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Builder & User Manual

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Crickmore Lab

WaterWorks

Original Design by Stephen Thornquist

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# Introduction

The Waterworks system can be used to probe the motivation of *Drosophila* by enabling the delivery of environmental challenges or use of optogenetic tools. This system features a 12-well behavioral arena that allows for testing of multiple animals simultaneously. The temperature within each well is set by the experimenter through a water system and can be acutely changed during an experiment to act as a threat. Below each well are three LEDs which can be used to activate optogenetic tools or deliver a light stressor.

This manual describes the overall design and individual components of the Waterworks system, with comprehensive instructions for building a new system from scratch. It then describes how to use the system to control the temperature and lights, and concludes with a section on troubleshooting. While originally designed to study *Drosophila* copulation, Waterworks is compatible with other insects and can be used for studying a variety of behaviors such as courtship, female receptivity, locomotion, and feeding.

# Design Overview

## Original Design Reported in Thornquist et. al, 2020

This apparatus was designed entirely by Stephen Thornquist and first reported in:

Stephen C. Thornquist, Kirill Langer, Stephen X. Zhang, Dragana Rogulja, Michael A. Crickmore, CaMKII Measures the Passage of Time to Coordinate Behavior and Motivational State, Neuron, Volume 105, Issue 2, 2020, Pages 334-345.e9, ISSN 0896-6273, https://doi.org/10.1016/j.neuron.2019.10.018.

(https://www.sciencedirect.com/science/article/pii/S0896627319308918)

A basketball net in an office

Description automatically generated

**Figure 1: Example System**

## Water System for Heat Threats

**Figure 2: Water System Schematic**



A schematic of the water system used to control the temperature is shown in **Figure 2**. It features two water sources: a room temperature reservoir and water bath that can be set to higher temperatures. If desired, the room temperature reservoir can be swapped for a second water bath (to allow for higher baseline temperature, such as when using thermogenetic tools). The behavioral chamber is designed so that a small reservoir of water is positioned beneath the floor of the wells, allowing heat to transfer from those reservoirs to the well and the animals inside. The system is designed so that the temperature of each well can be regulated independently by the experimenter manually turning two stopcocks that control whether the source of the water is the room temperature water reservoir or the water bath.

By default, the apparatus is set up so that room temperature water constantly flows to each of the wells until a temperature increase is initiated by the experimenter (**Figure 3A**). Water from the room temperature water reservoir travels into twelve paths each leading to one of the twelve “IN” stopcocks controlling the flow through a skinny tube leading to the water reservoir beneath a well (**Figure 3B, C**). The water then exits the well through a second skinny tube leading to one of the twelve “OUT” stopcocks controlling the flow back to the water reservoir (**Figure 3D, E**).



**Figure 3: Water Flow Diagrams**

A. Schematic depicting the path of water traveling from the room temperature reservoir to well 12. Room temperature water paths are highlighted in blue.

B. Water first travels from the reservoir to the “IN” stopcock.

C. If the stopcock is set to allow room temperature water flow, water then travels to the well.

D. Water leaves the well through separate tubing, traveling to the “OUT” stopcock.

E. If the stopcock is set to allow room temperature water flow, the water will then travel back to the reservoir.

F. While room temperature water is flowing through all wells, the master valve should be open, allowing hot water to circulate primarily in a loop from and back to the water bath.

The alternate water source, typically a water bath set to a noxious temperature, is set up the same way but with the addition of an extra route back to the bath, controlled by master valve (**Figure 3F**). This extra route is important because if all the wells are set to room temperature (as is the case when the system is not in use or when a heat threat is not acutely being delivered) then the water being pumped from the water bath will have nowhere to go and pressure will build. Such pressure could cause cracks in the stopcocks leading to leaks or burn out the pumps causing inefficient heat threats. Therefore, when no heat threat is being given the master valve is left open to allow the hot water to circulate back to the water bath.



**Figure 4: Stopcock Positioning**

The stopcocks allow the user to control the temperature of each well (**Figure 4**). There are two stopcocks for each well. The “IN” stopcock (labeled blue in the diagrams) controls the source of the water. The “OUT” stopcock (labeled in orange in the diagrams) controls where the water returns to. It is important that both stopcocks for a given well are set so that water comes from and returns to the same source, otherwise one water source will overflow. To give a heat threat, the user should first close the master valve so that all hot water is being pumped towards the behavior chamber, then the user simply turns the switch on both stopcocks corresponding to a given well (**Figure 5**).



**Figure 5: Delivering Threats**

## Circuit Controlling LEDs

To control the LEDs, the system uses an Arduino, a Raspberry Pi, and the user’s computer. One LED star containing 3 LEDs (typically red, green, and blue) is positioned below each well. The LEDs are in a circuit (described below) embedded in a PCB to which the Arduino is attached. The Arduino controls the voltage being sent through the circuit for each LED to turn them on and off, with each pin of the Arduino regulating a different LED. The Raspberry Pi is hardwired to the Arduino and serves as the interface between the Arduino and the user, relaying commands from the user’s computer to the Arduino to control the LEDs. By default, the Raspberry Pi is sending a constant signal to the Arduino to keep all the lights off, until a command specified by the user dictates they should turn on. The Raspberry Pi is also connected to the internet via an ethernet cable, allowing the user to send commands to it via Wi-Fi. The software package for Waterworks includes a python script that runs on the user’s computer that creates a graphical user interface (GUI) allowing the user control of the camera that records the behavior chamber (attached to the Raspberry Pi), the lights, and a set of timers which can be used to aid the manual delivery of heat threats.

In the case of incandescent lightbulbs, voltage and current have an Ohmic relationship (V=IR, where R is a linear function) and so regulating the voltage is a straightforward way to control the current going through the lightbulb, and therefore the brightness. LEDs instead have a non-linear relationship between voltage and current, where very small changes in voltage can result in huge changes in the current the LED tries to draw, which can result in LED burnout. This makes it exceedingly difficult to control the brightness of our LEDs just by manipulating voltage. So, to control our LEDs we use a BuckPuck which precisely controls the current sent to the LED. Each BuckPuck is designed to allow a certain amount of current to pass through the LED, for example, a 1000 mA BuckPuck which we use in the circuit with our red LEDs will keep the current passing through the LED at 1000 mA.



**Figure 6: LED Circuit with BuckPuck**

Each LED is controlled in parallel by a simple circuit that includes its own BuckPuck (**Figure 6**). On the BuckPuck, the LED+ and LED– refer to current sent to and received current from the LED, respectively. The BuckPuck can send current because it is connected to a power source via the Arduino. VIN+ and VIN- are the voltage coming from and back to the power source.

The CTL and REF pins on the BuckPuck regulate whether current is sent to the LED (and thus whether the LED is on). The REF pin is set to 5 mV. If the circuit board has power, then 5 mV will also be sent to the CTL pin. If the REF and CTL mV match, then the LED is off. If they don’t match (the Arduino is not sending a voltage to CTL pin) then the LED turns off. This is why if the circuit board is plugged in, but the Raspberry Pi is not, all the lights on the board turn on. This property also allows us to be able to dim the green LEDs via pulse width modulation (basically we can flicker the lights so fast that we cannot perceive them turning on and off, but the LED appears to dim).

# Components

This section identifies the parts and tools required to create a new Waterworks system.

## Parts Shopping List

Quantities listed build 1 box unless indicated by an asterisk:

\*Material can be used for multiple boxes

See accompanying excel spreadsheet for annotations.

It will be very helpful to order all the supplies from McMaster-Carr at once and keep them together with the packing slip. The stickers that have the line numbers on each part will allow you to refer to the packing slip to determine which part is which. There are pictures throughout the manual that will also be helpful for identifying parts.

### General Circuit Board Components

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Circuit Board | Circuit board from Gerber files | 1 | EasyEDA | <https://docs.easyeda.com/en/PCB/Order-PCB> |
| Arduino Mega | ARDUINO MEGA 2560 REV3 | 1 | Arduino Store | <https://store.arduino.cc/usa/mega-2560-r3> |
| Circuit Board Power Supply: AC/DC 12 V 100W Adaptor | Digi-Key # 2034-3899-ND | 1 | Digi-Key | <https://www.digikey.com/en/products/detail/cincon-electronics-co-ltd/TRH100A120-11E12-VI/9685152> |
| Circuit Board Power Supply: Power cord | Digi-Key # T1166-NA-ND | 1 | Digi-Key | <https://www.digikey.com/en/products/detail/AC-C7+NA/T1166-NA-ND/2743487?itemSeq=381403878> |
| Female DC Barrel Jack Connector | Digi-Key # CP-202A-ND | 1 | Digi-Key | <https://www.digikey.com/en/products/detail/cui-devices/PJ-202A/252007?s=N4IgTCBcDaIA4CsAEYAMYCCIC6BfIA> |
| 7 Pin  Single Row  2.54 mm Pin Pitch  Female Connectors | Digi-Key # S7040-ND | 36 | Digi-Key | <https://www.digikey.com/en/products/detail/sullins-connector-solutions/PPPC071LFBN-RC/810179> |
| AC 300V 10A 2 Pin 5mm Pitch PCB Mount Screw Terminal | Digi-Key # 102-6161-ND | 36 | Digi-Key | <https://www.digikey.com/en/products/detail/cui-devices/TB003-500-P02BE/10064085> |
| 2.54mm Single Row Male Pin Header Connector (1 x 40) | Digi-Key # S1012EC-40-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/sullins-connector-solutions/PREC040SAAN-RC/2774814> |
| 2.54mm Single Row Male Pin Header Connector (2 x 18) | Digi-Key # S2012EC-18-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/sullins-connector-solutions/PREC018DAAN-RC/2774876> |
| Power Strip with at least 8 AC Outlets | Ex. Digi-Key # HM3526-ND | 1 | Digi-Key | <https://www.digikey.com/en/products/detail/hammond-manufacturing/1580H10A1/2358802> |

### LEDs and Optics (LED Supply)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Custom 3-Up Luxeon High Power LED | LED #1 Deep-Red  LED #2 Green  LED #3 Blue[[1]](#footnote-1)  No jumpers  LEDs Addressable | 12 | LED Supply | <https://www.ledsupply.com/leds/custom-3-up-luxeon-high-power-led> |
| Carclo Lens | 10507 Carclo Lens – 3-Up Narrow Spot LED Optic | 12 | LED Supply | <https://www.ledsupply.com/led-optics/10507-carclo-lens-3-up-narrow-spot-led-optic> |
| BuckPuck DC LED Drivers – 700 mA | Connection : 7 Pin SIP  Dimming : Dimming (+$1.00)  Output Current : 700mA | 12 | LED Supply | <https://www.ledsupply.com/led-drivers/buckpuck-dc-led-drivers> |
| BuckPuck DC LED Drivers – 1000 mA | Connection : 7 Pin SIP  Dimming : Dimming (+$1.00)  Output Current : 1000mA | 24 | LED Supply | <https://www.ledsupply.com/led-drivers/buckpuck-dc-led-drivers> |

### Soldering Supplies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Soldering Iron with one tip | Ex. Metcal MFR Single Port System | 1\* | Metcal | <https://store.metcal.com/en-us/shop/soldering-desoldering/soldering-desoldering-systems/mfr-series/MFR-1120> |
| Soldering Iron Tip Replacement  (Only if you don’t have them) | Ex. Metcal MFR Hand Piece, Soldering Tip | 1\* | Metcal | <https://store.metcal.com/en-us/shop/soldering-desoldering/hand-pieces/MFR-H2-ST2> |
| Solder (Leaded or Lead-Free, user’s choice) | Ex. Kester Solder from Digi-Key  # KE1400-ND (Leaded) OR  # KE1137-ND (Lead-Free) | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/kester-solder/24-6337-8800/61656>  OR  <https://www.digikey.com/en/products/detail/kester-solder/24-7068-7601/738753> |
| Benchtop Solder Smoke Absorber | Ex. SRA Soldering Products AO486; Digi-Key # 2260-AO486-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/sra-soldering-products/AO486/10709947> |
| Soldering Stand (Recommended) | Ex. Aven Tools Soldering Stand with Dual Alligator Clips and Magnifying Glass  Digi-Key #243-1018-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/aven-tools/26000/600972> |
| Light/Magnifier (Recommended) | Ex. Stahl Tools HH3 Magnifying Lamp | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/stahl/374-700/10488171> |
| Black Wire | Remington Industries 22UL1007STRBLA  Digi-Key # 2328-22UL1007STRBLA-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/remington-industries/22UL1007STRBLA/11613841> |
| Red Wire | Remington Industries 22UL1007STRRED  Digi-Key # 2328-22UL1007STRRED-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/remington-industries/22UL1007STRRED/11615042> |
| Multimeter (Recommended) | Ex. Fluke-117, Digi-Key # 614-1011-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/fluke-electronics/FLUKE-117/1506333> |
| Wire Stripper and Cutter | Ex. Klein Tools 11057, Digi-Key # 1742-1270-ND | 1\* | Digi-Key | <https://www.digikey.com/en/products/detail/klein-tools-inc/11057/6804879> |

### Outer Behavior Chamber Supplies (McMaster-Carr)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| 1/8” Clear Cast Acrylic Sheet (Grey) | McMaster Carr #8505K722 | 2 | McMaster-Carr | https://www.mcmaster.com/catalog/127/3896/ |
| 1/8” Acrylic Sheet (Black) | McMaster-Carr #8505K742 | 4 | McMaster-Carr | https://www.mcmaster.com/catalog/127/3896 |
| Plastic Hinge with Holes | McMaster-Carr #1635A22 | 2 | McMaster-Carr | https://www.mcmaster.com/catalog/127/3185 |
| Steel Phillips Flat Head Screw | McMaster-Carr #90273A245 | 1\* | McMaster-Carr | https://www.mcmaster.com/catalog/127/3289/ |
| 18-8 Stainless Steel Narrow Hex Nut | McMaster-Carr #90730A011 | 1\* | McMaster-Carr | <https://www.mcmaster.com/catalog/127/3450> |

### Inner Behavior Chamber Supplies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| 1/16” Clear Acrylic | McMaster-Carr #8589K12 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/130/4105/8589K12> |
| 1/8” Clear Acrylic | McMaster-Carr #8560K257 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/130/4105/8560K257> |
| 1/8” Black Acrylic | McMaster-Carr #8505K742 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/3896> |
| 1/4” Clear Acrylic | McMaster-Carr #8589K82 | 1 | McMaster-Carr | <https://www.mcmaster.com/catalog/130/4105/8589K82> |
| Gasket Maker | McMaster-Carr #7660A21 | 1 | McMaster-Carr | <https://www.mcmaster.com/catalog/130/3941/7660A21> |
| Optical Sheet/Diffuser Paper[[2]](#footnote-2) | Inventables #23114-01 | 1 | Inventables | https://www.inventables.com/ |
| Nonwhitening Cement for Acrylic (Weld-On 4SC) | McMaster-Carr #7517A3 | 1\* | McMaster-Carr | https://www.mcmaster.com/products/weld-on-adhesives/ |

### Water Flow Supplies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Water Bath | PolyScience WB05A11B Digital General Purpose Water Bath, 5 L Capacity, 120V/60 Hz | 1-2[[3]](#footnote-3) | Poly  Science | <https://www.polyscience.com/general-purpose-water-baths/5-liter-general-purpose-water-bath> |
| Water Bath Cleaner (Recommended) | Ex. PolyScience Polyclear Clarifier | 1\* | Poly  Science | <https://www.polyscience.com/fluidaccessories/polyclean-clarifier-8-oz-case-12-x-8-oz> |
| Aquarium Tubing Clips for Water Bath  (Recommended) | Ex. Houkr Aquarium Adjustable Multifunction Clip Set (2Pcs) | 1 | Amazon | <https://www.amazon.com/Houkr-Aquarium-Adjustable-Multifunction-Water-Change/dp/B087M3PT2T/ref=cm_cr_arp_d_product_top?ie=UTF8> |
| Room Temperature Water Reservoir | Rubbermaid Ice Bin 12.1" x 5.5" x 6.12" | 1 | Amazon | <https://www.amazon.com/Rubbermaid-Ice-Bin-12-1-6-12/dp/B0010L1C28> |
| Aquarium Tubing Clips for Reservoir | MiguCo 4pcs Fish Tank Water Pipe Mounting Clip Aquarium Tube Clamp Hose Holder | 1\* | Amazon | <https://www.amazon.com/MiguCo-Water-Mounting-Aquarium-Holder/dp/B07WWLF783> |
| Water Pump | EcoPlus 728300 Pump, 158 GPH, Black | 2 | Amazon | <https://www.amazon.com/EcoPlus-Submersible-Aquarium-Fountain-Hydroponics/dp/B0018WVNXC> |
| High-Pressure Soft PVC Plastic Tubing for Air and Water, 3/8" ID, 5/8" OD, Clear | McMaster-Carr #5238K748 | 10 ft | McMaster-Carr | https://www.mcmaster.com/catalog/130/151/5238K748 |
| Plastic Barbed Tube Fitting for Air and Water, Straight Reducer, for 3/8" x 1/4" Tube ID | McMaster-Carr #5372K517 | 1 | McMaster-Carr | https://www.mcmaster.com/catalog/130/193/5372K517 |
| Clear Masterkleer Soft PVC Plastic Tubing for Air and Water, ¼” ID, 3/8” OD | McMaster-Carr  #5233K56 | 100 ft | McMaster-Carr | <https://www.mcmaster.com/catalog/127/149> |
| Wye Connector (10 pk) | McMaster-Carr #5372K186 | 3 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/193> |
| 3-way Stopcock w/ Swivel Male Luerlock (50 ct) | Ex. Smiths Medical #MX5311L | 1\* | Smiths Medical | https://www.smiths-medical.com/en-us/products/infusion/syringe-infusion/iv-disposable-components/small-bore-stopcocks |
| Tygon PVC Soft Plastic Tubing for Air and Water, Clear, 1/8" ID, 3/16" OD | McMaster-Carr  #6516T43 | 50 ft | McMaster-Carr | <https://www.mcmaster.com/catalog/127/150> |
| Plastic Barbed Tube Fitting for Air and Water, Tight-Seal, Adapter, for 1/8" Tube ID x 1/4 NPT Male | McMaster-Carr  #5463K439 | 3 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/194/> |
| Plastic Quick-Turn Tube Coupling, Sockets, for 1/4" Barbed Tube ID, Nylon | McMaster-Carr  #51525K216 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/257> |
| Plastic Quick-Turn Tube Coupling, Plugs, for 1/4" Barbed Tube ID, Nylon | McMaster-Carr  #51525K126 | 4 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/257/> |
| Plastic Quick-Turn Tube Coupling, Sockets, for 1/8" Barbed Tube ID, Nylon | McMaster-Carr  #51525K213 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/257/> |
| Plastic Quick-Turn Tube Coupling, Plugs, for 1/8" Barbed Tube ID, Nylon | McMaster-Carr  #51525K123 | 2 | McMaster-Carr | <https://www.mcmaster.com/catalog/127/257/> |
| 1-way stopcock with male and female leur lock | Ex. Qosina Corp 99759 (aka FisherScientific NC1503539) | 1 | FisherScientific | https://www.fishersci.com/shop/products/1-way-stopcock-female-luer-lo/NC1503539 |

### Raspberry Pi Components

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Raspberry Pi | Raspberry Pi 3 Model B Motherboard | 1 | Amazon | https://www.amazon.com/Raspberry-Pi-MS-004-00000024-Model-Board/dp/B01LPLPBS8 |
| Power Supply for the Raspberry Pi | Ex. CanaKit 5V 2.5A Raspberry Pi 3 B+ Power Supply/Adapter | 1 | Amazon | https://www.amazon.com/CanaKit-Raspberry-Supply-Adapter-Listed/dp/B00MARDJZ4 |
| Raspberry Pi Camera | Raspberry Pi Camera Module V2-8 Megapixel,1080p (RPI-CAM-V2) | 1 | Amazon | https://www.amazon.com/Raspberry-Pi-Camera-Module-Megapixel/dp/B01ER2SKFS |
| Micro SD Card | SAMSUNG (MB-ME32GA/AM) 32GB 95MB/s (U1) microSDHC EVO Select Memory Card with Full-Size Adapter | 1 | Amazon | https://www.amazon.com/Samsung-MicroSDHC-Adapter-MB-ME32GA-AM/dp/B06XWN9Q99 |
| IR 850 Filter | Ex. Gzikai 37mm IR 850 Glass Infrared X-Ray Filter 850nm IR Filter for Camera Lens Digital DSLR SLR. | 1 | Amazon | https://www.amazon.com/Gzikai-Infrared-Filter-Camera-Digital/dp/B0CL272KM7?th=1 |
| Desktop Monitor | Ex. Ex. PHILIPS 22 inch Class Thin Full HD (1920 x 1080) 75Hz Monitor, VESA, HDMI & VGA Port, 4 Year Advance Replacement Warranty, 221V8LN | 1 | Amazon | https://www.amazon.com/PHILIPS-Computer-Monitors-Replacement-221V8LN/dp/B0BRR4ZGNP?th=1 |
| HDMI Cord | Ex. Amazon Basics CL3 Rated High-Speed HDMI Cable (18 Gbps, 4K/60Hz) - 3 Feet, Pack of 2, Black | 1 | Amazon | https://www.amazon.com/AmazonBasics-High-Speed-HDMI-Cable-2-Pack/dp/B014I8SP4W |
| IR Lights | Ex. Phenas Home 48-led CCTV Ir Infrared Night Vision Illuminator Camera LEDs Lamp | 2 | Amazon | https://www.amazon.com/Phenas-48-led-Infrared-Vision-Illuminator/dp/B00GFDAJEI |
| AC/DC Adaptor 12V Power Supply | Ex. 12V Power Supply 7A 84W Security System Power Adapter, COOLM AC 100V-220V to DC 12 Volt DC 7 Amp Transformer for LED Strip Light CCTV Security System | 1 | Amazon | https://www.amazon.com/COOLM-Power-Adapter-100-240V-Output/dp/B07BVPJBCW |
| 1 to 2 Way DC Power Splitter | Ex. 2Pack 1 to 2 Way DC Power Splitter Cable Barrel Plug 5.5mm x 2.1mm for CCTV Cameras LED Light Strip and more | 1 | Amazon | https://www.amazon.com/2Pack-Power-Splitter-Barrel-Cameras/dp/B01M7N1GOH |
| Pi to Arduino Connector | Ex. Mediabridge USB 2.0 - A Male to B Male Cable (6 Feet) - High-Speed with Gold-Plated Connectors - Black - (Part# 30-001-06B) | 1 | Amazon | https://www.amazon.com/Mediabridge-USB-2-0-High-Speed-Gold-Plated/dp/B001MXLD4G |

### Miscellaneous

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Part | Description | Quant. | Source | Link |
| Tape | Ex. Daigger Label Tape | 1 | Daigger | https://www.daigger.com/label-tape-color-assortment-pack |
| 3D Printer | Ex. Lulzbot Taz Workhorse | 1\* | LulzBot | https://lulzbot.com/store/lulzbot-3d-printer-taz-workhorse-boxed-for-retail-na-kt-pr0051na?ref=KT-PR0051NA |
| 3D Printing Filament | Ex. HATCHBOX Matte PLA 3D Printer Filament, Dimensional Accuracy +/- 0.03 mm, 1 kg Spool, 1.75 mm, Black | 1\* | Amazon | https://www.amazon.com/HATCHBOX-Printer-Filament-Dimensional-Accuracy/dp/B09G7BXN2J |
| Set of Small Screwdrivers | Ex. 1-Piece Precision Screwdriver Set - Magnetic Mini Screwdrivers for Eyeglasses, Watches, Computers, Laptops, Phones - Phillips, Slotted, Torx, Non-Slip Handle, Portable Storage Bag Included | 1\* | Amazon | https://www.amazon.com/SEDY-Precision-Screwdriver-Magnetic-Screwdrivers/dp/B0B2NN9J6K |
| Absorbent Pads  (Recommended) | Ex. Amazon Basics Dog and Puppy Pee Pads with Leak-Proof Quick-Dry Design for Potty Training, Heavy Duty Absorbency, Regular Size, 24 x 23 Inches, Pack of 50, Blue & White | 1\* | Amazon | https://www.amazon.com/Amazon-Basics-Absorbency-Leak-proof-Quick-dry/dp/B07CWD5PX5 |
| Absorbent Cloths  (Recommended) | | Ex. Sophia's Secret Microfiber Cleaning Cloth - Multipurpose Reusable Kitchen Cloth - Highly Absorbent Lint Free Microfiber Dusting Cloth Rags for Car, House, Window - 8- 12x16, Pack of 8 | 1\* | Amazon | https://www.amazon.com/gp/product/B093KTHD3J/ref=ppx\_yo\_dt\_b\_search\_asin\_title?ie=UTF8&psc=1 |
| Support for Under the Circuit Board | We use the cardboard our fly food comes in, but you could also use a plastic tray (turned upside down), ex. iDesign Decorative Courtertop Vanity Tray Organizer for Bathroom, Bedroom, Closet, Entryway, The Clarity Collection – 8” x 8” x 2”, Clear | 1 | Amazon | https://www.amazon.com/InterDesign-Clarity-Cosmetic-Organizer-Products/dp/B012SBZ1EI |
| Hot Glue Gun Kit | Ex. Assark Glue Gun, Mini Hot Glue Gun Kit with 30 Glue Sticks for School Crafts DIY Arts Quick Home Repairs, 20W (Blue) | 1\* | Amazon | https://www.amazon.com/Assark-Sticks-School-Repairs-20W/dp/B09FYWQ44L |
| Tacky Glue | Ex. All-Purpose Tacky Glu | 1\* | Amazon | https://www.amazon.com/All-Purpose-Tacky-Metal-Glue-Household/dp/B0CLW5NZTR?th=1 |
| Fast Orange Hand Cleaner  (Highly Recommended) | Ex. Permatex Fast Orange Fine Pumice Lotion Hand Cleaner | 1 | Amazon | https://www.amazon.com/Permatex-Orange-Pumice-Lotion-Cleaner/dp/B07H3CCLJ4 |

## Selected Parts in Detail

### General Circuit Board Components

**Circuit Board**

**A screenshot of a computer

Description automatically generated with medium confidence**The circuit board was designed using EasyEDA: <https://easyeda.com/page/download>. It uses a DC power source to supply current, regulated by 36 buck pucks, to 12 3-color LEDs. An Arduino exerts control over the LEDs so that the user can determine the timing and duration of light provided to each well independently.

**Power Supply**

To power the circuit board, we use a 12 Volt, 8 Amp AC/DC power adaptor. Standard power outlets use alternating current (AC) while our circuit board uses direct current (DC) power. Most electronics use DC power because it provides a more consistent voltage.

A picture containing text, electronics, circuit

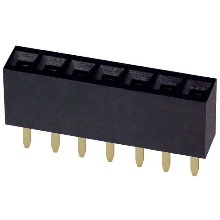
Description automatically generated**Arduino Mega**

A picture containing text, electronics, circuit

Description automatically generatedArduino is an open-source electronics resource that uses Arduino boards as hardware and the Arduino Software (IDE)[[4]](#footnote-4). The board is a microcontroller, meaning it generally works by taking inputs, processing them, and turning them into outputs. This makes them great for interfacing with sensors and LEDs. To interface with the Arduino so that you can view, edit, and upload the code it will use, you will need the IDE software[[5]](#footnote-5) and an adaptor to connect it to your computer (you can use the same adaptor from the parts list that you use to connect it to your raspberry pi if your computer accepts USB-A).

**DC Barrel Jack Connector**

Accepts power from a DC power supply and provides it to the rest of the circuit board. This connector has a 2.10 mm ID and a 5.5 mm OD.

**7 Pin Connectors**

These connectors will link the BuckPucks to the rest of the circuit board while allowing for easy replacement should a BuckPuck burn out or become damaged.

**Screw Terminals**

Screw terminals facilitate the connection between wires and the circuit board. The screw terminal is soldered to the board, while the wires can then be secured to the screw terminal by loosening the top screw, inserting the wire, then tightening the top screw. In our case the wires will be attached to the LED at the other end, so using a screw terminal allows for easy replacement of any damaged wires or LEDs.

**Male-Male Connectors**

These male-male connecters are used to connect the Arduino to the circuit board. The shorted pins will be inserted in to and soldered on to the circuit board. The Arduino can then be attached to or unattached from the larger pins.

### LEDs and Optics

**Diagram

Description automatically generatedCustom 3-Up Luxeon High Power LED**

**Function:** These take input current (which flows from positive to negative), pass it through a semiconductor (the diode) which outputs light (measured in lumens). Therefore, LED stands for Light Emitting Diode. Each LED star from LED Supply comes with three separate LEDs that can have different colors (determined by the temperature for white light or the material for other colors). These LEDs can either be connected in series or in parallel. If they are connected in series and you provide power, then you’re providing power to all of them. LEDs connected in series can only be lit/unlit all together. In this circuit configuration the LEDs come with a zero Ohm resistor (jumper) that connects the LEDs in series without causing you to lose charge. Instead, we want to be able control each LED individually, or in other words we want them to be individually addressable, which means we need them to be connected in parallel. If they are connected in parallel, then it does not have jumpers connecting the LEDs.[[6]](#footnote-6)

**Color Options:**Cool White, Neutral White, Warm White, Deep Red (627 – 670 nm), Red, Red-Orange, Amber (580 – 590nm), Green (520 – 580 nm), Cyan, Blue (460 – 490 nm), and Royal-Blue[[7]](#footnote-7)

**Brightness**: The light emitted by an LED is a function of the current passed through the diode. Our LEDs are the Luxeon Rebel type and either have 700 mA or 1000 mA running through them (which we regulate by the BuckPuck we include in the circuit). The 700 mA driver provides up to 3.2 V, producing a brightness of 189 lumens. The 1000 mA driver provides up to 3.4 V, producing a brightness of 236 lumens.

**A picture containing music, brass

Description automatically generatedCarclo Lens**

Text

Description automatically generated**Text

Description automatically generatedA picture containing text, metalware

Description automatically generatedFunction:** The lenses take the light emitted from the LED and reflect it into a beam. Some lumens are lost at this step (~88% make it through in our case). LED optics such as this can be used to either diffuse or focus the light coming from the LED. The beam angle is expressed as the full width half max (FWHM), which means it 50% of the light coming out of the LED is contained within that angle. On the LED Supply website it is formally defined as, “A measure of the width of a distribution defined as the separation of the data points on either side of the peak which have values equal to 50% of the maximum. For intensity distributions this is the angular range at which the intensity falls to half its maximum value.” The FWHM for the lens we use is 16.4°. Knowing this angle and the diameter of the behavior chamber (1 inch), we can use trigonometry to calculate the minimum distance above the lens the behavior chamber should be for the beam to illuminate the entire chamber, which is at least 3.5 inches.

1 inch

16.4°

3.5 inches

**BuckPuck DC LED Drivers**

BuckPucks are components that regulate voltage to control the amount of current sent to the LEDs in our circuit[[8]](#footnote-8). They are current limiting, preventing the LEDs from drawing too much power and burning out, and they allow us to control whether an LED is on, and how bright it, is by modulating the current sent to that LED. In our circuit, each LED has its own BuckPuck.

### Behavior Chamber Supplies

#### Acrylic Components:

The behavior chambers are mostly made from acrylic that has been laser cut according to a template.

**For future designs:** Use Maker Case (https://www.makercase.com/#/basicbox) to design boxes with edges that lock together. Compile designs in Adobe Illustrator. The laser cutter has a bed that is 18” x 32”, therefore the maximum size of acrylic from McMaster-Carr we can use is 12” x 24”, so you should set your artboard to be this size. Every piece that uses a different type of acrylic should be on a different artboard. If pieces using the same type of acrylic cannot fit in the 12” x 24” area, then they should be separated on to different artboards. You can then save as a pdf, and the different artboard will save as different pages. The instrumentation core has settings on file for different pieces of acrylic, but it is also good to annotate your designs with the settings you use.

#### Diffuser Paper:

Diffuser paper makes up the floor of the behavioral chamber. The choice of diffuser paper is important for two reasons: 1) it will allow light from the LEDs below to pass through and be spread evenly over the behavior well; 2) it allows the transfer of heat from the water reservoir to the behavior well. The exact diffuser paper you use can vary – and the one that we use will soon be discontinued so future iterations of this set-up will have to use something different. When choosing a new paper, make sure it sturdy enough to serve as a floor. It will be important to take new measurements when calibrating your system as the paper you chose will determine how much light and heat pass on to the animals in the behavior chamber.

# Preparing Materials

## Ordering the Circuit Board

1. Our circuit board was designed using EasyEDA which partners with JCLPCB for manufacturing. However, the Gerber Files can be sent to any company that manufactures PCBs.
   * How to order PCB (EasyEDA): <https://docs.easyeda.com/en/PCB/Order-PCB/>
   * JCLPCB website: <https://cart.jlcpcb.com/quote?edaOrderUrl=https%3A%2F%2Feasyeda.com%2Forder&electropolishingOnlyNo=no&achieveDate=72>
2. Upload the gerber files as a .zip file to the manufacturer’s website
3. The settings should automatically update or be as follows:

|  |  |
| --- | --- |
| Base Material | FR-4 |
| Layers | 2 |
| Dimensions | 241.55 x 484.12 mm |
| PCB Quantity | 5\* (or however many you want, order an extra or two in case you make a mistake) |
| Different Design | 1 |
| Delivery format | Single PCB |
| PCB Thickness | 1.6 |
| PCB Color | any (I selected black most recently, the prior version is in blue) |
| Silkscreen | white |
| Surface Finish | HASL (with lead) |
| Outer Copper Weight | 1 oz |
| Gold Fingers | No |
| Confirm Production File | No |
| Flying Probe Test | Fully Test |
| Castellated Holes | No |
| Remove Order Number | No |

## Laser Cutting the Behavior Chamber

*Prep:* The laser cutter is located in the Research Instrumentation Core on the HMS Quad. Contact Ofer or Pavel to discuss the project and schedule a training[[9]](#footnote-9). A Harvard ID is required to access the facility.

1. Open the page of the pdf containing the design you want to print in adobe illustrator.
2. Print the design and select the laser cutter as the printer.
3. View the design in the laser cutter software. Adjust the settings so that the piece you are cutting is where your acrylic is in the machine (it’s easiest to position both in the top right corner, leave 0.5” gap between the schematic and the corner in the software).
4. Adjust the settings for the laser according to the specifications in the pdf.
5. Place your piece of acrylic in the laser cutter.
6. Press play to print.

For the behavior chambers, you can discard all the cut-outs except for the doors in the door layer. You may want to cut an extra well bottom (layer 4), bath (layer 5), and bath floor (layer 6) as these are the most prone to fatal mistakes in the assembly process.

There are two options for the base: one for if you are going to be using LEDs/a circuit board underneath and one if you are not (i.e. you just want the heat capabilities and don’t need the lights). The only difference is whether it gives you a bottom to the base to print out that will fit in the sides.

## 3D Printing the Light Tubes and Door Handle

We use a 3D printer to manufacture the plastic columns that sit between the LEDs and the base of the behavior chambers. These columns keep the light contained so only the intended well becomes illuminated. You will need to print 12 tubes based on the WaterworksColumns.stl file. You may need to adjust the sizing of the tubes.

We also 3D print a door handle for the front of the box, this is optional.

# Assembly

## Circuit Board Assembly

1. Whenever you solder:
   1. Clear the area of unnecessary supplies and debris so you have ample room to work.
   2. Check that the soldering iron has the tip you want to use – the tip should be appropriately sized to the pieces you’ll be soldering (not too large or too small). Otherwise replace the tip (make sure the soldering iron is cool, then pull it off and switch it for one of the other tips).
   3. Turn on the soldering iron.
   4. Wet the sponge with some DI water (you may want to shake it off first in case there are some metal scraps).
   5. Turn on the carbon filter.
   6. Turn on the lights above the bench and surrounding the magnifying glass.
   7. Prep your supplies.
2. Install the 7 pin connectors:
   1. Working one at a time, place one of the 7 pin connectors into the appropriate slot.

Note that the board has 8 holes, but the BuckPuck only has 6 pins that are spaced for a 7 pin connector, so we use the 7 pin connector. This means there will be one empty hole. The hole that remains empty is the nearest to the bottom of the circuit board (assuming the board is oriented properly with the Arduino/Crickmore lab insignia in the top left).

* 1. Carefully flip the board over, holding the connector in place, such that now the header sits freely and upright on the bench top in its proper position (upright, not angled).
  2. Solder it to the board.
  3. Repeat until all are soldered on.

1. Install the DC barrel jack connector:
   1. Insert the piece so that connection is on the top of the board and facing the nearest edge.
   2. Flip the board over and solder from the bottom. You’ll need to use a lot of solder to fill the holes.
2. Install the male-male connectors that attach to the Arduino:
   1. Take the 2 x 18 connector and insert the shorter pegs from the bottom of the board into the digital pin section (the only section that has pins 2 x 2, all labels are written on the top side of the PCB). Solder from the top of the board.
   2. From the strip of 1 x 40 connector, break off a 1 x 8 piece. Coming from the bottom of the board, insert the shorter side of piece into the section labeled communication pins 14 – 21. Flip the board over and solder from the top of the board.
   3. From the strip of 1 x 40 connector, break off a 1 x 8 piece. Coming from the bottom of the board, insert the shorter side of the piece into the section directly to the left of the prior section, with pins labeled 0 – 7. Flip the board over and solder from the top of the board.
   4. From the strip of 1 x 40 connector, break off a 1 x 7 piece. Coming from the bottom of the board, insert the shorter side of the piece into the section directly to the left of the prior section, with pins labeled 8 – 13 and GND. Note there are 8 total holes in this section, you want to insert the connector so that it is shifted to the right by one pin, such that the AREF hole will not have a connection. Flip the board over and solder from the top of the board.
   5. From the strip of 1 x 40 connector, break off a 1 x 4 piece. Coming from the bottom of the board insert the shorter side of the piece into the section directly below the prior section, labeled power, with pins labeled: Vin, GND, GND, 5V. Note there are 6 total holes in this section, you want to insert the connector so that it is shifted to the right by two pins, such that the RESET and 3.3V holes will not have a connection. Flip the board over and solder from the top of the board.
3. Install the screw terminals:
   1. Working one or a few at a time, insert the screw terminal from the top on to the board. Make sure it’s all the way in – you might need to lightly hammer it in there.
   2. Flip the board over and solder it from the bottom.
4. Prepare and install the LEDs:
   1. Cut 36 ~1.5 inch pieces of red wire and 36 ~1.5 inch pieces of black wire.
   2. Strip a little piece of both ends of each piece of wire.
   3. Tin each wire: use a vice to hold the wire while you heat up one end of the wire and then coat the stripped portion in a little bit of solder. You only need to do this on one side of each wire.
   4. Tin the LEDs: heat up and then add a little bit of solder to each place where you would connect a wire to the LED (indicated by the bright red arrows in the picture below, there are 6 per LED: 3 + and 3 -).

A picture containing text, transport, weapon, wheel

Description automatically generated

* 1. Now you can attach the wires to each LED: head up the solder you put on the LED and gently place the tinned wire on top. Let cool, then give a small tug to make sure its secure.
  2. Once you’ve attached wires to all the LEDs, place one on each of the circles in the center of the PCB.
  3. Rotate the LED so that the wires for a given color LED are near the screw terminals for that color LED. Note the + and the - wires are on either side of the LED, not the same side. Glue down the LED with hot glue.
  4. Secure all wires to the corresponding screw terminals: loosen the screw on top with the mini screwdriver, insert the wire on the side, and tighten. Pay attention to the + and – signs.
  5. Plug in all the BuckPucks – the red and blue LEDs get the 1000 mA BuckPucks and the green LEDs have 700 mA BuckPucks.
  6. The time to troubleshoot LED problems is now. It will be very helpful if you have a power source you can control the voltage of or a low voltage power source so that you can see whether each LED is functioning without it being too bright. You may have to re-solder some wires if you see something is not lit.
  7. Attach the Carlco lens to each LED, you may want to glue this down with hot glue or some other adhesive. I’ve found tacky glue works best because it doesn’t dry too fast. And once dry it will hold the lens in place without being permanent like super glue, so you can remove the lens if you need to repair a connection to the LED.

## Behavior Chamber Assembly

This is going to be messy. You should wear proper PPE (lab coat, gloves) and have plenty of clean-up supplies handy. Fast Orange Hand Cleaner will get liquid gasket off your skin after some effort, water will just spread it everywhere. Nothing gets it out of clothes that I know of. **Read all the steps in their entirely before starting.**

1. The first thing we need to do is seal the bath layer and the bath floor layer together using Weld-On 4SC fast setting solvent cement. This sets in under 5 mins, so you need to work very quickly. Spread it on the bath layer, then press the bath floor layer on top. Hold the layers together tightly with clamps or heavy objects. Let them stay like that for **72 hours**.
2. After those layers have set, depending on how well you did with step 1, you may want to also put just a tiny amount of gasket in each water bath hole (imagine you were caulking the gap between a countertop and the wall). Do not block the small circular holes in the bath floor. A well will sit on top of each water reservoir, you can check how it will be positioned by placing the well walls layer on top. Do not get any liquid gasket on the bath floor where the well will be positioned – it will block the light from your LEDs.
3. Now you need to attach the well bottom (diffuser paper) layer to the bath layer. Spread liquid gasket over the bath layer (which should be fused with the bath floor layer now from step 1). Use your gloved finger to get an even layer. Change your gloves, don new gloves, then insert screws into the four corner holes from the bottom up so the plate can lay flat on your work surface with the gasket-side up. Place the diffuser paper on top of the liquid gasket and rub all over so that the two adhere (the screws will help keep the layers aligned at this stage). Carefully remove the screws and clean them off. Let the whole thing set **at least overnight**.
4. Flip the behavior chambers over so that the bottom is up. Screw in the 1/8” threaded barbed fitting (McMaster-Carr #5463K530) into the bath floor layer.
5. Align the tube lock layer on the bath floor layer. My preference is to insert the 3D printed columns now, but you can do it later. The align the tube layer on that. Insert screws in all the holes. Use a tape over the screw heads to secure the screws.
6. Flip the chamber over (top is now up) and apply the thumb nuts to secure the layers in place.
7. Flip the chamber over (bottom is now up). Cut 24 pieces of 1/8” tubing (the skinny tubing) about 12-16” long depending on how far away you want the stopcocks to hang (they will go on one end of this tubing). Attach each piece of tubing to one of the barbed fittings. Make sure they are secure or secure them with liquid gasket, hot glue, or something else (again I don’t recommend anything too permanent in case you need to replace this tube at some point). Let the adhesive set.
8. Flip the chamber over (top is now up). Remove the knurled nut and add the other acrylic layers in order. Replace the knurled nut.
9. Add the 3D printed columns to the bottom if you have not done so already. Gently squeeze the clips, insert, and release. Rotate the column if needed so the clip spring out and into place.

## Assembling the Outer Box Base

1. Take the front piece of the base (engraved with labels and has 24 holes). Carefully thread each skinny tube through the appropriate hole. The tube should come from the well with the corresponding number (wells on the behavior chamber are labeled [1 2 3 4; 5 6 7 8; 9 10 11 12] the same as this plate). The tube on the left side of the well will be designated the “IN” and the right-side tube will be designated the “OUT”.
2. Then assemble the sides, and base if you are using. Place the top plate on last. It may be hard to snap it in, try flipping it or the other pieces around to make them fit better. Be careful not to snap the acrylic (which can happen if you apply too much pressure). Secure everything with good quality tape (Diagger brand is my preference).

## Connecting the Behavior Chamber to Water

This is also going to be messy. You should wear proper PPE (lab coat, gloves) and have plenty of clean-up supplies handy. I recommend having absorbent pads and cloths in arms reach, and a large rectangular bucket to sit the equipment in (I use the Nalgene we use for dishwashing/autoclaving). Refer to **Figure 2** for a diagram and **Figure 7** for a picture of some of the parts.

1. If you have access to some cups or small trays to hold your connectors that would be helpful. Retrieve and unpackage at least:

* 12 Plastic Quick-Turn Tube Coupling, Sockets, for 1/8" Barbed Tube ID, Nylon (McMaster-Carr #51525K213). This is for the “IN” tubing.
* 12 Plastic Quick-Turn Tube Coupling, Plugs, for 1/8" Barbed Tube ID, Nylon (McMaster-Carr #51525K123). This is for the “OUT” tubing.
* 24 3-way Stopcocks
* 36 Plastic Quick-Turn Tube Coupling, Plugs, for 1/4" Barbed Tube ID, Nylon (McMaster-Carr # 51525K126). This is used for both “IN” and “OUT” tubing.
* 12 Plastic Quick-Turn Tube Coupling, Sockets, for 1/4" Barbed Tube ID, Nylon (McMaster-Carr # 51525K216). This is for “OUT” tubing.

1. Set-up 12 stopcocks in the “IN” configuration and 12 stopcocks in the “OUT” configuration (**Figure 7**).



**Figure 7 Stopcock Configuration**

1. Connect each stopcock to an appropriate skinny tube exiting the behavior chamber.
2. Now you’re going to assemble the main tubing (use **Figure 2** as a reference). Start with the “IN” tubing first and continue until all the lines have converged to a single input line. Work as follows:
   1. Attach the main tubing (the whole roll you ordered, McMaster-Carr #5233K56) to the connector on the stopcock for well 1 IN that is parallel to the connector attached to the skinny tubing. Using scissors, cut the piece so it’s maybe 2-3 inches long.
   2. Insert the left branch of the Y connector into that cut piece.
   3. Attach the main tubing to the to the connector on the stopcock for well 5 IN that is parallel to the connector attached to the skinny tubing. Cut so that it comfortably reaches the Y connector now attached to well 1.
   4. Insert the right branch of the Y connector attached to well 1 into that cut piece.
   5. Attach the main tubing (the whole roll you ordered) to the connector on the stopcock for well 9 IN that is parallel to the connector attached to the skinny tubing. Using scissors, cut the piece so it’s maybe 2-3 inches long.
   6. Insert the right branch of the Y connector into that cut piece. You should now have 2 Y connectors on the main tubing.
   7. Attach the main tubing to the bottom branch of the Y connector linking wells 1 and 5. Cut so that it comfortably reaches the open left branch of the Y connector now attached to the tubing for well 9.
   8. Insert the left branch of the Y connector attached to the tubing for well 9 into that cut piece. The input tubing for Wells 1, 5, and 9, should now all be linked and converge to a single Y connector with just the bottom branch open.
   9. Repeat this process for the “IN” tubing for wells (2, 6, and 10), (3, 7, and 11), and (4, 8, 12). You should end up with Y connectors with just the bottom branch.
   10. Place the water reservoir for the room temperature water to the left side of the behavior box.
   11. Cut a ~2 inch piece of the large high pressure tubing.
   12. In the box with the pump, find the adaptor that fits in the high-pressure tubing. Attach that to the pump and connect it to the high-pressure tubing.
   13. Insert the largest barbed connector into the high-pressure tubing (McMaster-Carr #5238K748) to the high-pressure tubing.
   14. Attach the main tubing to the other end of that connector and run it to the Y connector for all the inputs so that it hangs pretty loosely.
   15. Cut the main tubing and attach it to the Y connector.
   16. Repeat the same process (steps a through i) for the open connection on the “IN” stopcocks. The left/right branch instructions will be flipped for this set because it’s essentially a mirror image of what you just did (do what seems logical and you will be fine). You should again end up with a single Y connector with just the bottom branch open.
   17. Place the water bath for the hot water to the right side of the behavior box.
   18. Repeat steps m through o to set up the hot water pump.
   19. Now you’re going to the basically the same thing for the OUT valves. Start with the connecting the tubing parallel to the skinny tubing connection (just like we did for the IN valve, but it will be the mirror).
   20. You’ll end up with one Y connector again. Connect main tubing to this, run it loosely back to the room temperature water reservoir (so that water coming out would be deposited into the reservoir). Secure it with tape temporarily or just let it sit there.
   21. Now do all the connections that are left (perpendicular to the skinny tubing). You should end up with a single Y connector again.
   22. Connect main tubing to this, run it loosely back to the hot water bath (so that water coming out would be deposited into the reservoir). Secure it with tape temporarily or just let it sit there.
3. Now we need to create the master valve. Grab a Y connector. Find the tubing connected to the hot water pump. Go maybe 1/3 of the way down, past the edge of the bench top, somewhere near where all the stopcocks are hanging. Cut the tubing (hold both ends so you don’t lose track of them) and put the right branch of the Y connector into the cut side coming from the pump and the bottom branch into the cut side going to the stopcocks.
4. Attach the main tubing to the left branch of that Y connector and run it back to the hot water bath. Secure it with tape temporarily or just let it sit there.
5. Grab the one-way stopcock and attach a barbed connector to each end.
6. Go to the main tubing you just cut on step 6, maybe 2-3 inches away from the Y connector. Cut the tubing (hold both ends so you don’t lose track of them) and attach them back together with the one-way stopcock you just prepared. It doesn’t matter which way you attach it.
7. Now you’re going to lock all the output tubes down with the aquarium clips and tape.
8. You may want to tape some of the other tubing down, so it doesn’t get pulled.
9. Now you can test the equipment for leaks. Make sure everything related to the circuit board and anything electrical is far away. Assume water will spill and take the proper precautions so you don’t electrocute yourself.
10. Fill the water reservoir and water bath with water. Get the power strip and plug the two pumps and the water bath into the it. If something goes wrong, you will only have to hit the switch on the power strip to shut down both pumps.
11. Plug in the power strip and turn it on. Now you need to wait and watch for a while. It may take time for the water to start to flow (see the troubleshooting section on air bubbles below). Once you see water returning to the baths at a consistent pace, remove any remaining air bubbles. Wait another 10 mins. If there are no leaks at this point, you’re all set. If there are leaks find the source and address them. If it’s a problem with the behavior chamber you may have to redo steps 1-3 of the behavior assembly section.

## Preparing the Arduino and Raspberry Pi

1. Load the Waterworks\_Red\_Green\_Blue.ino on to the Arudino.
2. Load all the code from the Pi Package folder on to the Raspberry Pi.
3. Troubleshoot – there might be some issues with just copying over the code to the Pi. The Arduino should be fine. You can tell if the Pi is not controlling the Arduino properly if everything is powered on but the LEDs will not turn off. If your method for installing software on the Pi is not working and you have access to a functioning Pi with the software, trying instead creating using an image (see below).

#### Creating and Using an Image of an Operational Raspberry Pi

The easiest way to get the software on to a new Pi, or repair a corrupted Pi, is by creating an image of a functional Pi with the software installed. An image is essentially a clone. Because of that, your backup file will be the size of the card you’re copying, not the size of what’s actually on the card. So you need to create the image on a microSD card or hard drive that is at least the same size as the one you are copying. You’ll also want to clear any unnecessary videos and files from the clone to make the process smoother. In my experience the copying process will take many, many hours so it’s best to do this overnight.

See <https://www.tomshardware.com/how-to/back-up-raspberry-pi-as-disk-image> for more information on how to create an image. We have an image already in the Crickmore Lab on a red SSD that you can use. See <https://thepihut.com/blogs/raspberry-pi-tutorials/17789160-backing-up-and-restoring-your-raspberry-pis-sd-card> or other websites for how to write an SD card from an image.

## Final Assembly

1. Place the circuit board on its long-term stand (either cardboard or plastic) if it isn’t on one already. And position it wherever you’re going to keep it in the lab.
2. Attach the Pi to Arduino connector to the Arduino.
3. Carefully attach the Arduino to the circuit board (make sure all the pins are lined up.
4. Place the behavior chamber inside the base on the circuit board. You must make sure all the columns are lined up perfectly over their respective LEDs and may need to remove the sides of the base or ask for help from a lab mate to do this successfully.
5. If it feels unsteady, use some tape to secure the base to the circuit board.
6. Retrieve the front/door layer for the top, 2 plastic hinges with holes, 8 steel Phillips flat head screws, and 8 18-8 stainless steel narrow hex nuts. The hinges of the door will be on the left, check which is the top and which is the bottom of the door frame by making sure you can fit the door frame into the jigsaw cut-outs on the base. Attach the door to its frame with the hinges on the left side and screws pointing in (nuts will be on the inside of the box, the door will open outward).
7. Hot glue on your door handle if you are using one (you can do it once the top of the outer box is assembled but it’s easiest while the door and frame can lay flat).
8. Assemble the top, checking each side first to make sure you can fit the bottoms of the sides in the base and the tops of the sides come together so you can put the top on the box. Secure everything with tape.
9. Place the top on the base. It should sit within the jigsaw cut-outs. You may need to apply some pressure or flip pieces. Be careful not to apply too much pressure or you will crack the acrylic of the base (it’s easier to do than you might think).
10. Now we will prepare the Raspberry Pi. Insert the microSD card into the Raspberry Pi (if you haven’t done so already). Place the Raspberry Pi on the top of the box top.
11. Carefully attach the camera to the Raspberry Pi. For help, see <https://projects.raspberrypi.org/en/projects/getting-started-with-picamera> or <https://www.raspberrypi.com/documentation/accessories/camera.html> (contains a video). Lightly secure the camera over the cut out in the top of the top with tape (you will need to adjust this once you have the camera turned on and can see the positioning).
12. Attach a power source to the Raspberry Pi (do not plug the power source in yet).
13. Attach the Pi to Ardunio connector to the Raspberry Pi.
14. Attach the keyboard and mouse to USB ports on the Raspberry Pi.
15. Put the desktop where you want to keep it. Plug the desktop in to a power source (you can plug everything into the same power strip as the water pumps unless otherwise noted) and use the HDMI cable to connect it to the Raspberry Pi. Turn the monitor on.
16. If you are using an ethernet cable to provide the Raspberry Pi with internet access, attach that now and connect it to the ethernet port in your wall.
17. You may now plug in your Raspberry Pi to an outlet NOT on the power strip and let it boot.
18. Connect your two IR lights to the 1 to 2 way DC power splitter and then to the 12V power supply. Plug them in and position them so they are pointed at the base. You may need to elevate them with a wooden block.
19. Attach the IR filter to the inside of the top of the box with tape so that it covers the whole the camera is inserted into. Secure with tape, but make sure the tape is not blocking the camera’s view.
20. Using the command line on the Raspberry Pi, and the command (or something similar):

Raspivid -o Desktop/video.h264

Visualize what your camera will record. Reposition the camera and the IR lights as needed. Secure everything with good quality tape.

1. At this point your set up is pretty much not going to move, so tape anything now that needs to be secured more.
2. I use 12 rolls of multicolor tape to create labels for each well and place a label on the behavior chamber next to the well, on the tubing next to the “IN” stopcock for the well, and on the tubing next to the “OUT” stopcock for the well.

## Preparing the Computer that will Control the Raspberry Pi and Arduino

The code that goes on the computer is in the Pi Control folder. You should copy the whole folder over. You may need to install some Python packages depending on what comes with your computer (you will need to install Python if you don’t have it already). This code was developed and tested using Python 2.7, new versions of Python may not be compatible.

You will need to install gstreamer (<https://gstreamer.freedesktop.org/>) if you want to be able to stream video to the laptop.

# Using Waterworks for Behavioral Experiments

## Starting the Software & Setting up for Experiments

Before you start a batch of heat threat experiments, you should fill the baths (some water will evaporate over time) and flush the lines. There is a significant portion of water stored in the tubing and wells when you are not running experiments that will cool to room temperature. Running hot water through the lines will push this water out so that it can be heated in the bath, getting the water bath working and preventing a drastic drop in water temperature when you start giving threats. It will also make you aware of any air bubbles that may have gotten into the system so you can flush those out as well. See the air bubbles subsection in the standard maintenance portion of this manual (below) if you end up with bubbles you can’t get out.

To flush the lines, you’re simply going to close the master valve, then switch each of the wells to hot water (as if you were giving heat threats to every well, see **Figure 5**). I open them all in order (so that every well has hot water), then close them all in the same order. The time it takes to open all of them is sufficient to chase the old water out. You can now open the master valve and begin your experiment after the water bath has reached the desired set point. Check again for air bubbles and remove any that have appeared after this step.

Our software is run on a user’s computer by a shortcut called ‘pis’ in the command line. You can just also start the software by executing the pi\_control.py code in the Pi Control software folder. When you start the software you will see this GUI:

A screenshot of a computer

Description automatically generated

If this is the first time you are using that Pi and user computer together, skip below to the subsection on using the address manager to set the IP address then return to this point. Select the Pi you want to use from the dropdown menu and press connect. For this example, I will be using a Pi named Heimlich (after the caterpillar in Disney’s A Bug’s Life). You will then see this GUI:

A screenshot of a phone

Description automatically generated

Select the protocol you want to use for controlling the lights (see below for more details) or you can leave it on paired pulse if you’ll only be using heat threats. Press run protocol when you are ready. If this is the first experiment of the day or you just restarted the terminal, all the lights on board will briefly flash. IF THEY DO NOT FLASH, YOU HAVE NOT PROPERLY CONNECTED TO THE PI and need to troubleshoot the connection.

You should now see a GUI that looks like this:

A screenshot of a computer

Description automatically generated

The left portion of the GUI controls the LEDs and is explained below on the section using the lights for optogenetics. The Well Timers on the right side can be used for any experiment for timing anything you’d like (they’re just stopwatches, they don’t have any effects). Note the destroy button does not function. The start video section on the far right allows you to control the camera. If you want to record the experiment, type in the name you’d like to save this experiment as and check the save video box. Note that the Pi only has so much storage so if you are saving videos you’ll need to periodically delete them to free up space (see the post-hoc analysis section below). Then click start video. You should see the desktop connected to the Pi switch to a view of the behavior chamber (if not see the camera troubleshooting section). You may now load your flies into the wells and begin your experiment. Press stop video when you are done.

### Using the Address Manager to Set the IP Address:

If this is the first time a Raspberry Pi and user computer are used together, you will need to add the IP address of the Pi to the address book in the Pi Control software on that computer. The software already has some IP addresses in it – you can delete or edit them in the address manager as well. The IP address will also change and need to be updated if you plug the ethernet cable into a different jack or BCH updates it’s internet (this has happened before when they did an entire building power shutdown).

1. Initiate the software.
2. Click (anywhere) on the GUI that appears.
3. On the menu bar click address manager.
4. You can then edit or add addresses.
5. Close the GUI.
6. Quit the terminal (actually quit it from the menu bar, don’t just close out).
7. Restart the terminal and initiate the software – your Pi with the proper address should now appear from the dropdown menu.

## Using the Lights for Optogenetics

There are many options for controlling the lights, two are significantly more useful than the others: paired pulse and the stimulus constructor. Those are the ones I will describe here.

### Important notes for all protocols:

* Everything needs to be set to 0 except the color you are sending a command for.
* You cannot send commands for multiple colors at the same time.
* You cannot send the same command to multiple wells at the same time. Instead, you must type in the well number, hit send command, then type the next well number… and so on. If you do this too fast the command may not send properly, take a 1-2 second pause between commands (the perfect amount of time to start a timer?).
* For any given well, the most recent command will override any previous command.
* Only the green LED can change intensity. This is set by clicking the Update intensity values button, entering a new intensity, and clicking apply. The window will not go away but you will see in the terminal that the command has been sent. This sets all green LEDs to the same intensity; you cannot individually set the intensities. 0.178 is the default value.
* If you want the light to go on right away use a very small (0.001 ms) instead of 0.
* A pulse can be any length you want (including on the hours timescale).
* Things start to break down after 2.5 hrs of running continuously (the video might stop saving, the lights might overheat etc.). But it’s fine to run discontinuous experiments for many hours.
* You should pick a consistent naming scheme and stick with it – this will make it easier to convert videos in a batch later. I name all my videos in a format with the date, my initials, the first initial of the name of the box, and the number video I’m running on that box that day. For example, 20240129\_LMH\_1 indicates I recorded the video on 1/29/2024, that it was me who recorded (LM, Lauren Miner), the video was recorded on Heimlich (H) and it is the first video of the day (1).

Paired Pulse Experiments

All optogenetic experiments where you need one pulse (or the light continuously on for some period, the pulse doesn’t have to be short) or two pulses can be controlled using the paired pulse protocol. That is the protocol automatically selected for you on the dropdown menu when you start the program, so you just need to hit “run protocol”. To control the lights you’ll be using the left half of the window. You’ll see three columns (red, green, blue) referring to the three colors we standardly use. If you built the box with different colors those words won’t change, so you’ll have to figure out which corresponds to which.

A screenshot of a computer

Description automatically generated

To send a pulse type fill out the boxes in a single column, type in the well number, then press send command. The command will appear to the right in the command history section (if it doesn’t then hit send again). If you only want one pulse leave the rest duration and second pulse boxes set to 0.

### Stimulus Constructor

If you want to design your own stimulus that doesn’t fit within the paired pulse mode, use the stimulus constructor. To initiate the constructor, check the box next to run protocol then hit run protocol:

A screenshot of a phone

Description automatically generated

This will open the following window:

A screenshot of a computer

Description automatically generated

In the center at the top, just to the right is the new stimulus button. Click that to open this window where you can name the stimulus you want to create:

A screenshot of a computer

Description automatically generated

Then click new block to design your stimulus:

A screenshot of a computer

Description automatically generated

Click create block and then save stimulus to save your protocol. You can then select this protocol and send commands with the new command button.

## Delivering Heat Threats

Before you do any experiments with heat threats you need to determine what temperature to set the water bath to. See the confirming well temperature subsection in the standard maintenance portion of this manual (below).

You can use any protocol to run an experiment where you’ll only be delivering heat threats. In this case hitting run protocol just gives you access to the well timers and the camera control.

To deliver a heat threat, you need the master valve to be closed and the well to be switched to hot. Typically, we close the master valve right before delivering the first heat threat and then leave it closed until the last heat threat is delivered. I start the heat threat at the exact time (e.g. 8:00 min) and close it at the exact time (e.g. 9:00 min). You could also do something like start it 5 seconds before (e.g. 7:55 min) and stop it 5 seconds before (e.g. 8:55 min) if you wanted to account for the time it takes the well to heat up. That small of a delay has not affected behavior at all that I’ve seen, but you should note what you do in the methods and be consistent about it across all your experiments.

## Post-Hoc Analysis

Every time you run an experiment a log is created in the Pi Control software on the user’s computer. Create a folder called ‘log’ if it isn’t there and is giving you an issue. This log will record when timers were stopped and started and when commands were sent.

When you record a video and have checked the save video box, the saved video will be on the Raspberry Pi. You can view the contents of the Raspberry Pi by typing ls into the command line. Videos are saved as .h264 files and typically need to be converted before you can play them on a user computer. You can convert videos one by one or in a batch with the convert.sh code that comes with the Waterworks software you install on the Pi. Both methods rely on MP4Box, which requires a one-time install using the command:

sudo apt-get install -y gpac

To convert a single video (named pivideo in this example) then use the command:

MP4Box -add pivideo.h264 pivideo.MP4

To convert a batch of videos all starting with the same prefix, such as 202312 (for example, because I start all my videos with the date in YYYYMMDD format this would convert all videos from December 2023):

bash convert.sh 202312

You can access the videos on the Pi using the cyberduck software on a user computer (<https://cyberduck.io/>), which will allow you to download and delete files on the Pi. Files on the Pi need to periodically be deleted so it doesn’t run out of space. If the Pi is full, it will just stop saving videos and you will not get a warning message.

# Standard Maintenance

## Confirming Well Temperature

It is recommended that about every two weeks the temperature in the wells is checked using a thermocouple thermometer. Begin by filling the bath, flushing the lines, and allowing the bath to reach room temperature, just as you would prior to the first experiment of the day. Close the master valve, give a heat threat to the first well, and insert the thermocouple so that most of the sensor region is pressed against the floor (it may take some fiddling to get the position right). The temperature will begin to climb, rapidly at first and then it will level off. The well temperature is considered the temperature that it levels off at (which can be observed as toggling back and forth between two readings, e.g., 41.1 to 41.0 to 41.1, or a pause on a temperature of a few seconds. It should reach this point within 20-30 seconds. Repeat this process for all wells, keeping an eye on the temperature of the bath.

All the wells should read roughly within a degree of each other. If they are universally below the expected temperature, this likely means the bath is set too low. If they suddenly take too long to reach temperature, this likely means the pump is weak and should be replaced soon. However, you may also encounter problems with individual wells. A tell-tale sign that there is a problem with water flow to a given well is that the output of water back to the bath is very weak compared to a functioning well. Calcification or buildup of algae is the most likely culprit, and this can be addressed by looking at the tubes and replacing them as needed. If the problem tube goes to multiple wells, this could explain a few, but not all wells, having an issue. Kinks in tubing are another, less common, source of water flow issues.

## Bath & Tube Maintenance

Water baths should be kept filled to prevent pump burn out or bubbles in the wells. If bubbles do get in the wells, gently lift the behavior chamber, and rotate it around like a nutator. You might do this while giving a heat threat to just the well of concern (this will increase the water pressure to that well). You might also lift all the tubing so that gravity carries the bubbles along the lines. See the air bubbles section for more details.

Use deionized or distilled water to fill the baths, as this will limit microbial growth. Commercial water bath treatments are available to limit microbial growth, ethanol also seems to work. The best fix, however, is just to replace the problematic tubing, especially for treating calcification. DO NOT USE VINEGAR. Vinegar will de-calcify a little bit, but not nearly as effectively as needed and more importantly it will eat away at the liquid gasket that creates the seal between wells.

### Leaks

The most common source of leaks from the external tubing area is an imperceptible crack in a stopcock. This is caused by excessive pressure going to the stopcock for an extended period of time, i.e., leaving the master valve closed overnight when the box is not in use. It can be prevented by making sure to always open the master valve after completing an experiment, so the hot water has somewhere to go, and the pressure is relieved.

To replace a stopcock (best with two people):

1. Turn off the power to the pumps.
2. Get a needle-nose plier and a replacement stopcock.
3. Put an absorbent pad or bucket underneath you.
4. Turn the stopcock on the box so that hot water is not flowing (it’s likely already in this position, do the same for the new stopcock.
5. Pinch the tubing where the hot water comes from to restrict as much water flow as you can, twist off the old stopcock just from this connection and twist on the new one.
6. Pinch the tubing coming from the behavior chamber to stop as much water flow as you can, twist off the old stopcock from this connection and twist on the new one. Turn the valve of the new stopcock so that hot water flows to/from the well (this will prevent water from shooting out the other end). Turn the valve of the old stopcock (now connected to only one tube) the same way so that the off is in the same direction as the remaining tube.
7. Pinch the tubing coming from the room temperature water source (the only remaining tube), twist off the old stopcock and twist the new stopcock on to the connector to the remaining tube (this should be the stopcock connection that swivels so you don’t have to turn the whole piece).

### Air bubbles

The simplest way to get air bubbles out is to give a heat threat to the well with the issue. Because there is a higher pressure coming from the hot water pump forcing water through only one well as opposed to the room temperature water pump whose force is divided between twelve wells, this is usually sufficient to chase the bubbles out if you leave it for a few minutes. If it doesn’t work on its own, then you’ll want to tilt the inner behavioral chamber in different directions to “loosen” the bubble and get it moving towards the out tubing for that well (tilt the box so gravity makes the bubble move closer to the out tubing). You may also want to lift the main tubing for all the wells up and down a few times to get bubbles out of them.

In drastic cases you’ll need to gently force bubbles out of the water reservoir. Take the outer box top off so you have unrestricted access to the base and the behavior chamber. Unscrew each of the nuts. Hopefully the screws won’t fall out because there should be some tape on the screwheads underneath from when the box was built, but if they do then you’ll need to retrieve them after and might have to take apart the base. Remove top layers of the behavior chamber so that the diffuser paper well bottom layer is exposed. Set the stopcocks to deliver a heat threat to the well with the issue. Identify where the “OUT” tubing connection is underneath. Using two fingers push the air towards the “OUT” tubing like you were popping a pimple (gross but accurate). Be patient and don’t apply to much pressure or you could damage the diffuser layer. You could also try flipping the entire behavior chamber upside-down if it’s not working, but then you’ll need to realign the columns after. When the bubble has been removed, re-attach and secure all the layers of the behavior chamber and re-attach the outer box top.

## Cleaning

Depending on the behavior you are testing, you may need to clean your behavior chamber. If you are just running mating behavior it will be fine for months, and you only need to clean it if the camera’s vision is impeded because the well door is not clear.

Take the outer box top off so you have unrestricted access to the base and the behavior chamber. Unscrew each of the nuts. Hopefully the screws won’t fall out because there should be some tape on the screwheads underneath from when the box was built, but if they do then you’ll need to retrieve them after and might have to take apart the base. Remove top layers of the behavior chamber so that the diffuser paper well bottom layer is exposed. You can clean the acrylic layers you removed with warm soapy water and use a rag to wipe off the diffuser paper layer. I do not use ethanol to clean any part of the chamber because flies find it aversive. When you are done, re-attach and secure all the layers of the behavior chamber and re-attach the outer box top.

# Troubleshooting

## Connection Issues

If on the computer you use to control the Pi and Arduino, you hit run protocol and nothing happens, look at the terminal. The most common connection issue will appear as an “ssh error” where in the terminal you’ll see a line that says:

AttributeError: ‘Raspberry\_Pi’ object has no attribute ‘ssh’

An example of which is below

A screenshot of a computer program

Description automatically generated

This means that something is not connected to the internet, most likely your computer.

1. If you’re in the Crickmore Lab, make sure your Wi-Fi is set to TCH (which requires specific security software and a BCH ID and password to access) not chbguest. If you’re not in the Crickmore Lab, make sure the Raspberry Pi and your computer are on the same internet system. Quit the terminal and try again.
2. If that doesn’t work, it could be that either the Raspberry Pi is not connected to the internet, or you have the wrong IP address for it. Using the terminal on the Raspberry Pi, type in the command line:

ifconfig

This will bring up a series of text. In the second “paragraph”, on the second line you should see something like:

Inet addr: 10.32.64.168

That is your IP address. If you don’t see that or it gives you some error, then the Pi is not connected to the internet. Try re-attaching or replacing the ethernet cable or trying a new jack. Always shutdown your Raspberry Pi before unplugging or plugging in any cables with the command:

sudo shutdown –h now

If the Pi is connected to the internet and you have an IP address for it, check it against the IP address you are trying to connect to. In the screenshot of the terminal from the user computer above, you can see the after the first line it says:

(’10.32.64.168’, ‘Flick’)

10.32.64.168 is the address of the system named Flick (named after the character in Disney’s A Bug’s Life) that I am trying to connect to. These two addresses should match. If they don’t, update the address for the Raspberry Pi via the address manager (which comes up on the top bar when you click the GUI for controlling the boxes, see the section on using the address manager under the section on using Waterworks for behavioral experiments), quit the terminal and try again.

## Camera Issues

### The “start video” command gives a black screen on the monitor

1. First check whether this is a problem with the IR:
   1. Gently open un-tape the camera from the top of the box and point it at yourself… if you suddenly see an image then it’s a problem with the IR.
   2. Check if it is a problem with the IR lights by using a different light you know works connected to that light’s original power source. If this works, then it’s either your lights are burned out or the power source is burned out. Use the working light connected to the power source in question and/or the working power source connected to the light in question to figure out which.
   3. If it is not a problem with the lights, it is likely a problem with the filter on the top inside of the box. The filters need to be IR 850. In the past IR 910 filters have been known to filter out too much of the light and you get a black screen.
2. Check that the camera is enabled.
   1. From the desktop view:
      1. Click the raspberry pi icon in the top left.
      2. Click “Preferences” from the drop-down menu.
      3. Click “Raspberry Pi Configuration” from the drop-down menu.
      4. Select the “Interfaces” tab.
      5. First on this tab is the camera, make sure the enable bubble is selected.
   2. From the command line view:
      1. Use the following command to access the configuration settings for your pi:

sudo raspi-config

* + 1. Select the camera option.
    2. Select enable.
    3. When you exit, it will ask you to reboot, do so.

1. Check whether the camera is capable of taking a photo.
   1. Use the command:

raspistill -o testimage.jpg

* 1. If it works you should see the image appear on the monitor briefly, then the image will appear in the directory, which can be observed using the command ls. Either way continue to step 4.

1. Check whether the camera is capable of taking a video.
   1. Use the command.

Raspivid -o testvideo.h264

* 1. If it works you should see the video record for the specified time and see the saved video in the directory.

1. Update the pi:
   1. Use the command:

sudo apt-get update

* 1. Use the command:

sudo apt-get update

1. Check that the camera hardware is installed properly.
   1. Is the ribbon connector attached in the right direction?
   2. Is the ribbon connector firmly seated and straight in its socket?
   3. Is the camera itself connected to the little PCB it’s on?
2. Reinstall the same camera (just in case).
3. Replace the camera with one that you know works.
   1. If camera replacement doesn’t work, there is probably an issue with the larger pi.

## General Pi Issues

### Software

First, double check that your microSD card is properly in its slot and hasn’t popped out (pushing it in pops it out). Next try powering down the pi, unplugging everything, and letting it sit for a bit before restarting it. To safely shut off a pi, use the command:

sudo shutdown –h now

While annoying, it’s true that most software issues not solved by a restart can be solved by wiping the SD card (or getting an entirely new one) and reinstalling the software (i.e., starting over). This is true when just some functions are not working and if the SD card has been corrupted (in which case just use a new one). If restarting did not work, try to find distinguish whether the problem is with the Raspberry Pi itself or with the microSD card in the Raspberry Pi. Visually inspect the Raspberry Pi for water damage and make sure it’s getting power – the lights on the pi can inform you of its status (<https://pimylifeup.com/raspberry-pi-red-green-lights/>). See the hardware section below for more. To check the SD card, try it in a different pi you know is functional if that’s an option for you. Make sure you safely power down any Raspberry Pi before removing or inserting an SD card.

If you’re still troubleshooting and think it’s the SD card, the best solution is to get a new SD card and use an image from a working pi to set it up. See the subsection “Preparing the Arduino and Raspberry Pi” of the assembly portion of this manual.

#### Black monitor

If your problem is that the monitor is always black, it could be that the monitor did not have power when the pi was turned on (such that the pi booted as ‘headless’). Unplug everything, make sure the monitor is on and has power, then plug the pi back in.

### Hardware

1. Check each connection (or just the connection to the problem part). Unplug it, use a duster to clean it off, and re-connect that wire. If you can source the problem, then just replace that cable entirely.
   1. Before unplugging the power cable, shut down your Raspberry Pi with the command:

sudo shutdown –h now

1. Check the power source for the Raspberry Pi. Some power sources have a red light that should be on when it’s plugged in. All Raspberry Pis have two little lights on them – one green and one red. When plugged in and powered properly, the red light should be on.
   1. If the monitor says directly (top right corner) that the pi is not receiving enough power and replacing the power supply does not solve this issue, the problem is with the pi itself. You must replace the pi.
   2. The green light being on but not the red light indicates the pi is not receiving enough power (the red light must be on)

## Lighting Issues

### Lights spontaneously flash

The Raspberry Pi exerts control over the lights telling them to remain off (which is why if you unplug the pi all the lights turn on). So, this could mean there is power issue with the Raspberry Pi (more likely if this is an old board). Otherwise, if the board has had water on it recently, this could indicate a short, which you likely can’t fix without replacing the whole PCB. If it is a new board, check for bad solder joints or a problem with the code on the Raspberry Pi or Arduino.

### Light(s) will not turn off

#### If this is all the lights on the board:

1. Check that the software on the Raspberry Pi works. Plug a different, functioning board/Arduino into the Raspberry Pi in question and see what happens. If that option is not available, check if commands generally work on the Raspberry Pi (can you start the video etc.) to see that the software was downloaded properly. If nothing or multiple things don’t work, reinstall the software and/or see the pi troubleshooting section. Don’t leave the power to the lights on while you do this, it might get hot.
2. Check that the code on the Arduino is installed and working properly. You can put this Arduino on an otherwise working pi and board or put an Arduino from an otherwise working set-up on this set-up to determine if the issue is the Arduino or something else. Otherwise, reinstall the software on the Arduino.
3. If that still doesn’t work, check that everything is soldered properly (good solder joints at the Arduino connection and no solder is touching the next bit of solder). If this is following an incident of water on the board, it could help to just heat up each bit of solder to re-establish any broken connections.

#### If this is just one light or a few:

1. First try unplugging the BuckPuck, leave it unplugged for a while (overnight – multiple days), then replace it with a brand-new buck puck.
2. If that doesn’t work, the quick and dirty solution is to just unplug the BuckPuck for that LED and just don’t use it. In most cases, this is what I would recommend. You could try to find the source of the issue, which could be a short or a bad solder joint/other connection. Unless it’s a new circuit board, it’s most likely a short. If it’s a short you’re probably out of luck so I’ll refer you back to the quick and dirty solution. If it’s a fixable connection issue, you can try to find and address it (by re-soldering a wire or tightening a screw terminal somewhere).

### Light(s) will not turn on

#### If this is all the lights on the board:

1. Check the power source for the lights. Replace it with a new or working power source (the IR lights use the same power source; you could steal that if you don’t have a new one handy just to check).
2. If this is a new board, you soldered something wrong. First check that everything is soldered in the right place and attached properly. If that doesn’t solve it, go through under an illuminated microscope, and check your solder joints, starting with the barrel jack and Arduino connections.
3. If this is an old board… something happened to it… probably water…RIP. You could check a few LEDs to confirm that they’d work just to be sure, and you could check the solder joints as described in step 2. But probably it’s a loss and you should scrap it for whatever parts work and assemble a new PCB.

## Water flow issues

### Water is not flowing at all

#### If water is not flowing at all because you just plugged in a pump, or the bath was empty while a pump was running, it will sort itself out I promise. There is also nothing you can do really to help it, it’s because there is an enormous air bubble in the lines somewhere (probably immediately after the pump). The air bubble may be so big you can’t tell it’s an air bubble because you can’t see the start or end of it. It is not worth taking a part the tubing to try to let the air bubble out, you’ll just create a mess. Make sure the water bath is full and leave it plugged in. Come back in an hour or two.

### Water is not flowing to a particular well

You might not notice this problem until you do a temperature check or notice weird behavior in a well (hopefully it’s the former). The problem is almost certainly that there is a clog in the line, it’s just a matter of where…

#### If this is a brand-new box you just built:

Sorry to say but most likely you got some super glue/liquid gasket/plastic cement where it was not supposed to be and that’s blocked the water flow. The most likely place for this to happen is at the connector from the smallest tubing into the acrylic. Unfortunately, the only way to try to solve it is to take the whole thing apart and try to clear out that connector with a needle or pin. If that doesn’t work, you’ll need to remake those layers of the behavior chambers.

#### If this is an old box:

Firstly, I would not recommend trying to find the blockage by using any kind of dye in the water. Secondly, make sure you get the electronics far away before you try messing with any of the tubing. You’ll want gloves, absorbent pads, and ideally a bucket (the large rectangular Nalgene containers used for autoclaving are ideal because you can sit all the tubing and even the box inside it to contain the spill). Usually, you can tell where the problem is by the appearance of the tubing. Dark red or black in the main tubing is algae, try replacing that (wear gloves). Calcification is the next most common problem – the tubes will be an opaque white color and may even feel hard. That is usually a problem with the skinny tubing, and it could be that the calcification is blocking the tube or the connector to the behavioral chamber plate. Try replacing the skinny tubing first and then try clearing out the connector with a needle or pin. If you can’t find an issue with the tubing by eye and are fairly convinced that it’s something with the water reservoir in the behavioral chamber itself, the only solution is to replace that portion of the chamber with new parts.

1. LEDs can be any three colors, these colors are selected most often [↑](#footnote-ref-1)
2. The exact material we use will soon be discontinued. See the detailed parts section below for advice on selecting a new option. [↑](#footnote-ref-2)
3. If you plan on using two water baths order two and double the water bath clips. Then omit the room temperature water reservoir and water reservoir clips. [↑](#footnote-ref-3)
4. https://www.arduino.cc/en/guide/introduction [↑](#footnote-ref-4)
5. https://www.arduino.cc/en/software [↑](#footnote-ref-5)
6. For more about LEDs see this guide: https://www.ledsupply.com/blog/what-you-need-to-know-about-leds/ [↑](#footnote-ref-6)
7. For more information visit: https://www.ledsupply.com/leds [↑](#footnote-ref-7)
8. http://www.ledsupply.com/blog/understanding-led-drivers/ [↑](#footnote-ref-8)
9. https://instrumentation.hms.harvard.edu/new\_users/ [↑](#footnote-ref-9)