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

Electronic Supplementary Information

A Microfluidic Device and Instrument Prototypes for the Detection of Escherichia coli in Water Samples using a Phage-Based Bioluminescence Assay

Luis F. Alonzo*, Troy C. Hinkley, Andrew Miller, Ryan Calderon, Spencer Garing, John Williford, Nick Clute-Reinig, Ethan Spencer, Michael Friend, Damian Madan, Van T. T. Dinh, David Bell, Bernhard Weigl, Sam R. Nugen, Kevin P. Nichols, Anne-Laure M. Le Ny*

Intellectual Ventures Laboratory, 14360 SE Eastgate Way, Bellevue, WA 98007, USA

Now: Global Health Labs, 14360 SE Eastgate Way, Bellevue, WA 98007, USA

E-mail: luis.alonzo@ghlabs.org, annelaure.leny@ghlabs.org

Supplemental Figures and Data

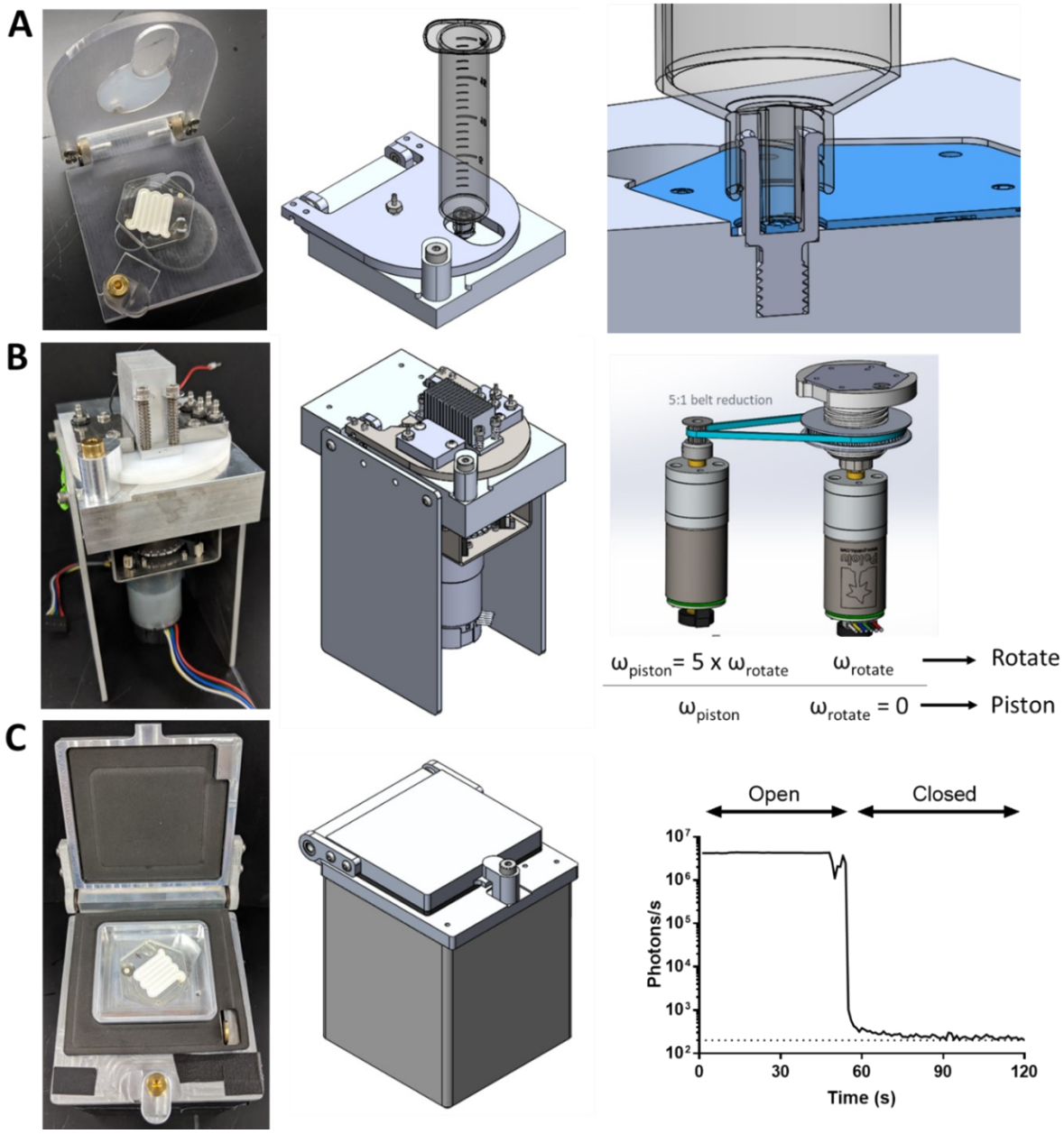


Fig. S1. Instrumentation prototypes. (A) The filtration platform is a 3D printed clamp assembly for the dead-end filtration of high volume water samples. A custom-built world-to-chip interface, compatible with standard Luer Lock connections, was designed as a reusable feature to enable cost savings on the disposable microfluidic device. The CAD file, 'Filtration.STEP', includes necessary information to replicate this build. (B) The liquid handling platform is a mechanical assembly capable of rotating the microfluidic device in two axes. To achieve a pistoning motion, the powered rotating portion of the second motor is held fixed ($\omega_{rotate} = 0$) while the movement of the powered rotating portion of the first motor (ω_{piston}) induces the z-axis motion of the stage holding the microfluidic device. The system can facilitate a rotational motion of the stage without the z-axis motion when the first motor (ω_{piston}) and the second motor (ω_{rotate}) induce controlled rotational rates. The first motor spins the powered rotating portion at an angular rate that is five times that of the powered rotating portion spun by the second motor when the belt provides a 5:1 belt reduction. As such, the belt moves at the same angular rate as the powered rotating portion of the second motor. The CAD file 'Liquid_Handling.STEP' includes necessary information to replicate this build. (C) The detection platform is a light-tight reading chamber for the measurement of the luminescent signal via a photomultiplier tube. PMT signal read-out increases due to ambient light contamination during the insertion of the microfluidic device, but this effect is reverted within 30 s after the lid is closed. The CAD file 'Detection.STEP' includes all of the necessary information to replicate this build.

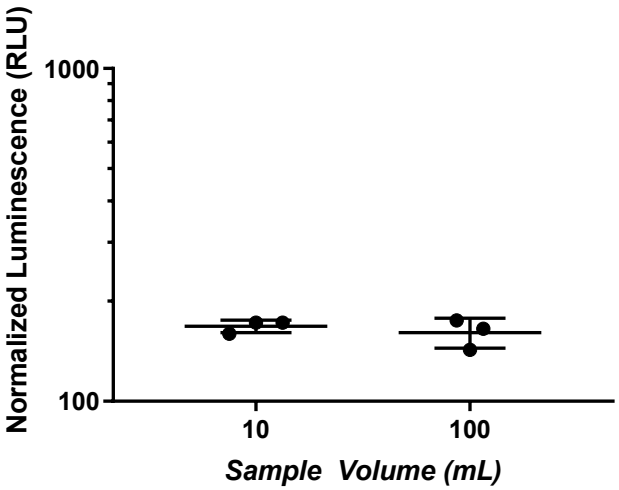


Fig. S2. Assay performance comparison between 10 and 100 mL sample volumes containing 906 *E. coli* cells. No significant difference was observed (n = 3 , p > 0.05).

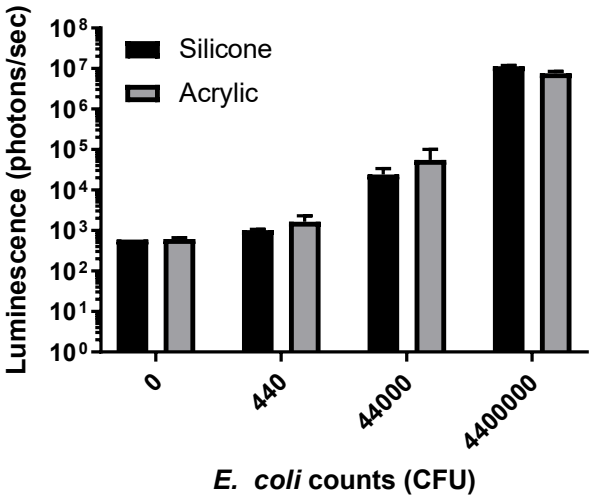
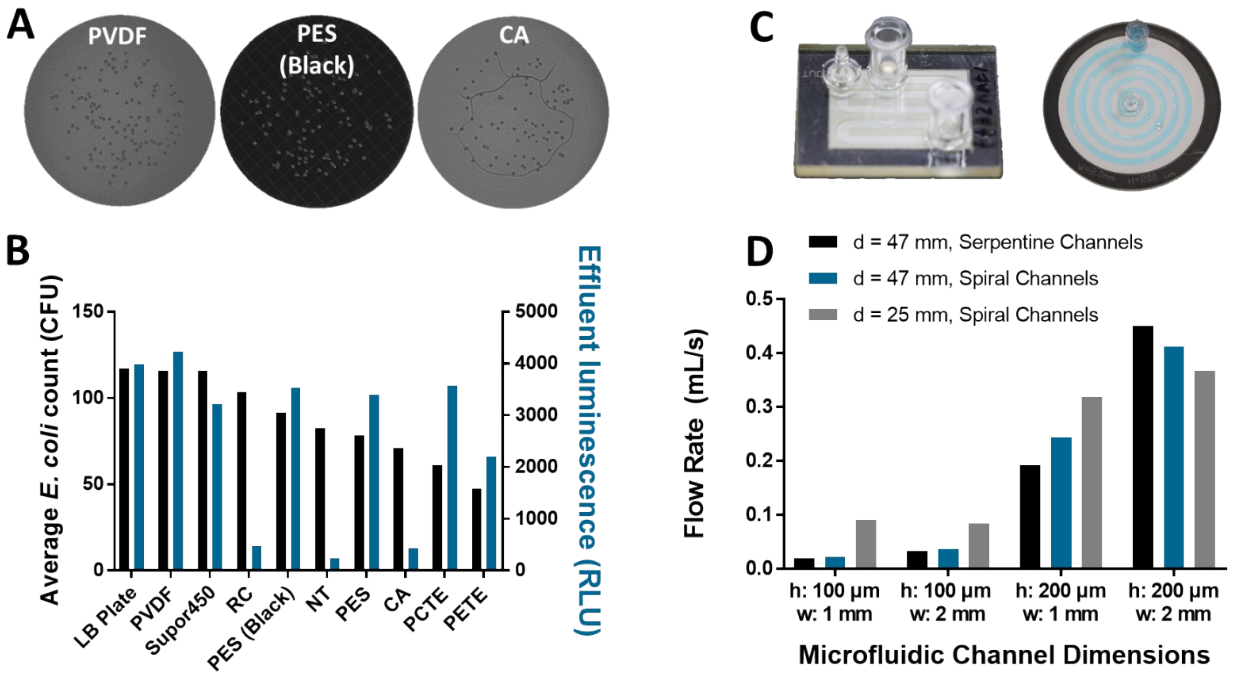


Fig. S3. Assay performance comparison between silicone-based and acrylic-based adhesive-bonded devices. No significant difference was observed (n = 3 , p > 0.05).



E

Diameter (mm)	Flow Rate (µL/s)
1	1.06
2	2.10
4	7.14
6	9.35
8	12.5
10	15.8

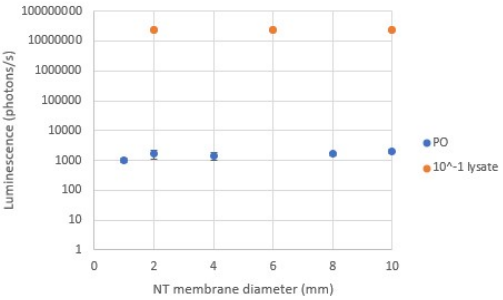


Fig. S4. Microfluidic device prototype optimization via functional subunit devices. (A-B) Microfluidic device material selection experiments to determine cell viability (*E. coli* CFU count) and protein adsorption (reporter luminescence) of a variety of filter membranes using coupons. A PVDF membrane for the sample filtration area was selected due to its biocompatibility and low protein adsorption and an NT membrane due to its high protein adsorption. (C-D) Microfluidic device channel geometry and PVDF membrane area variations to optimize sample filtration rate using early prototype laminate devices. A range of microfluidic channel dimensions and overall membrane filtration areas were tested to balance device size with filtration performance. A minimum filtration threshold of 0.4 mL/s was set to achieve filtration of 100 mL of lab spiked samples within 5 mins. The final channel height of the injection molded device used in this study (500 µm), determined based on mold manufacturing tolerance requirements, is greater than the range tested in preliminary experiments ensuring fast and efficient sample filtration. (E) Detection area NT membrane variations to optimize filtration rate and bioluminescence detection. A range of NT membrane diameters were tested to balance device filtration performance, device size, and minimal impact on luminescence signal output. At the dimensions tested, there was no significant effect on luminescence signal.

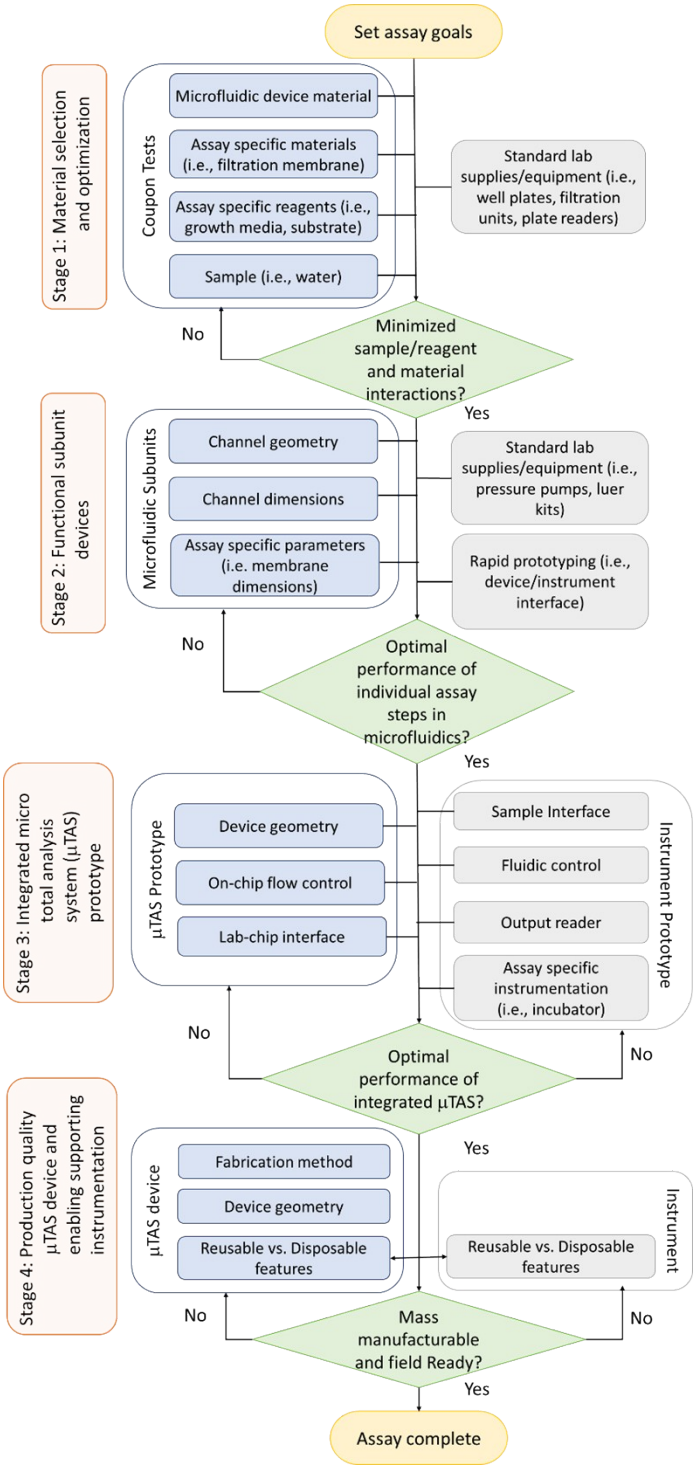


Fig. S5. Product development process for a field-ready microfluidic device. This flowchart describes a general microfluidic device and instrument design and optimization process. Specifically to our assay: Step 1: Coupon tests were performed to determine enzyme binding to thermoplastic materials. Step 2: Laminate microfluidic module was tested to verify E. coli isolation from water sample. Step 3: Laminate integrated device was developed and validated against our previously published detection of E. coli in water sample assay. Step 4: Final laminate device was modified to allow for an injection molded fabrication process for the detection of E. coli in water sample. The microfluidic platform was intentionally designed to be user-friendly and operated in the field. However, additional development efforts on reagent stability and packaging, specifically phage and substrate solution lyophilization, are of interest to enhance the prospect of a portable system

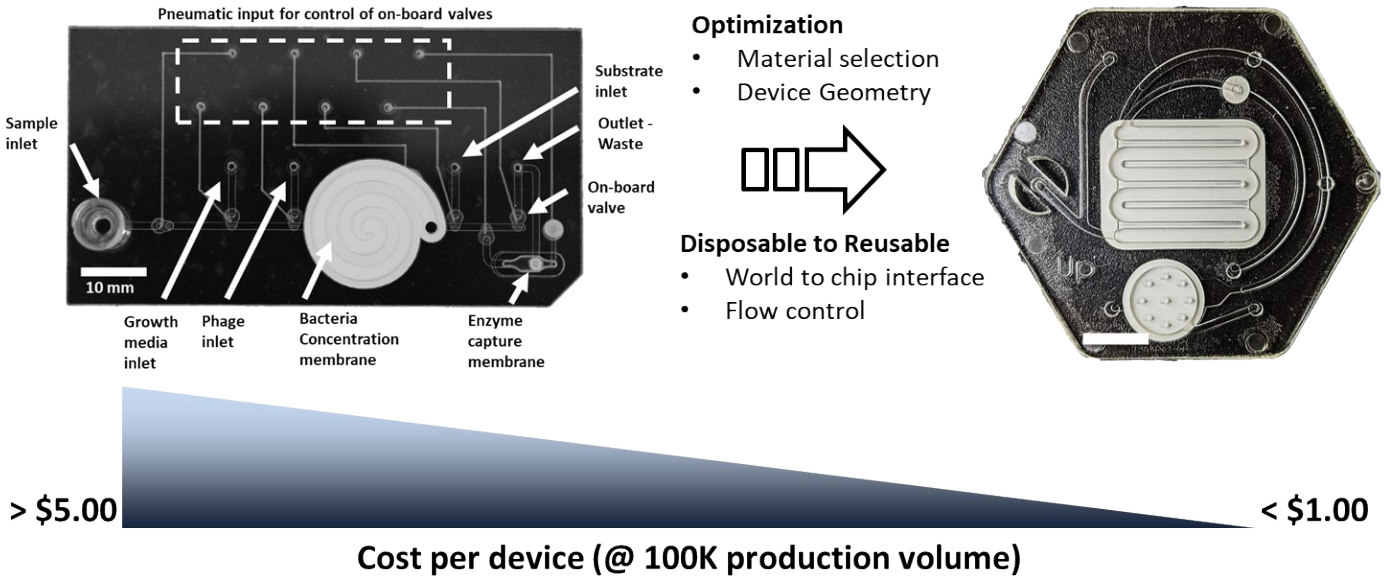


Fig. S6. Changes in microfluidic device architecture/geometry over the development cycle led to a final design that was simplified and ameanable to scalable manufacturing methods, resulting in a significant reduction of per unit cost. Scale bar = 10 mm.

Supplemental Methods

Filtration platform

The accompanying file, "Filtration.STEP", is a 3D computer-aided design (CAD) representation of the filtration platform prototype described in the main text of this article. By sharing this file, we intend to assist in identifying the components required, both commercially available and custom made, to replicate this early version of the filtration prototype. Minor alterations to the design will need to be performed by the reader to accommodate the platforms' use with the injection-molded version of the microfluidic device described in the main text of this article. The table below (Table S1) further details the source of commercially available parts and the manufacturing technique used to produce any custom-made parts. The CAD file should also serve as a guide to assist with assembly of the prototype.

Filtration.STEP	Qty	3D printed	Laser Cut	Machined	Commercially Av.	Material or Vendor
037442	1	x				VeroClear
037619	1					VeroClear
037537	1			x		Stainless Steel
037664	2	x				VeroClear
037665	1	x				VeroClear
4406T550	1				x	McMaster-Carr
6338K561	2				x	McMaster-Carr
90145A483	6				x	McMaster-Carr
95446A530	1				x	McMaster-Carr
90480A195	1				x	McMaster-Carr
036856	2	x				VeroClear
91253A165	4				x	McMaster-Carr
037539	1		x			Silicone

Table S 1. List of parts required to assemble a filtration platform prototype. Vendor information and catalog number are provided for commercially available parts. Manufacturing method and material used are provided for custom-made parts.

Once assembled, to operate the filtration platform, a connection to a negative pressure source (i.e., vacuum) via Tygon tubing is required. Visual inspection of the sample volume during filtration will determine when the vacuum should be shut off.

Liquid handling platform

The accompanying file, "Liquid_Handling.STEP", is a 3D computer-aided design (CAD) representation of the liquid handling platform prototype described in the main text of this article. By sharing this file, we intend to assist in identifying the components required, both commercially available and custom-made, to replicate this early version of the liquid handling prototype. Minor alterations to the design will need to be performed by the reader to accommodate the platforms' use with the injection-molded version of the microfluidic device. The table below (Table S2) further details the source of commercially available parts and the manufacturing technique used to produce any custom-made parts. The CAD file should also serve as a guide to assist with assembly of the prototype.

Liquid_Handling.STEP	Qty	3D printed	Laser Cut	Machined	Commercially Av.	Material or Vendor
036765	1			x		Aluminum 6061-T6
036757	1			x		Delrin 150 NC010
034946/6338K436	2			x	x	McMaster-Carr
034879/1375K53	1			x	x	McMaster-Carr
1375K320	1				x	McMaster-Carr
4843					x	Pololu
036834	1			x		Stainless Steel AISI 304
036787	1			x		Stainless Steel AISI 304
036789	1			x		Stainless Steel AISI 304
EE_SX4162_P2	2				x	DigiKey
92196A076	6				x	McMaster-Carr
EE_SX4163_P2	1				x	DigiKey
036819	1			x		Stainless Steel AISI 304

036831	1			x		Aluminum 6061-T6
63604-512	1				x	DigiKey
037724	1			x		Copper
036838	1		x			Silicone
9657K267	4				x	McMaster-Carr
036853	2			x		Aluminum 6061-T6
036856	2			x		Aluminum 6061-T6
90145A83	2				x	McMaster-Carr
6338K561	2				x	McMaster-Carr
036953	2		x			Silicone
035121	2			x		Aluminum 6061-T6
92196A151	4				x	McMaster-Carr
4406T550	5				x	McMaster-Carr
037351	1			x		Aluminum 6061-T6
95446A530	1				x	McMaster-Carr
037626	1			x		Aluminum 6061-T6
037625	1			x		Stainless Steel AISI 304
2826	1				x	Pololu
95630A650	1				x	McMaster-Carr
037630	1			x		Aluminum 6061-T6
037697	2			x		Stainless Steel AISI 304
1679K87	1				x	McMaster-Carr
92949A146	23				x	McMaster-Carr

Table S 2. List of parts required to assemble a liquid handling platform prototype. Vendor information and catalog number are provided for commercially available parts. Manufacturing method and material used are provided for custom-made parts.

Once assembled, to operate the liquid handling platform, the following is required: an electrical power connection, a computer with LabView software, a motor controller to control DC motors (i.e., #3285, Pololu Robotics & Electronics), a TEC controller, a negative pressure source (i.e., vacuum) via tygon tubing, and liquid reagent reservoirs via tygon tubing. A stand-alone executable file, “Motor Positioner DM.exe”, is provided as an example of a user interface for the optimization of various operational parameters (e.g., stage rotation, stage pistoning, etc) and monitoring the performance of the platform (Fig. S7) Final parameters will need to be defined by the reader. Dispensing and removal of reagents is controlled through the operation of the vacuum source and should be verified visually after every step during the optimization stages. Additional modifications are required to fully automate this process.

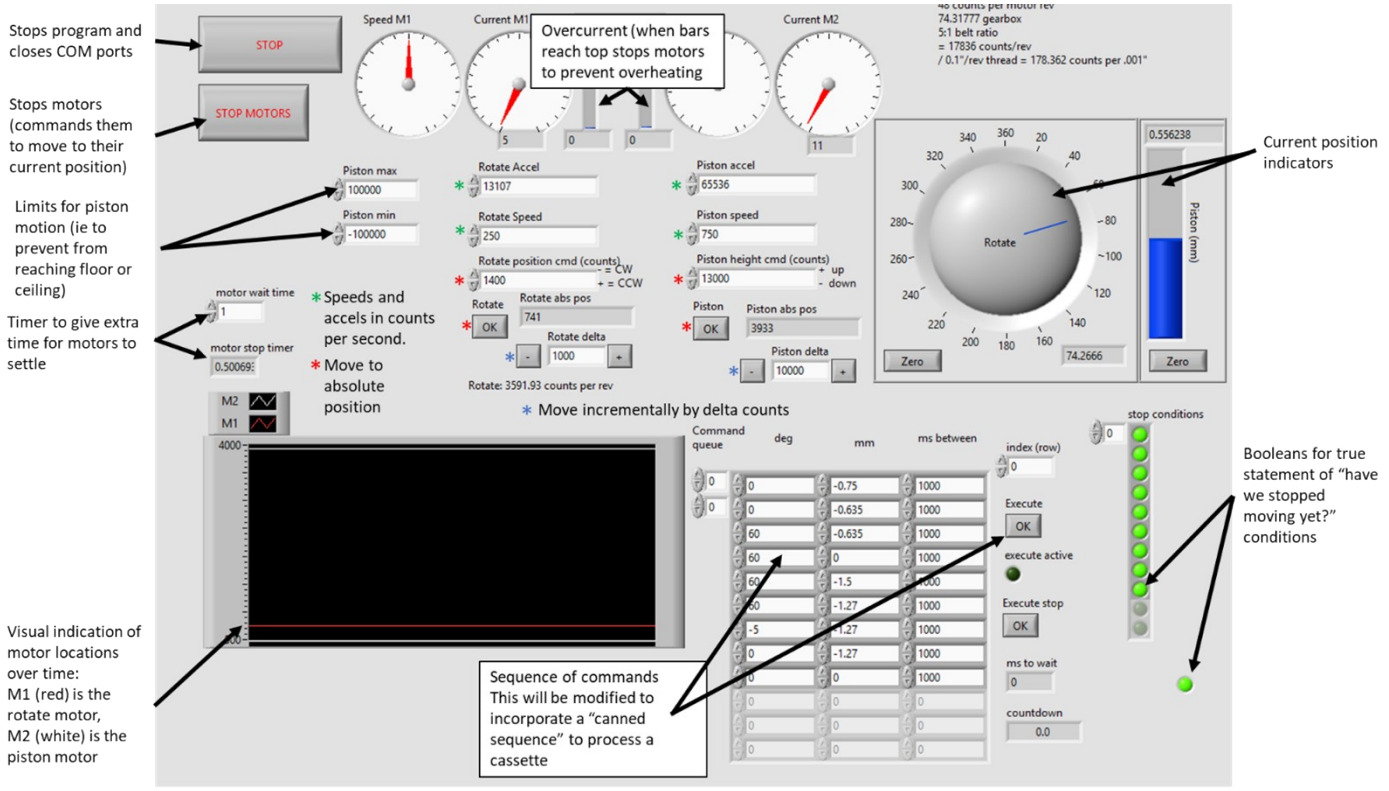


Fig. S7. Screen shot of Motor Positioner DM GUI with overlayed explanations and clarifications.

To increase throughput, our current instrumentation prototypes can be adapted to address these limiting incubation steps. For example, the Peltier cell on the liquid handling platform can be separated and redesigned to be shared amongst multiple microfluidic devices tested in parallel, increasing the number of samples that can be processed at a given time.

Detection platform

The accompanying file, "Detection.STEP", is a 3D computer-aided design (CAD) representation of the detection platform prototype described in the main text of this article. By sharing this file, we intend to assist in identifying the components required, both commercially available and custom-made, to replicate this early version of the detection prototype. Minor alterations to the design will need to be performed by the reader to accommodate the platforms' use with the injection-molded version of the microfluidic device. The table below (Table S3) further details the source of commercially available parts and the manufacturing technique used to produce any custom-made parts. The CAD file should also serve as a guide to assist with assembly of the prototype.

Detection.STEP	Qty	3D printed	Laser Cut	Machined	Commercially Av.	Material or Vendor
036225	1			x		Aluminum 6061-T6
036222	1			x		Aluminum 6061-T6
036226	2			x		Aluminum 6061-T6
036242	1			x		Stainless Steel AISI 304
036241	1			x		Brass
036239	1			x		Brass
036420	1			x		Aluminum 6061-T6
036442	1		x			Rubber
036443	1		x			Rubber
036489	1			x		Aluminum 6061-T6
95649A018	3				x	McMaster-Carr
PDM03-9107-USB	1				x	ET Enterprises
92196A074	5				x	McMaster-Carr
037351	1				x	Aluminum 6061-T6

95446A490	1				x	McMaster-Carr
92949A146	8				x	McMaster-Carr
9440T110	2				x	McMaster-Carr
97395A485	2				x	McMaster-Carr
9654K945	1				x	McMaster-Carr
037551	1	x				VeroBlack
92196A160	4				x	McMaster-Carr

Table S3. List of parts required to assemble a detection platform prototype. Vendor information and catalog number are provided for commercially available parts. Manufacturing method and material used are provided for custom-made parts.

Once assembled, to operate the detection platform, the following is required: an electrical power connection and a computer with ET Enterprises software. Following the PMT's manufacturer recommended protocol, the user must ensure that the timing between addition of substrate reagent and luminescence measurement is well regulated to obtain consistent results between different sample measurements.