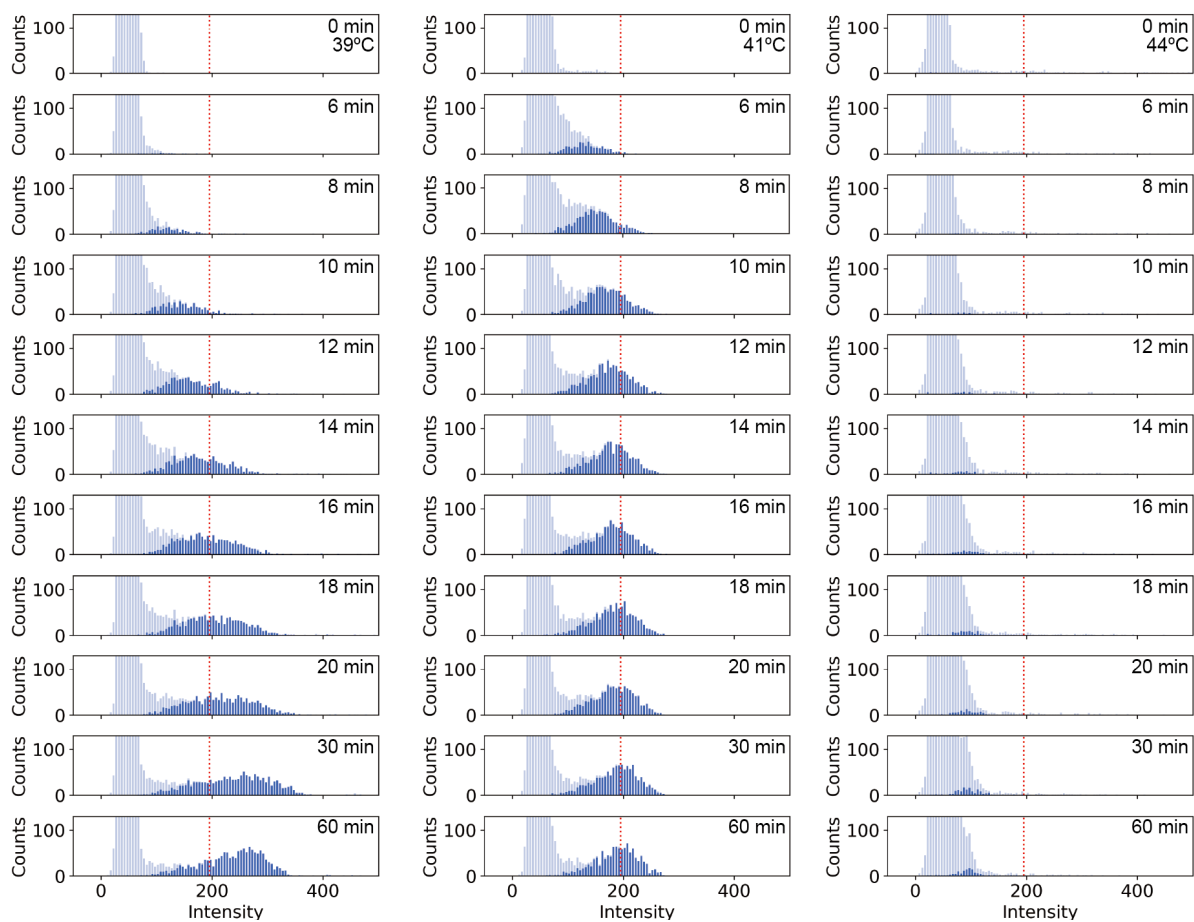
Supplementary Fig. S1

In-house fabricated microchamber chip used in this study. (A) Overview of chip assembly and digital assay procedure. (B) Multi-image array of the flow cell of the microchamber chip. Bright field (Gray), Cy5 setting (Red, for Alexa Fluor 647 fluorescence) and GFP setting (Green, for Mango fluorescence) of the microscope is shown. (C) Magnified image of the area of yellow rectangle in (B).



Supplementary Fig. S2

Fraction of positive microchambers when RT incubation time before starting the reaction at 39°C was varied. Left: no template control, right: 4 pM template condition.



Supplementary Fig. S3

Histogram of fluorescence intensity during timelapse measurement. Vertical red dotted line indicates the mean + 15SD threshold signal level at 0 s. Related to Fig. 3.

Name	Sequence [†]	Base pairs	Reference
SARS-Cov-2 N Gene Template	ctgagggagccttgaatacaccaaaagatcacattggcaccgcgaatcctgctaacaatgctgc aatcgtgctacaacttctcaaggaacaacattgccaaaaggcttctacgcagaagggagcag aggcggcagtcgaagccttctcgttctctcatcagctagtcgcaacagttcaag	181	GenBank OU610953.1
SARS-Cov-2 Mango-Fwd Primer	GGCACGTACGAATATACCACATACCAAACCTTCCTTCGTACGTGCCctgagg gagccttgaatacaccaaaagatcac	78	This work
SARS-Cov-2 T7-Rev Primer	GAATTTAATACGACTCACTATAGGGAGACCcttgaactgttgcgactacgtgatga ggaacgag	64	This work
Chlamydia CDS2 Template	caaatatcatctttcggttgcgtgtcctgtgaccttcattatgtcggagctgagcaccctaggcg ttgtactccgtcacagcgttgcctgaagcagctgcggggtattttaaaagggttgagcgttg tagtcctgcttgagagaacgtgcgggcgattgccttaa	172	NCBI Reference Sequence NC_001372.1
Chlamydia Mango-Fwd Primer	GGCACGTACGAATATACCACATACCAAACCTTCCTTCGTACGTGCCttaagg caaatcgccgcacgttctctcaagc	78	Ref. S1 ¹
Chlamydia T7-Rev Primer	GAATTTAATACGACTCACTATAGGGAGACCaaatatcatctttcggttgcgtgtc ctgtg	62	Ref. S1 ¹
HIV pol Template	tggcagtattcattcacaattttaaaagaaaaggggggttggggggtacagtgcaggggaaa gaatagtagacataatgaacagacatacaaaactaagaattacaaaaacaaattacaaaa attcaaaatttcggg	141	GenBank AF033819.3
HIV Mango-Fwd Primer	GGCACGTACGAATATACCACATACCAAACCTTCCTTCGTACGTGCCtggcag tattcattcacaattttaaaagaaaagg	80	Ref.S2 ²
HIV T7-Rev Primer	GAATTTAATACGACTCACTATAGGGAGACCcccgaaaattttgaattttgtattt gtttttg	64	Ref.S2 ²
Dengue Template	aacagcatattgacgtgggagagaccagagatcctgctctcctcagcatattccaggcac agaacgcgagaaaaatggaatggtgctgttgatcaacagggttc	107	GenBank AY662691.1
Dengue Mango-Fwd Primer	GGCACGTACGAATATACCACATACCAAACCTTCCTTCGTACGTGCCcagcat attgacgtgggagagaccagagatcc	79	Ref. S3 ³
Dengue T7-Rev Primer	GAATTTAATACGACTCACTATAGGGAGACCgaacctgttgattcaacagcaccatt ccatttt	63	Ref. S3 ³
Zika Template	tctcttgagtgcttgattctactcatggtgcaggagggttgagaagagaatgaccacaaa gatcatcatgagcacatcaatggcagtgctgtagtcatgatctgggaggattttcaatgagtg acctggccaagc	142	GenBank LC002520.1
Zika Mango-Fwd Primer	GGCACGTACGAATATACCACATACCAAACCTTCCTTCGTACGTGCCctctcttg gagtgcttggtgattctactcatggt	78	Ref. S4 ⁴
Zika T7-Rev Primer	GAATTTAATACGACTCACTATAGGGAGACCgcttgccagggtcactcattgaaaat cctc	60	Ref. S4 ⁴

Supplementary Table S1. Oligonucleotide sequences used in this study.

[†]Mango and T7 sequences are highlighted in capital letters.

Experiment	Number of microchambers		
	Positives	Total	% of positives
SARS-CoV-2 day 1 negative control	9(12)	34927	0.026(0.034)
SARS-CoV-2 day 2 negative control	0(7)	35560	0.000(0.020)
SARS-CoV-2 day 3 negative control	1(4)	36790	0.003(0.011)
Chlamydia day 1 negative control	1(129)	34578	0.003(0.373)
Chlamydia day 2 negative control	0(22)	35991	0.000(0.061)
Chlamydia day 3 negative control	97(176)	33823	0.287(0.520)
HIV day 1 negative control	0(4)	31080	0.003(0.013)
HIV day 2 negative control	2(5)	33756	0.006(0.015)
HIV day 3 negative control	5(19)	35203	0.014(0.054)
Dengue day 1 negative control	1(9)	35443	0.003(0.025)
Dengue day 2 negative control	0(1)	33866	0.000(0.003)
Dengue day 3 negative control	0(13)	33482	0.000(0.039)
Dengue day 4 negative control	0(10)	33064	0.000(0.030)
Dengue day 5 negative control	0(2)	32141	0.000(0.006)
Dengue day 6 negative control	0(5)	36715	0.000(0.014)
Zika day 1 negative control	0(3)	38537	0.000(0.008)
Zika day 2 negative control	0(0)	31197	0.000(0.000)
Zika day 3 negative control	0(198)	29667	0.000(0.667)
Zika day 4 negative control	6(9)	38004	0.016(0.024)
Zika day 5 negative control	0(5)	34961	0.000(0.014)
Zika day 6 negative control	1(8)	35381	0.003(0.023)

Supplementary Table S2. Number of positive counts in negative control experiments.

Numbers before omitting the obvious false positives discriminated by eye are shown in parentheses.

Supplementary References

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- S4. A. Abd El Wahed, S. S. Sanabani, O. Faye, R. Pessoa, J. V. Patriota, R. R. Giorgi, P. Patel, S. Bohlken-Fascher, O. Landt, M. Niedrig, P. M. Zanotto, C. P. Czerny, A. A. Sall and M. Weidmann, *PLoS Curr*, 2017, **9**.