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

Supplementary Information

Rapid sample preparation for detection of antibiotic resistance on a microfluidic disc platform

Alexandra Perebikovsky,*[a] Yujia Liu,[b] Alexander Hwu,[c] Horacio Kido,[d] Ehsan Shamloo,[c] Dian Song,[d] Gabriel Monti,[f] Oren Shoval,[d] Dan Gussin,[e] and Marc Madou,*[a,c,d]

[a]Department of Physics and Astronomy, University of California, Irvine, 4129 Frederick Reines Hall, Irvine, CA 92697, United States

[b]Department of Materials Science and Engineering, University of California, Irvine, 544 Engineering Tower Irvine, CA 92697, United States

[c]Department of Chemical and Biomolecular Engineering, University of California, Irvine, 916 Engineering Tower, Irvine, California 92697, United States

[d]Department of Mechanical Engineering, University of California, Irvine, 4200 Engineering Gateway, Room W3311, Irvine, CA 92697, United States

[e]Toolbox Medical Innovations, 1965 Kellogg Ave, Carlsbad, CA 92008, United States

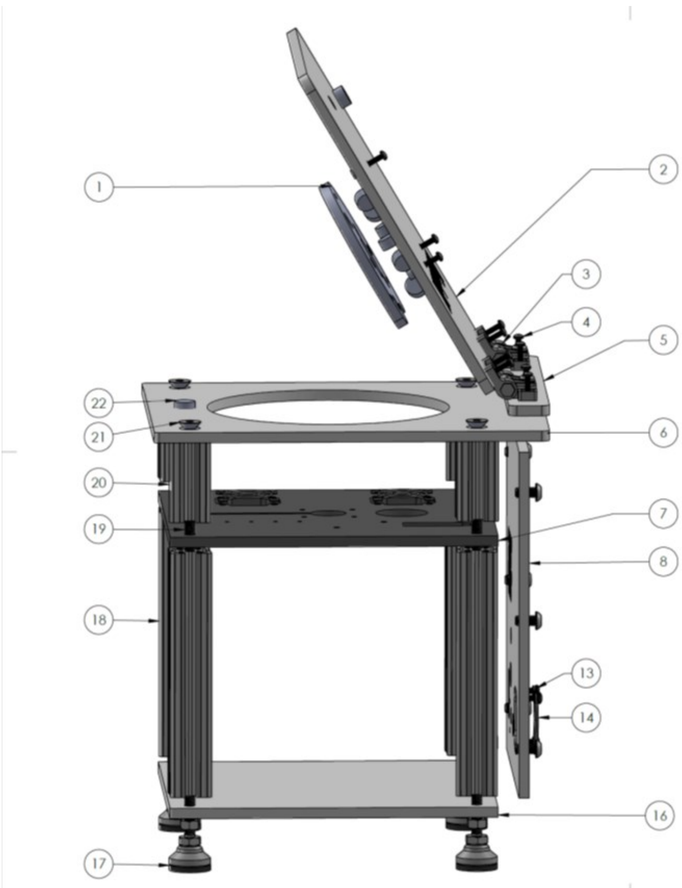
[f]Department of Urology, University of California, Los Angeles, 200 Medical Plaza Driveway 140, Los Angeles, CA 90095, United States

Supplementary Section 1

The Spinbox

The main mechanical assemblies of the spinbox are shown below, including the full list of materials needed to build each assembly. The outer housing (not shown) is made from laser-cut PMMA and is affixed to the outer shell. The touchscreen user interface, raspberry pi, and electronics are also not shown below.

1.1 Housing



ITEM NO.	DESCRIPTION	QTY.
1	Holder, Acylic T0.25in, Magnet	1
2	Panel, Acrylic T0.25in, Lid	1
3	Hinge, Adjustable Friction, Black	2
4	Screw, Pan Head, Phillips, Metric	11
5	Separator, Acrylic T0.25in, Hinge Support	1
6	Panel, Acrylic T0.25in, Top	1
7	Panel, Acrylic T0.25in, Main Platform	1
8	Panel, Acrylic T0.25in, Back	1
9	Panel, Acrylic T0.25in, Controller Mount (Not Shown)	1
10	Panel, Acrylic T0.25in, Heater (Not Shown)	1
11	Separator, Acrylic T0.25in, Front	1
12	Screw, Socket Cap, Button-Head, 18-8 SS	18
13	Screw, Pan, Head, Phillips, 6-32	4
14	Guard, SS, 40mm	3
15	Nut, Hex, 6-32	4
16	Panel, Acrylic T0.25in, Bottom	1
17	Feet, Swivel Leveling, Cushioned, Nickel-Plated, L1.25in_Thread 1/4-20	4
18	Extrusion, Aluminum T1in, L6in	4
19	Set Screw, Point, Cup, 18-8 SS	4
20	Extrusion, Aluminum T1in, L2in	4
21	Screw, Flat Head, Hex Drive, Passivated 18-8 SS	4
22	Magnet, Neodynum,	8
23	Spacer, Plastic, White, T0.25in_ID6_OD10	3

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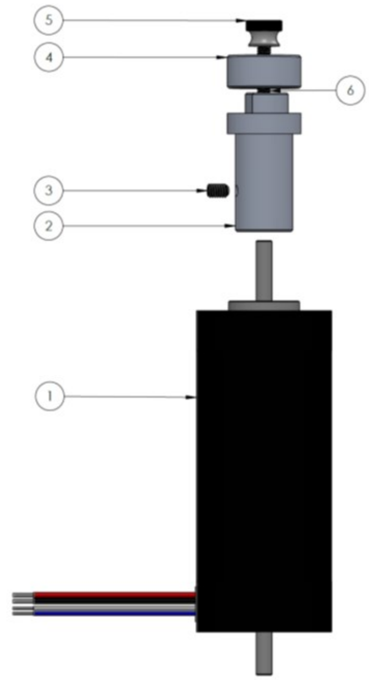
TITLE: Subassembly, Housing

DWG NO. 01-001 | A3

1.2 Motor Assembly



ITEM NO.	DESCRIPTION	QTY.
1	Motor, BLY174D	1
2	Spin Chuck, Aluminum, L0.25in	1
3	Set Screw, Point, Cup, Alloy Steel, L0.25in	1
4	Retainer, Aluminum, OD0.9in	1
5	Thumb Nut, Flanged, Round,	1
6	Set Screw, Point, Cup, Alloy Steel, L1in	1

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TITLE: Subassembly, Motor	
DWG NO. 01-002	A3
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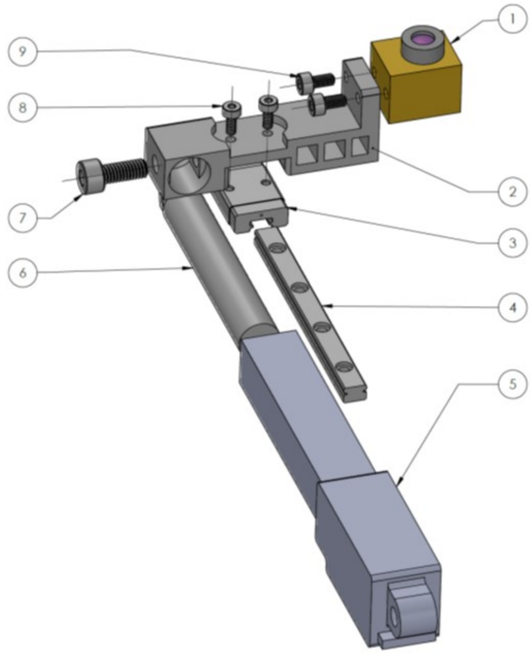
1.3 Laser Assembly



ITEM NO.	DESCRIPTION	QTY.
1	Laser	1
2	Mount, Laser Holder	1
3	Carriage, Ball Bearing, Miniature	1
4	Carriage, Threaded Hole	1
5	Actuator, Linear, 50mm	1
6	Actuator, Linear, Stroke	1
7	Screw, Head Cap, Socket, M4	1
8	Screw, Head Cap, Socket, M2	2
9	Screw, Head Cap, Socket, M2.5	2

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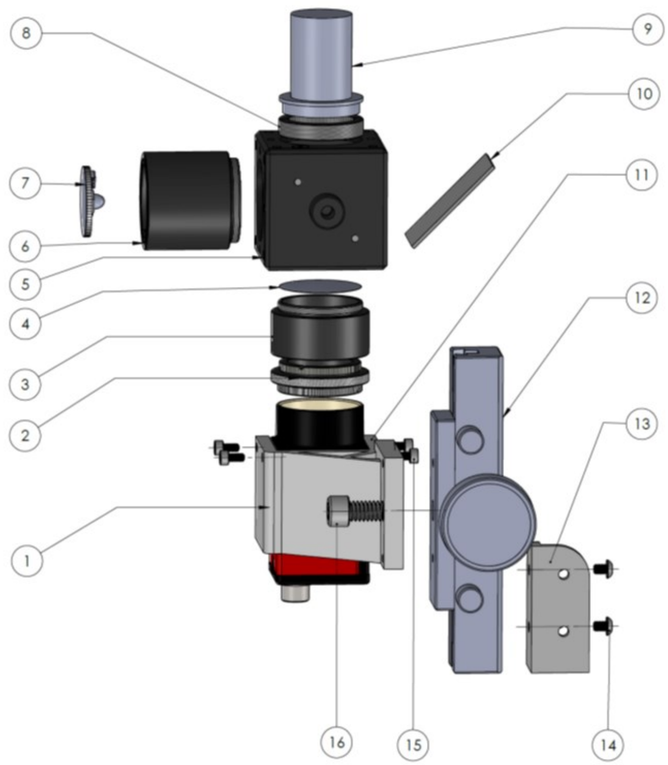
TITLE:

Subassembly, Laser

DWG NO. 01-003 | A3

SCALE 1:2 | SHEET 1 OF 1

1.4 Optics Assembly



ITEM NO.	DESCRIPTION	QTY.
1	Camera, Mako-G, C-Mount	1
2	Adapter, Camera	1
3	Adapter, Filter Cube	1
4	Lens, Filter	1
5	Cube, Filter Holder	1
6	Adapter, Filter Cube to LED	1
7	PCB, LED, Excitation	1
8	Adapter, Filter Cube to Lens	1
9	Lens, Magnification	1
10	Holder, Camera, Mako	1
11	Holder, Camera, Mako	1
12	Mount, Holder, Camera, Adjustable	1
13	Mount, Panel, Holder, Camera	1
14	Screw, Socket Cap, Button Head	5
15	Screw, Head Cap, Socket, M2.5	4
16	Screw, Head Cap, Socket, 1/4-20	1

REV:

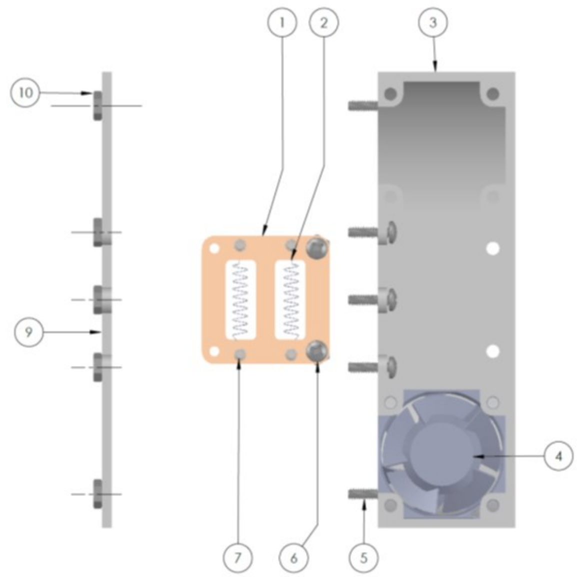
Subassembly, Optics

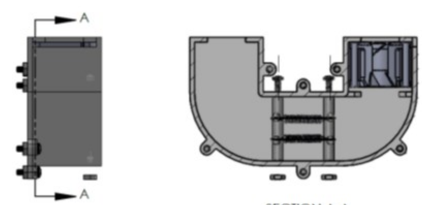
01-004

A3

1.5 Heater Assembly

ITEM NO.	DESCRIPTION	QTY.
1	Holder, Mica, Resistor, Coiled	2
2	Resistor, Coil, NiCh, 30G	4
3	Holder, Housing, Heater	1
4	DC Fan, 24VDC, W40mm_T28mm,	1
5	Screw, Pan, Head, Phillips, 6-32	6
6	Screw, Pan Head, 18-8SS, 6-32	2
7	Connector, Rivet, OD0.12in	8
8	Spacer, Aluminum, Unthreaded	6
9	Cover, Housing, Heater	1
10	Nut, Hex, 6-32	8





SECTION A-A

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TITLE:

Subassembly, Heater

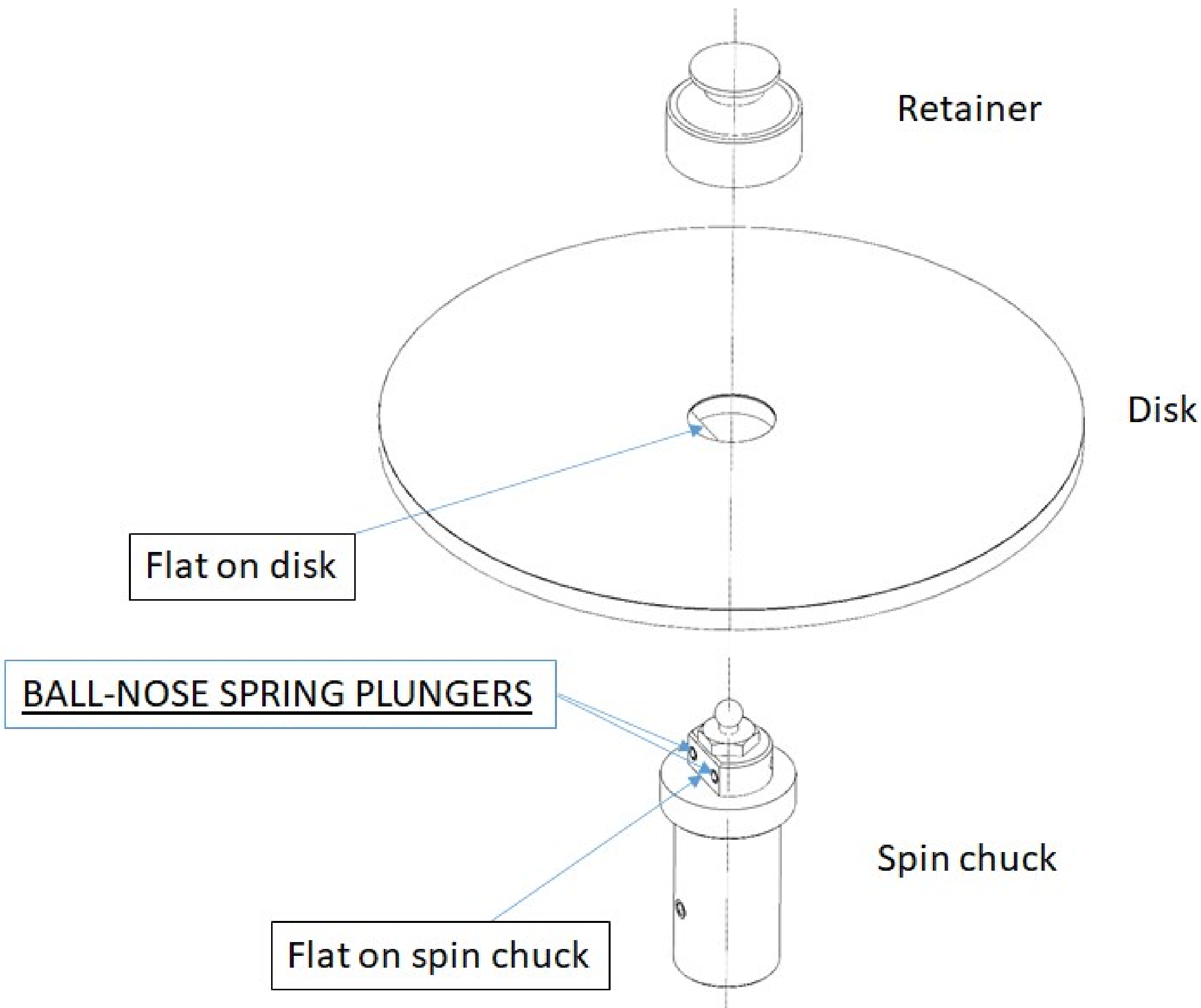
DWG NO. 01-005 A3

SCALE 1:2 SHEET 1 OF 1

Supplementary Section 2

Spinchuck for enhanced angular alignment

For experimental testing, a custom spinchuck was developed with a D-shape so that the disc was angularly aligned in the same position during each test. Two ball nose spring plungers were used to hold the disc in place and prevent movement during spinning. This allowed for precise angular positioning during both laser valve actuation and fluorescent imaging.

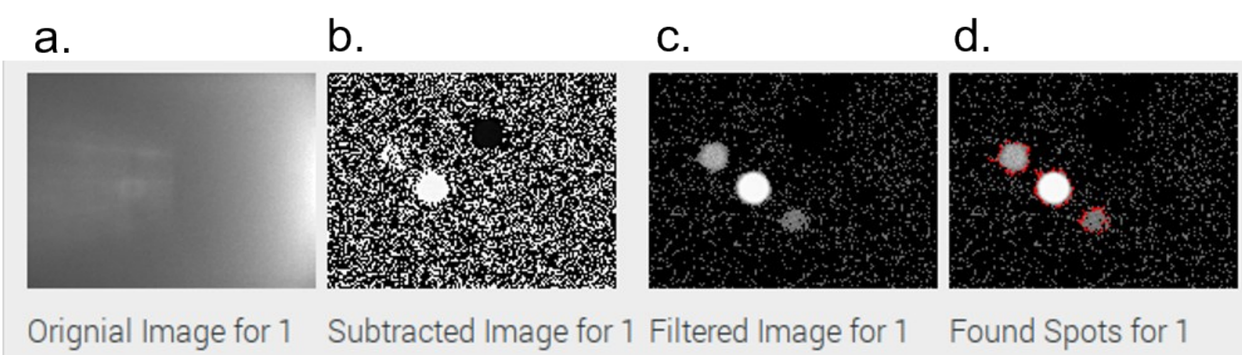


Supplementary Section 3

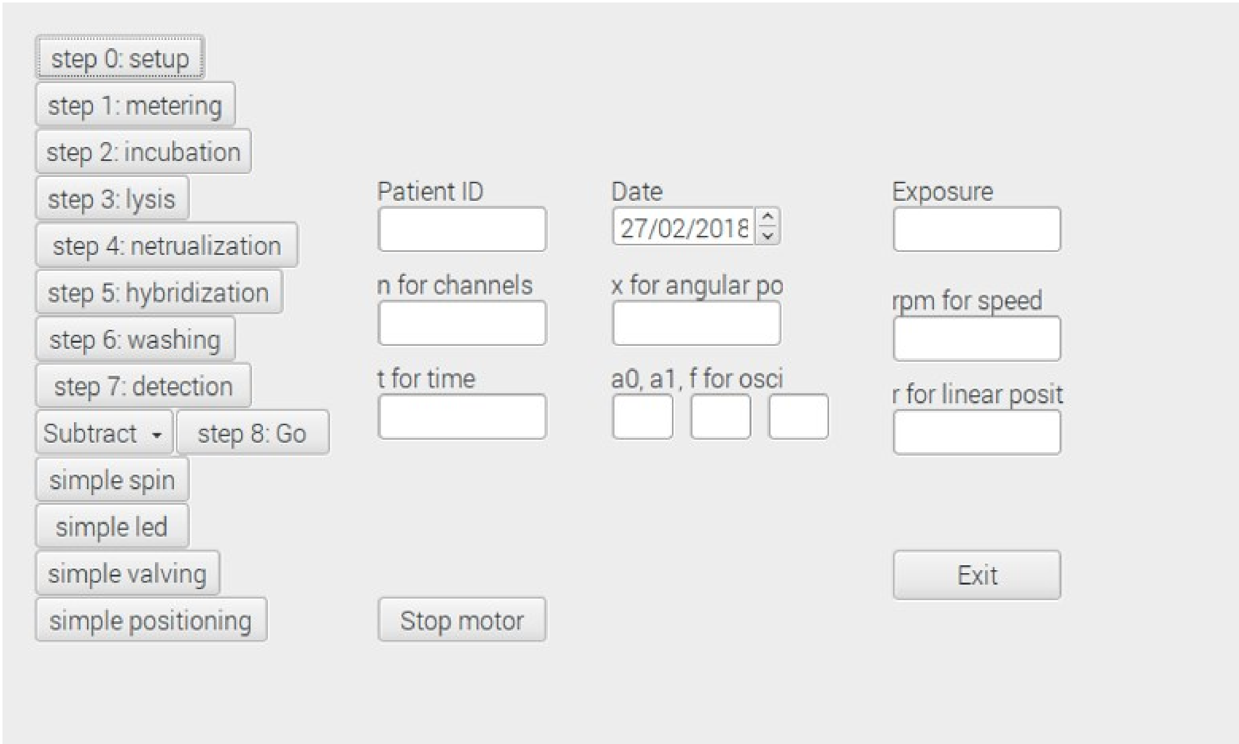
Programming Algorithm and Graphical User Interface

The software that controls the machine is based on a Python3 program with a set of libraries including pyqt4 as the graphical user interface. It has the following functions:

1. Controlling the movement of the main, brushless DC motor. This includes spinning at a specified RPM, acceleration/deceleration (RPM/s), oscillation at a specified angle and frequency (Hz), and angular positioning.
2. Controlling the linear actuator to move the laser to a certain radial position. A linear actuator is used here to carry a 5W blue light laser diode. A PWM code is sent from the raspberry pi to the actuator through GPIO pins on the Raspberry Pi. The motor and linear actuator are moved correspondingly to target the laser at specific valving position.
3. Controlling the laser with desired power. The laser is controlled by an PWM code to adjust the power for fast and accurate actuation of the valve.
4. Controlling the excitation led to light up the detection area. A GPIO code is integrated to control a 1W led excitation source for the integrated fluorescent microscope.
5. Controlling the custom-built fluorescent microscope to take picture. Through an ethernet cable, the camera is controlled through Pymba (https://github.com/morefigs/pymba), a python wrapper for Allied Vision's Vimba C API. The library enables control over camera parameters such as exposure and gain.
6. Processing the image with OpenCV code. OpenCV-python is an open source computer vision library that was used to detect and measure the fluorescence intensity of each probe binding area. Pictures of the detection area before and after hybridization are taken by the custom built fluorescence microscope camera for comparison. To process the fluorescent image (a), each image is first background subtracted (b). Then the resulting image is smoothed with a Gaussian blur algorithm. A threshold is applied to remove any spikes from the picture which may be falsely recognized as detection spots (c). Finally, the “findContours” function is used to outline the perimeter of the fluorescent spots and the intensity of each pixel is summed up as the total fluorescence intensity (d).



The software has a user friendly interface with one integrated function that runs all the assay steps and an optional developer mode for more advanced settings and parameters.


step 0: setup
step 1: metering
step 2: incubation
step 3: lysis
step 4: netrualization
step 5: hybridization
step 6: washing
step 7: detection
Subtract
step 8: Go
simple spin
simple led
simple valving
simple positioning
Patient ID
n for channels
t for time
Stop motor
Date
27/02/2018
x for angular po
a0, a1, f for osci
Exposure
rpm for speed
r for linear posit
Exit