Image-based Feedback and Analysis System for Digital Microfluidics

Supplementary Information

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In addition to this file, we have included

1) **A video to describe on how to setup the image-based feedback software**
2) **.STL files for printing the pogo pin holder (Fig. S1)**
3) **A .ZIP file containing the software to execute the automation and the feedback system and the enzymatic assay**
4) **BOM list for the automation system – control board and pogo pin**

Reagents and Materials

All general-use reagents were purchased from Sigma, unless specified otherwise. *E.coli* DH5α and BL21(DE3) strains and original pET16b vectors were generously donated from Dr. Vincent Martin. The restriction enzymes and T4 ligase used for cloning were obtained from Thermo (Waltham, MA). DNA oligos used in PCR amplification were synthesized by Thermo. Miniprep kits (Cat no. BS413) and gel extraction kits (Cat no. BS354) were purchased from BioBasic (Amherst, NY). β-glucosidase substrate 4-Nitrophenyl β-D-glucopyranoside (pNPG) was purchased from Carbosynth (cat no. EN03181, San Diego, CA).

Microfluidics device fabrication reagents and supplies included chromium-coated with S1811 photoresist on glass slides from Telic (Valencia, CA), indium tin oxide (ITO)- coated glass slides, R_S=15-25Ω (Cat no. CG-61IN-S207, Delta Technologies, Loveland CO), 3M HFE 7500 Engineering fluid from MG Chemicals (Burlington, ON), FluoroPel PFC1601V from Cytonix LLC (Beltsville, MD), MF-321 positive photoresist developer from Rohm and Haas (Marlborough, MA), CR-4 chromium etchant from OM Group (Cleveland, OH), and AZ-300T photoresist stripper from AZ Electronic Materials (Somerville, NJ). Transparency masks for device fabrication were printed from CADArt (Bandon, OR) and polylactic acid (PLA) material for 3D printing were purchased from 3Dshop (Mississauga, ON, Canada).

Device Design, Fabrication, and Assembly

Two digital microfluidic device geometries were used for this study which were made using Autocad 2017. Design #1 consisted of a linear array of electrodes with one reservoir electrode and design #2 consisted of driving electrodes separated by gaps of 20 μm; electrode patterns and dimensions are listed in Table S1.

Device fabrication followed procedures previously published.[1] Briefly, chrome substrates were patterned using photolithography, developing, etching, and stripping methods. After patterning, these were coated with Parylene- C (~5 μm) and FluoroPel 1601V (180 nm). Parylene was applied by evaporating 15 g of parylene C dimer in a vapour deposition instrument (Specialty Coating Systems, Indianapolis, IN) and the hydrophobic FluoroPel 1601V (Cytonix, Beltsville, MD, USA) was spin coated (1500 rpm, 30s) and post-baked on a hot plate (180°C, 10 min). Unpatterned top plates were formed by spin-coating ITO with FluoroPel 1601V (as with bottom substrates).

Devices were assembled with the ITO top-plate and a patterned bottom plate separated by a spacer formed with one or four pieces of double-sided tape (70 or 280 μm respectively) Droplets were sandwiched between these two plates and were actuated by applying electric potentials between the two plates. Each electrode was connected to a contact pad (not shown in Table S1 for simplicity) that is interfaced with our pogo pin connector. Droplet motion was managed using our automated imaging feedback system. All reagents were manually loaded into the reservoirs using a pipettor.

Molecular cloning

The gene sequence for the *Thermobaculum terrenum* β-glucosidase (BGL1) was obtained from NCBI (GenBank accession number WP_041425608.1) and was synthesized by Gen9 (now part of Ginko Bioworks) in a pGm9-2 backbone (sequence of BGL1 – Fig. S3.). The gene was amplified by PCR with primers (shown below) introducing a 5' XbaI and a 3' BamHI restrictions sites.

Forward:

5'- TGACTGACTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGA CCCGTATGAAGATCCGC - 3'

Reverse:

5' - GCATGCATGGATCCCTACAGGGTCAGACCATGACCG - 3'

Individual PCR reactions consisted of 10 μL 5X Phusion buffer, 1 μL dimethylsulfoxide (DMSO), 20 ng template DNA, individual dNTPs and primers to a final concentration of 200 μM and 0.5 μM, and distilled water up to 50 μL. The following PCR thermocycling conditions were used: initial denaturation at 98 °C for 30 s followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s and extension at 72°C for 30 s/kb, and a final extension step at 72°C for 10 min. PCR products were loaded into a 0.8% agarose gel in TAE buffer and resolved at 130 V for 30 min. The corresponding bands were extracted using a gel extraction kit. The gene was then digested using XbaI and BamHI restriction enzymes and ligated into a linearized pET16b vector backbone (see plasmid map – Fig. S4). The ligation product was transformed into chemically competent *E.coli* DH5α cells and plated on LB plates containing 100 μg/mL ampicillin (Amp). For transformation, 100 μL of thawed competent cells were mixed on ice with 100 ng of

the ligation product. This mixture was heat-shocked at 42°C for 60 s after which cells were placed on ice for 1 min for recovery. 900 µL of LB were added to the transformation mixture and the cells were incubated at 37°C for 1 h. 200 µL of this mixture were plated onto selective media. The following day, single colonies were inoculated in 5 mL of LB Amp media overnight and plasmids were extracted using a BioBasic miniprep kit. Finally, proper insertion of the gene was verified by digesting 2 µg of plasmid with XbaI and BamHI and running the product on a 0.8% agarose gel to look for the correct insert band size.

Protein expression

The plasmid containing the cloned BGL1 gene was first transformed into E.coli BL21(DE3) for recombinant expression. The transformed cells were inoculated overnight in a 5 mL pre-culture. The following day, the culture was diluted to OD 0.05 in a 100 mL starter culture and grown at 37°C with 200 rpm shaking. Upon reaching OD 0.4, expression of the BGL1 gene was induced by addition of 1 mM IPTG and induction was carried out under the same growth conditions for 8 hours. The final induced culture was centrifuged at 4000 rpm for 5 min and the supernatant was discarded. The cell pellet was resuspended in 2 mL lysis solution per 50 mL of initial culture. The lysis solution comprises 1 mg/mL lysozyme, 25 U/ml benzonase and 1 mM phenylmethanesulfonylfluoride (PMSF). Lysis was carried out for 30 min at room temperature and the lysates were diluted 100-fold in assay buffer containing 50mM sodium-citrate at pH 7 and stored at 4°C before the assay.

BGL off-chip assay

In the assay, nine reactions consisted of equal volume of cell lysate and 4 mM of p-nitrophenyl-β-D-glucopyranoside (pNPG) dissolved in the assay buffer. At 30 min intervals, 134 μL from a reaction were added to 67 μL of a 300 mM glycine-NaOH solution in a transparent flat bottom well plate to stop the reaction. Absorbance at 405 nm was immediately acquired after stopping each reaction on a TECAN infinite M200 plate reader with the following settings: 9 nm bandwidth, single reads per well, 25 flashes per reading, and 0 ms of settle time. Reactions with absorbance units > 4 were diluted and the final absorbance was calculated from the diluted sample. The assay was repeated in triplicate and lysates from a transformed culture with an empty pET16b plasmid were used as a negative control.

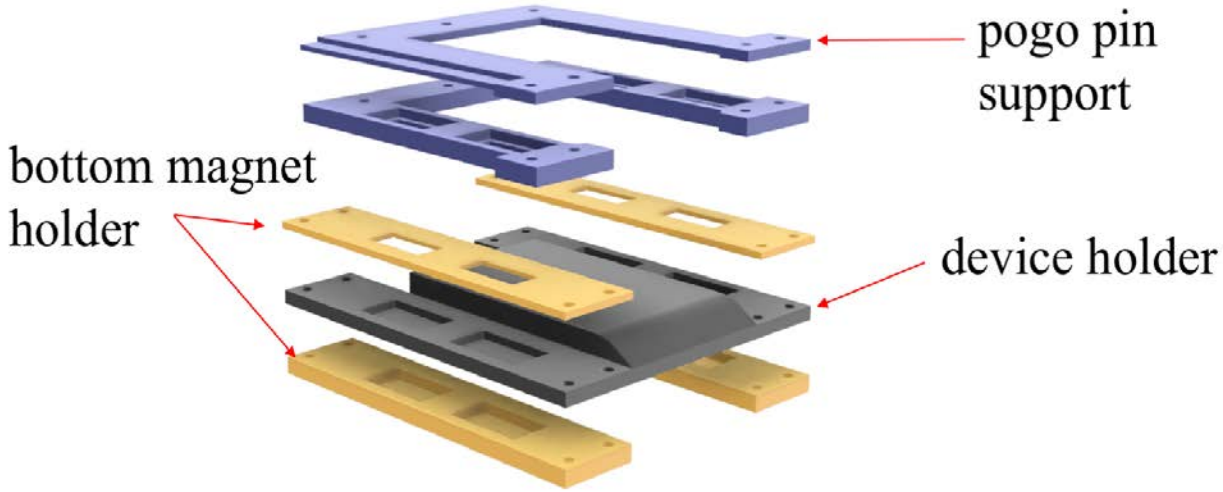


Fig. S1 – A schematic showing the 3D printer holder for the pogo pin and the DMF device. The top holder (shown in blue) is used to support the pogo pin holder. This top holder also consists of rectangular magnets that will attract to the bottom holder (shown in gold) with the device inserted in between the top and bottom holder (shown in gray).

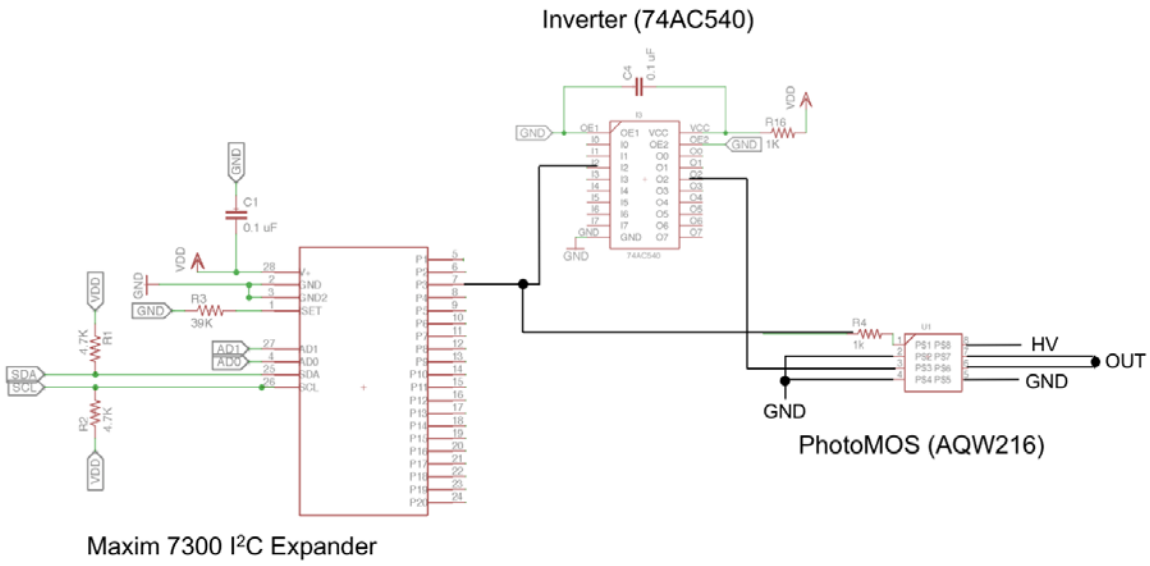


Fig. S2 – A circuit diagram showing the connectivity of one output. The software uses I^2C communication protocol to deliver a user-configurable high (5V) and low (0V) signals to the Arduino (not shown). The data (SDA) and clock (SCL) signals are delivered to a Maxim I/O expander with an address AD0 and AD1 and the output of the expander is connected to a PhotoMOS switch and inverter. Each switch contains two optical photodiodes that will be used to deliver two logic states: high (i.e. ~100 V) and low (i.e. 0 V). The inverter is used to prevent any short circuit at the output of switch. The output of the switch is connected to a pogopin board that houses 104 spring loaded pins (see BOM list for parts).

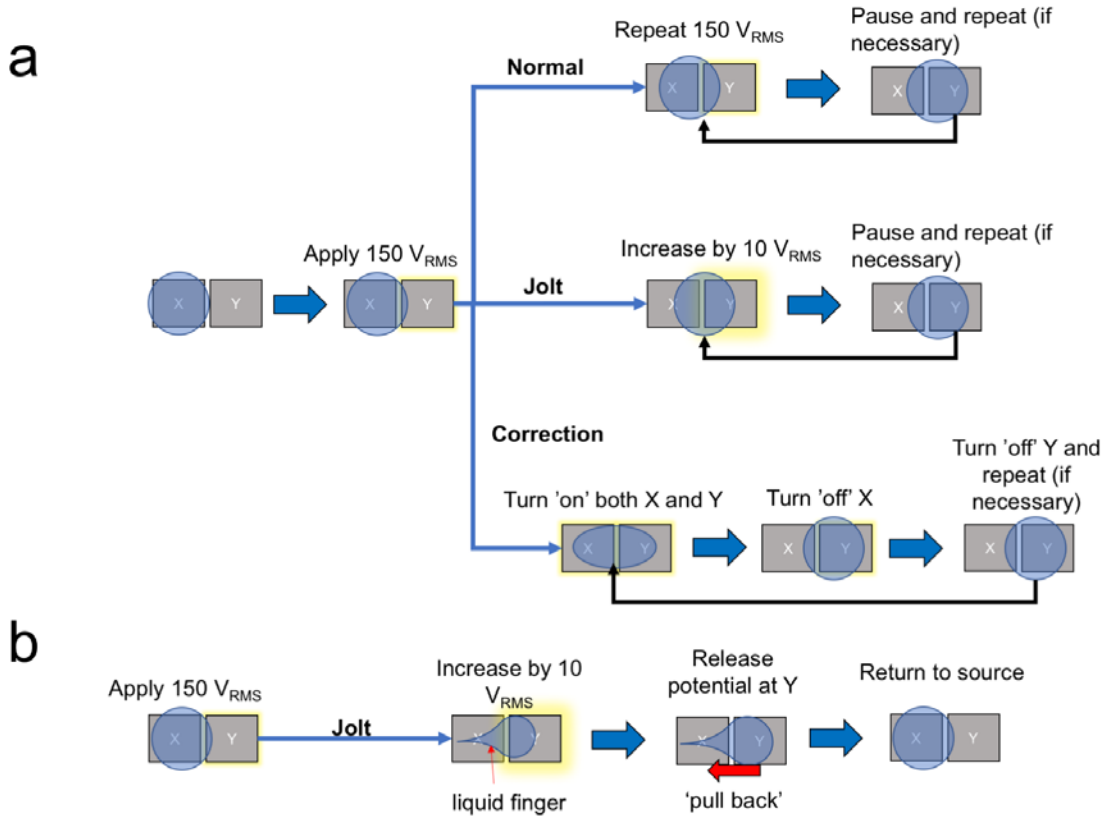


Fig. S3 – (a) A schematic showing the actuation schemes tested with our imaging feedback system. In the normal scheme, an additional 150 V_{RMS} potential was applied to the destination without increasing the voltage. In the jolt scheme, the voltage was increased by 10 V_{RMS} (or set by the user) for each actuation cycle. In the correction scheme, both source and destination electrodes were activated at the reference potential of 150 V_{RMS}. We also tested the combination of jolt and correction (not shown) which starts with the correction scheme and then increases the reference voltage (150 V_{RMS}) by 10 V_{RMS} to the destination electrode Y at the end of the correction scheme (not shown). (b) A schematic showing the 'pull-back' problem frequently demonstrated using the jolt scheme with highly viscous biological liquids.

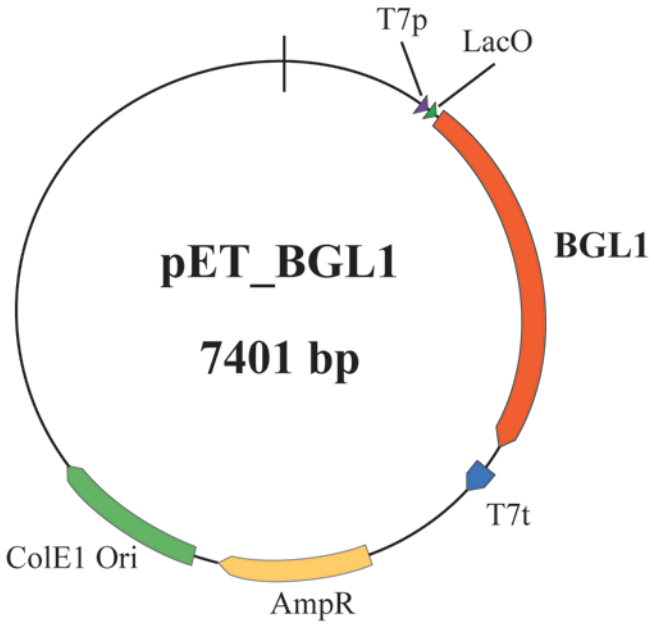


Fig. S4 – A plasmid map of pET_BGL1 consisting of a pET16b backbone with BGL1. Other parts in this plasmid consists of a T7 promoter and terminator with ColE1 origin of replication and ampicillin resistance.

ATGAAACATCTGGTTACCACACTGCTGTTTGGTCTGGTTTTTGCAGCAGCAGTTCGTGCACAG
AGCAGCCGTGTTGATAGCCTGCTGGCAGCAATGACCCTGGCAGAAAAAATCAGCCTGCTGC
ATGGTGCAATTGATCCGACCGGTGAAGCCGGTGCAGGTTATGTTCCGGGTGTTCCGCGTCTG
GGTATTCCGGCAATGCGTCTGACCGATGGTCCGGCAGGTATTCGTACCCGTCATCCGGCAAC
CGCACTGCCAGCACCGGTTGCACTGGCAGCCAGCTTTGATCCGGAACTGGCATATCGTTATG
GTCATGTTATGGGTATTGAAGGTCGTGCACGTCGTCATGAAGTTCTGCTGAGCCCGATGGTT
AATATTGTTCGTGTTCCGGAAGCAGGTCGTAACTTTGAAACCTTTAGCGAAGATCCGCTGCT
GAGCGCAGAAATGGTTGCAGCCGAAGTGCGTGGTATTCAGGATGCAGGTATGATGGCAACC
GTTAAACATTATGTGGCCAACAACTTTGAGAATGATCGTATGCGTGTTAATGTGGTTGTTGA
TGAACGTACCCTGCGCGAAATTTATCTGCCTGGTTTTGAAGCAGCCATTAAAGCGGGTGCAG
CAGCCGTTATGTGTGCATATAATCGTGTGAATGGTCCGTATGCCTGTGATAATGAAATGCTG
CTGACCGATATTCTGCGTGATGAATGGGGCTTTGAAGGTTGGGTTATGACCGATTGGTTTGC
AGGTCATAGCCTGGAAAGCCTGGTTCGTGGTCTGGATCAAGAAATGCCTGGTTATACCATTC
CGTTTAGCAGTCCGGATATGCCGCTGGCACCGGCAGTTTTTGCCGATAGCCTGTTAGCCGCA
GTTGAAAGCGGTCGTATTGATGAAGCCTATGTTGATCGTGCAGTTCGTCGTATTCTGGTTCAG
ATGGAACAGTTTGGCCTGCTGGATGGCGAAAGCACCCCTCCGGAAATTAACATTGAAGCAC
ATGCAGCCGTTGCACGTGAAGTTGCAGAAGCAGGCGCTGTTCTGCTGCGTAATGAAAATCAG
ACCCTGCCGCTGAGCGAACGTGATCTGCAGCATCTGGTTGTTATTGGTCCGACCGCAACACG
TCCGCTGATTGGTGGTGGCGGTAGCAGTCGTGTTCAGCCGTTTCGTACCACAAGCACTCTGG
CAGCACTGCAAGAACTGGCAGGTCCGCAGGCACAGATTCGTTATGTGCCAGGTATTGATCTG
GATGGTATGCCGGTTCCGAGCAGCGCACTGCGTACACCGGATGGTCAGCCTGGTCTGCTGCG
CCAGGGTGCAGATGGTACAACCCAGGTTGATGCACAGCTGGATTTTACCGGTGAACGTGCAC
TGCCTCCGGGTAGCCAGTGGACCTGGACCGGTACACTGACCGCACCGACCGCAGGCGTTTAT
GAACTGAAACTGCAGACAGCCGGTGGTGTTGGCACCCTGAGCATTGATGGCCAGCCGGTTCT
GCGTACCGGTATGTTTTTTAGTGATGCAAGCCTGATTCCGACAGCAGATAGTCTGGAAAATG
CAACCTATCGTATTGAACTGCAGGCAGGCCAGCAGCTGAGCCTGACCGTTCAGATTAGCGGT
CAGGTGCCGAGCCTGCCGTTTCTGCCTGCCGGTCAGAATCCGGTTCAGGTTCGTCTGGCATG
GGTTACACCGGAACGTCGTGCAGCCTTTCTGGAAGAAGCAGCGGAAGCAGCACGTGCAGCA
CATGCCGCAATTGTTTTTGTTTATGAAGAGGGCACCGAAGGTCGCGATCGTGAAACCCTGGC
ACTGCGTCCGGATCAGGATGCCCTGGTTGAAGCAGTTGCCGCAGCAAATCCGCGTACCACCG
TTGTTATGAATGTTGGTGCACCGACACTGATGCCGTGGGCAGAACGTGTTGGTGCCATTCTG
CTGATGTGGTATCCGGGTCAAGAAGGTGGTTGGGCCACCGCAGATGTGCTGCTGGGTCGTGC
AAATCCGGCAGGCCGTCTGCCGGTTACCTTTCCGCGTCGTGCCGAAGATGCTCCGACCGCCA
GTCCGGAACGTTATCCTGGTGTTGATCTGACAGCACGTTATGATGAAGGCATTTTTGTTGGTT
ATCGTTGGTATGATGCCCAGCAGATTGAACCGCTGTTTCCGTTTGGTCATGGTCTGAGCTATA
CCACCTTTGCCTATGAAAATCTGCGTGTTGAACCTGATGGTGATGGTTTTGTTGTTCGTTTTG
TGGTGCGTAATACCGGTGATCGCGCAGGTAGTGATGTTCCGCAGGTTTATCTGGGTCCGCCT
GAAAATCCGCCTGTTCCGATGGCCGTTCGTCAGCTGGTTGGTTTTCGTCGTGTTACACTGGCA
CCTGGTGAAGCACAAGAAGTTACCGTTCGTATCGATGGTCGTGCCCTGAGCTATTGGAGCGT
TGAAGATCATGCCTGGGTTAAAGCAACCGGTCGTCGTACCCTGTATGTGGGTGCAAGCGCAC
GCGATCTGCGCCTGCAGACCGAAATTGATGTTCAGTAG

Fig. S5 – Sequence of BGL from *Thermobaculum terrenum*.

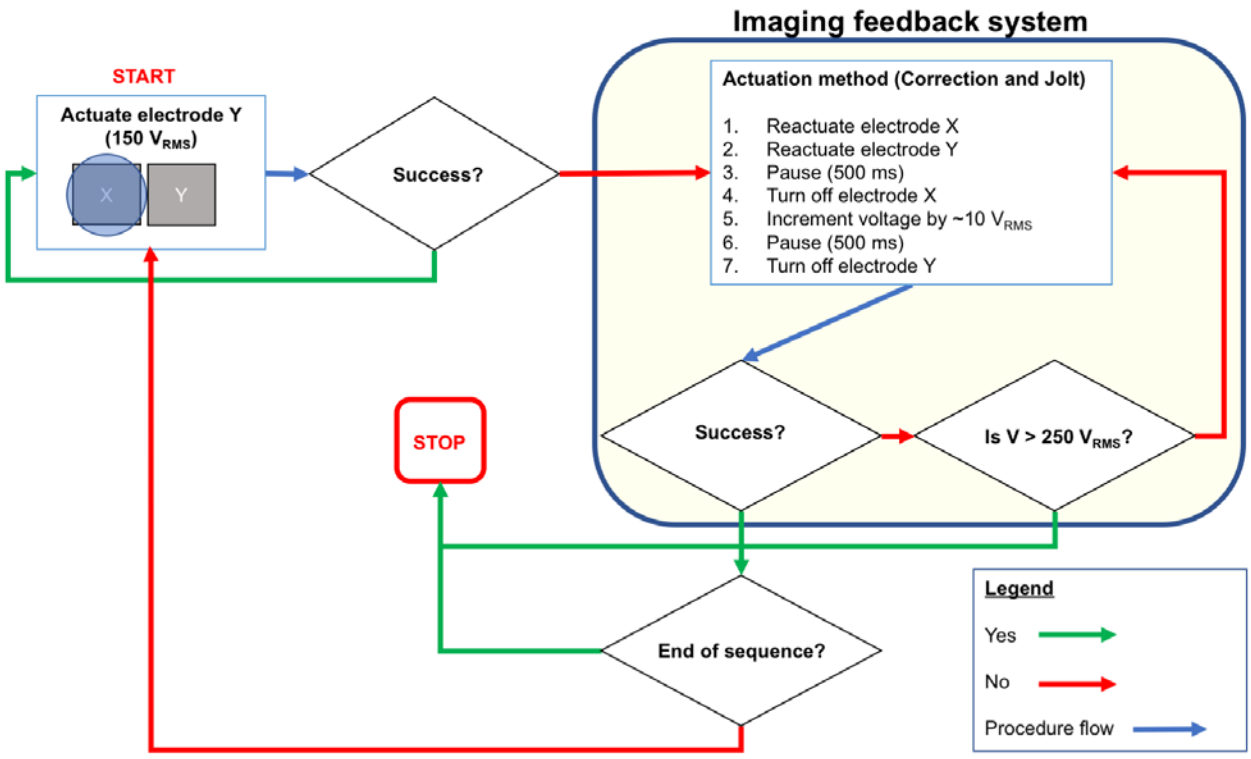


Fig. S6 – Flowchart summarizing the algorithm used to manage the image-based feedback system. Droplets are actuated with a 150 V_{RMS} AC signal with 15 kHz. The imaging feedback system is initiated if the droplet does not move to the destination electrode (shown as Y). The actuation method is a feedback scheme to move the droplet onto Y (see methods). As an example, the schematic shows the procedure for the jolt and correction actuation scheme. This method can be switched to only jolt or correction depending on the user selection at the beginning of the program setup. If droplet movement failed, the algorithm will continue with the actuation scheme until the voltage surpasses 250 V_{RMS} or if the droplet has moved to the electrode Y. If the droplet movement is successful, the algorithm continues with the droplet movement sequence unless the sequence is finished.

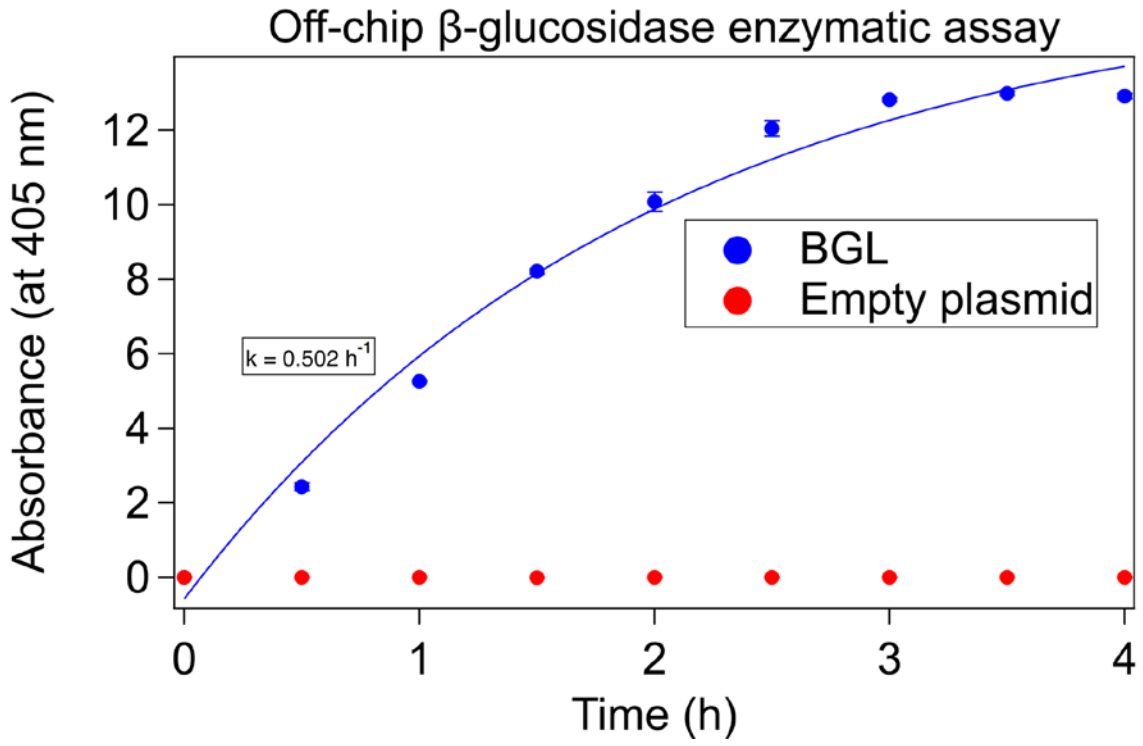
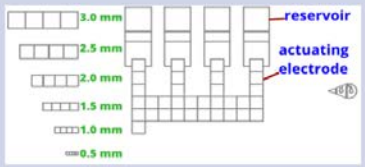
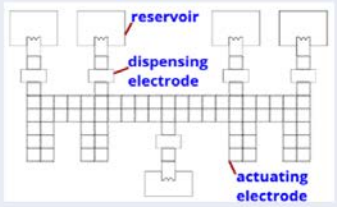


Fig. S7 – Off-chip enzymatic assay with an absorbance readout as a function of time were collected every 30 min. Nine reactions containing equal volume of lysate with enzyme and substrate were mixed in a well of a 96 well plate. At 30 min intervals, the reaction was arrested by glycine-NaOH solution and an absorbance measurement was acquired from the product formation of pNP which gave a yellow colour. Each experiment was repeated three times, and error bars are +/-1 SD.

Table S1 – DMF devices used in this study

Devices	Reservoir dimension (mm)	Actuation electrode (mm)	Dispensing electrode (mm)
Device #1 	4.5 x 4.5	2.25 x 2.25	Same as actuation
Device #2 	8.0 x 6.0	2.48 x 2.48	4.25 x 2.25

1. S. C. C. Shih, I. Barbulovic-Nad, X. Yang, R. Fobel and A. R. Wheeler, *Biosens. Bioelectron.*, 2013, **42**, 314-320.