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Builder & User Manual

[Draw your reader in with an engaging abstract. It is typically a short summary of the document.   
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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 decision of a fly when drives are put in conflict. This system features a 12-chamber behavioral arena that allows for testing of multiple mating pairs simultaneously. The temperature within the chamber is set by the experimenter, making it compatible with thermogenetic tools. The temperature can also be acutely changed during the experiment to act as a threat. The current system allows for two temperature options during any given experiment, controlled manually by the experimenter. In addition to modulating temperature, the waterworks system also features three LEDs positioned underneath each chamber which can be used to activate optogenetic tools genetically encoded in the fly or to deliver a light stressor.

This manual describes first describes the components and design of the waterworks system, complete with comprehensive instructions to build a new system from scratch. It then describes how to use the system to control both temperature and lights. Finally, it discusses how to analyze and plot data obtained using this system. While originally designed to study drosophila mating termination, it is amenable for studying a variety of *Drosophila* behaviors such as courtship and female receptivity.

# Design Overview

## Circuit controlling LEDs

## Water system for heat threats



A schematic of the system for controlling the temperature is above. It features two water sources: a room temperature reservoir and water bath that can be set to given temperature. If desired the room temperature reservoir can be swapped for a second water bath. The system is designed such that room temperature water constantly flows from the room temperature water reservoir into twelve paths each leading to one of the twelve “in” stopcocks controlling flow through a small tube leading to a well. The water then exits the well through a second small tube leading to one of the twelve “out” stopcocks controlling the flow out and back to the water reservoir.



Diagram

Description automatically generatedThe 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 valve. The stopcocks allow the user to choose whether the water comes from the room temperature or hot water source.

Diagram

Description automatically generatedThe system normally runs room temperature water, so when there are no heat threats given that valve is left open so that hot water can return to the bath even when all the routes to the wells are closed. This prevents excess pressure from building up when the box is not in use, such pressure could cause cracks in the stopcocks leading to leaks or burn out the pumps causing inefficient heat threats. When giving a heat threat this valve can be closed to make sure all the hot water is sent to the wells. To give a heat threat, the user simply turns the switch on both stopcocks corresponding to a given well.

# Components

This section identifies the parts and tools required to create a new waterworks behavioral arena.

## Parts Shopping List

Quantities listed build 1 box unless indicated by an asterisk:

\*Material can be used for multiple boxes

Also see accompanying excel spreadsheet

### 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/ |
| 3D printing stuff | McMaster-Carr # |  | McMaster-Carr |  |

### 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 | 2 | 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 |
| Cardboard box or other support for underneath the circuit board | We use the cardboard tray that our fly food comes in | 1 |  |  |
| Small screw driver |  |  |  |  |

## 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, 7 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.

**Arduino Mega**

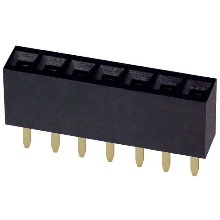
A picture containing text, electronics, circuit

Description automatically generatedA 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), written in the Arduino programming language[[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, see and edit code you will need the IDE software[[5]](#footnote-5) and an adaptor to connect it to your computer (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).

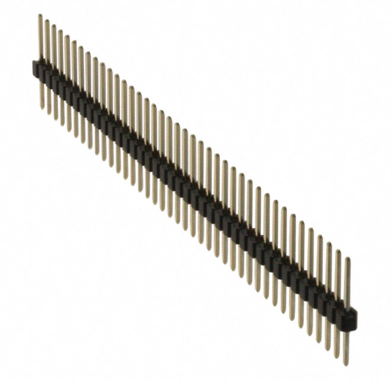
**DC Barrel Jack Connector**

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 connect the buck pucks to the rest of the circuit board while allowing for easy replacement should a buck puck 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. 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 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**

Text

Description automatically generatedBuck pucks 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 buck puck and each buck puck has 7 inputs (hence 7 pins). LED+ and LED– send current to and receive current from the LED, respectively. Control is an input that comes from {?}. The output current of the buck puck will be 0 when the control is high. Reference is an output that provides the operating voltage for our Arduino. Vin+ and Vin- are the current coming from and back to the power source.

### 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 transfers the heat from the water reservoir below 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. You may also less or more light and heat passing through depending on your choice. It will be important to take new measurements when calibrating your system.

# 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 New\_Gerber\_Waterworks.zip to the manufacturer’s website (gerber files must be formatted as a zip file)
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 will be where your acrylic is in the machine.
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.

## 3D Printing the Light Tubes

We use a 3D printer to manufacture the

# 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). I prefer the #### tip.
   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 the lit 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.
   2. Note that the board has 8 holes, but the buck puck 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).
   3. 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).
   4. Solder it to the board
   5. Repeat until all are soldered on
3. 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
   3. You’ll need to use a lot of solder to fill the holes
4. 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.
5. 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.
6. 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:

## Behavior Chamber Assembly

## Preparing the Arduino and Raspberry Pi

#### Creating an image of an operational 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.

## Preparing the computer that will control the pi and Arduino

# Using Waterworks for Behavioral Experiments

## Starting the Software & Setting up for Experiments

## 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.

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.

## 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).

Before you start a batch of heat threat experiments, you should 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). 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.

Diagram

Description automatically generatedTo 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.

# 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 the majority 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 build up 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. Use DI 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.

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 up all the tubing so that gravity carries the bubbles along the lines.

### 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 really drastic cases you’ll need to gently force bubbles out of the water reservoir

# Troubleshooting

## Connection Issues

If on the computer you use to control the Pi and Arduino, you hit

## 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 (unlikely) 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 image (i.e., starting over). This is true of some function just not working, or if the SD card has been corrupted (in which case just use a new one).

If that didn’t work, try to find distinguish whether the problem is with the raspberry pi or with the microSD card in the raspberry pi. Visually inspect the pi for water damage and make sure it’s getting power – the lights on the pi can inform you of it’s 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 a option for you. Make sure you safely power down any 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 pi with the command:

sudo shutdown –h now

1. Check the power source for the pi. Some power sources have a red light that should be on when it’s plugged in. All 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 have to 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 has to be on)

## Lighting Issues

### Lights spontaneously flash

The 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 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 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 pi works. Plug a different, functioning board/Arduino into the pi in question and see what happens. If that option is not available, check if commands generally work on the 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 buck puck, 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 buck puck 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. It’s probably a short and 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 it following the procedure for when one light is out (below).

### 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

#### 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.

#### If 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 box is old:*

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)