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1   **TITLE:**
2   Fabrication and Testing of Miniature Automatic Photophoretic Trapping Rigs
3
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22   **SUMMARY:**
23   This work describes and characterizes the fabrication of miniature automatic photophoretic
24   trapping rigs.
25

26   **ABSTRACT:**
27   This paper presents an automated, rapid-fab-compatible, photophoretic trap test rig to enable
28   the democratization and crowdsourcing of volumetric display research. The rig can be
29   constructed within 2 h using a laser cutter, 3-dimensional (3D) printer, and common hand tools.
30   In its current form, the rig can be used to test the following critical parameters: particle type, trap
31   type, numerical aperture, and airflow at a rate of approximately 250 samples per hour. With
32   minor modification, the rig can be made to test an even larger set of parameters, such as laser
33   power and laser wavelength, depending on the user's needs. The rig can use machine vision for
34   automated data capture and analysis. The operation and construction of the test rig are
35   described with concise, easy-to-follow steps. Results from a four-unit test rig 'farm' covering the
36   power and particle type parameters are reported. This platform will broaden the scope and
37   composition of optical trap display parameters and researchers through accessibility and
38   democratization.
39

40   **INTRODUCTION:**
41   The optical trap display (OTD) makes possible the display geometries seen in science fiction. It
42   operates by trapping a particle through photopheresis and illuminating the particle[1-4]. Then,
43   dragging that particle through space forms an image in the air that the viewer perceives as
44   continuous per the persistence of vision[5]. This screenless 3D technology allows it to display

45 geometries such as long-throw projections, tall sand tables, and wrap-around displays[1]. These
46 geometries are uniquely compelling because they require no screen and create content that can
47 be seen from virtually every angle.
48
49 Researchers at Brigham Young University found initial success in their first-generation
50 photophoretic trapping system by using a beam expander and galvanometer scanners, along with
51 several mirrors and one or more spherical lenses to create a photophoretic trap through spherical
52 aberration[1,4]. This first-generation trapping rig also contained RGB (red-green-blue) lasers to
53 allow for precise colored display illumination. Using this trapping system, OTDs are created by
54 moving a single particle through a convoluted path. This approach limits the size of images to
55 under a cubic centimeter and limits the complexity of real-time images to wireframes and other
56 sparse content[6,7]. Furthermore, the scaling of this technology is limited by the inconsistency of
57 photophoretic trapping[8]. If a single trap/particle system can be optimized, scaling the display
58 could be achieved by replicating an optimized trap and synchronously trapping and scanning
59 multiple particles[9]. Any problems with a single trap will be compounded in a multi-trap system,
60 so careful optimization of trap and particle parameters is critical.
61
62 The optimization of an individual trap/trapping system requires extensive testing to be done for
63 every parameter of the photophoretic trapping system[7]. Such parameters include particle type
64 (substance, shape, size), laser power, laser wavelength, and numerical aperture (focal length,
65 diameter, tilt). Testing and experimentation through trial and error for each parameter will
66 optimize individual traps and multiple synchronous traps. Still, they will require large amounts of
67 data to be collected.
68
69 In the past, the research and testing process for optimizing photophoretic trapping through
70 spherical aberration has only been done by a handful of researchers across the world[1-10]. Until
71 recently, researchers at Brigham Young University have relied on a single, large, expensive
72 trapping system to collect the data needed, which caused the process of testing and gathering
73 data to be slow[1,7]. However, since introducing optical trap displays as a solution for 3D
74 visualization in 2018[1], individuals of all age groups and from several continents have expressed a
75 desire to participate in the research. Because of the generated interest in OTDs, researchers have
76 wanted to find a way to allow all interested parties to participate in the research process.
77 Previous generations of photophoretic trapping rigs, which contained beam splitters and
78 galvanometers, were too expensive and time-consuming to mass-produce and crowdsource[1,6],
79 so a different solution was needed.
80
81 A new miniature photophoretic trapping rig has been developed, which allows all interested
82 parties to participate in research and quickly test and gather data for all significant parameters
83 mentioned above. They can be fabricated rapidly by anyone who has access to a 3D printer and
84 laser cutter. This design attempts to minimize cost and complexity, mitigate risk, and maximize
85 automation, interconnectivity, and flexibility (**Figure 1**). The new rig employs the most
86 straightforward optical setup for photophoretic trapping possible: a single laser and lens[10]. The
87 small rigs are simple to use once set up and can test at a rate of approximately 250 attempts per
88 hour.

89

90 The data collected from these rigs from the tests of future citizen scientists and researchers will
91 significantly help develop photophoretic trapping in its use for 3D visualization by allowing for
92 the optimization of trapping parameters and individual traps.

93

94 **PROTOCOL:**

95

96 **1.   3D printing and laser cutting of the required materials**

97

98 1.1.   3D print the materials listed below

99

100 1.1.1. Print the lens holder using a filament FDM (Fused Deposition Modeling) 3D printer (see
101 **Table of Materials**) as per the information provided in **Supplementary File 1**. This lens holder is
102 for a 30 mm (diameter) lens (**Figure 2**).

103

104 NOTE: The file can be easily customized to fit other lenses.

105

106 1.1.2. Next, print out the cantilever platform and the holder (**Figure 2**) (**Supplementary File 2**
107 and **Supplementary File 3**).

108

109 NOTE: This can take ~2-14 h, depending on the detail of the prints. The detail of the prints should
110 not affect the trapping rate if enough of the trapping substance has been placed (step 5.3.4).

111

112 1.2.   Using a 3D laser cutter (see **Table of Materials**), cut out the rig pieces (**Figure 3**)
113 (**Supplementary File 4-5**).

114

115 NOTE: This cut can be done on any material of quarter-inch thickness, but wood is the
116 recommended material. **Supplementary File 4** contains the required rig pieces that fit on a 12 x
117 12 in piece of wood. **Supplementary File 5** includes an optional light shield/blocker.

118

119 1.3.   Using a glass cutter (see **Table of Materials**), cut a standard test tube (~2.5 cm in
120 diameter) roughly in half so that the half with two open ends is ~6.5 cm long.

121

122 **2.   Assembling the wooden rigs**

123

124 NOTE: While assembling the wooden rig, instructions in steps 2.1 -2.5 may say "slide," but pieces
125 may require more force to be appropriately positioned and built.

126

127 2.1.   Place the base piece down with the Y emblem facing up (**Figure 1A**).

128

129 2.2.   Hold the two long side pieces on either side of the base while the first laser holder is slid
130 in to place on one end and the first test tube holder on the other side (**Figure 1B,C**).

131

132 2.3.   Off to the side, slide both electromagnet holders onto the camera holder. Ensure that the

133 magnet holders are separated by ~1 cm on each side (**Figure 1D**).
134

135 2.3.1.   Slip the magnet holders and the camera holder as a unit next to the first test tube holder,
136 spaced. Hence, 1 cm between the test tube holder and the first electromagnet holder (the edge
137 of the camera holder past the electromagnet holder can serve as a reference for 1 cm) (**Figure**
138 **1E**).
139

140 NOTE: Both the camera holder and light shield are not required for the primary use of the
141 miniature trapping system, but both are recommended for the uniformity of the rig setup.
142

143 2.4.   Next, place the second test tube holder after both electromagnet holders so that there is
144 ~1 cm space between the second test tube holder and the second electromagnet holder.
145

146 NOTE: In total, between the two test tube holders, there is ~4 cm of space (the camera mount
147 has a width of 4 cm, for a positioning reference) (**Figure 1F**).
148

149 2.4.1.   If using the optional light shield/blocker (**Supplementary File 5**), slide the light shield onto
150 the opposite end of the test tube holders and electromagnet holders from the camera holder;
151 this will help center and align the test tube and electromagnet holders.
152

153 2.5.   Slide the second laser holder into place; no exact distance is required. The recommended
154 distance is 3-4 cm, but this may need to vary depending on the length of the laser.
155

156 2.6.   If desired, an optical rail (see **Table of Materials**) can be slid under all the holders to align
157 other elements of the trapping system. This will be particularly useful for aligning the lens with
158 the laser and test tube (**Figure 1G**).
159

160 2.7.   Place the electromagnet (see **Table of Materials**) into the electromagnet holders (**Figure**
161 **1H**).
162

163 **3.   Connection of the circuit with the specified microcontroller board**
164

165 3.1.   Plug in a computer monitor, keyboard, and mouse to the microcontroller board (see **Table**
166 **of Materials**). Startup the microcontroller board and ensure that the operating system is
167 functioning. No changes in the original microcontroller configurations are required, although
168 both VNC (virtual network connection) and SSH (secure shell) can be selected if desired. This
169 would allow remote access to the microcontroller.
170

171 3.2.   Build the electromagnet controlling circuit using a voltage regulator (see **Table of**
172 **Materials**), a breadboard, and a few wires (**Figure 4A**).
173

174 NOTE: All pin numbers for the microcontroller board are the GPIO (general purpose input and
175 output) pins.
176

177 3.2.1. Place the voltage regulator in the breadboard so that each pin is in a different row to
178 allow for proper usage.
179
180 3.2.2. Wire the input pin of the voltage regulator to one of the 5V power pins on the
181 microcontroller board.
182
183 3.2.3. Wire the adjust pin of the voltage regulator to GPIO 23 on the microcontroller board.
184
185 3.2.4. Connect the input wire of the electromagnet to the output pin of the voltage regulator.
186 Then connect the output wire of the electromagnet to a ground pin on the microcontroller; this
187 is best achieved when using another row in the breadboard to connect the two using an
188 additional wire.
189
190 **4.   Uploading the code for operating the system**
191
192 NOTE: For uploading the code, either step 4.1 or step 4.2 needs to be followed. Step 4.1 provides
193 instructions for the simple version of the code that does not use a camera. Step 4.2 provides
194 instructions for the version that uses a camera.
195
196 4.1.   Perform the steps in alignment with the instructions provided in **Supplementary File 6**.
197
198 4.1.1. Open the terminal and navigate to the location to store the needed files. Create a new
199 directory on the microcontroller board by typing the terminal command **'mkdir'** followed by the
200 desired directory name. This directory will be used to store the files for running the trap rig.
201
202 4.1.2. Insert **Supplementary File 6** into the new directory. See the readme section at the
203 beginning section of the file for details. After changing the test number to the desired amount,
204 the program is ready to run.
205
206 NOTE: This file contains one needed variable called num_tries which controls how many tests to
207 do in one run. This file always includes a few pauses, which can be shortened for quicker tests.
208
209 4.2.   Run and view SQLite on the microcontroller board following the steps below. This requires
210 **Supplementary File 7-11** and a camera and the relevant expertise.
211
212 4.2.1. Install the needed database libraries onto the microcontroller board by typing in the
213 terminal **'Sudo apt-get install SQLite browser'** and '**Sudo apt-get install sqlite3**'. This will allow
214 the microcontroller board to automatically store all data from the tests using **Supplementary File
215 9**.
216
217 4.2.2. Save **Supplementary File 11**, the camera script as main.py on the camera. This can be
218 done through a file explorer or the integrated development environment (IDE) developed for the
219 camera (see **Table of Materials**).
220

221 NOTE: IDE is recommended because it allows users to see the camera's output, which helps when
222 ensuring that the camera is focusing correctly.
223
224 4.2.3. Connect the camera to the microcontroller board. Use 4 pins, including the ground pin
225 from the camera. Connect the ground pin must to the ground of the microcontroller board. The
226 following pins should match as mentioned below:
227
228 4.2.3.1.   Connect Camera pin 8 to GPIO 19: This pin sends the results of each trap back to
229 the pi.
230
231 4.2.3.2.   Connect Camera pin 9 to GPIO 17: This pin gives the camera permission to start
232 searching.
233
234 4.2.3.3.   Connect Camera pin 7 to GPIO 5: This is the camera status pin.
235
236 4.2.4. Create a directory to store all the files. After creating this directory, save the files as
237 provided in **Supplementary File 7-10** in the directory; rename them readme.txt, main.py,
238 electromagnet.py, and test_insert.py, respectively.
239
240 4.2.5. Read through **Supplementary File 7** (ReadMe.txt).
241
242 NOTE: The readme file gives a good explanation of what each file does and the changes
243 that might be needed on each file, such as the directory path of the database.
244
245 4.2.6. Open the Database viewer installed in step 4.2.1. Click the **new database** button and save
246 the database in the same directory as the other files. The new database must match the name of
247 the database file found in test_insert.py.
248
249 4.2.7. Inside the database, create a table inside the new database to save the data. The database
250 has 5 fields, parameter_type, trapped, testname, testnum, and rigID.
251
252 NOTE: The table sections must be precise as specified or more changes will need to be made in
253 Main.py and test_insert.py.
254
255 **5.   Test Preparations**
256
257 5.1.   Prepare the lens by placing the lens inside the lens holder. Ensure that the lens stays inside
258 the holder during testing. Some hot glue might be needed here.
259
260 NOTE: The rig requires using a spherical bi-convex to ensure the proper formation of trapping
261 regions.
262
263 5.2.   After preparing the lens (step 5.1), place the lens holder on the optical rail and the laser
264 (see **Table of Materials**) in the laser holder.

NOTE: The suggested laser in the list of materials requires no calibration before use. Safety glasses must be worn anytime the laser is in use.

5.2.1.   Using the lens and the laser or other light source, find the focal point of the laser and slide the lens holder along the optical rail until the focal point is centered over the electromagnet.

NOTE: This step is critical for trapping; if the focal point is not centered over the electromagnet, the cantilever platform will not raise particles into the focal point.

5.2.2.   Mark this point with a pencil on the wooden base for future reference.

NOTE: Each lens already comes with a focal length measurement, but these measurements are not always correct.

5.3.   Prepare the trapping cite

5.3.1.   Ensure that the laser is properly turned off again.

5.3.2.   Using a hot glue gun, glue a small button magnet (see **Table of materials**) of the same polarity as the electromagnet onto the flat surface of the platform so that the electromagnet will repel the platform.

NOTE: The polarity of the magnets must be matched appropriately so that the platform will be repelled by the electromagnet, pushing the particles into the beam of the laser so that trapping can occur.

5.3.3.   Take the 3D-printed, cantilever-like platform and coat the platform in black aluminum foil, which protects the platform from melting.

NOTE: Regular foil can be used, but it causes too much glare for the camera system to be used. Try using the black foil tape (see **Table of materials**), which works fine with the camera (**Figure 5A**). Foil is recommended because it can be easily replaced for testing other substances, but similar products can be used if desired.

5.3.4.   After covering the platform with the aluminum foil, place the selected particle type chosen by the user for testing on the slanted side of the platform (see **Table of Materials** for particle type options or see **Figure 6A**).

5.3.5.   Gently insert the cantilever arms into the circular holder so that the magnet side is facing out. Then gently insert the test tube into the same holder. If this has been done correctly, the magnet will almost be touching the glass (**Figure 5B**).

5.3.6.   Place the test tube onto the test tube holders so that the platform is centered over the

309 electromagnet. If the magnet has been appropriately attached to the cantilever platform, the
310 cantilever should appear to be in an upward position repelled by the electromagnet.

311

312 5.4.   Place the camera in the camera holder to capture any traps that occur above/around the
313 platform. Then double-check all other positionings (**Figure 1I-J**).

314

315 **6.   Beginning the test**

316

317 NOTE: For testing, either step 6.1 or step 6.2 needs to be followed.

318

319 6.1.   If using instructions from step 4.1, press **start** in the file or start the file normally from the
320 terminal.

321

322 6.2.   If using instructions from step 4.2, start this test from the terminal with the parameters
323 as mentioned below.

324

325 6.2.1. Using terminal commands, run the file system once inside the proper directory with the
326 command "**python3 main.py test_num parameter_type exact_parameter**". The file main.py is
327 provided in **Supplementary File 8**.

328

329 6.2.2. Replace the **test_num** with the number of tests desired. Replace the **Parameter_type** by
330 the type of parameter the test is focused on.

331

332 NOTE: For example, if tests were being done to determine what powered laser is best,
333 Parameter_type would be replaced by laser_power, and the exact_parameter would be replaced
334 by the optical output power of the current laser.

335

336 **REPRESENTATIVE RESULTS:**
337 The principal result achieved by following the above protocol is creating a miniature
338 photophoretic trapping rig, which can quickly test various parameters. So far, these rigs have
339 been used to test two critical parameters, laser power, and particle type. The parallel running of
340 multiple miniature rigs has allowed researchers to collect data with much larger sample sizes in
341 a much quicker period of time.

342

343 The first test, conducted while developing the above protocol, was a laser power test. A single
344 miniature rig was used during this test, without the camera detection system, as it had not yet
345 been developed; instead, step 4.1 from the protocol was used. This required a researcher to be
346 present for the data collection of all trap detections. The goal of this test was to determine the
347 ideal laser power output for trapping to occur. By placing an optical attenuator (variable neutral
348 density filter) between the laser and lens on the rig, the optical power of the laser was varried.
349 **Figure 7** shows the results of this experiment. A high optical power output corresponded with a
350 higher rate of trapping. The laser at full power had the highest recorded trapping rate for this
351 test. However, this test was limited to one laser with a maximum optical power of ~120 mW.

352

The second test was to determine which material or substance would have the highest rate of trapping. This test was conducted using a singular miniature test rig without the camera detection system. Ten different particles were tested with a sample size of 100 attempts for each particle (all the substances tested can be found in the **Table of Materials** with their descriptions). The sample size was limited to 100 because of the need to have a researcher watch each attempt for data collection. All the necessary data was collected in two working days. **Figure 6A** shows the results of the particle type test. Of the 10 materials/particles types tested, it was found that diamond nanoparticles (55-75%) and printer toner were the two best particle types with rates of 14% and 10%, respectively (**Table 1**).

After the first two tests, researchers felt limited to a singular rig that required active watching during testing; this led to step 4.2 outlined in the protocol. This option includes a camera detection system, which enables users to run several miniature test rigs at a time and does not require a user present for testing.

For testing this new camera system, a modified re-test of the particle type test was conducted. Only a few different particle types were selected from the 10 used initially to be re-tested for this new particle type test. The selected particles underwent a new round of testing. Using a test rig "farm" of four miniature test rigs, each of the selected particles had a total test sample size of 4,000 attempts. Once again, all necessary data was collected in two full workdays (**Table 2**). The primary purpose of this particle type re-test was to test the new camera system. This test allowed for comparing results from the initial particle type test, with a researcher reporting the traps, to the results from the camera detection system. The test results were slightly different than the original tests but still comparable (**Figure 6B**). The best particle type from the initial test, diamond nanoparticles 55-75%, was still the best in the re-test but did have a slightly lower trapping rate than before. The difference in results is most likely due to a bigger sample size and an imperfect camera detection system. Although the results for this particle test were slightly different than expected, when testing other parameters where the material stays constant, such as laser power or lens focal length, the results gathered by the camera script will be reliable.

The results from all three tests conducted are relative to the rigs they were performed on, but the trends found in the data will prove to be true when tested on other more precise photophoretic test rigs. The miniature test rigs are not meant to replace other test rigs entirely. Still, they are meant to allow researchers to explore all parameters and possibilities quickly and efficiently in edisonian (trial and error) testing to find trends and discoveries for further research on more precise rigs.

FIGURE AND TABLE LEGENDS:

Figure 1: Rig progression with the completed miniature photophoretic test rig. The figure corresponds with step 2 and its sub-steps. (**A**) Shows step 2.1. (**B**) Demonstrates step 2.2, the base with the two long sides. (**C**) Shows step 2.2, the rig's frame, a base with both sides, and the first laser holder and test tube holder. (**D**) Step 2.3 shows the camera holder combined with both electromagnet holders. (**E**) Step 2.3.1 offers the combination of Figures 1C,D. (**F**) Step 2.4, the

second test tube holder and the second laser holder have been added. (**G**) The optional light shield and optical rail have been added. (**H**) The electromagnet is placed in its holder. (**I**) The laser and the test tube have been placed in their holders. (**J**) This shows the entire completed test tig without the power source for the microcontroller board.

Figure 2: 3D-printed pieces. Included in this figure are the lens holder, circular platform holder, and cantilever platform. The design for the 3D-printed lens holder can be found in **Supplemental File 1**. This lens holder, when printed, is for a lens of 30 mm in diameter. **Supplementary File 2-3** contain the designs for the platform holder and platform. The platform holder has four sets that the platform can use, but for the rig to work as designed, the platform should use the holes indicated in the figure.

Figure 3: Labeled laser cut pieces. This figure labels the pieces of **Supplementary File 4**, which contains the file for all the laser-cut pieces except for the optional light shield. After printing, there should be 1 base, 2 sides, 2 laser holders, 2 test tube holders, 2 electromagnet holders, and 2 camera holders (only one is necessary). The optional light shield can be found in **Supplementary File 5**.

Figure 4: Voltage regulator and electromagnet circuit. (**A**) For reference, when building the circuit. The voltage regulator has 3 pins, an adjust, input, and output. (**B**) This figure shows the completed circuit described in step 3.

Figure 5: Platform preparations and test-tube trap cite. (**A**) Before testing can be done, the platform must be prepared. The reservoir of particles where the laser will shine to pick up particles will be placed on the platform immediately before testing. Black aluminum foil should be placed on the platform before the particles. This prevents the laser from melting through the platform. (**B**) During testing, the actual trapping of particles occurs within the test tube, ensuring consistent automatic motion of the platform for each trap attempt.

Figure 6: Particle type test (manual) and (camera). (**A**) A test of 10 different particles was conducted to find the particle with the best trapping rate. (**B**) A second particle type test was conducted with a camera detection system. Only 4 of the original 10 particles were tested.

Figure 7: Laser power test results. The rate of trapping for different laser power levels was measured during the laser power test. The higher powers produced higher rates of trapping.

Table 1: Results of the particle type test that would have the best trapping rate. The total sample size of 100 attempts was performed in 4 sets of 25 for each material.

Table 2: Results of the particle type test conducted with a camera detection system. Data collected from SQLite database. Data was compiled initially in 4 sets of 1000 for a sample size of 4000 per material. Individual records for each set were not compiled from SQLite; only the total percentages were compiled.

Supplementary File 1: File_1-Lens Holder.stl. This contains the 3D printing file for the lens holder (see **Figure 2**).

Supplementary File 2: File_2-Platform.stl. This contains the 3D printing file for the cantilever platform (see **Figure 2**).

Supplementary File 3: File_3-Platform Holder.stl. This contains the 3D printing file for the platform holder (see **Figure 2**).

Supplementary File 4: File_4-Rig Pieces.odg.This contains the laser-cutting file for the rig pieces (see **Figure 1** and **Figure 3**).

Supplementary File 5: File_5-Light Shield.odg. This contains the laser-cutting file for the optional light shield/blocker.

Supplementary File 6: File_6-Opt1.system.py. This contains the entire code for the use of the instruction from step 4.1.

Supplementary File 7: File_7-Opt2.Read Me.txt. This contains the readme file with a few details for **Supplementary Files 8-11.**

Supplementary File 8: File_8-Op2.main.py. This contains the main script for the instructions found in step 4.2.

Supplementary File 9: File_9-Opt2.electromagnet.py. This contains the script for step 4.2 that controls the electromagnet.

Supplementary File 10: File_10-Opt2.test_insert.py. This contains the script for step 4.2 that automatically uploads data into the database.

Supplementary File 11: File_11-Opt2.camera_controller.py. This contains the script that needs to be uploaded to the camera during step 4.2.2.

DISCUSSION:
The present protocol contains several essential steps that are critical to the automatic running of the trapping rig. First, the electromagnet must be appropriately attached to the microcontroller board through the specified circuit. Without the electromagnet, the total utility of the miniature test rig is lost. The electromagnet controls each trapping attempt by raising the particle reservoir on the cantilever platform up into the path of the laser. Each trap attempt is another cycle of raising and lowering the platform.

The camera is only used in step 4.2 as described in the protocol, but it is critical for that option. Step 4.2 requires a camera to detect if a particle has been trapped, allowing data collection from multiple rigs. If the camera is not attached correctly, the rig will not be able to attempt any

485   trapping.

486

487   The third and most critical step, step 5.2.1, is aligning and focusing the laser. The lens must be
488   placed so that the focal point occurs over the electromagnet. The cantilevered platform will pass
489   through the focal point over the electromagnet, allowing for particles to trap. Suppose the focal
490   point is not centered above the middle of the electromagnet. In that case, it becomes challenging
491   to ensure that the cantilever platform carrying particles will pass through the focal point to create
492   traps. This can lead to a lack of traps. It is also essential that the platform is elevated over the
493   electromagnet so that the laser path is not constantly contacting the platform. This can cause the
494   camera to report false positives. To more easily adjust the focal point's location, it is suggested
495   to use an optical rail in the setup of the rig; this will allow users to easily slide the lens holder
496   backward or forward to position the focal point properly. The laser and test tube/cantilever part
497   are already aligned if the rig has been appropriately built; the use of the optical rail will keep the
498   lens aligned with the other parts.

499

500   Two separate options are detailed in the Protocol, step 4.1 and step 4.2. The first option, step
501   4.1, is the original simple way to run the miniature tapping rig. This option relies on the human
502   eye to detect particles instead of a camera system. This option is best for collecting smaller sets
503   of data quickly or in situations where a live demonstration is desired. The first option was used
504   during the first two experiments before the second option was created. The second option, step
505   4.2, uses a camera for automatic detection and trapping, allowing thousands of tests to be run
506   and entered into a database without any human supervision. The accuracy of the camera
507   depends on the exact testing condition; certain more reflective materials, when tested, appeared
508   to have a less accurate trapping rate when compared to similar tests done with human detection.
509   However, several parameters in the camera script can be changed to increase the camera's
510   accuracy. The exact accuracy of the camera is something that can be improved, but it is also not
511   a significant concern because the miniature rigs are meant for initial testing. The second option
512   can also be easily modified to run two test rigs off a single microcontroller board; details for that
513   modification are included in **Supplementary File 7**.

514

515   Current work is developing a more exact and consistent form of automatic trap detection through
516   machine learning. This new machine learning detection system, when finished, will use
517   convolutional neural networks to better detect trapped particles with a much higher rate of
518   accuracy (above 95%), further bolstering the use and effect such miniature testing rigs can have
519   on the future of photophoretic trap display research.

520

521   In its current base form, the miniature trapping rig is limited in a few ways. These miniature rigs
522   are unable to create actual OTDs by scanning the particle after a trap has occurred. The design
523   also limits the possibility of scanners being added for future use in creating OTDs. Another
524   limitation of the design is the need for additional components for a specific test to occur. For
525   example, a variable optical attenuator was used to collect the data sets at different optical output
526   power levels during the laser power test. Similarly, if a researcher wished to test laser wavelength
527   in a future test, they would require several other lasers of comparable optical power with
528   different wavelengths in addition to the laser used in this work. The rig would most likely require

529 additional modifications to hold each laser, this process would limit the speed at which such a
530 test could be conducted, but it would still be possible. This design is also determined by the need
531 to 3D print a new lens holder for each lens. The design and application are also limited to
532 spherical biconvex lenses, which produce spherical aberration to form regions where trapping
533 can occur.
534
535 Going forward, future applications include continued testing and optimization of photophoretic
536 trapping parameters. As briefly mentioned above, the miniature trapping rig could easily be
537 modified into a basic inexpensive OTD system by adding scanners for the y-axis and x-axis control.
538 The electromagnet-controlled particle delivery used in the miniature trapping rig could also be
539 implemented in future advanced OTD systems.
540
541 The miniature trapping rig is ultimately unique and distinct in this field of research because it can
542 be inexpensively and quickly fabricated, allowing for rapid mass testing. These rigs are meant to
543 be lean systems designed for the initial testing and optimization of photophoretic trapping
544 parameters. An individual rig can test at a rate of ~250 attempts per hour. Many other types of
545 photophoretic trapping systems or rigs have been developed to have better automatic systems
546 or accomplish more by scanning the particle to create an image after a successful trap[1,8]. These
547 miniature trapping systems are not meant to replace the use of such systems. They are intended
548 to quickly test parameters and conditions of photophoretic trapping to give researchers a better
549 understanding of what makes for good photophoretic trapping. The miniature trapping rig will
550 democratize photophoretic trap research and allow for a new wave of Edisonian experimentation
551 and progression in this field of research.
552

557 **DISCLOSURES:**
558 The authors have nothing to disclose.
559

560 **REFERENCES:**
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577   Volumetric display by movement of particles trapped in a laser via photophoresis. *SPIE*
578   *Proceedings*. **10723**, 02 (2018).

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582   manipulation of airborne particles with a bottle beam. *Optics Express*. **19** (18), 17350-17356
583   (2011).

584

Figure 1
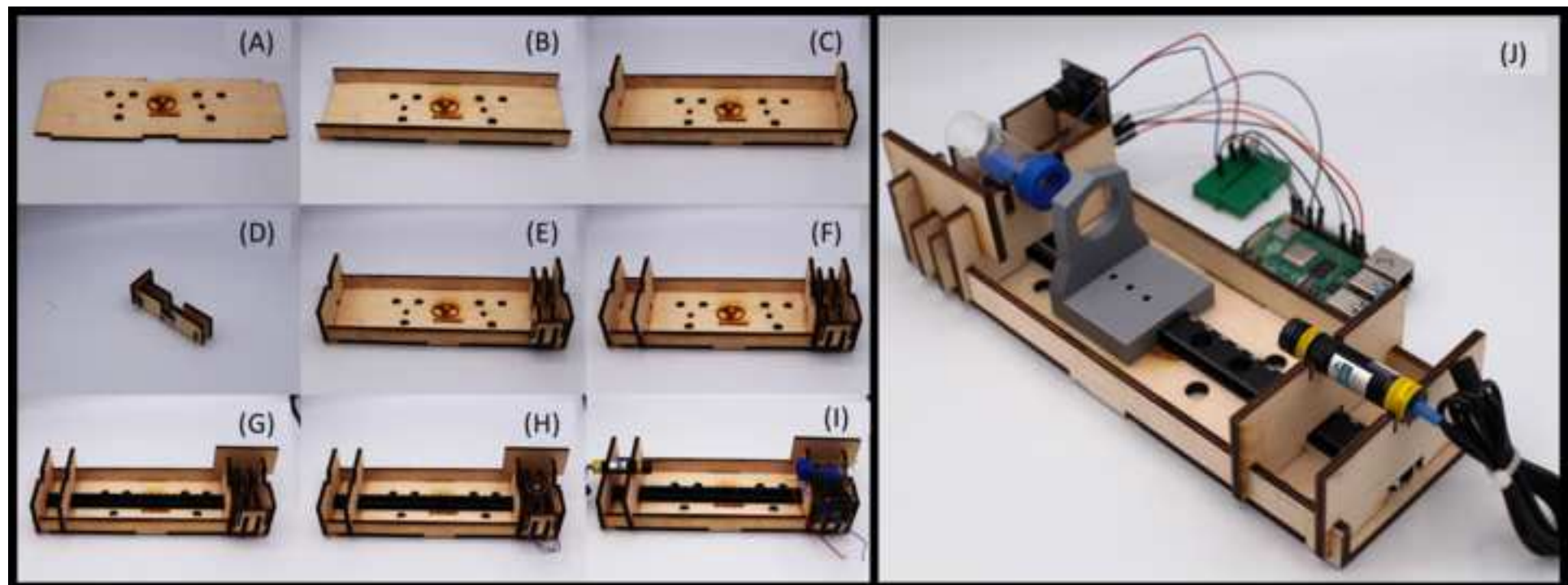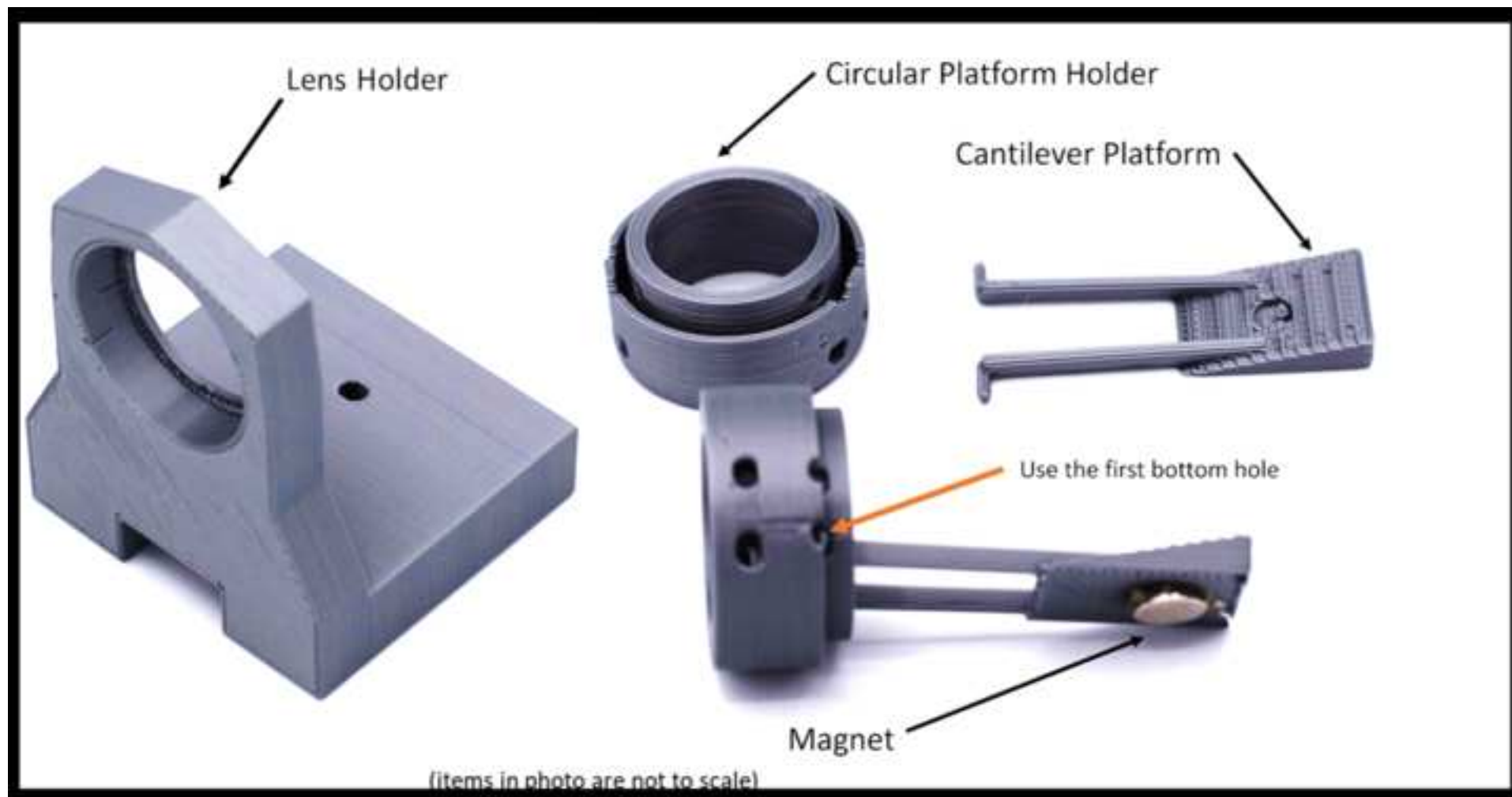
Figure 2   Click here to access/download;Figure;Figure 2.png ⬇

Lens Holder

Circular Platform Holder

Cantilever Platform

Use the first bottom hole

Magnet

(items in photo are not to scale)

Figure 3

Base

Sides

Laser
Holders

Electromagnet
Holders

Camera
Holder
(only 1 needed)

Test Tube
Holders

Figure 4

Click here to access/download;Figure;Figure 4.png

(A)

Adjust

V-Input

V-Output

(B)

Figure 5

(A)

(B)

Figure 6

Click here to access/download;Figure;Figure 6.png ⬇

(A) Average Trapping Rate of Each Substance (Manual Detection)
(B) Average Trapping Rate of Each Substance (Camera Detection)

Figure 7

Trapping Rate According to Power Level

Table 1
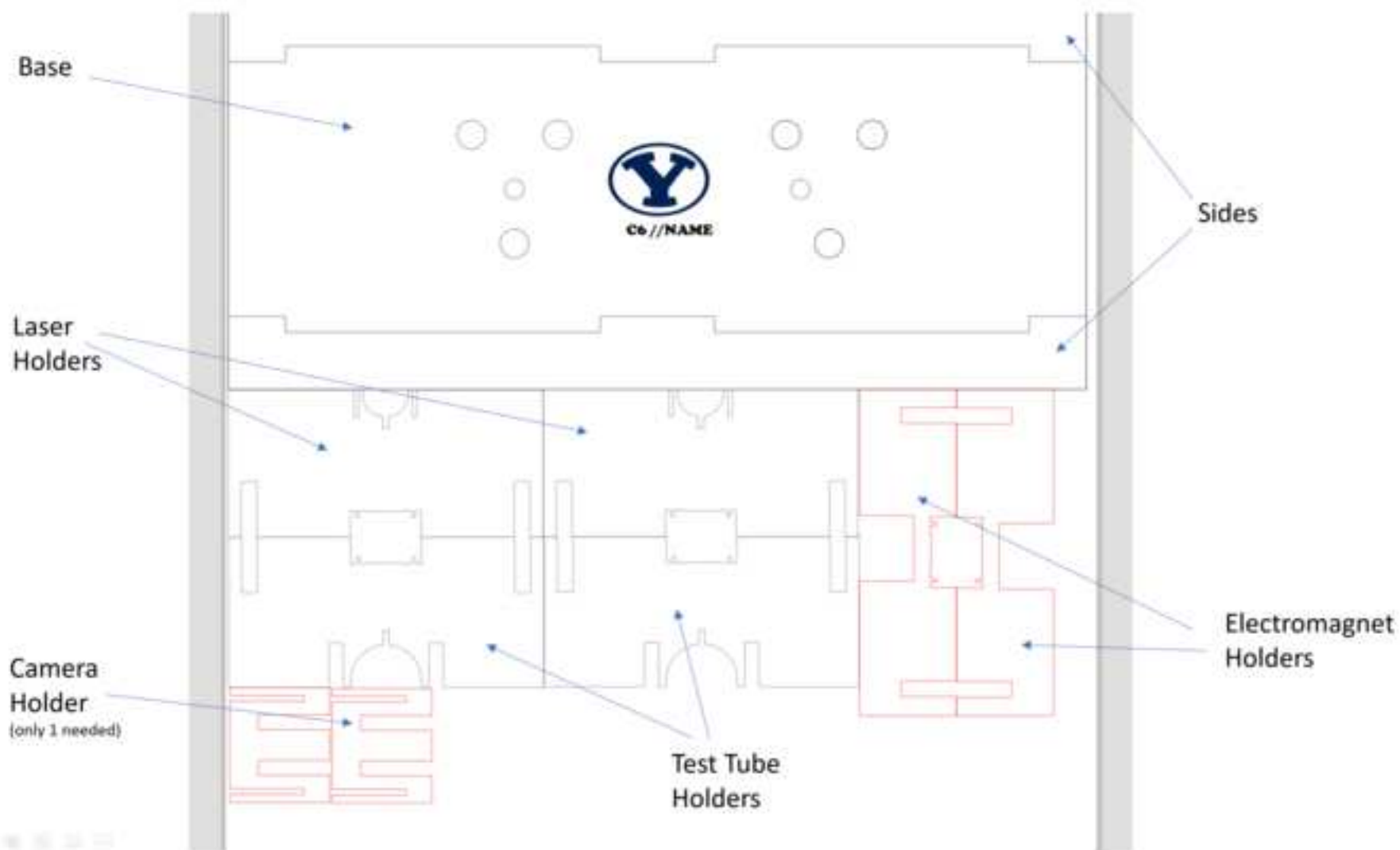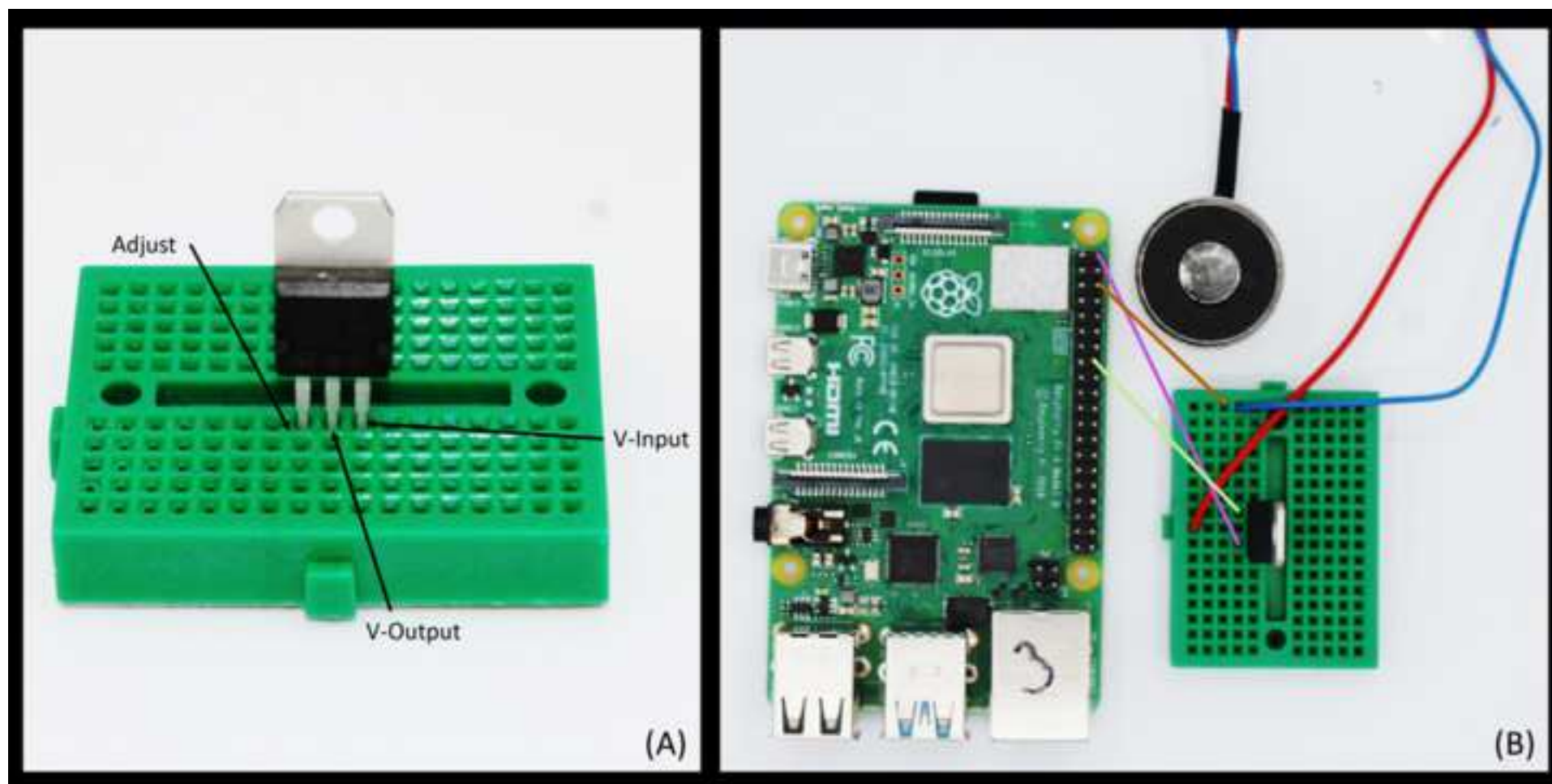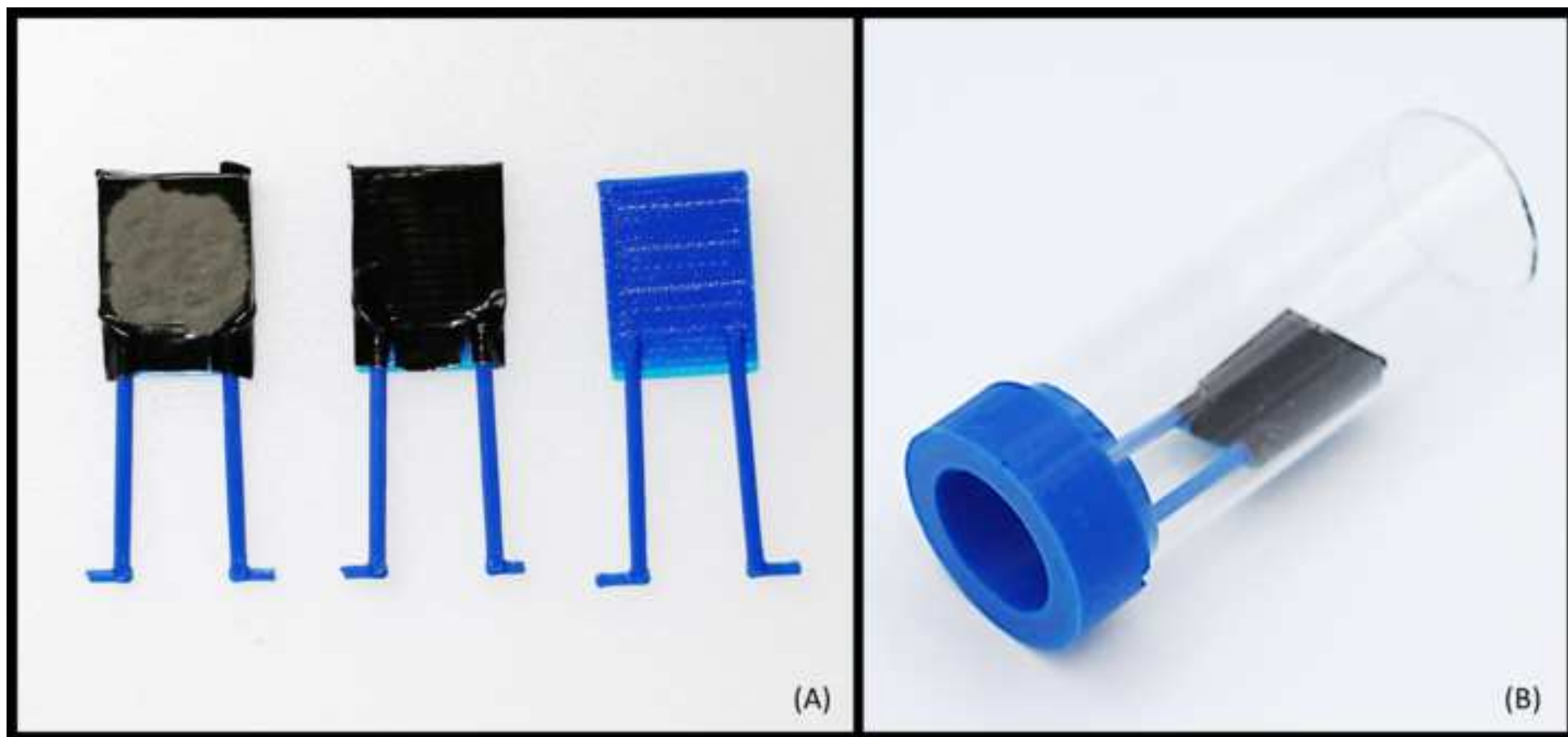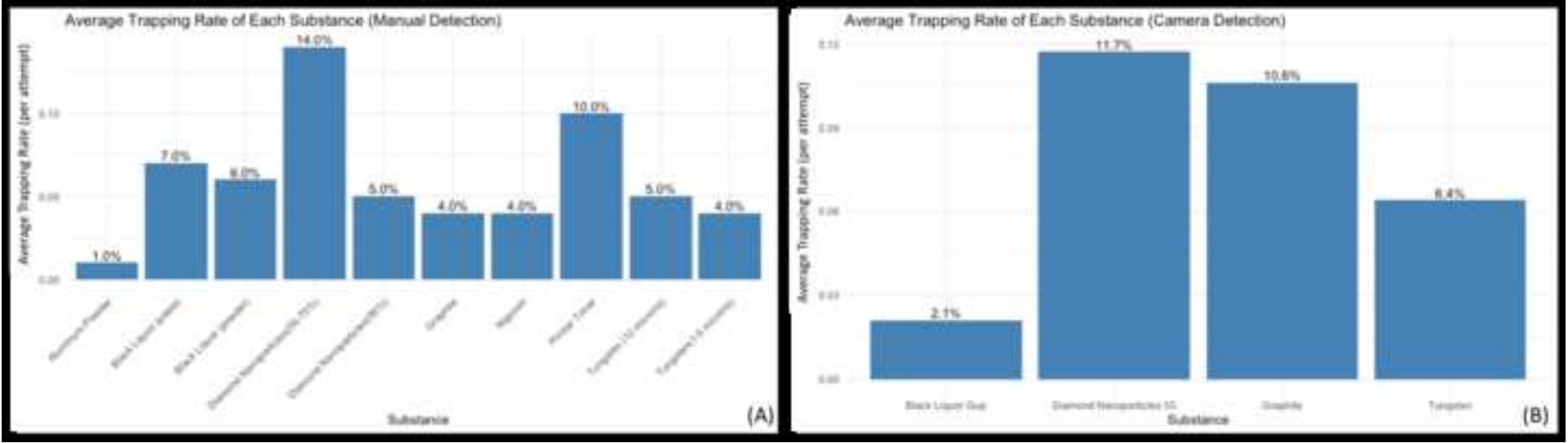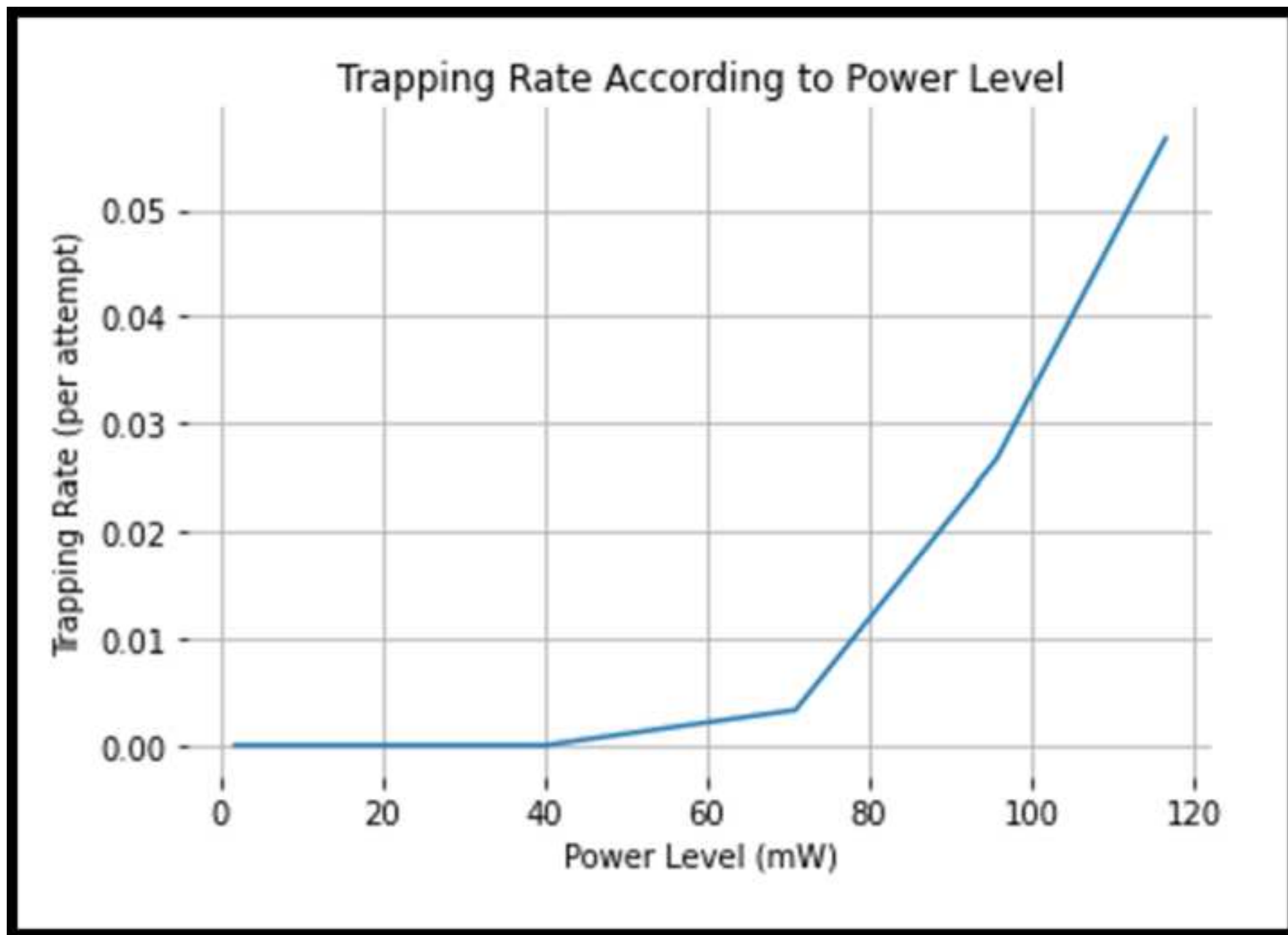
Black Liquor (powder)	Black Liquor (paste)	Tungsten (12 microns)	Tungsten (1-5 microns)
1	2	2	1
1	3	2	0
2	1	1	1
2	1	0	2
6	7	5	4
6.00%	7.00%	5.00%	4.00%

Aluminum Powder	Printer Toner	Graphite	Diamond Nanoparticles (95%)
0	3	2	1
1	2	1	2
0	3	1	1
0	2	0	1
1	10	4	5
1.00%	10.00%	4.00%	5.00%

Diamond Nanoparticles (55-75%)	Nigrosin
5	2
3	1
3	1
3	0
14	4
14.00%	4.00%

Table 2

Black Liquor Paste	Diamond Nanoparticles 55-75%	Graphite
2.10%	11.70%	10.60%

Tungsten (12 microns)

6.40%

Click here to access/download

Table of Materials

63113_R2_Table of Materials_FINAL.xlsx

Revisions Rebuttal Document

Reviewer #1:

We are very grateful for the comments and concerns expressed by Reviewer 1. Your comments allowed us to better clear up some of the misconceptions and confusing points of the paper. The comment about our claims on testing laser power and wavelength were particularly helpful. Because of your comments we were able to clear up and better explain our claim of testing laser power. Your concerns of laser alignment and calibration also allowed us to better describe the laser alignment process for the miniature trapping rig. We believe that we have addressed all you concerns and that in so doing have clarified several points of possible confusion and unclarity in out paper. Thank you.

- Major concerns
- *While the procedure is clearly explained, and seems reproducible, the problem of the laser calibration and alignment should be addressed.*
    - **Many lasers do require specific calibration and alignment procedures, but we've chosen a laser for our experiment that requires no calibration. However, this wasn't properly explained in the paper, so your comment was very helpful in avoiding possible confusion for future readers. We have addressed the issue of calibration in step 5.2**
    - "NOTE: The suggested laser in the list of materials requires no calibration before use." (line 227)
- *The authors claim that the system can test laser power and wavelength, however no control of these parameters are presented. The laser recommended has a fixed wavelength and power.*
    - **This comment was very eye-opening, We did not mean to make the claim to have control over laser wavelength with our miniature trapping rigs. We do mention in a few places that these rigs could be easily modified to test the effect of laser wavelength. This point has been more adequately addressed in the paragraph of limitations we have added. Our paper does claim to have tested the effect of laser power on photophoretic trapping, in our representative results we have specified the use of an optical attenuator (variable neutral density filter) that allowed us to vary the laser power. This was also added to the materials table.**
    - "Another limitation from the design is the need of additional components for specific test to occur. For example, during the laser power test a variable optical attenuator was used to collect the data sets at different optical output power levels. Similarly, if a researcher wished to test laser wavelength in a future test, they would require several different lasers of comparable optical power with different wavelengths in addition to the laser listed in the list of materials. The rig would most likely require additional modifications to hold each laser" (lines 423-429)
    - "By placing an optical attenuator (variable neutral density filter) between the laser and lens on the rig, the optical power of the laser was able to vary." (lines 294-296)

- *In addition, the trap will be very sensitive to the laser/lens/cantilever alignment. How could the person ensure the focal point is correctly formed and positioned? If this is not the case, the experiment cannot be replicated, and the data collected not valid.*
    - **The alignment is very important to the successful reproduction of this experiment. To help future readers better understand how to align the laser and more detail has been added in the discussion paragraph that discusses aligning the lens with the laser and cantilever. We have also specified in step 5.1 the type on lens required for trapping to occur.**
    - "The lens must be placed so that the focal point occurs over the electromagnet but more specifically so that the cantilever platform will pass through the focal point over the electromagnet, allowing for particles to trap. If the focal point is not centered above the middle of the electromagnet, it becomes very difficult to ensure that the cantilever platform carrying particles will pass through the focal point to create traps. This can lead to a lack of traps. It is also important that the platform is elevated over the electromagnet so that the laser path is not constantly contacting the platform. This can cause the camera to report false positives. In order to more easily adjust the focal point's location, it is suggested to use an optical rail in the setup of the rig, this will allow users to easily slide the lens holder backwards or forwards to position the focal point properly. The laser and test tube/cantilever part are already aligned if the rig has been properly built, the use of the optical rail will keep the lens aligned with the other parts." (lines 392-402)
    - "NOTE: The rig requires use of a spherical bi-convex, to ensure the proper formation of trapping regions." (Line 223)

- *I am also concern about the trapping rate according to the substance since this later should be very well characterized to have any interest. For example, in figure 11, author have tested "aluminum powder" which is very vague. For the result to be useful, the scientific community need to know the particle size and distribution. Some material can also have different coloration according to their level of oxidation which would be important to know.*
    - **Thank you for this important concern, we of course want this paper and the results to be reproducible, so this comment was very helpful in reminding us to include all particle types in the materials table. The details of substances used such as average particle size should be available in the description section of the materials table. A sample of the distribution or particle placement used during testing can already be seen in Figure 5(a).**

- *Minor concerns*

L37 "Our group found success", rephrase.

L39 "an aberration trap", can you please define the meaning

L41 "a complicated path" → convoluted path

L44 "curious nature of photophoretic trapping" Why exactly is that "curious"?

L52 "Edison-style testing" → trial and error

L54 "large amounts of data to achieve" → to be collected.

L61 "Because of this we wanted" → because of this interest, we wanted…

- o **We are grateful for these concerns and suggestions for way in which we could improve our paper. We made changes to increase clarity, these included correctly rewording such phrases like in line 39 to avoid confusion. The use of "curious" was removed on line 44.**
- o L38 "Researchers at Brigham Young University found initial success"
- o L40 "a photophoretic trap through spherical aberration[1,4] "
- o L43 "a convoluted path"
- o L53 "Testing and experimentation through trial and error"
- o L55 "to be collected. "
- o L61 "Because of the generated interest in OTD"

Reviewer #2

    We are very appreciative of the comments and concerns expressed by reviewer 2. We believe that the concerns expressed have helped us clear up why parts of the protocol are written they way they are. These comments were also very useful in giving us a new idea for future tests to be conducted on the trapping rig, namely a test on differently detailed 3D prints. The comments have also given us several things to consider in order to improve our design and protocol for future use.

- • *The results of the testing show what appears to be a relatively low trapping rate (<10%). This can be attributed to several factors, including positioning of the focal point of the beam on the cantilever platform. The authors did mention that the quality of the cantilever with the lowest setting would be sufficient for the 3D printed components. Could this affect the trapping efficiency even with the foil coating? Does the foil cover mimic the rough surface below? Have the authors considered a reflow or epoxy to smooth the cantilever surface? Could a metallic shim be glued to the surface instead of the foil? I'm not sure if the surface roughness is a contributing factor to the trapping efficiency but this may be system parameter worth exploring.*
  - o **Thank you for your comment. It was very eye-opening to comments or questions that future readers might have. As of yet we have not done any official testing to see if surface roughness is a contributing factor to trapping efficiency, this will be something we look into in future tests. The foil listed in the material list is a black foil tape that does not easily mimic the rough surface without sufficient forces. Your comment was very useful in rewording/adding to our protocol to help others reproduce our work. We have not investigated using a metallic shim, but we do better explain why we use foil.**
  - o "NOTE: Regular foil can be used but it causes too much glare for the camera system to be used, instead try using the black foil tape in the materials table which works fine with

- o "3D print the materials listed below. NOTE: This can take anywhere from 2 to 14 hours depending on the detail of the prints. The detail of the prints should not affect the rate of trapping if enough of the trapping substance has been placed in step 5.3.4" (lines 84-86)

General Editorial Comments:

Thank you for the general editorial comments, all the comments have been addressed.

- *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*
  - o **The document has been proofread for grammar and spelling issues**
- *Please provide references for lines: 49-58.*
  - o **References were added for lines 49-58, specifically see lines 51 and 58**
- *Please define abbreviations upon first use. For example, 3D, GPIO, etc.*
  - o **All abbreviations are now defined upon first use**
- *JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. For example: Prusa printer, Raspberry Pi, etc.*
  - o **All commercial language that would require (™) or (®) and company names have been removed and replaced with generic terms, this includes Prusa, raspberry pi, and OpenMV.**
- *Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). Please avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol.*
  - o **The text has been revised to avoid all instances of personal pronouns. All instances of "could be", "should be" and "would be" have been removed with the exception of an example explanation in step 6.2.1 on lines 276-277**
- *Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.*
  - o **Protocol has been reviewed and deemed sufficient for reproduction of this experiment**

- *Steps 3.1, 4.1.1, 4.2.1, 4.2.6: Software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc.).*
    - **Steps 3.1, 4.1.1, 4.2.1, 4.2.6 have been edited to be more detailed and explicit.**
    - "3.1. Plug in a computer monitor, keyboard, and mouse to the microcontroller board. Start up the microcontroller board and ensure that the operating system is functioning. No changes in the original microcontroller configurations are required, although both VNC (virtual network connection) and SSH(secure shell) can be selected if desired. This would allow remote access to the microcontroller."
    - "4.1.1 Open the terminal and navigate to a the location you would like to store the needed files. Create a new directory on the microcontroller board using the terminal command "mkdir" followed by the desired directory name. This directory will be used to store the files for running the trap rig."
    - "4.1.2. Insert file 5 into the new directory. This file contains one needed variable called num_tries which controls how many tests to do in one run. This file always contains a few pauses which can be shortened for quicker tests. See the readme section at the beginning section of file 5 for details. After changing the test number to the desired amount, the program is ready to run."
    - "4.2.6 Open the Database viewer installed in step 4.2.1. Click new database and save the database in the same directory as the other files. The new database must match the name of the database file found in test_insert.py. Inside the database create a table inside the new database to save the data. The database has 5 fields, parameter_type, trapped, testname, testnum, and rigID. NOTE: The sections of the table must be exactly as specified or more changes will need to be made in Main.py and test_insert.py."
    -
- *Step 5.2: How was the lens prepared? Please mention.*
    - **The lens preparation takes place in step 5.1. Both steps have been slightly edited to better communicate this.**
    - "5.1. Prepare the lens by placing the lens inside the lens holder. Some hot glue might be needed to ensure that the lens stays inside the holder during testing. NOTE: The rig requires use of a spherical bi-convex, to ensure the proper formation of trapping regions."
    - "5.2. After preparing the lens at stated in step 5.1, place the lens holder on the optical rail and the laser in the laser holder. NOTE: The suggested laser in the list of materials requires no calibration before use."
- *Please ensure that the highlight is up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.*
    - **The highlighted portion of the protocol was reviewed to ensure that all necessary information for highlighted steps is included in the highlighted portion.**

- *Please try to reduce the total number of Figures to 5-6. You may please combine few Figures and include them as individual panels, (A), (B), (C), etc. For example, Figures 2-3 can be combined, 6-7 can be combined, 8-9 can be combined, 11-12 can be combined. Accordingly, please revise the Figure references in the manuscript text and also the legends.*
  - **Reduced the number of figures to 7 by combing figures 2-3, 11-12, 6-7, 8-9 and 1 and 5. Figure references throughout the paper have been changed to reflect the new figures.**
- *Figure legends 2, 3: Please use only one abbreviation for the same terminology throughout the entire manuscript, either 3d or 3D.*
  - **All abbreviations are now uniform across the entire paper.**
- *Figure 10, 11, 12: Please provide trapping rate units in all the graphs.*
  - **Units have been provided for all graphs**
- *All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Each Figure Legend should include a short description of the data presented in the Figure and relevant symbols.*
  - **Each figure legend has been changed to include a short description of the data presented (for applicable figures)**
- *Please include a paragraph on (1) Limitations of the method, (2) Any future application of the technique in the Discussion section.*
  - **A specific paragraph on the Limitations of the method has been added (starting on line 419)**
  - **A specific paragraph on future applications has been added (stating on line 432)**
  - "In its current base form, the miniature trapping rig is limited in a few ways. These miniature rigs are unable to create actual OTDs by scanning the particle after a trap has occurred. The design also limits the possibility of scanners being added for future use in creating OTDs. Another limitation from the design is the need of additional components for specific test to occur. For example, during the laser power test a variable optical attenuator was used to collect the data sets at different optical output power levels. Similarly, if a researcher wished to test laser wavelength in a future test, they would require several different lasers of comparable optical power with different wavelengths in addition to the laser listed in the list of materials. The rig would most likely require additional modifications to hold each laser, this process would limit the speed of which such a test could be conducted, but it would still be possible. This design is also limited by the need to 3D print a new lens holder for each lens. The design and application is also limited to spherical bi-convex lenses which produce spherical aberration to form regions where trapping can occur."
  - "Going forward future applications include continued testing and optimization of photophoretic trapping parameters. As briefly mentioned above, the miniature trapping rig could easily be modified into a basic inexpensive OTD system by adding scanners for y-axis and x-axis control. The electromagnet-controlled particle delivery used in the miniature trapping rig could also be implemented in future advanced OTD systems."

- *Please do not abbreviate the journal titles in all the References. Also include volume and issue numbers for all References.*
  - **All journal titles have been unabbreviated, and volume and issue numbers have been added for applicable references.**
- *Please ensure that the table of materials contains all the essential supplies, reagents, and equipment used in the study. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.*
  - **The Materials table has been resorted(alphabetically) and all materials needed to reproduce the experiment have been included.**
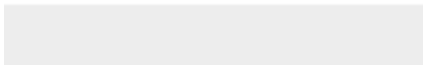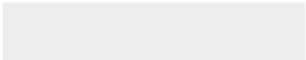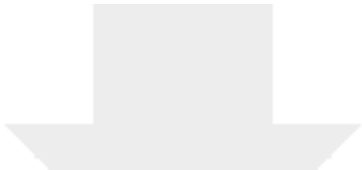
Click here to access/download
Supplemental Coding Files
File_1-Lens Holder.stl
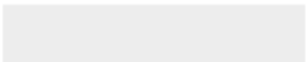
Click here to access/download
Supplemental Coding Files
File_2-Platform.stl
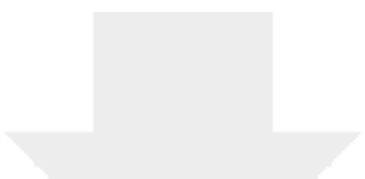
Click here to access/download
Supplemental Coding Files
File_3-Platform Holder.stl

Click here to access/download
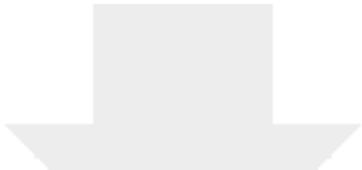**Supplemental Coding Files**
File_4-Rig Pieces.odg

Click here to access/download
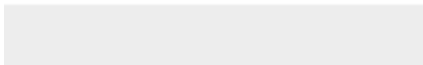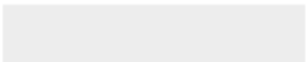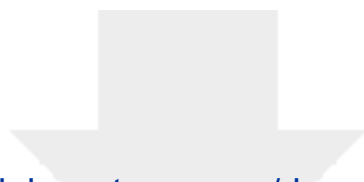**Supplemental Coding Files**
File_5-Light Shield.odg

Click here to access/download
Supplemental Coding Files
File_6-Opt1.system.py

Click here to access/download
Supplemental Coding Files
File_7-Opt2.ReadMe.txt

Click here to access/download
Supplemental Coding Files
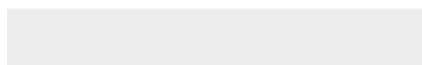File_8-Opt2.main.py

Click here to access/download
Supplemental Coding Files
File_9-Opt2.electromagnet.py

Click here to access/download
Supplemental Coding Files
File_10-Opt2.test_insert.py

Click here to access/download
Supplemental Coding Files
File_11-Opt2.camera_controller.py