

## Supporting Information

# Nucleic Acid Target Sensing Using a Vibrating Sharp-Tip Capillary and Digital Droplet Loop-Mediated Isothermal Amplification (ddLAMP)

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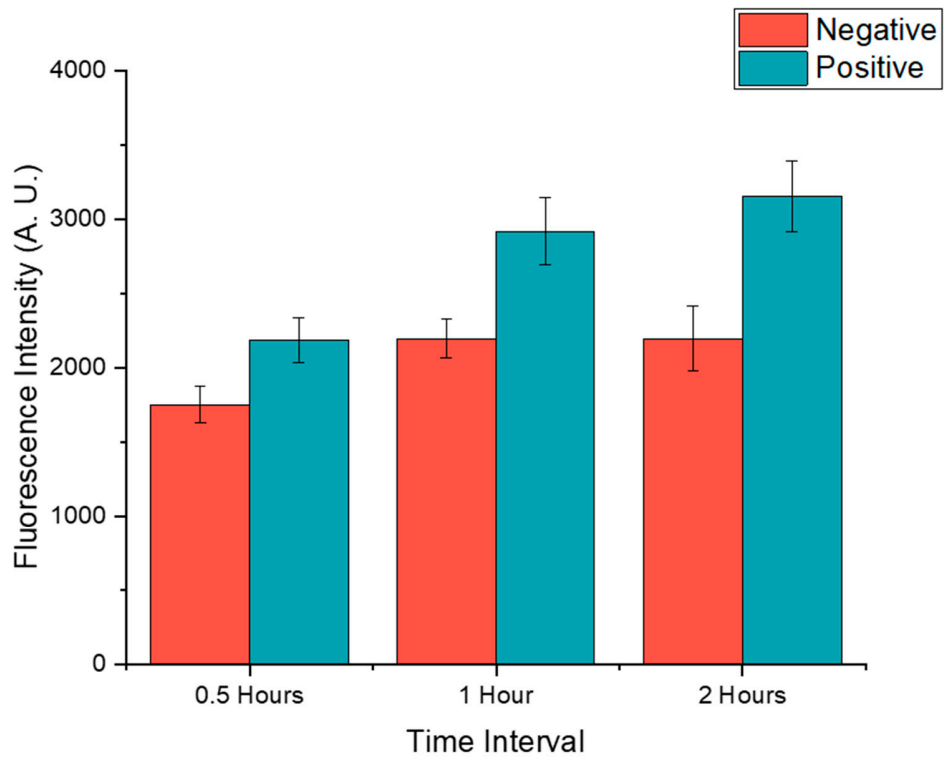


Figure S1: Comparison of fluorescence intensity difference using different reaction time intervals.

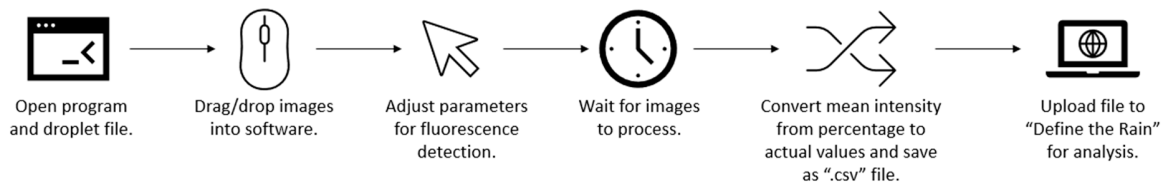


Figure S2: General overview of CellProfiler image processing.

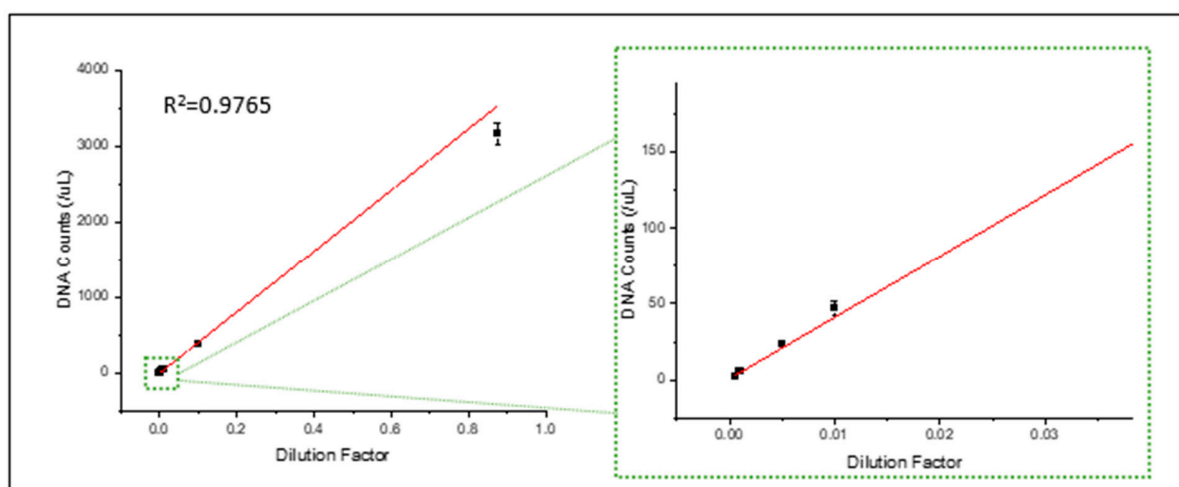


Figure S3: Linear relationship of manual image analysis with expanded view of low concentration for *Vibrio cholerae*.

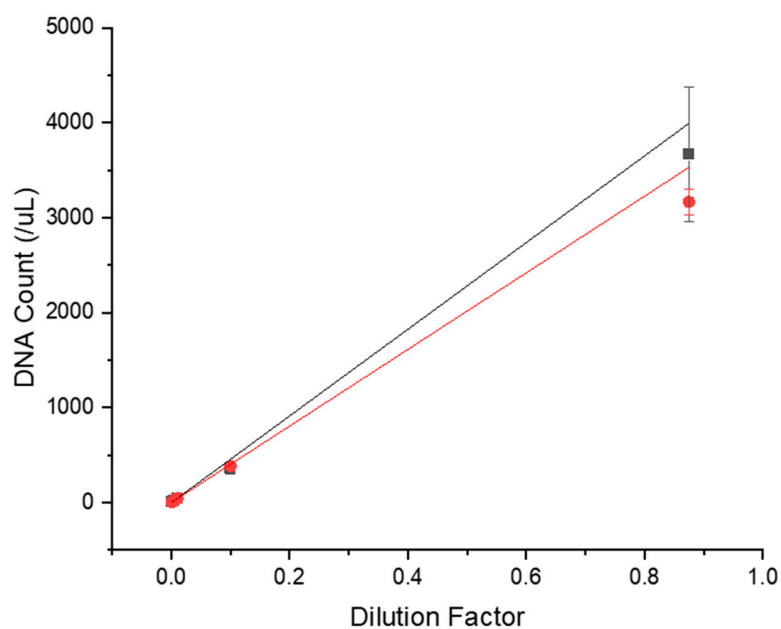


Figure S4: Comparison of image analysis methods for *Vibrio cholerae*. (Black square – automated image analysis; Red circle – manual image analysis)

Table S1: List of primers used.<sup>1</sup>

Primer Reference	Sequence (5' to 3')
Listeria Monocytogenes Loop-B	TGCATAGCCGACATGAGAGG
Listeria Monocytogenes Loop-F	CATCTTTCAAAGGCGTGGC
Listeria Monocytogenes F3	GGCAACCTGCCTGTAAGTTG
Listeria Monocytogenes B3	TGCCTCCCGTAGGAGTCT
Listeria Monocytogenes FIP	GCCCATCTGTAAGCGATAGCCGAACCGGGGCTAATACCGAA
Listeria Monocytogenes BIP	AATGGCCTACCAAGGCAACGATGTCTCAGTCCCAGTGTGG
Vibrio Cholerae Loop-B	CTTCTGTGTGGTATGCCAATAT
Vibrio Cholerae Loop-F	--
Vibrio Cholerae F3	GCTTTAATGGTACGGGTACT
Vibrio Cholerae B3	CCGTGGATTTGGCATCTG
Vibrio Cholerae FIP	TAATCAAAGCCAACGTTAGCAGCCTGGATTGAGTGATTAAAACT TG
Vibrio Cholerae BIP	TATGCTCAATGATAGCTGGTTCCTTGTAGGTTGCCGTTGTT
Shigella Sonnei Loop-B	--
Shigella Sonnei Loop-F	CTCACAGCTCTCAGTGGC
Shigella Sonnei F3	GCTGGAAAACTCAGTGCCT
Shigella Sonnei B3	GGAACATTTCCTGCCCCA
Shigella Sonnei FIP	CATGTGAGCGCGACACGGTCACAGCAGTCTTTCGCTGTTG
Shigella Sonnei BIP	ATCTCCGGAAAACCCTCCTGGTAGCGCCGGTATCATTATCGA

Table S2: Droplet size comparison of a 20- $\mu\text{m}$  tip operating at 98.4 kHz and 3.5 V<sub>pp</sub>.

Pulse Frequency (Hz)	Droplet Radius ( $\mu\text{m}$ )	Droplet Volume (nL)
5	$142.7 \pm 3.5$	$12.2 \pm 0.9$
10	$112.3 \pm 2.9$	$5.9 \pm 0.5$
20	$88.2 \pm 2.2$	$2.9 \pm 0.2$
50	$71.4 \pm 0.5$	$1.53 \pm 0.03$
100	$55.7 \pm 0.3$	$0.72 \pm 0.01$
200	$42.8 \pm 0.3$	$0.328 \pm 0.006$
500	$28.2 \pm 0.3$	$0.094 \pm 0.003$
1000	$19.6 \pm 0.3$	$0.032 \pm 0.001$
2000	$11.3 \pm 0.3$	$0.0061 \pm 0.0005$
4000	$7.5 \pm 0.5$	$0.0018 \pm 0.0003$
5000	$5.4 \pm 0.6$	$0.0007 \pm 0.0002$

Table S3: Droplet size comparison of a 20- $\mu\text{m}$  tip operating at 98.4 kHz and 4.0 V<sub>pp</sub>.

Pulse Frequency (Hz)	Droplet Radius ( $\mu\text{m}$ )	Droplet Volume (nL)
5	$174.4 \pm 2.3$	$22.2 \pm 0.9$
10	$138.1 \pm 1.3$	$11.0 \pm 0.3$
20	$109.5 \pm 0.5$	$5.50 \pm 0.07$
50	$80.3 \pm 0.7$	$2.17 \pm 0.06$
100	$63.4 \pm 0.4$	$1.07 \pm 0.02$
200	$49.0 \pm 0.3$	$0.494 \pm 0.008$
500	$32.6 \pm 0.4$	$0.145 \pm 0.006$
1000	$23.0 \pm 0.3$	$0.051 \pm 0.002$
2000	$13.8 \pm 0.3$	$0.0109 \pm 0.0006$
4000	$9.2 \pm 0.4$	$0.0033 \pm 0.0004$
5000	$6.5 \pm 0.4$	$0.0012 \pm 0.0002$

Table S4: Data summary of DNA counting methods for *Vibrio Cholerae*.

Analysis Method	Dilution Factor	DNA Count (/μL) Reservoir 1	DNA Count (/μL) Reservoir 2	DNA Count (/μL) Reservoir 3	DNA Count (/μL) Reservoir 4	DNA Count (/μL) Average
<i>Fully Automated</i>	0.0005x	2.3	2.1	2.4	2.7	2.3 ± 0.3
	0.001x	5.9	5.7	5.2	5.7	5.6 ± 0.3
	0.005x	22.7	23.6	23.7	23.0	23.3 ± 0.5
	0.01x	48.4	42.5	44.3	48.9	46.0 ± 3.1
	0.1x	425.5	349.4	279.6	384.3	359.7 ± 61.8
	0.875x	3142.2	2994.4	4460.2	4057.9	3663.7 ± 709.4
<i>Fully Manual</i>	0.0005x	2.6	2.3	2.5	2.8	2.5 ± 0.2
	0.001x	6.2	5.8	5.4	5.6	5.7 ± 0.3
	0.005x	22.8	25.4	23.7	23.7	23.9 ± 1.1
	0.01x	50.2	42.2	44.8	51.6	47.2 ± 4.5
	0.1x	366.5	338.3	407.4	425.3	384.4 ± 39.4
	0.875x	2976.5	3257.0	3147.4	3283.7	3166.1 ± 139.5

Table S5: Data summary of DNA counts at different concentrations for *Shigella sonnei*.

Dilution Factor	DNA count (/μL) Reservoir 1	DNA count (/μL) Reservoir 2	DNA count (/μL) Reservoir 3	DNA count (/μL) Reservoir 4	DNA count (/μL) Average
0.0001x	5.4	6.4	7.4	7.1	6.6 ± 0.9
0.001x	64.8	58.8	65.7	61.3	62.6 ± 3.2
0.01x	748.2	708.4	788.6	664.5	727.4 ± 53.2
0.1x	5932.6	6686.3	5767.8	6313.6	6175.1 ± 410.4

## References:

1. Curtin, K.; Wang, J.; Fike, B. J.; Binkley, B.; Li, P. A 3D printed microfluidic device for scalable multiplexed CRISPR-cas12a biosensing. *Biomed. Microdevices*. **2023**, 25, 34. DOI: 10.1007/s10544-023-00675-x.