Supplementary Materials for

## A digital microfluidic system for serological immunoassays in remote settings

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Fig. S1. Quality control procedure 1. (**A**) Portions of maps of capacitance measured for two devices (i and ii), with heat map ranging from ≤ 80 fF (dark red) to ≥ 400 fF (white). (**B**) Distribution of capacitance (median ± 1.5 interquartile range) for the electrodes in devices i and ii. Broad distributions with outliers below the cutoff (for example, Device i) indicate potential failure points; such devices were discarded. Note that in previous systems (ref. *33-34*), device failure was a common occurrence; the combination of QC-1/QC-2 reported here eliminates it entirely.
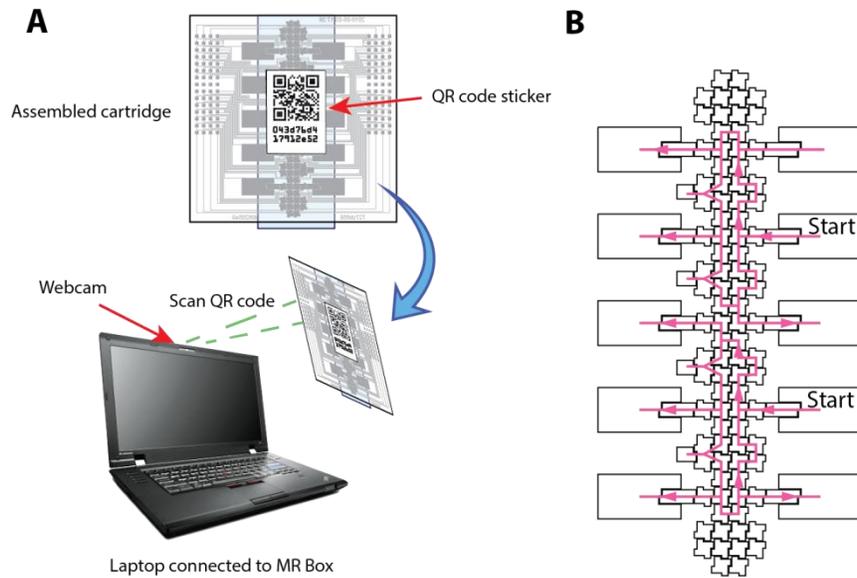
Fig. S2. Quality control procedure 2. (**A**) Quick response (QR) code on the back of the DMF cartridge is scanned (represented by green dashed lines) into the laptop webcam and the device ID is entered into the cartridge metadata. (**B**) Schematic of droplet paths for QC-2. Two droplets (4-6 µL ea.) of PBS buffer are loaded into reservoirs and moved across the driving electrodes in a counter-clockwise fashion in the assay region (pink arrows). Cartridges with inoperable electrodes were discarded. Note that in previous systems (ref. *33-34*), device failure was a common occurrence; the combination of QC-1/QC-2 reported here eliminates it entirely.
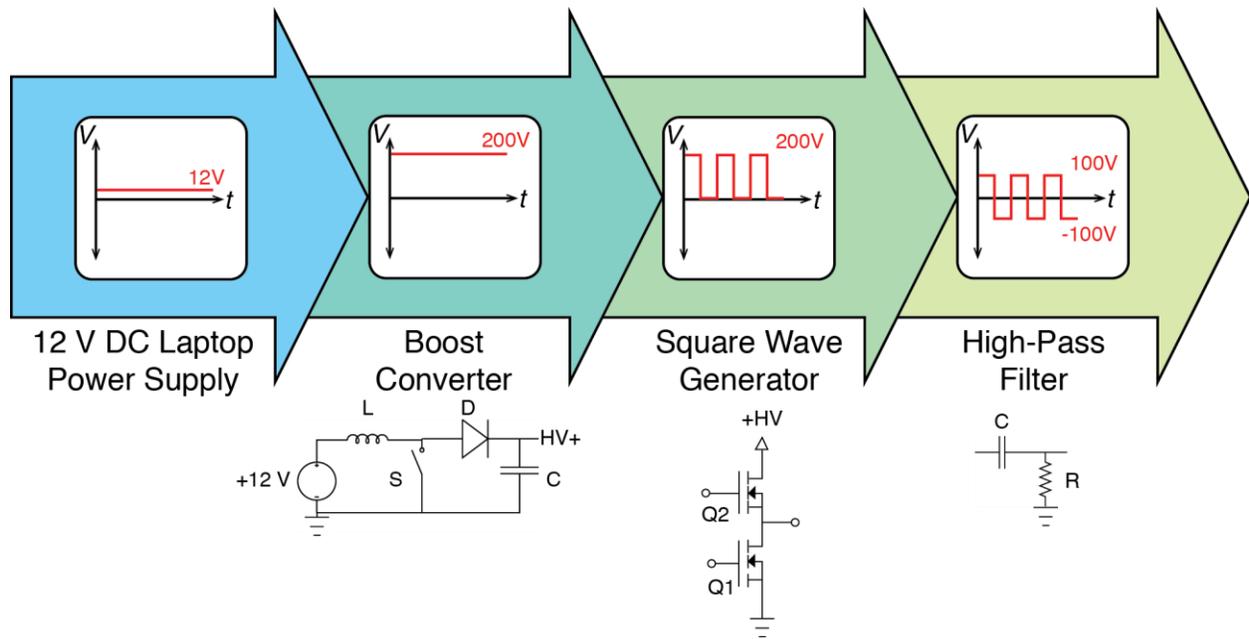
Fig. S3. Integrated control system. Diagram of the integrated amplifier with AC signal generation embedded in the open-source DropBot 3.0 control system (http://microfluidics.utoronto.ca/dropbot/). DC voltage is supplied to the MR Box from a 12 V laptop power supply. A (step-up) boost converter amplifies the +12 V input to +60-240 V and then a square wave generator creates a unipolar square wave using two n-channel power MOSFETs in totem-pole configuration. Filtering through a high-pass filter with a cutoff frequency of 0.8 Hz generates a bipolar AC signal, which is used to bias driving electrodes during droplet movement.

Inactivated   **Activated**

Magnetic particles   DMF top plate   Magnetic particles

Droplet

DMF bottom plate

Magnetic lens

Magnet

PLA housing

Tilt bracket

Servo motor

TowerPro MG995   TowerPro MG995
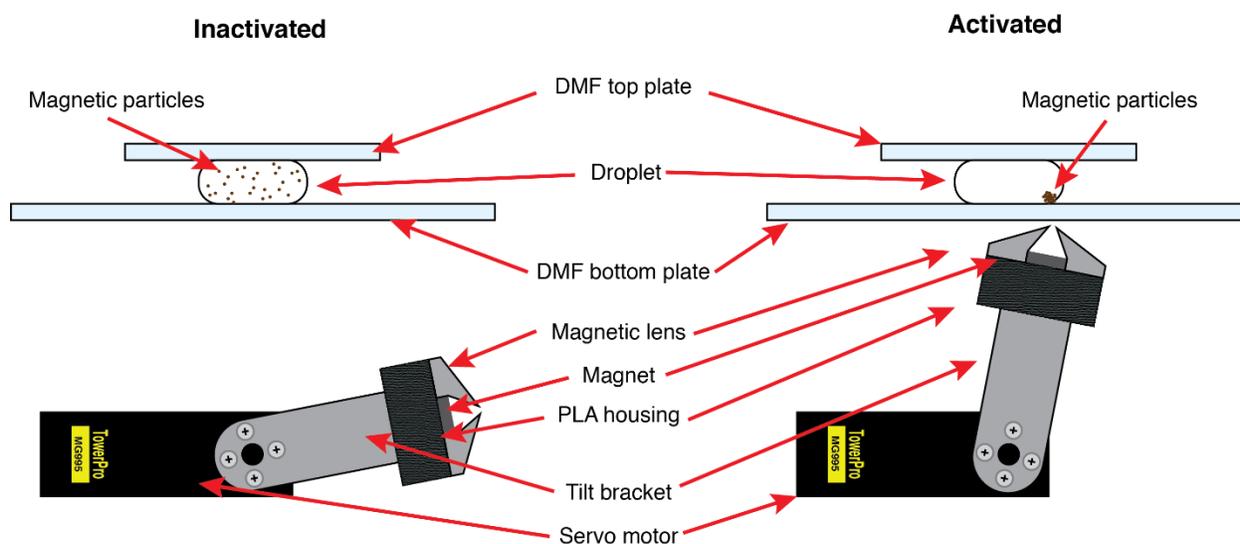
Fig. S4. Magnetic separation. Cartoon of the magnetic lens system illustrating the inactivated (left) and activated (right) states. The magnet and magnetic lens are mounted in a 3D-printed PLA housing and affixed to a tilt bracket. The servo motor controls the orientation of the magnet and lens with respect to the DMF cartridge (comprising a DMF top plate and DMF bottom plate). Magnetic particles within the droplet align with the magnetic field, such that they can be separated from droplets when the system is activated. Note that this mechanism is smaller, lighter, and consumes less power than the original vertical/step-motor system (ref. *33-34*). Cartoon not to scale.
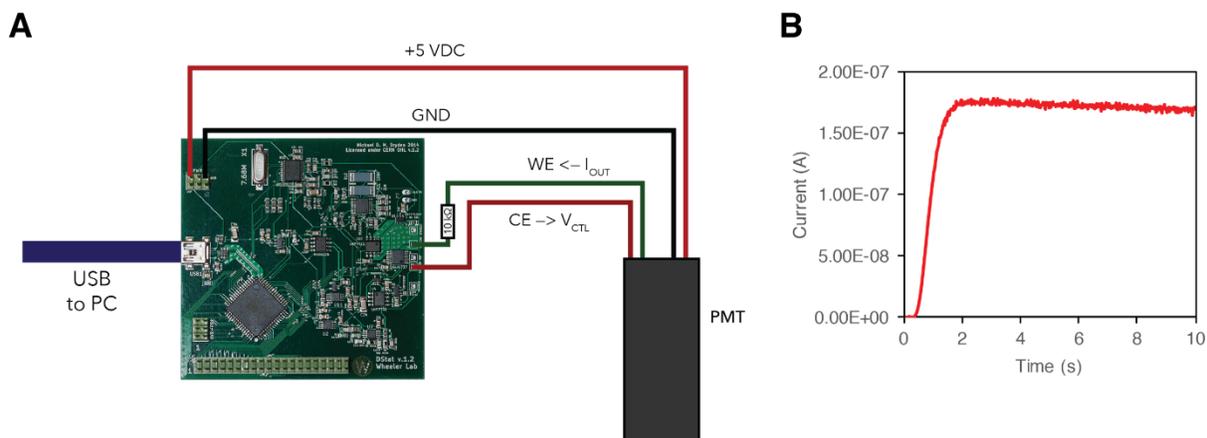
Fig. S5. PMT control. (**A**) Schematic and picture illustrating how the open-source DStat potentiostat (http://microfluidics.utoronto.ca/dstat) is used to control the PMT for chemiluminescent measurements. The DStat supplies +5 V DC and ground (GND) to the PMT. PMT control voltage (V_{CTL}) is set by the counter electrode (CE) and the output current I_{OUT} is measured by the working electrode (WE). (**B**) PMT current response from the DStat measuring a droplet containing a proxy-mix of immunoassay reagents (luminol, H_2O_2, and horseradish peroxidase, HRP). DMF systems with integrated PMTs described previously (ref. *33-34*) relied on third-party counter-timers and software that were not easily integrated with the MicroDrop control software, making portable operation nearly impossible.
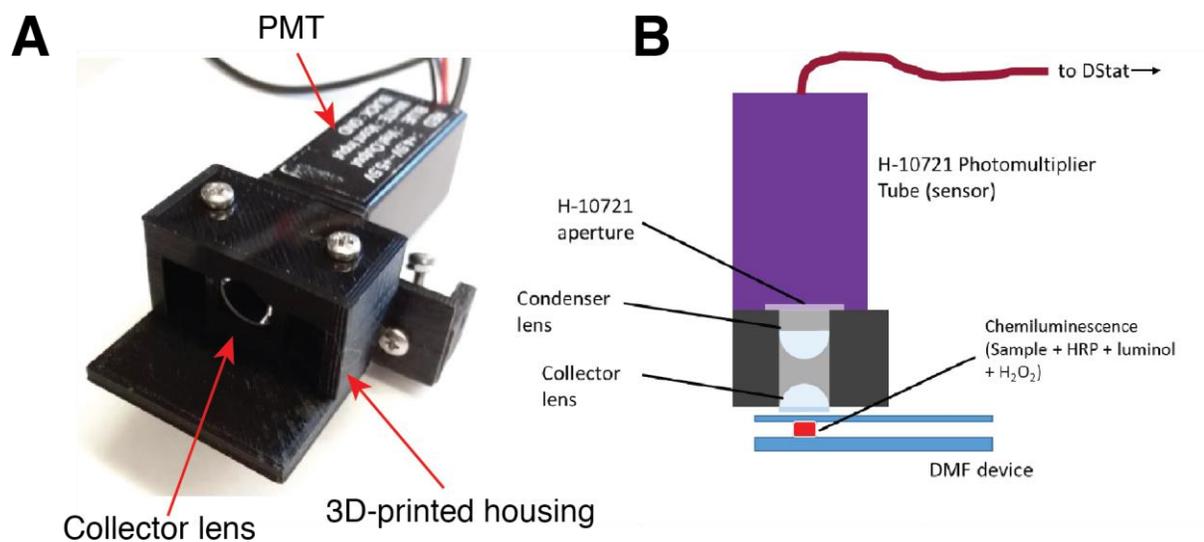
Fig. S6. Detection. (**A**) Photograph of the PMT coupled to the 3D-printed lens housing showing the collector lens. (**B**) Cartoon of a cross section of the PMT, lenses, and DMF device. The collector and condenser lens project light emitted from the chemiluminescent reaction into the aperture of the PMT. DMF systems with integrated PMTs described previously (ref. *33-34*) relied on direct contact between a moveable PMT and the device, a source of common mechanical failure.

**Manual Droplet Programming**
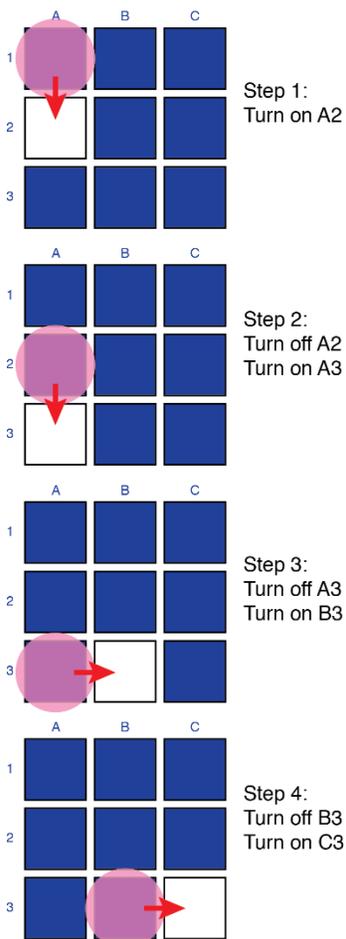
Click-and-Drag Routing

Auto-routing

Step 1:
Turn on A2

Step 2:
Turn off A2
Turn on A3

Step 3:
Turn off A3
Turn on B3

Step 4:
Turn off B3
Turn on C3

Step 1:
Click and drag
from A1 to C3
via A2, A3,
and B3

Step 1:
Select A1 and
C3
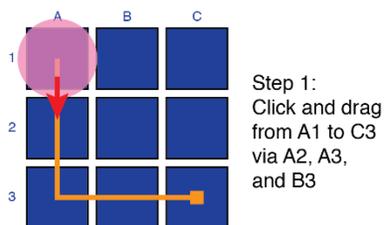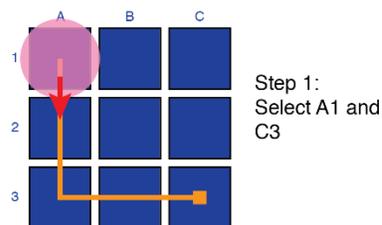
Immunoassay Protocol

	Number of steps of protocol
Manual droplet programming	**1746**
With path routing	**123**

Fig. S7. Droplet programming. Cartoon illustrating three types of droplet-movement programming in the open-source MicroDrop 2.0 software platform (http://microfluidics.utoronto.ca/dropbot/). Manual programming (left) was the only mechanism reported for the previous versions of this system (ref. *64*). In this mode, the user must provide the electrode-state of each electrode (on=white; off=blue) in each step. MicroDrop 2.0 provides two new mechanisms – Click-and-drag (middle) and Auto-routing (right). In click-and-drag mode the user defines the path the droplet will take as a single step. In auto-routing mode, the user defines the source and the destination and the software generates a path, avoiding the paths of other droplets. By using path routing, the time and attention required to generate an immunoassay protocol decreases by an order of magnitude compared to manual programming (from 1746 to 123 steps). Programming required much more time on the older systems, making "on the fly" protocol-modifications nearly impossible.
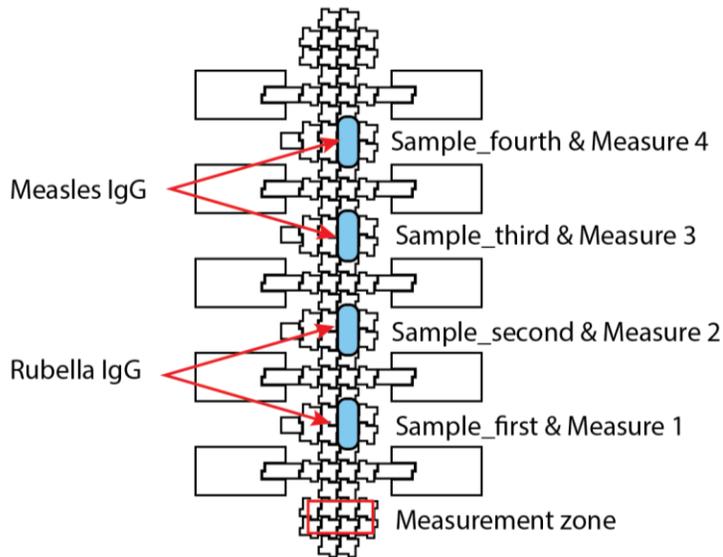
Fig. S8. Assay metadata. (**A**) Cartoon illustrating the layout of the assays for a 4-plex immunoassay. Droplets are measured in order from bottom to top. (**B**) Screenshot of user-editable metadata. Metadata collected for each cartridge included device ID, sample ID, and labels for the PMT measurements. Fields are filled by scanning a unique QR code associated with each cartridge with a webcam during QC-2 (see fig. S2). The previous version of MicroDrop, described in ref. (*64*), did not store user-defined metadata, making it impossible to connect device performance metrics with assay performance.
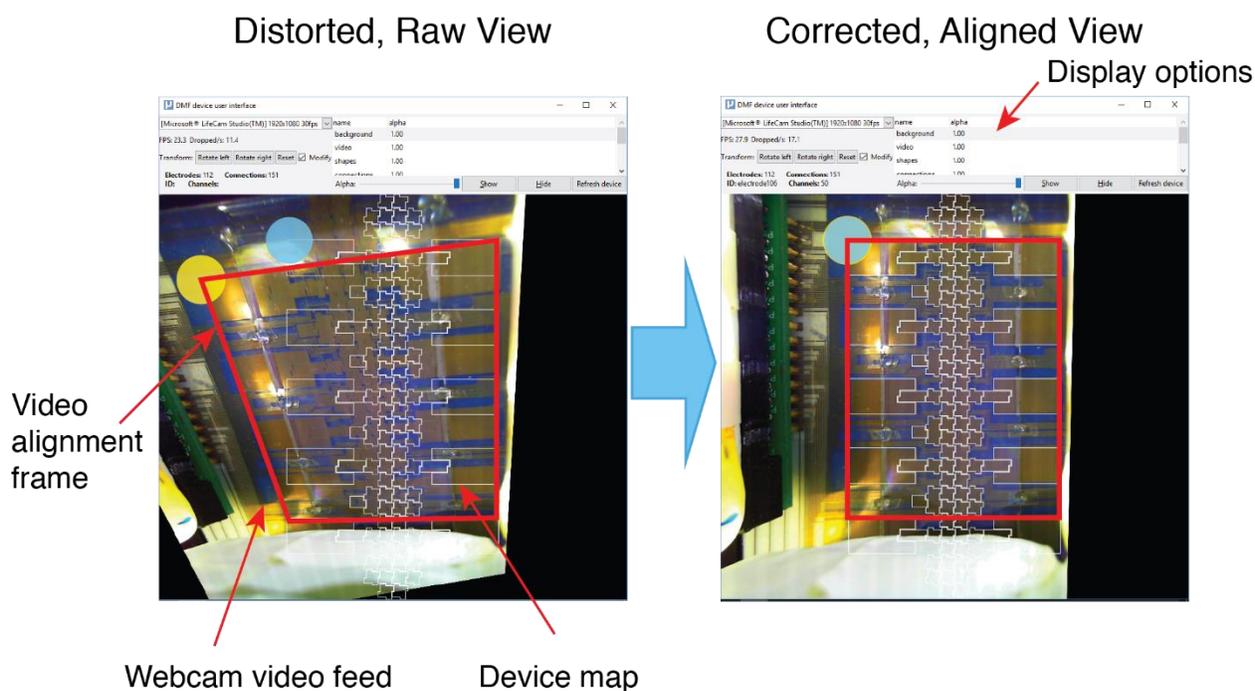
Fig. S9. Device alignment. Click-and-drag webcam-device alignment in the open-source MicroDrop 2.0 software platform (http://microfluidics.utoronto.ca/dropbot). Screenshots of the distorted, raw feed collected from the web cam (left), and after translation and transformation to match the device map (right). A video alignment frame (red outline) defines four points on the raw feed. By clicking and dragging, the user matches a video-element on the video (yellow circle) to its corresponding point on the device map (blue circle). Display options for turning on various layers, including background, video, and device map shapes, are available to the user. Note that these features are new to MicroDrop 2.0. A previous version of this system (ref. *33-34, 64*), did not allow real-time image manipulation, resulting in substantial rates of device load-failure.
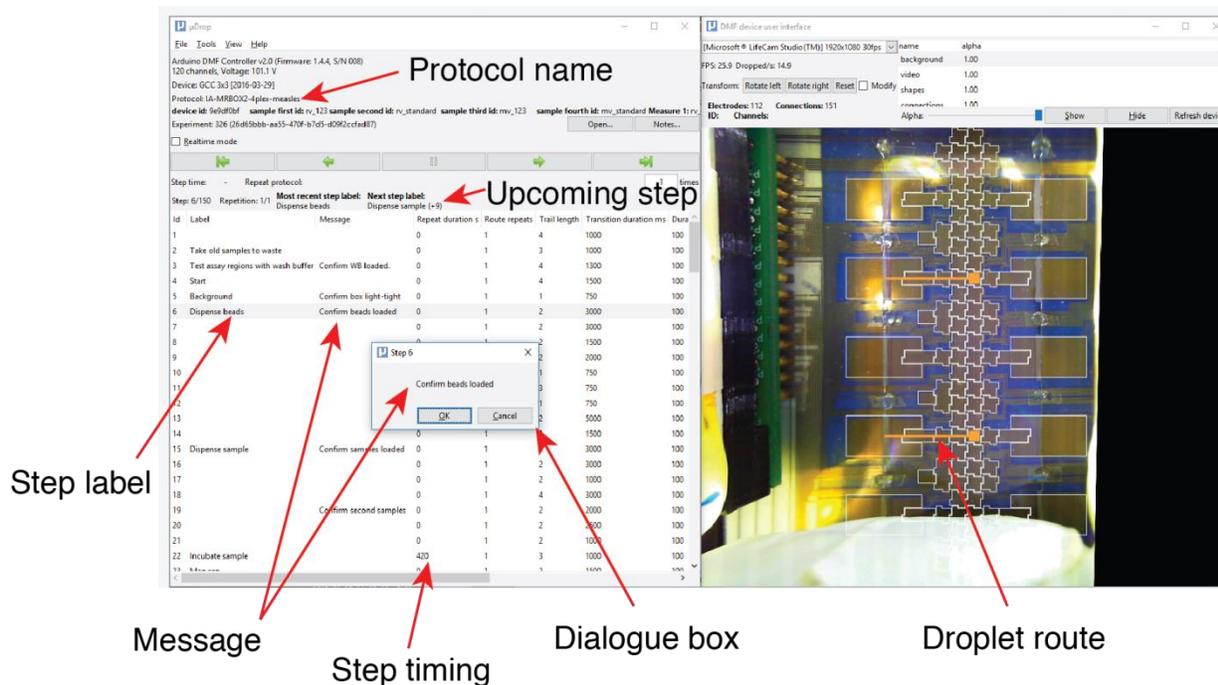
Fig. S10. Program interface. Screenshot of open-source MicroDrop 2.0 software platform (http://microfluidics. utoronto.ca/dropbot) interface, highlighting protocol management features. Left: user-defined step labels identify the step in the protocol and are reported on the display to communicate each upcoming step. The duration of any repetitive step can be defined by the step timing (in seconds). A message can be set to appear in a dialogue box that prevents the protocol from proceeding without user intervention. Right: Web-cam view of device, illustrating droplet routes generated in the graphical interface, defining the movement of droplets in each step. The previous version of the software, as described in ref. (*64*), only allowed for user-control of only the duration of each electrode-actuation step, with no connection to higher order procedures (for example, mixing and incubating). Programming required much more time on these older systems, making "on the fly" protocol-modifications nearly impossible.
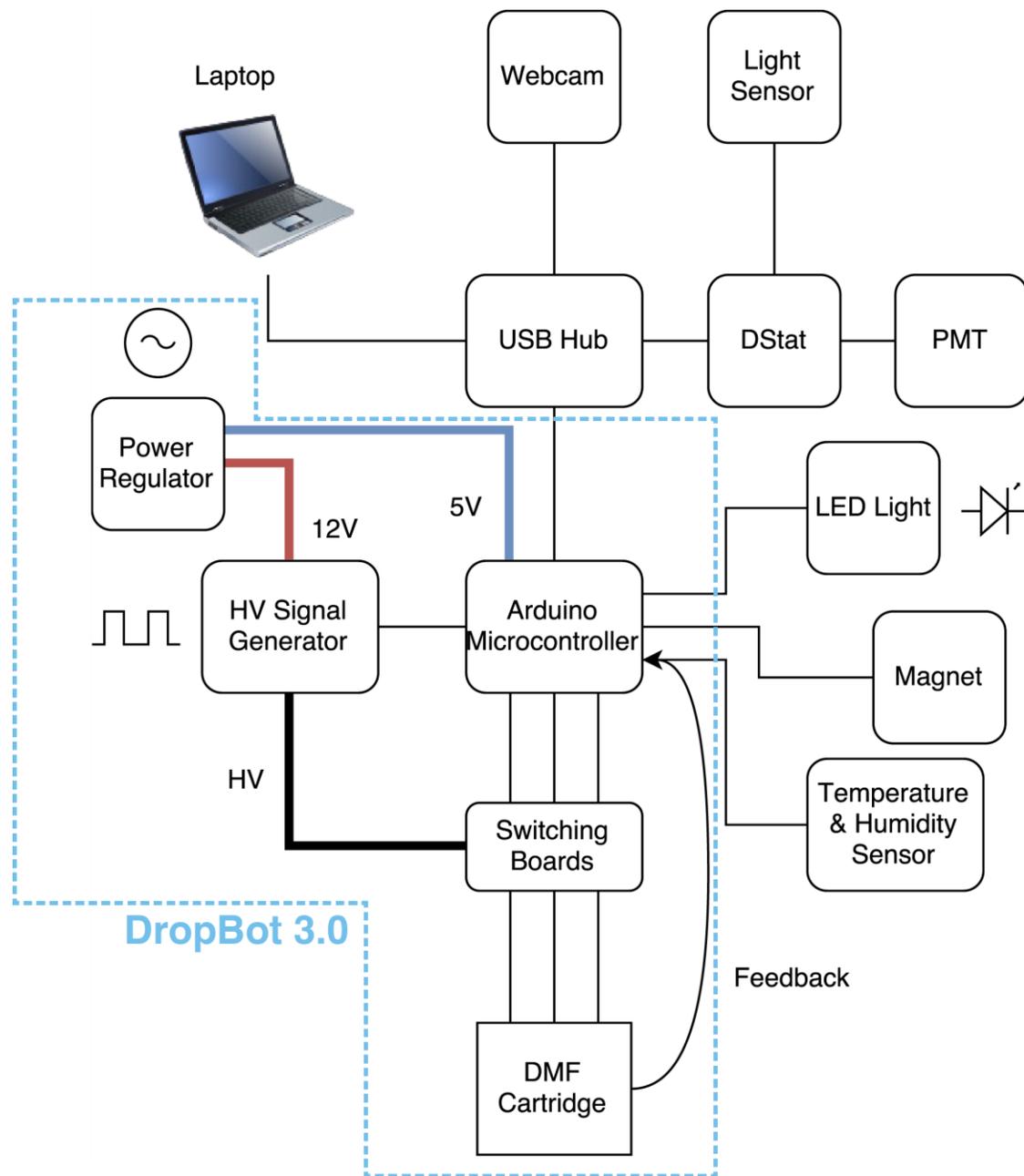
Fig. S11. MR Box connectivity diagram. An Arduino microcontroller is used to regulate the high voltage (HV) signal generator and switching boards for moving droplets on the DMF cartridge. The microcontroller measures feedback and adjusts output voltages accordingly. The microcontroller also controls the states of the LED lights, motorized magnet, and temperature and humidity sensors. Power is supplied by a 12 V DC laptop power regulator. A USB hub connects a laptop computer to the micro a webcam the DStat, which controls the PMT and interfaces with a light sensor lock. Elements that comprise the open-source DropBot 3.0 (http://microfluidics.utoronto.ca/dropbot/) are enclosed in blue dashed lines.
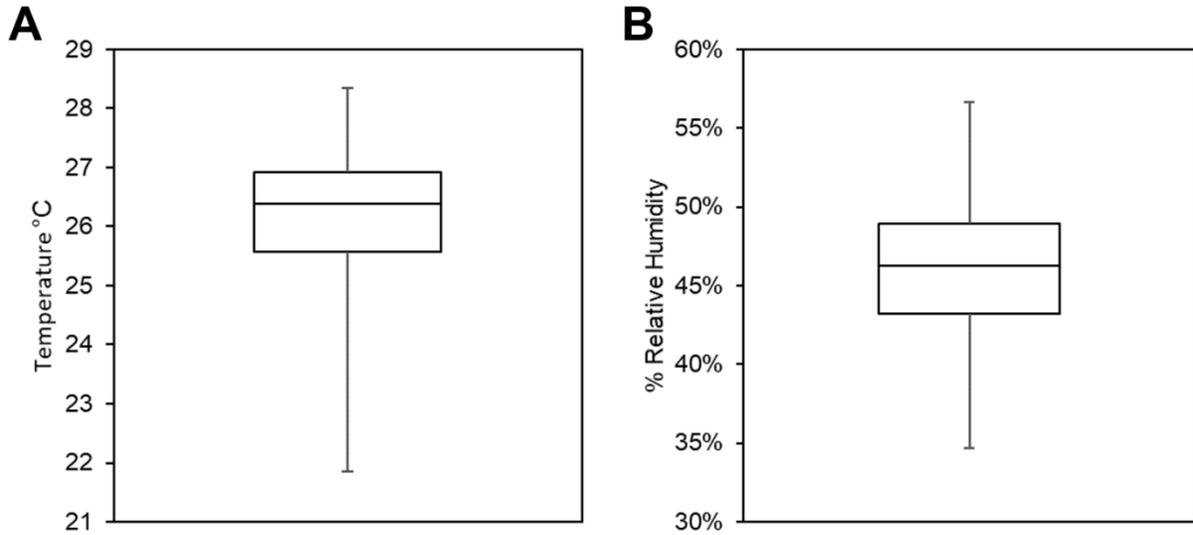
Fig. S12. Temperature and humidity. Boxplot summary of temperature (**A**) and relative humidity (**B**) readings collected during all of the experiments conducted in the field trial in Kakuma, Kenya. The box extends from the lower to upper quartile values, with the line indicating the median, and the whiskers representing the range of the data (*n*=2,030).

Table S1. MR Box bill of materials. All prices USD.

Description	Supplier	Quantity	Unit Cost	Cost
Casing				
3mm acrylic plate, black, 500 mm x 300 mm	Plastic World	8	$3.68	$29.44
Vinyl tape, black	ULINE	1	$6.99	$6.99
custom 3D-printed side connector, 3-sided	In-house	22	$0.03	$0.66
custom 3D-printed side connector, 2-sided	In-house	30	$0.02	$0.60
surface mount hinges 1" x 1"	McMaster-Carr	2	$1.43	$2.86
M3 x 16 mm machine screws	McMaster-Carr	100	$8.85 / 100 pcs	$8.85
M3 x 20 mm machine screws	McMaster-Carr	25	$5.13 / 50 pcs	$2.57
M3 x 35 mm machine screws	McMaster-Carr	4	$4.57 / 25 pcs	$0.73
MM-B-16 magnet mounts	K&J Magnetics	26	$3.00	$78.00
Device Manifold				
Machined aluminum manifold	Department of Chemistry, University of Toronto	1	$20.50	$20.50
Pogopin PCB connector	Assembled in-house	1	$68.50	$68.50
Zinc-Plated Music Wire, 1.5" Long,.312" OD, .023" Wire Diameter,	McMaster-Carr	2	$6.52 / 12 pcs	$1.09
1/4-20 hex shoulder screws, 3/4" shoulder 3/4" thread	McMaster-Carr	4	$8.65	$34.60
1/4-20 wing nut	McMaster-Carr	4	$6.37 / 5 pcs	$5.10
3/8"-screw washer	McMaster-Carr	4	$8.00 / 25 pcs	$1.28
Magnetic separation				
BZ084 neodymium permanent magnet, 3" x 1/2" x 1/4", 0.33T	K&J Magnetics	1	$13.81	$13.81
machined AISI 1080 steel lens pair	Department of Chemistry, University of Toronto	1	$41.00	$41.00
custom 3D-printed magnet holder, PLA	In-house	1	$0.27	$0.27
TowerPro MG955 servo	SainSmart	1	$5.99	$5.99
MG995 servo 2-DOF tilt bracket set	Creatron Electronics	1	$8.74	$8.74
Optics and sensing				
Honeywell Sensing and Productivity Solutions HIH6030-021-001	DigiKey	1	$9.59	$9.59
ACL1210U-A - Aspheric Condenser Lens, Ø12 mm, f=10.5 mm, NA=0.54, ARC: 350-700 nm	ThorLabs	2	$27.30	$54.60
DStat	Open Hardware	1	$120	$120.00
Custom 3D printed optics housing	In-house	1	$0.34	$0.34
8mm (185-850nm), Current Output Type PMT Module, #83-901	Edmund Optics	1	$1,075	$1,075.00
Lighting & Webcam				
Logitech Webcam HD Pro C920	Amazon.com	1	$62.76	$62.76
Susay Waterproof LED Light Strip 12V with 300 SMD LED 3258 Cool White	Amazon.ca	11	$0.03	$0.33
White LED 24 SMD 3528 Panel Car T10 Festoon Dome Bulb Adapter Internal	Amazon.ca	1	$2.91	$2.91
DropBot Control Electronics				
Switching board (DropBot v2)	Assembled in-house	3	$238.69	$716.07
HV Signal Generator		1	$82.80	$82.80
			Total	$2,455.98