CHARISMA Imaging Robot User Manual

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Introduction

CHARISMA (**C**aenorhabditis **H**ealthspan and Lifespan **A**cquisition by **R**obotic **I**maging of a **S**erially **M**onitored Plate **A**rray) is a platform for automatically imaging an array of plates for the purposes of long-term monitoring of *C. elegans* populations or individuals. Part 1 of this manual describes hardware construction and requirements. Part 2 of this manual describes the use of a Matlab Graphical User Interface (GUI) to control the system and automate image acquisition and data analysis.

Hardware Requirements

The required parts are listed in "ImagingRobotChecklist.xlsx." Additional recommended tools and materials are described in Part 1: Before You Begin.

Software Requirements

Computer (minimum system requirements)
Windows 7 64-bit
Intel Core i7-2600 CPU @ 3.40 GHz
8 GB Ram

Software
Matlab 2014a license or newer

Part 1: Hardware

Here we describe how to construct the CHARISMA imaging robot. Please see the associated excel file for a complete list of parts required to construct the system.

Before You Begin

To complete construction of the CHARISMA hardware, you will want to have the following materials and tools handy:

- **Drill press and hand drill and set of drill bits**
- **A portable vice**
- **Tap wrench with 4-40 and 8-32 taps**
- Soldering iron and solder wire
- 16-gauge electrical wire
- Wire cutters with crimping ability (Recommended Pair)
- Electrical tape
- A set of hex and flathead screwdrivers
- A multimeter

Items in **bold** are not required if custom hole drilling/tapping and cuts are performed by a local machine shop. Underlined items are highly recommended if not required for successful construction of the robot.

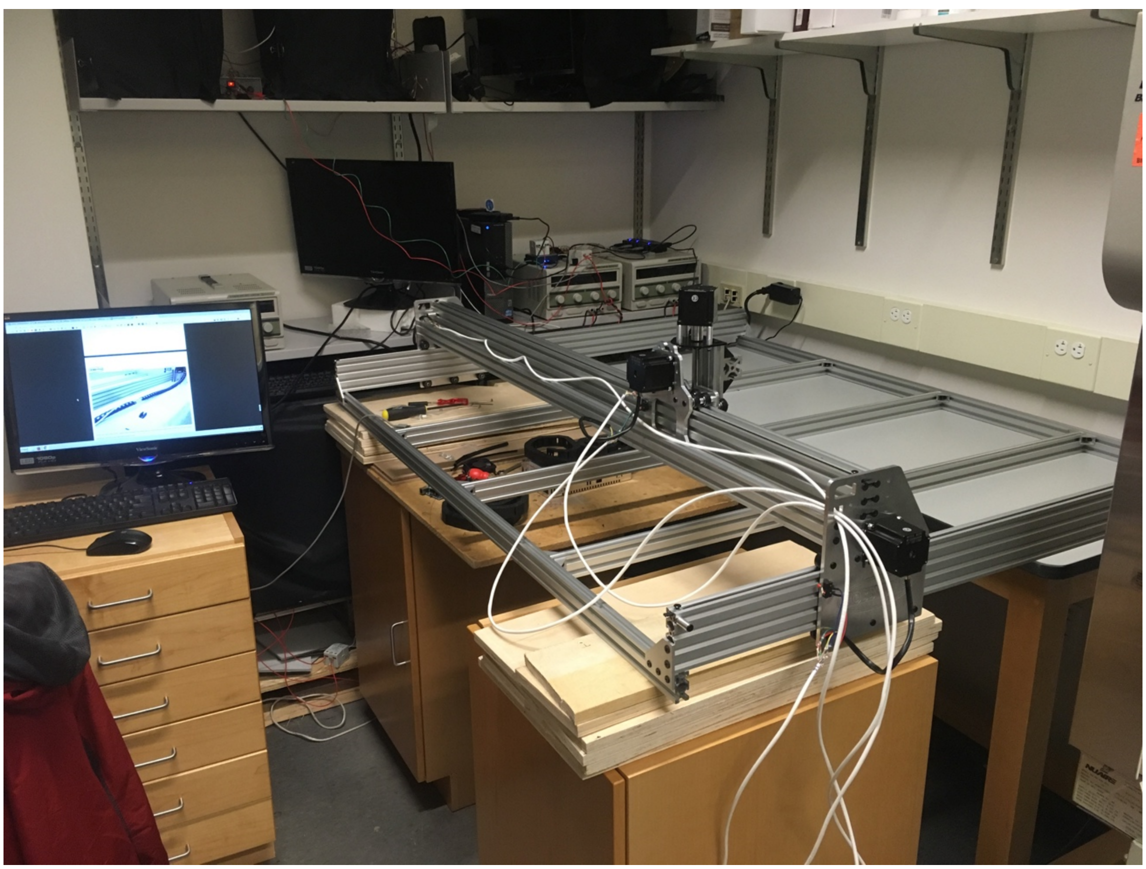
CNC Robot

We use a DIY CNC machine purchased from SMW3D: https://www.smw3d.com/ox-cnc-kit/

We ordered a custom footprint of 1500 mm (X-axis) x 1250 mm (Y-axis), which is sufficient to image 91 plates. Note: the maximum CNC footprint available from SMW3D is 1500 mm x 1500 mm, which corresponds to a capacity of 111 plates.

Note: The CNC robot can rest either on lab benches or tables (as shown below) or a platform can be built out of 1.5" aluminum extrusion into the light and temperature enclosure (see Table of Contents). **It is recommended to construct the enclosure prior to assembling the robot, regardless of whether the robot will rest on a table or aluminum extrusion platform.** Constructing the enclosure will be more difficult with the robot already assembled, as it is difficult to move the robot once assembled. The benefit of using aluminum extrusion is that multiple platforms can be constructed, allowing for multiple robots to run in the same enclosure (at least two).

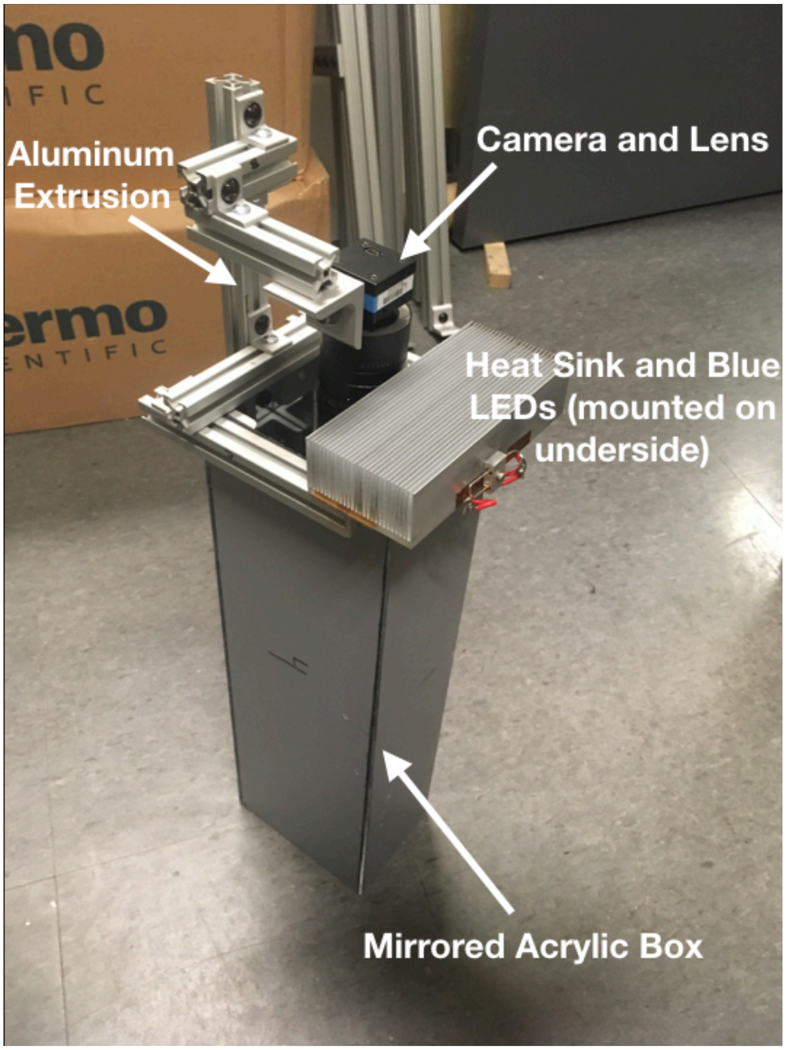
To construct the machine, follow the build instructions supplied by SMW3D with the exception of not adding the spindle to the z-axis stage. Once everything else is constructed, the custom gantry we designed which holds the camera, blue and red LEDs, and mirror box can be mounted to the z-axis stage (see next section).



The large format CNC comes with 3 cross struts (~4' long 20 mm aluminum extrusion) to support the body of the machine (shown above). We recommend purchasing at least two additional cross struts to support the acrylic platform on which the plates will rest. These additional struts should be fastened to the CNC using T-nuts and corner brackets sized for 20 mm extrusion. All 5 struts should be approximately evenly spaced across the width of the CNC machine.

Custom Gantry

In this section we will describe how to construct the custom gantry which consists of a camera, illumination, stimulation LEDs, and mirror box for focusing the stimulation light onto the plates (shown below).



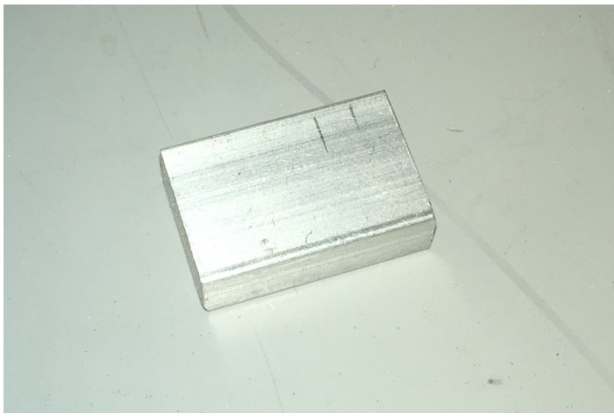
The gantry consists of the following:
1) **Mirrored acrylic box** (four panels glued together, 2 pieces 18.5" x 4.125", 2 pieces 18.5" x 5.5") which generates a uniform blue illumination at the plate. A small hole should be drilled in one panel of mirrored acrylic for the red LED wires.
2) **Red illumination LEDs** attached on the inside of the bottom of the mirror box.
A red LED strip should be cut with a scissor to fit inside the mirror box. The strip should be coated with white silicone to diffuse the light.
3) **Digital camera** (we use 5 megapixel USB camera manufactured by The Imaging Source) with lens (25 mm focal length)

4) **Three high-power blue LEDs** attached to an aluminum heat sink. Each LED requires two 4-40 nylon screws to attach it to the heat sink. The heat sink therefore needs to be tapped with two 4-40 screw holes for each LED. Superthin insulating tape needs to be placed between the LEDs and the heat sink prior to holes being drilled (this is critical). A thermal fuse box should be attached with another 4-40 screw, so the heat sink requires a total of seven 4-40 screw holes drilled and tapped. A computer fan should be fastened to the heat sink with silicone and wired in parallel with red illumination LEDs to prevent overheating.
5) **Thermal fuses** attached to the heat sink to prevent overheating in the case of electrical or software error. Two thermal fuses should be wired in parallel with each other and in series with the blue LEDs.
6) **20 mm aluminum extrusion** supports to mount camera, heat sink, and attach the custom gantry to the robot z-axis stage. Required pieces: 1 x 24" long, 3 x 6.5" long, 2 x 3" long and 1 x 7" long.

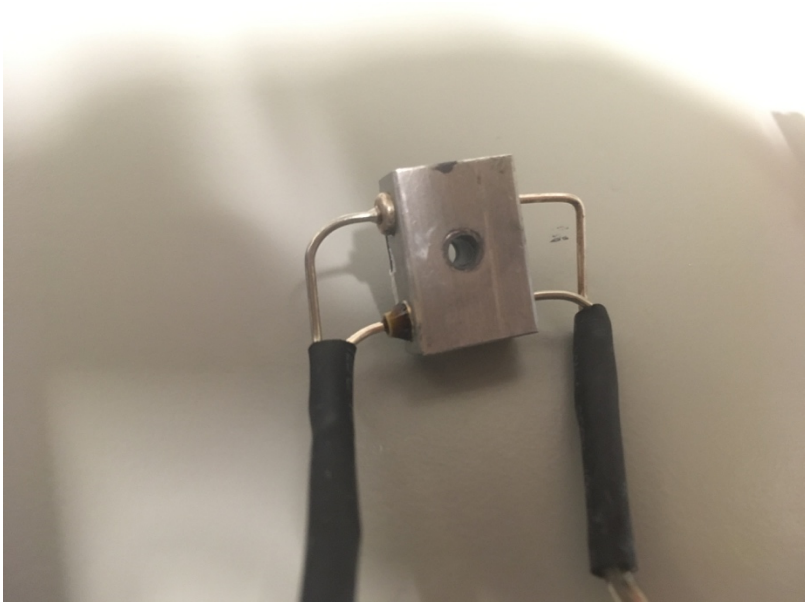
The following pages contain detailed instructions on construction of the custom gantry.

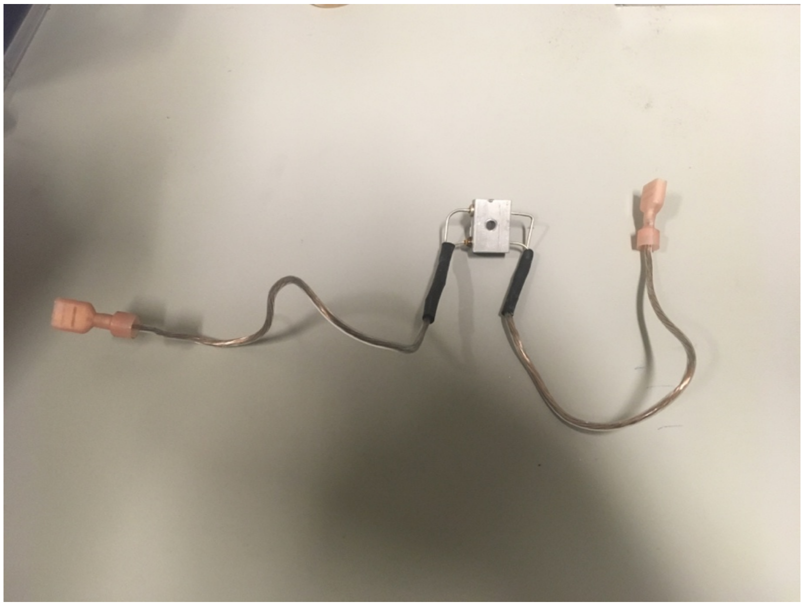
Thermal Fuse Box Construction

Begin with an aluminum block of size 1/2" x 3/4" x 1/4". We have this piece cut from an aluminum bar of size 1/2" x 1/4" x 1'.
Drill three holes in the block: 2 holes with a #17 drill bit and 1 hole with a #32 drill bit as shown. The large holes should be located on the 1/4" x 3/4" face of the block. The small hole should be located on the 1/2" x 3/4" face of the block.



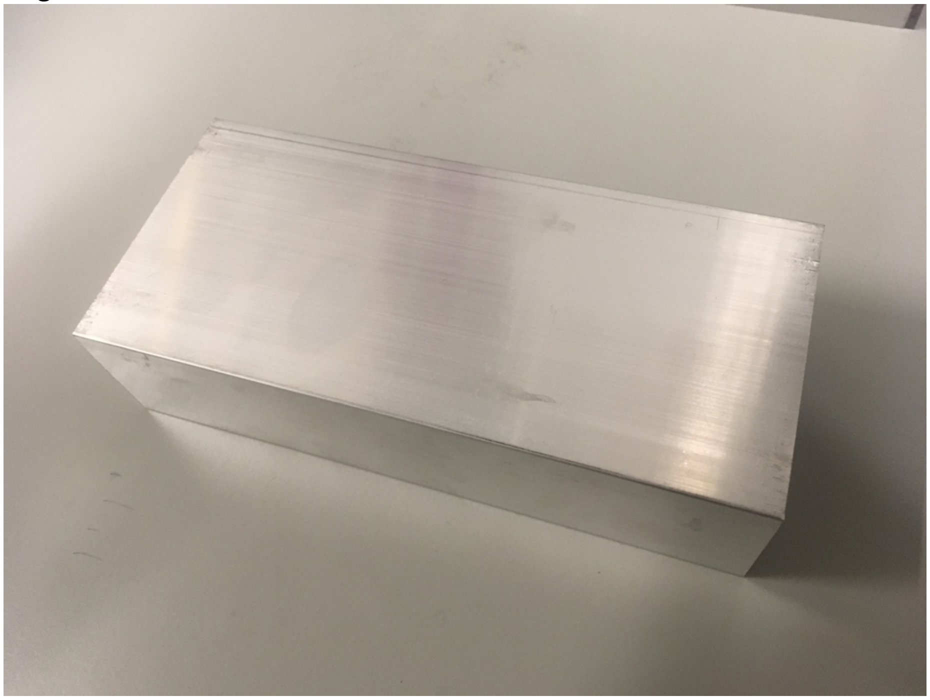

Next, insert two thermal fuses through the large holes and solder these in parallel to a 16 gauge wire. Finally, add a quick connector by crimping it to the ends of the wire.



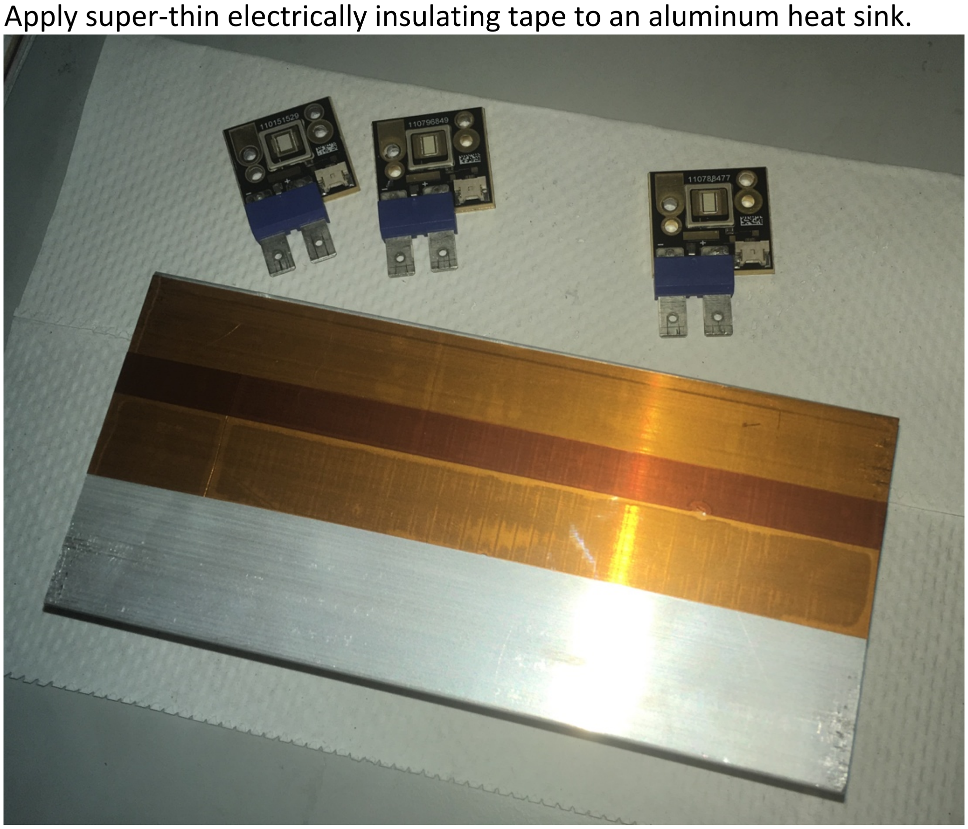


Blue LED Heat Sink Construction

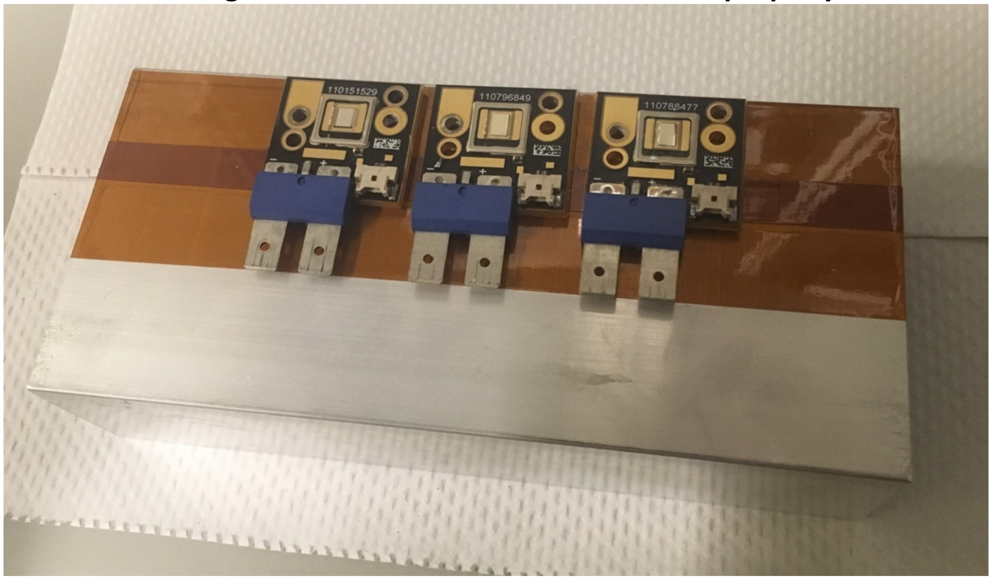
Begin with an aluminum heat sink.



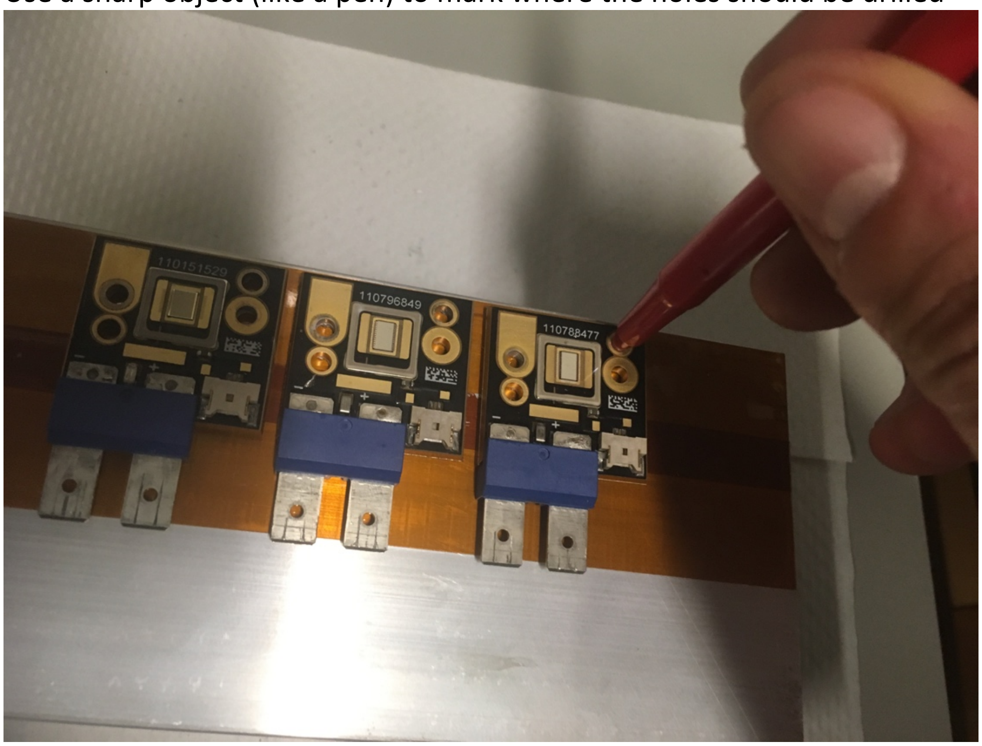
Apply super-thin electrically insulating tape to an aluminum heat sink.



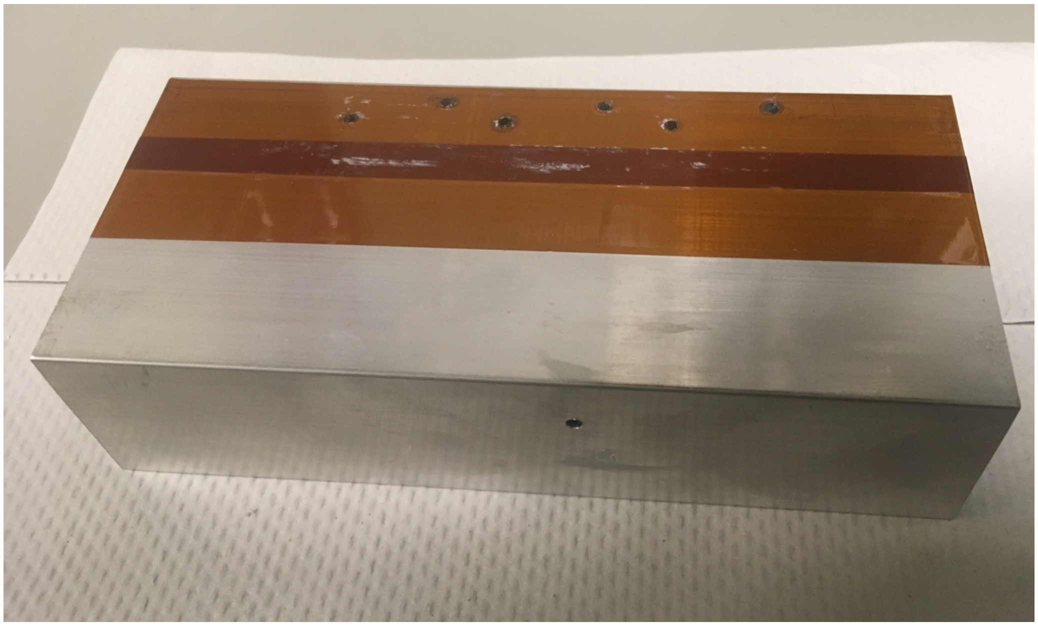
Align 3 blue LEDs as shown. **The LEDs should not be touching in their final positions. If the LEDs are touching each other the device will not work properly.**



Use a sharp object (like a pen) to mark where the holes should be drilled

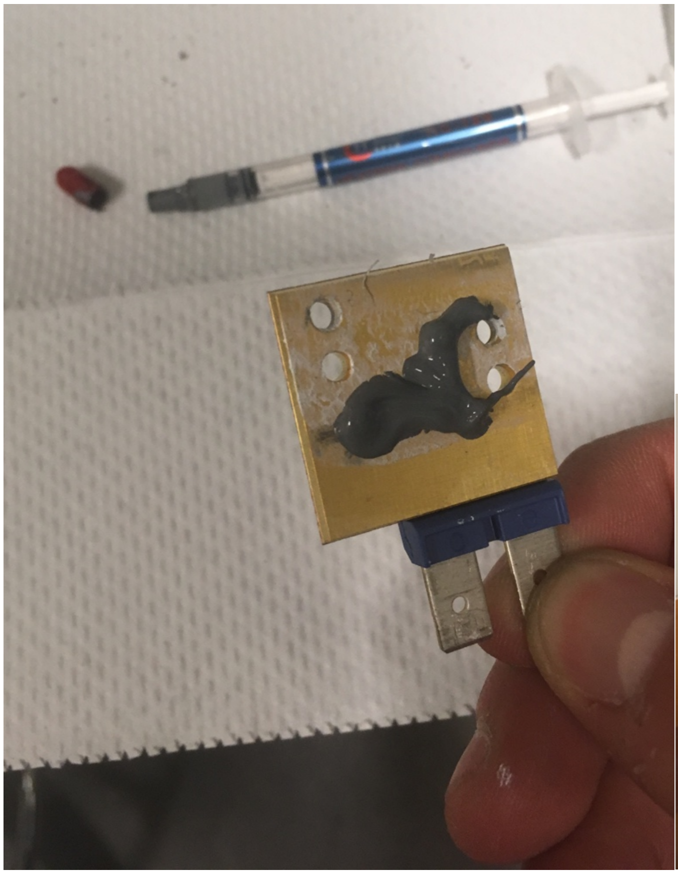


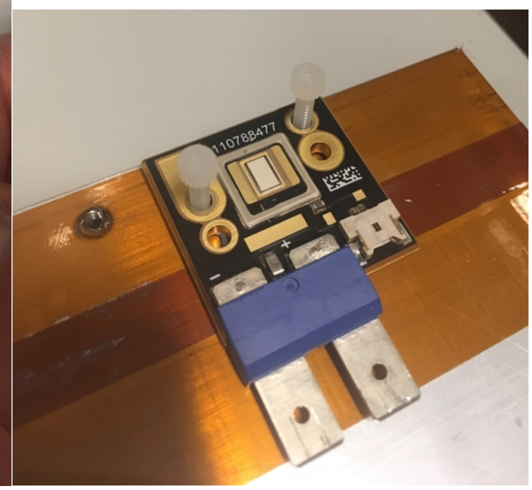
Drill the 6 holes for the LEDs with a #43 drill bit. These holes will be tapped with 4-40 tap. Drill a 7th hole on the side of the heat sink.



Drill 2 holes on the opposite side of the heat sink as shown with #29 drill bit. Ensure these holes are at least 4.25" apart. This hole will be tapped with 8-32 tap. Tap all holes with the appropriate tap.

Apply a dab of heat sink compound to the back of each blue LED. Fasten the 3 blue LEDs to the heat sink using 1/2" long 4-40 nylon screws.

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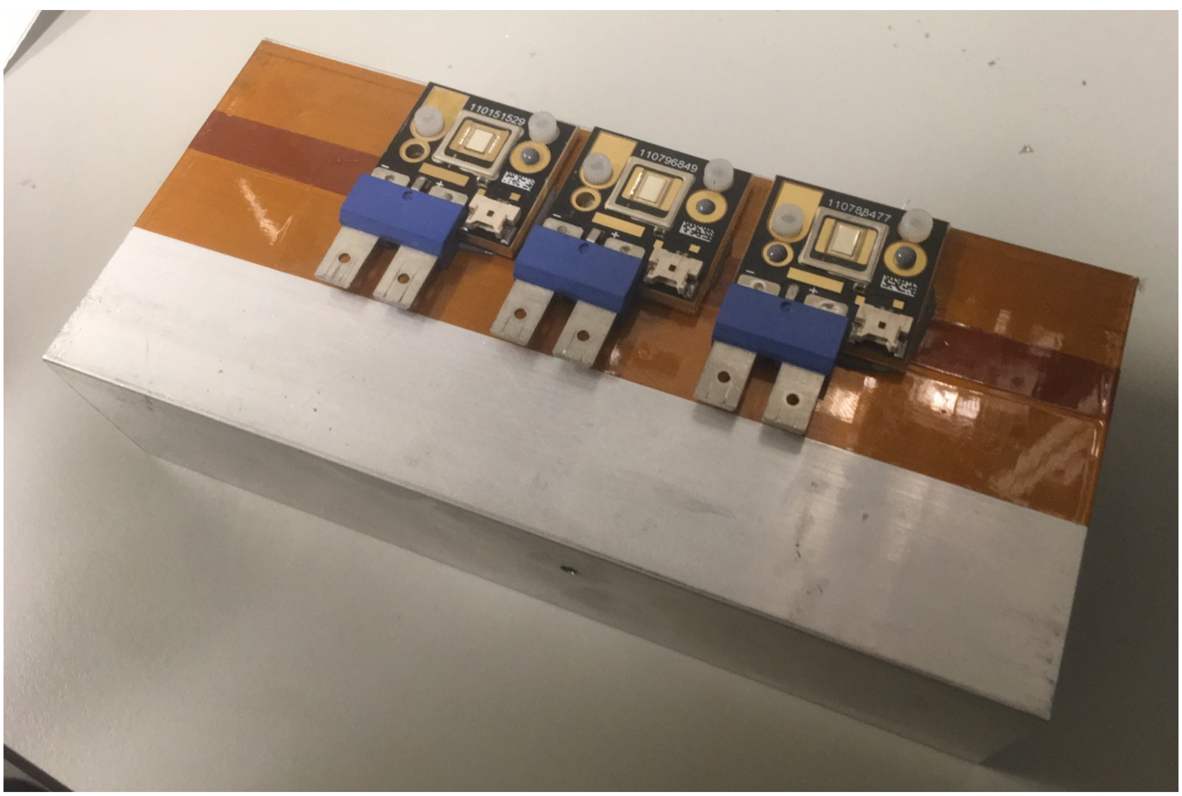
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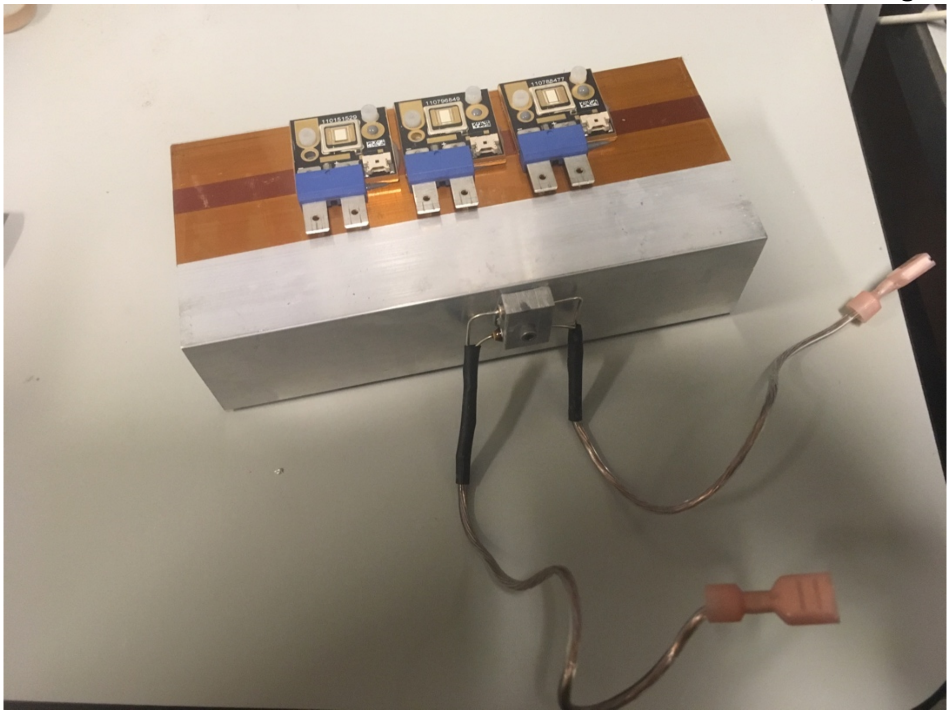
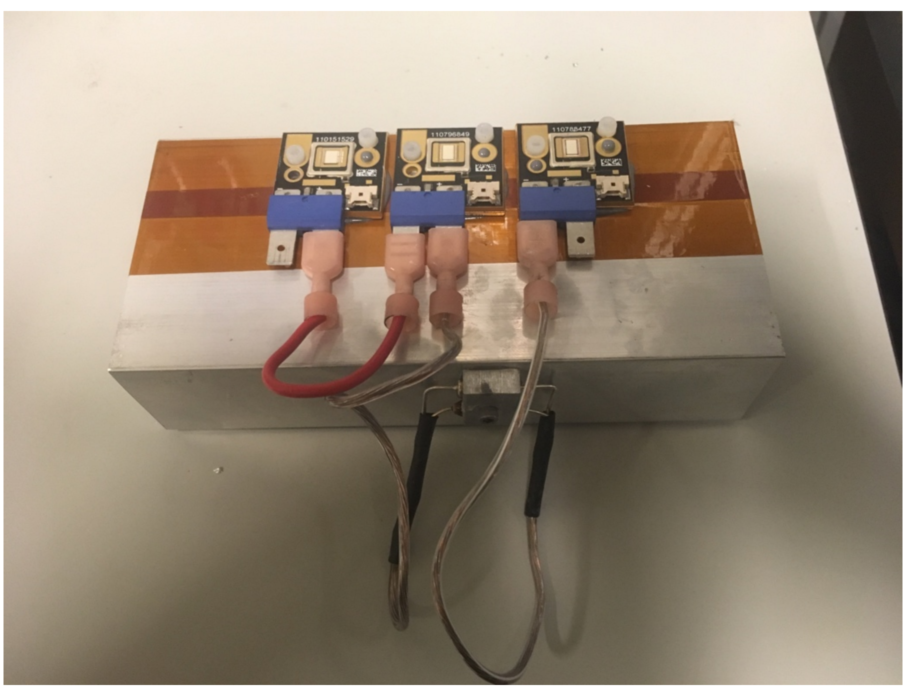
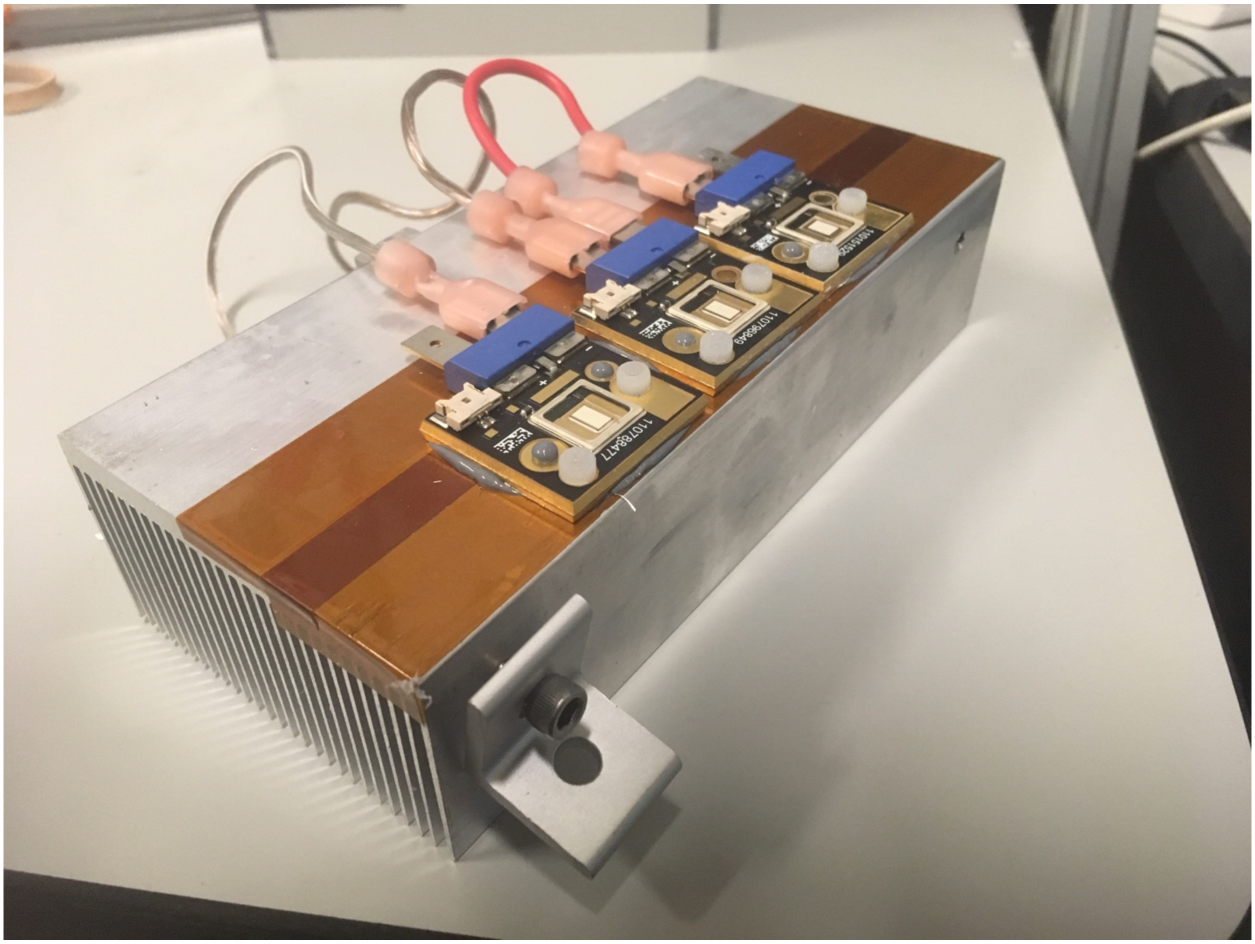
Fasten the thermal fuse box to the side of the heat sink with 1/2" long 4-40 metal screw.

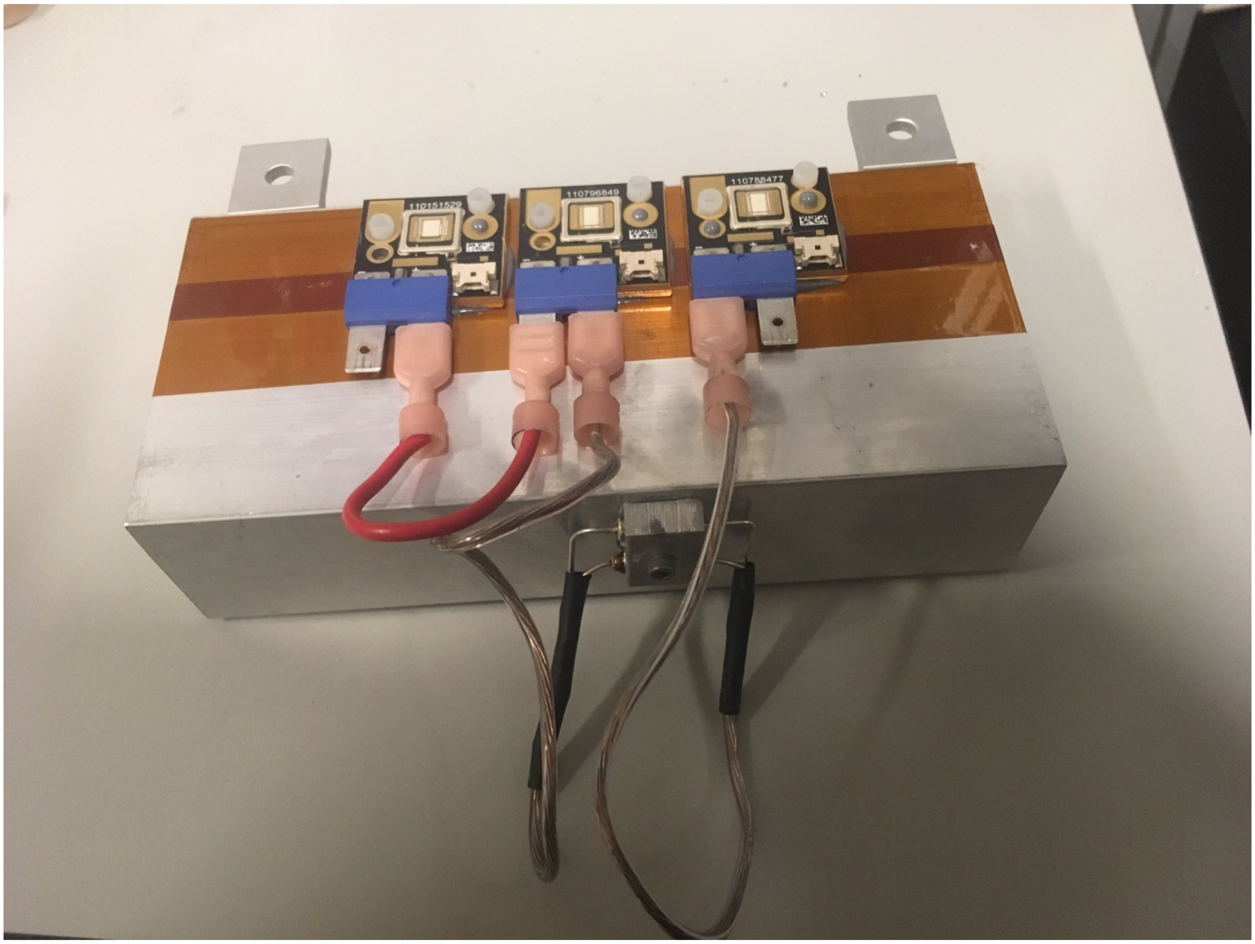


Connect the thermal fuse box wire terminals between the 2nd and 3rd LEDs. Connect the 1st and 2nd LEDs with another 16 gauge wire fitted with quick connectors. Always ensure the positive terminal of one LED is connected to the negative terminal of another LED. The direction of current in these LEDs will be from right (3rd LED) to left (1st LED).



Fasten two 20 mm corner brackets to the other side of the heat sink with 1/2" long 8-32 metal screws.





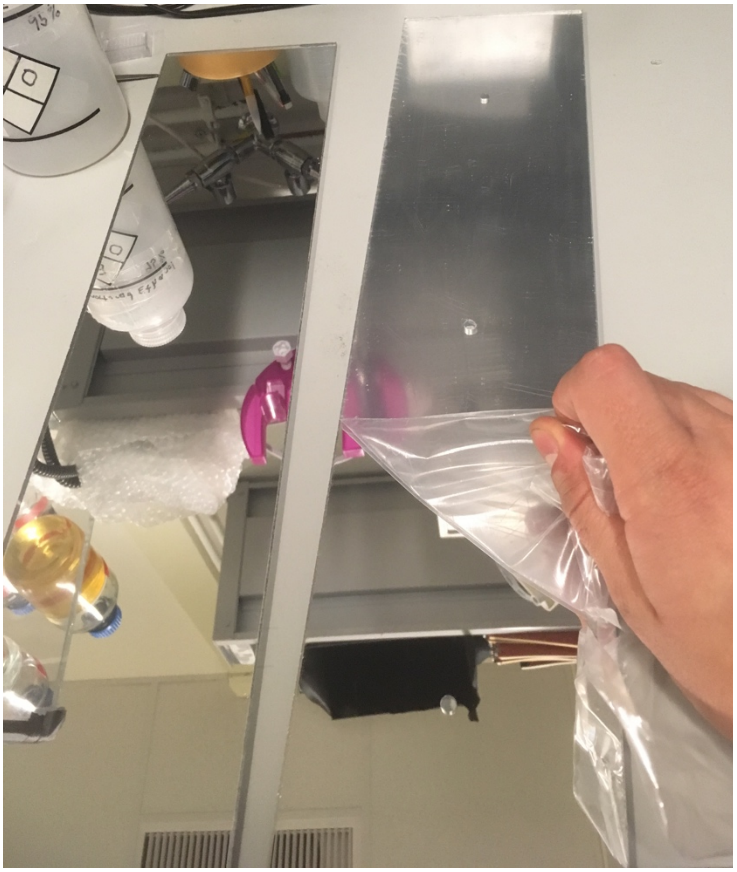
This completes the blue LED heat sink setup. A computer fan can be added later to the ventilation side of the heat sink. It is most convenient to attach the fan after the heat sink is mounted to the mirror box.

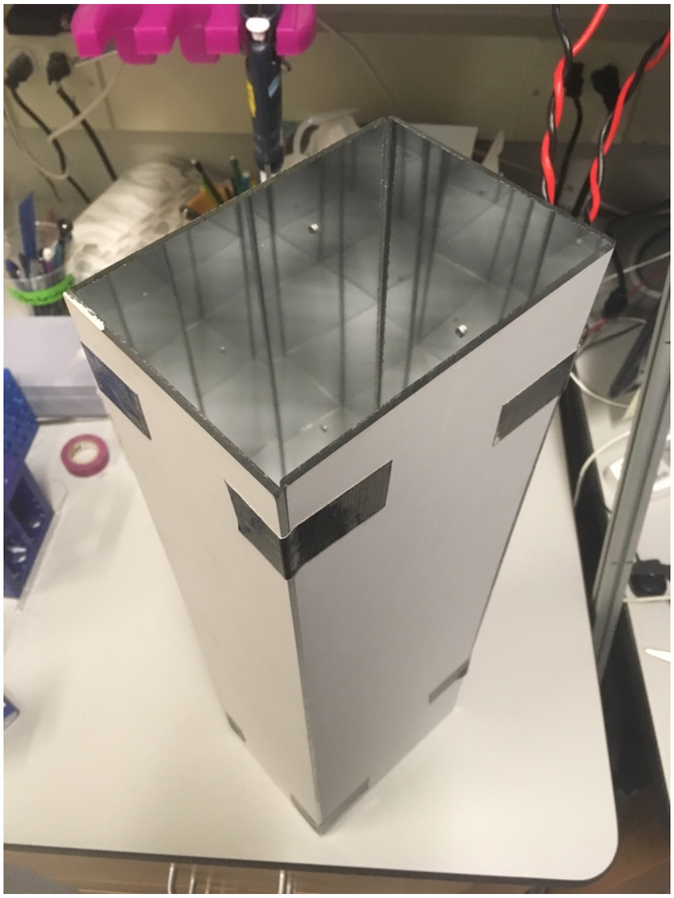
Mirror Box Construction

Drill 3 holes in one of the 18.5" x 4.125" pieces of mirrored acrylic. Use a #9 drill bit. Holes should lie on the center line of the acrylic. The M5 screws attaching the mirror box to the 20 mm extrusion backbone go through these holes.

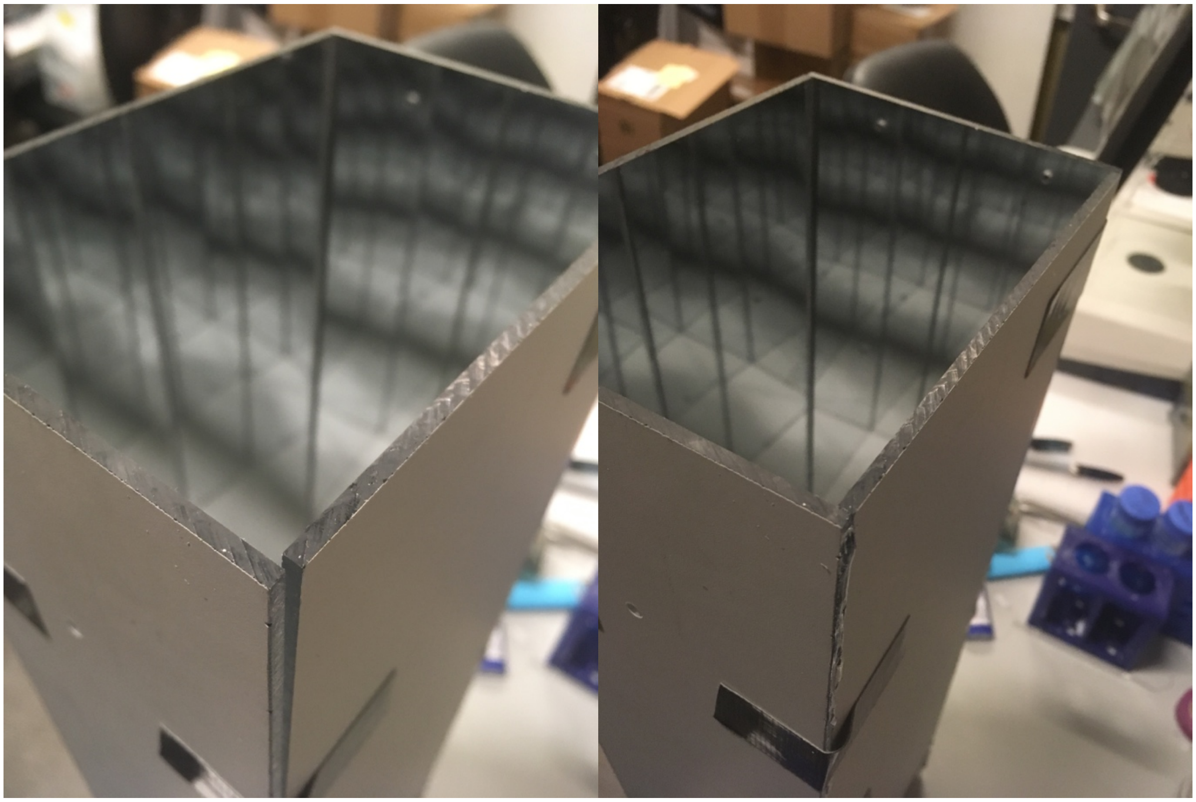
Drill 1 hole in one of the 18.5" x 5.5" pieces of mirrored acrylic. Use a #28 drill bit. This is the exit hole for the red illumination LED wires.

Remove the protective covering from each piece of mirrored acrylic and construct the box using tape on each vertex.

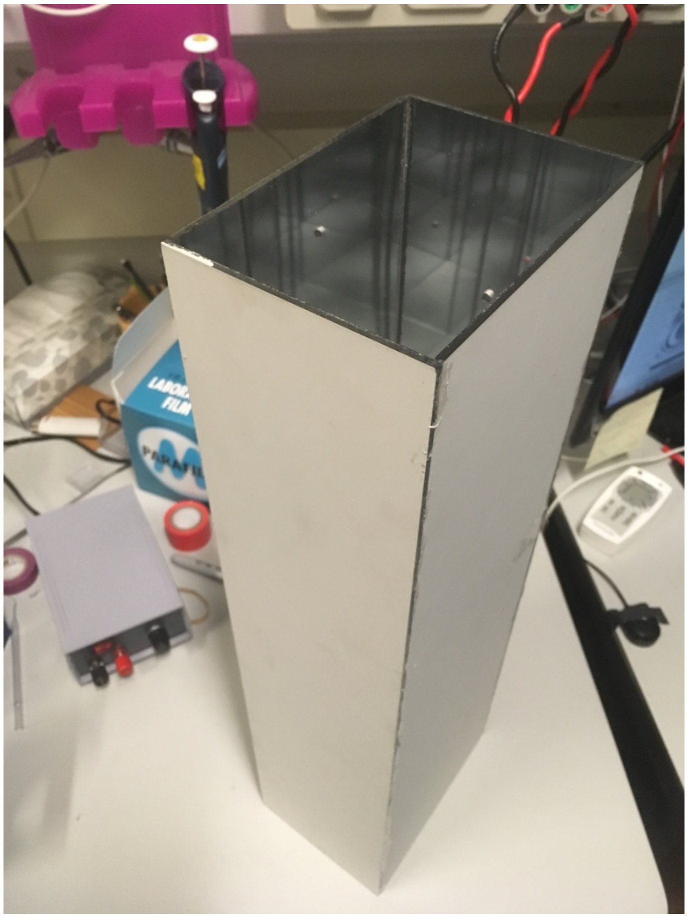




Add acrylic glue along each edge to finish construction of the box. Start in the middle of each edge and let the fast-acting glue set. Once the middle of each edge is set, you may proceed to glue each vertex. The acrylic may bow a little bit, so you will have to glue each vertex individually by adding some glue and then pressing the two pieces together until the glue sets (15-30 seconds). Do this for all eight vertices of the box to ensure it fits together snugly.



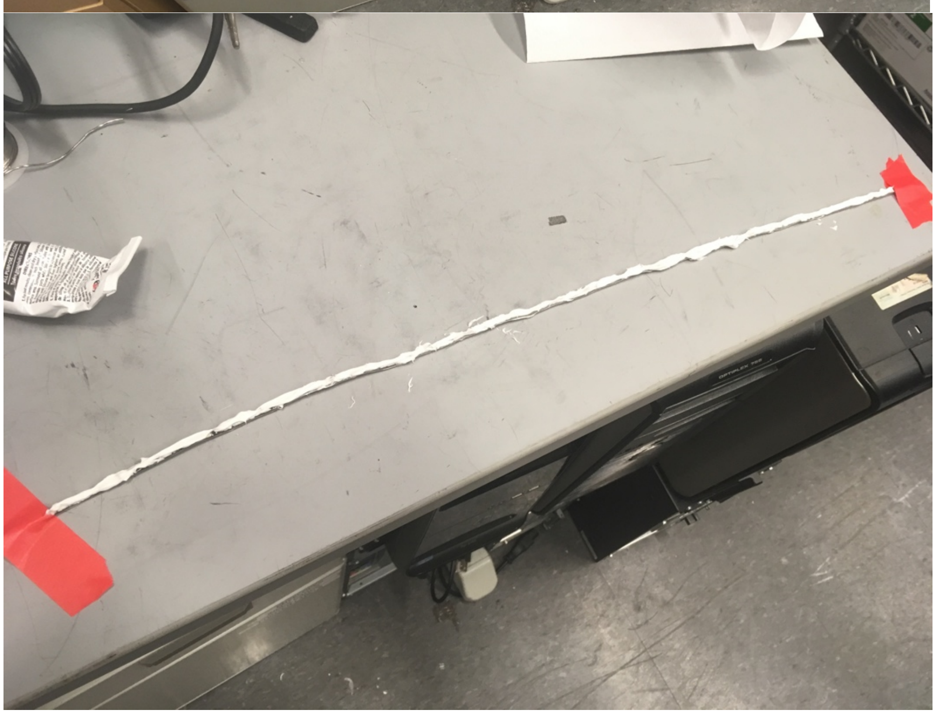
Once all the glue has set, you may remove the tape if desired.



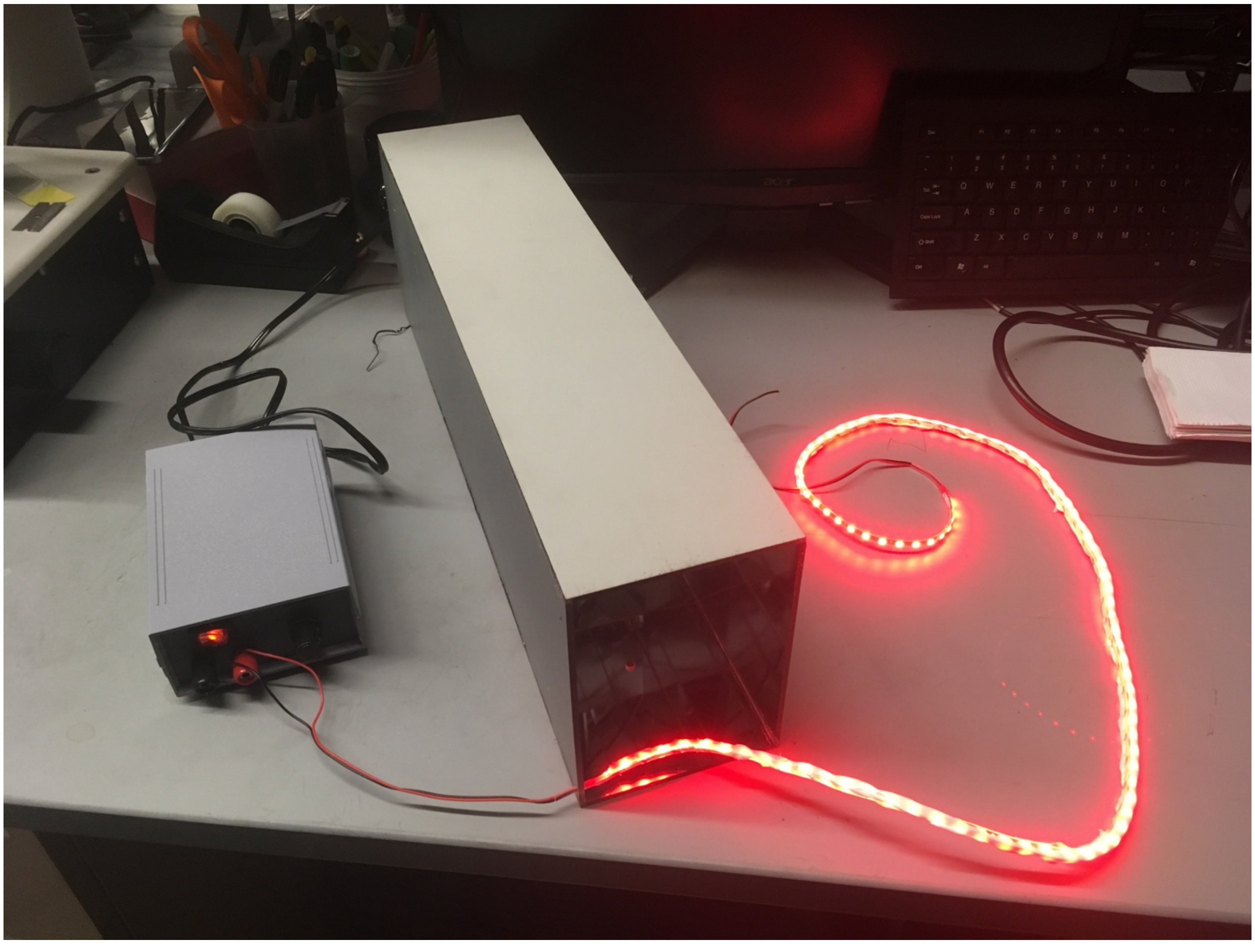
Once complete, ensure that a 24-well plate fits inside the box. There should be a few millimeters of give on each side of the 24-well plate.

Next, coat the red LED strip with white silicone. This serves to diffuse the red light from the point sources of the LED strip. Use may a metal spatula to evenly distribute the silicone across the LED strip.

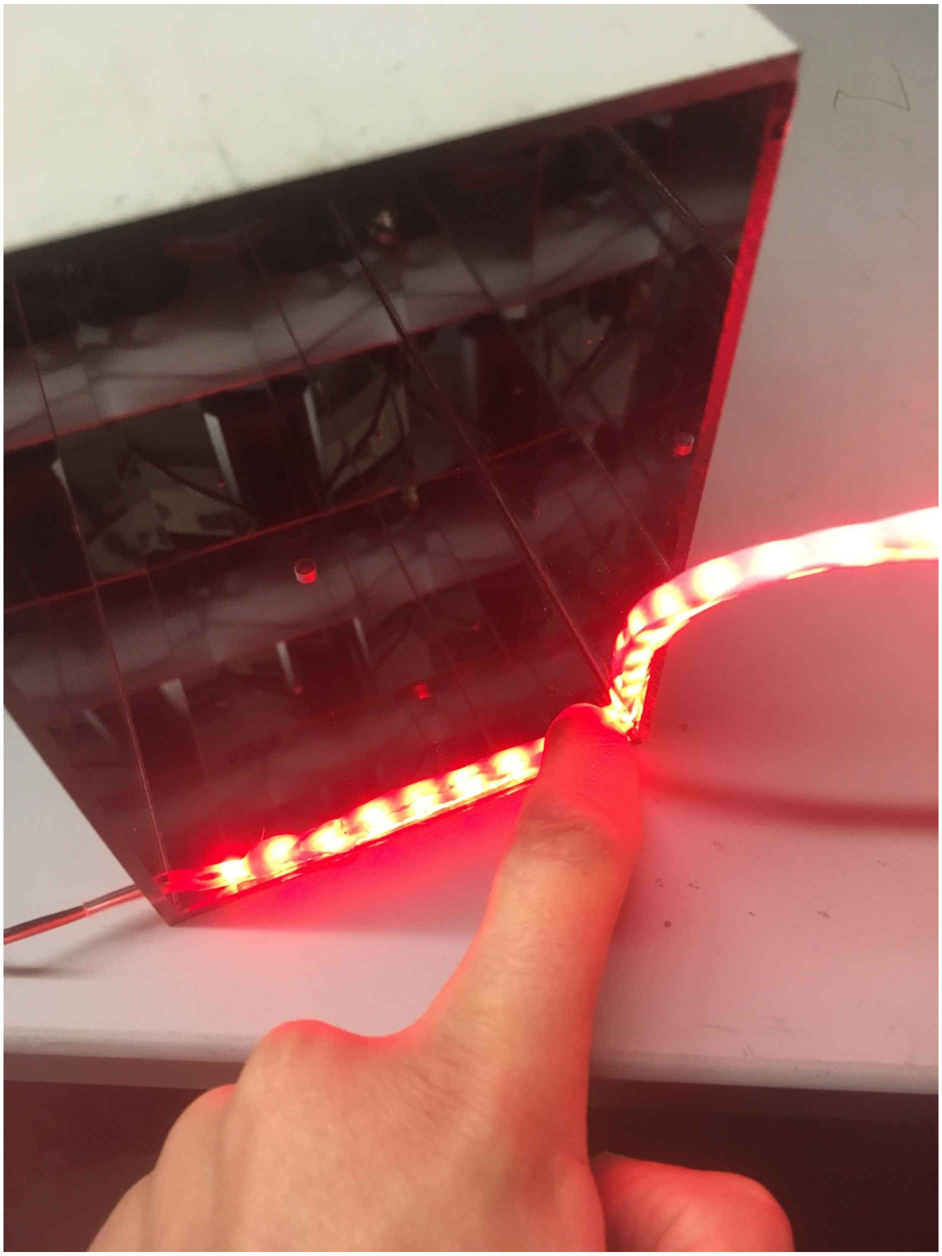

Weller
WHITE
ALL PURPOSE
RTV
Sealant
& Adhesive

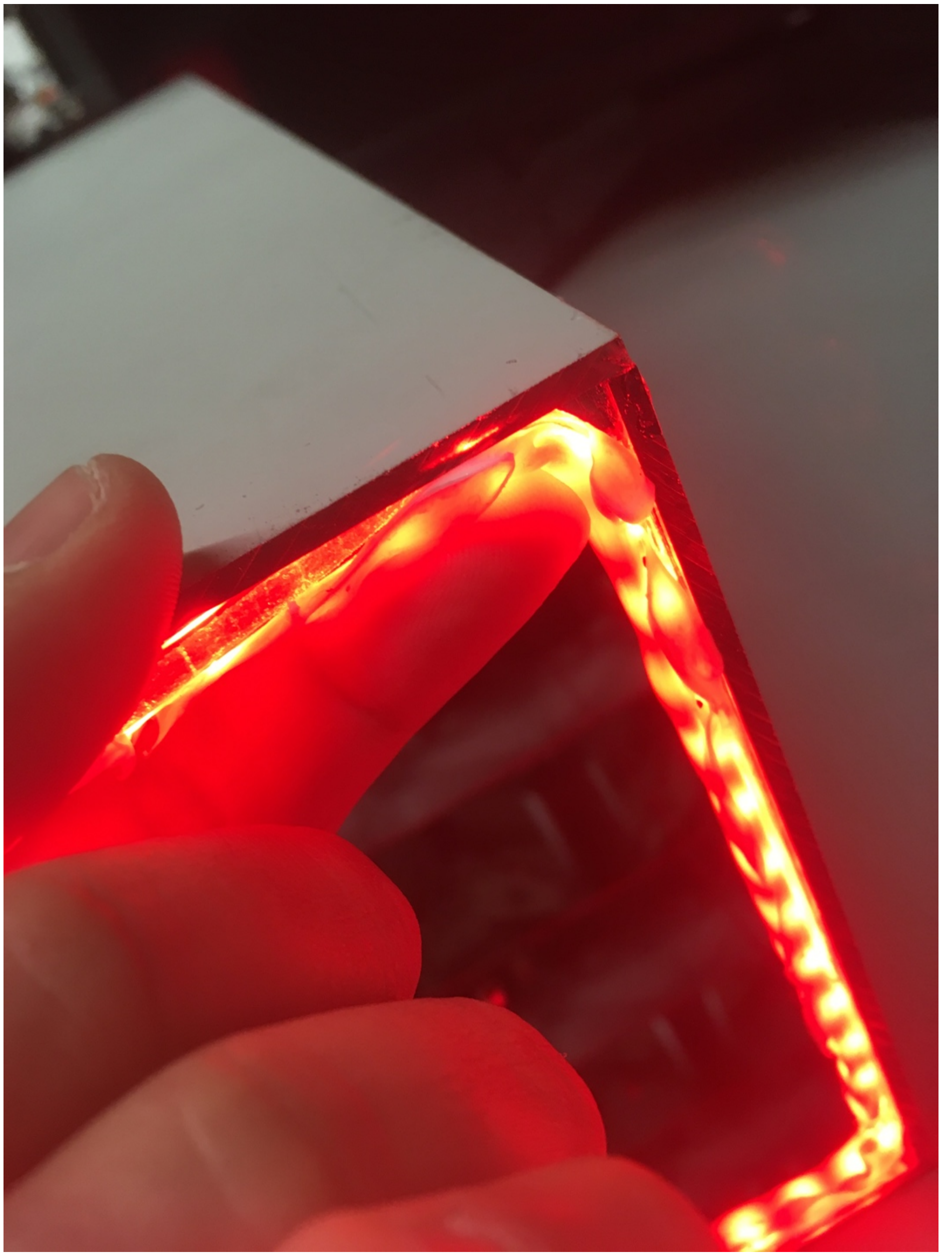


After allowing the silicone to dry overnight, guide the positive and negative terminals of the red LED strip through the small hole on the bottom of the mirror box, and connect the terminals to a 12V power supply if desired. Having the LEDs plugged in and turned on will help when trimming to fit the mirror box.

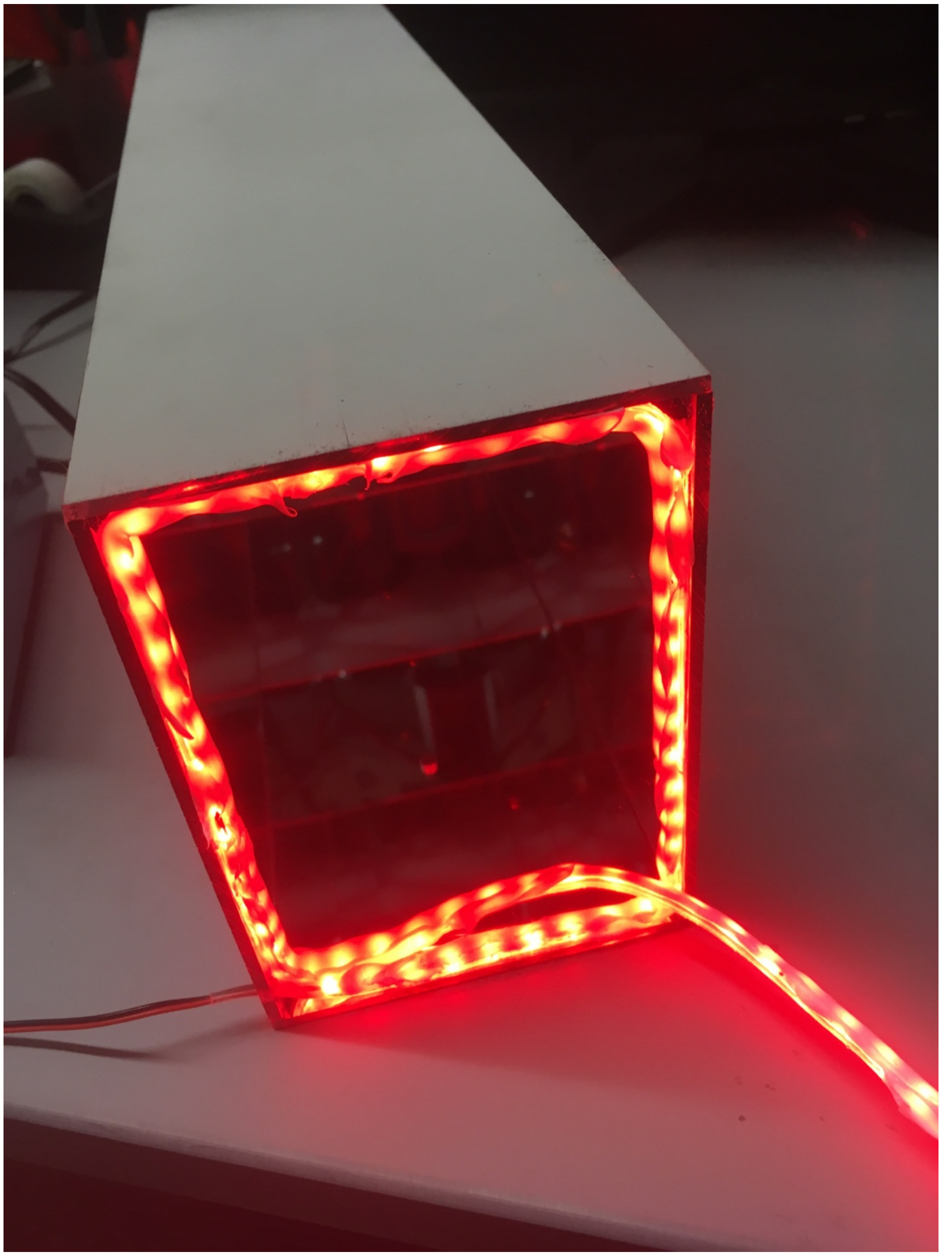


Remove the adhesive backing from the LED strip and fasten the strip to the inside of the mirror box. Ensure that the adhesive on the back of the LED strip makes strong contact with the inside of the mirror box and that the LEDs fit snugly.

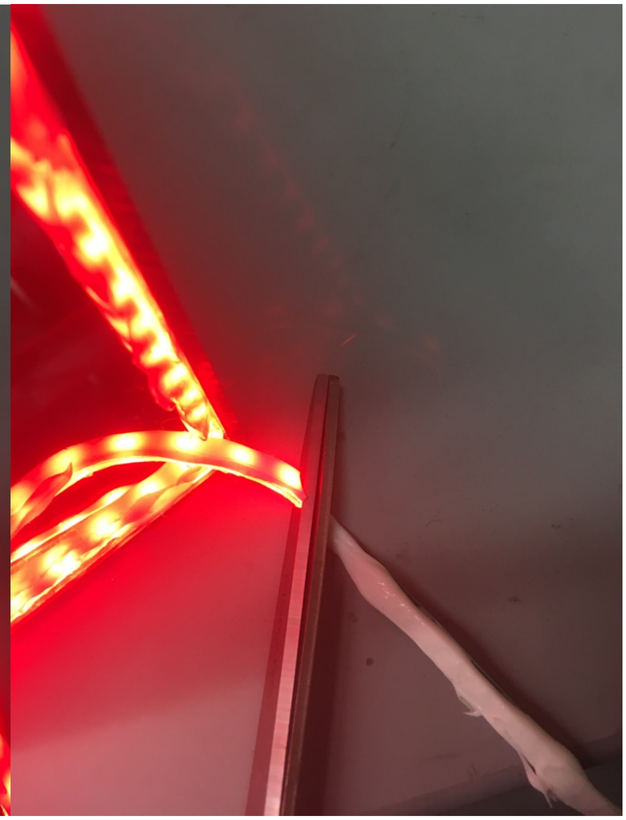


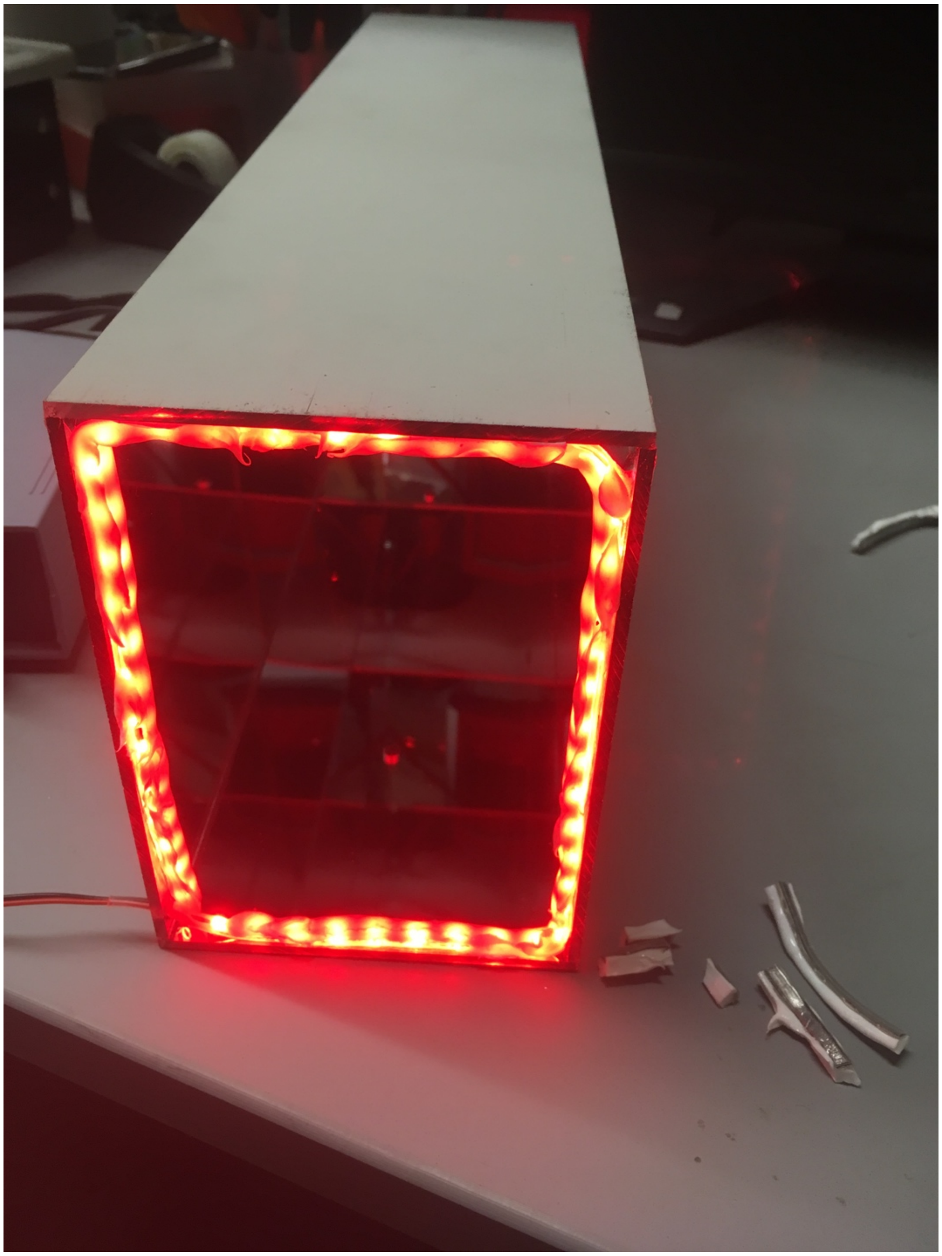


Once the LEDs have been wrapped around the entire box, there will be significant overhang.



The overhanging LED should be trimmed off with a scissor. It is best to begin trimming at the far end of the overhang and slowly trim off excess until you reach the point where the LEDs wrap around the box once with no overhang.





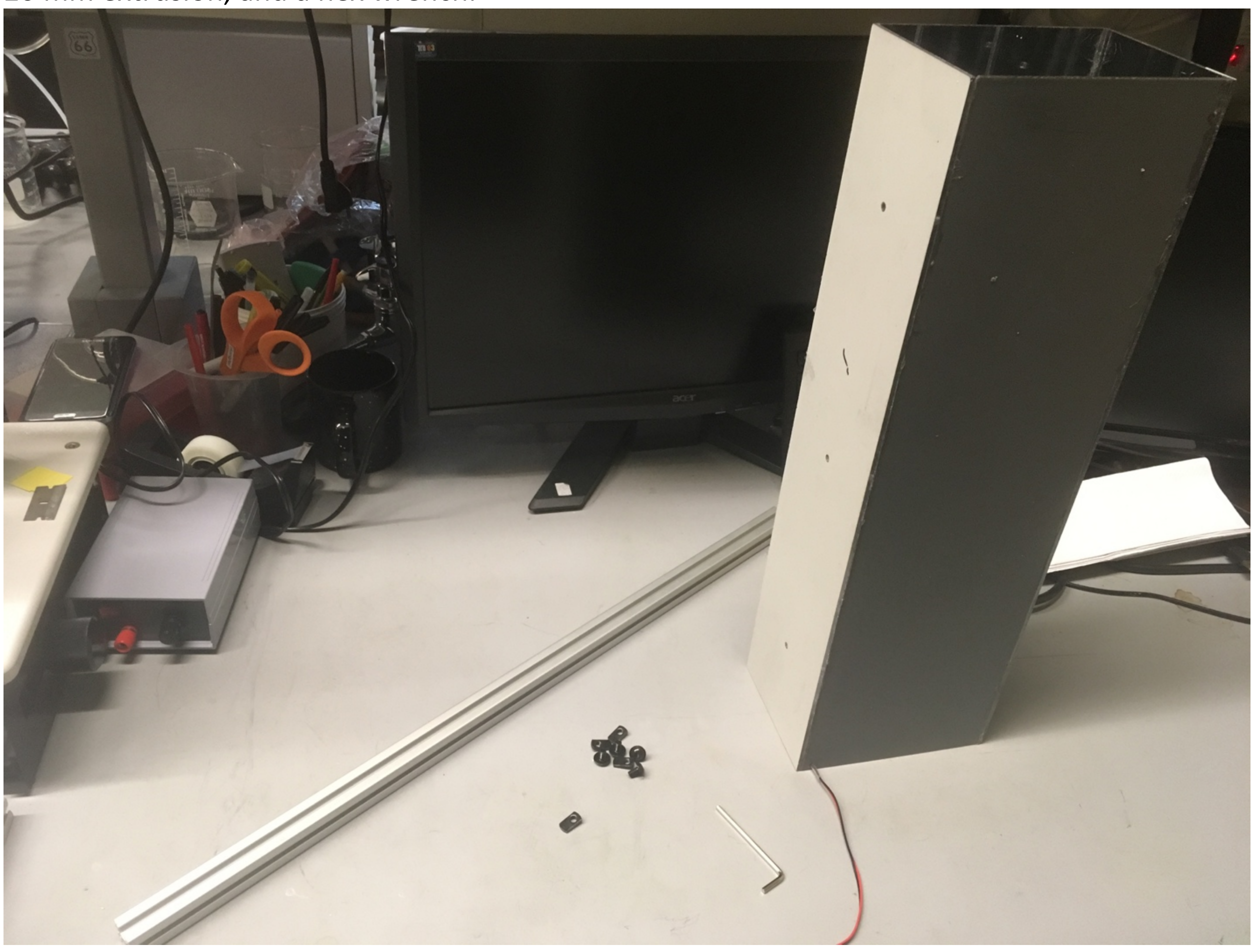
Note: You may wish to permanently fasten the red LED strip to the mirror box by applying small amounts of acrylic glue.

This concludes construction of the mirror box.

Assembling Custom Gantry

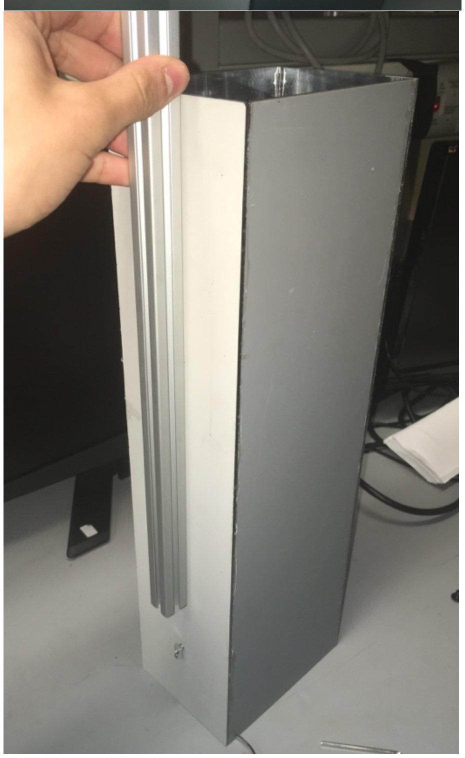
Once the mirror box and blue LED heat sink are assembled, it is time to combine these parts and attach them to a 20 mm aluminum extrusion backbone which can be mounted to the CNC robot.

Begin with the mirror box, a 24" long piece of 20 mm aluminum extrusion, 3 T-nuts for use with 20 mm extrusion, and a hex wrench.



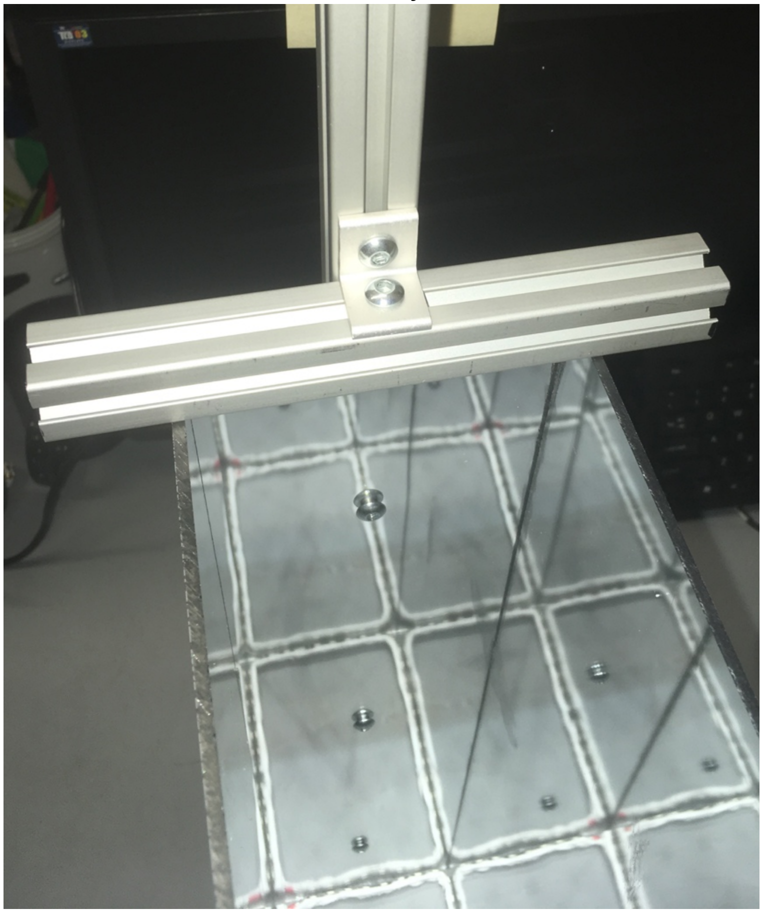
Slide the screws through the three holes in the back of the mirror box, and attach the T-nut loosely, as shown below.

Next, slide the aluminum extrusion down the mirror box over the three T-nuts.

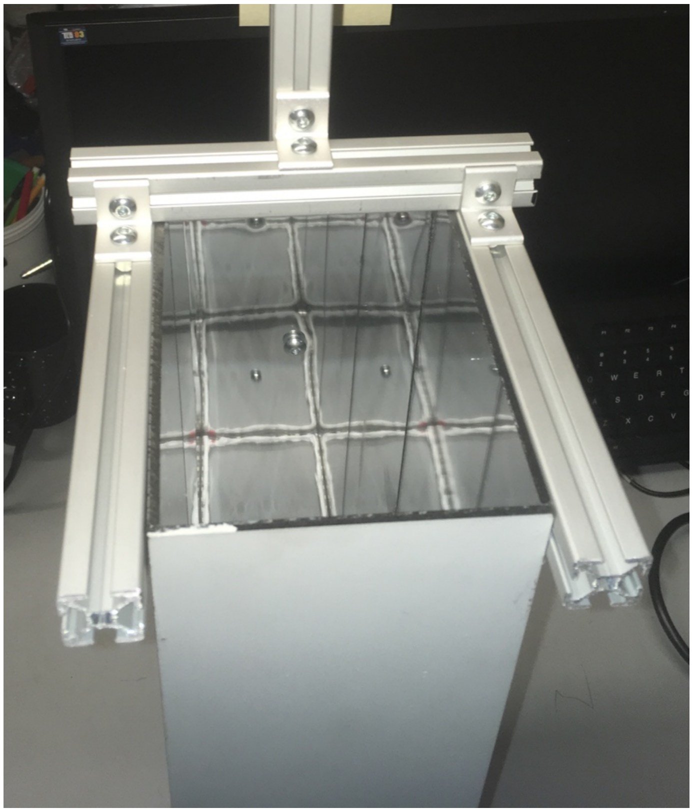


Next, tighten the three screws to secure the 20 mm extrusion backbone to the mirror box. The 24" backbone should not touch the ground when the mirror box is resting on a flat surface.

Next, using a 20 mm corner bracket and T-nuts, fasten a 6" long piece of 20 mm aluminum extrusion to the 24" backbone just above the mirror box.

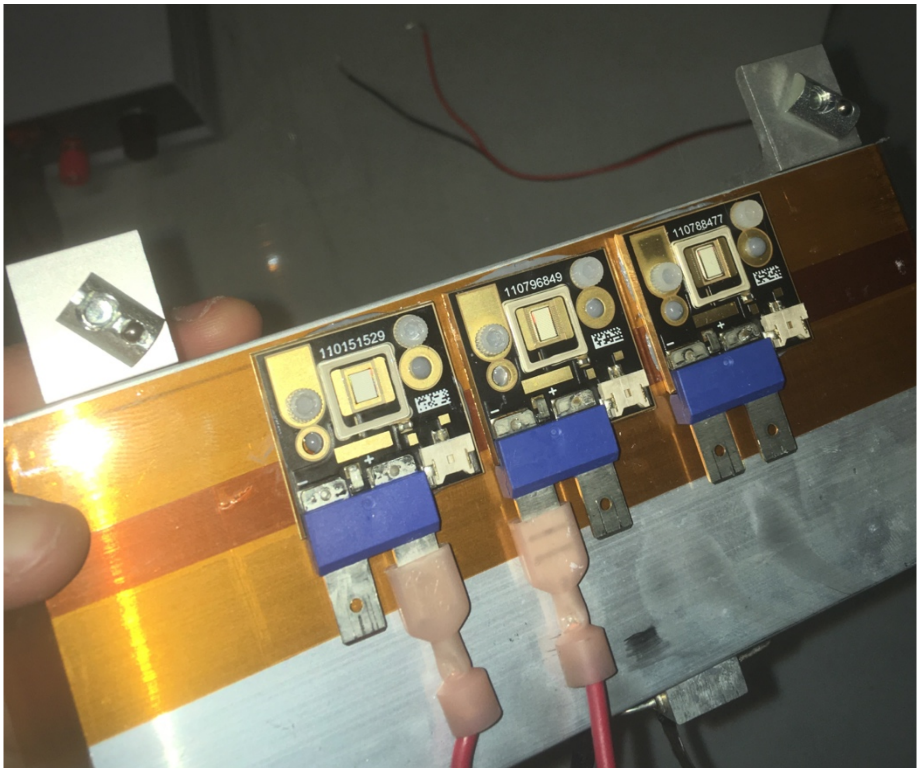


Next, attach two more ~6" long pieces of 20 mm aluminum extrusion to either end of the piece fastened perpendicularly to the 24" backbone. These pieces should be approximately flush with the mirror box.

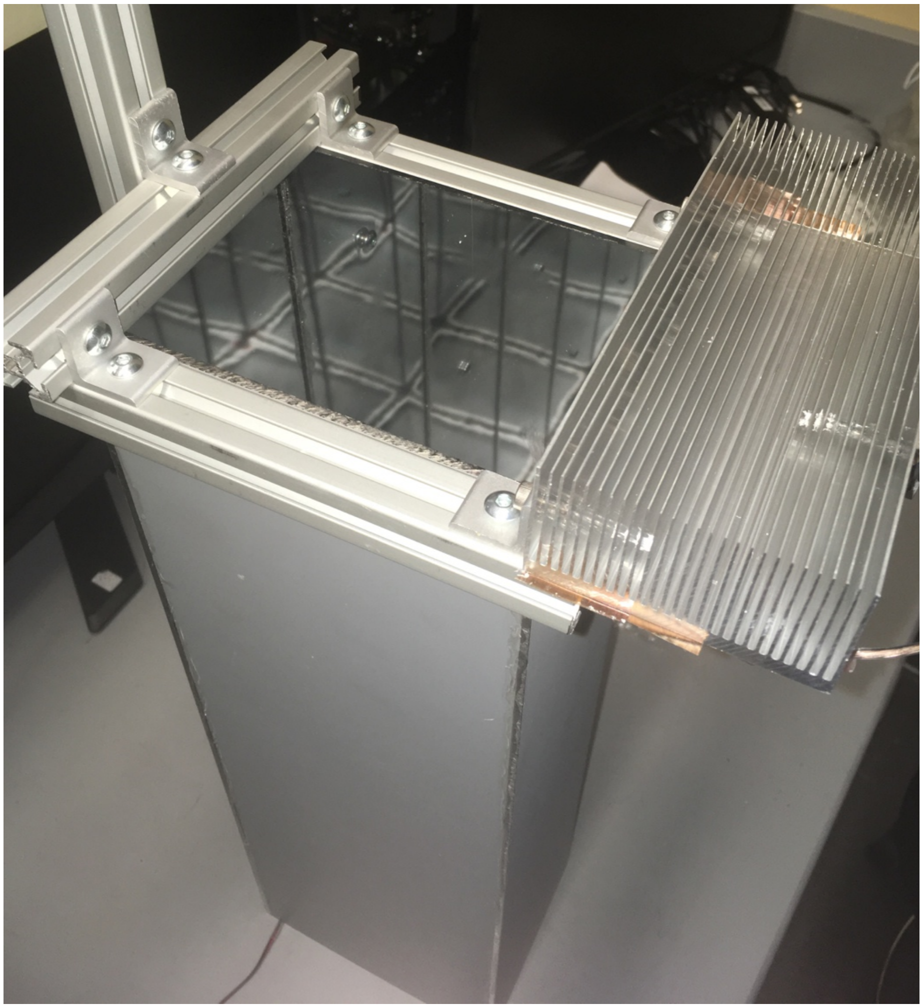


You may wish to raise this crossbar if necessary to help sliding the blue LED heat sink onto the 20 mm extrusion in the following few steps.

Place M5 screws through the corner brackets attached to the blue LED heat sink and loosely attach 20 mm-appropriate T-nuts.

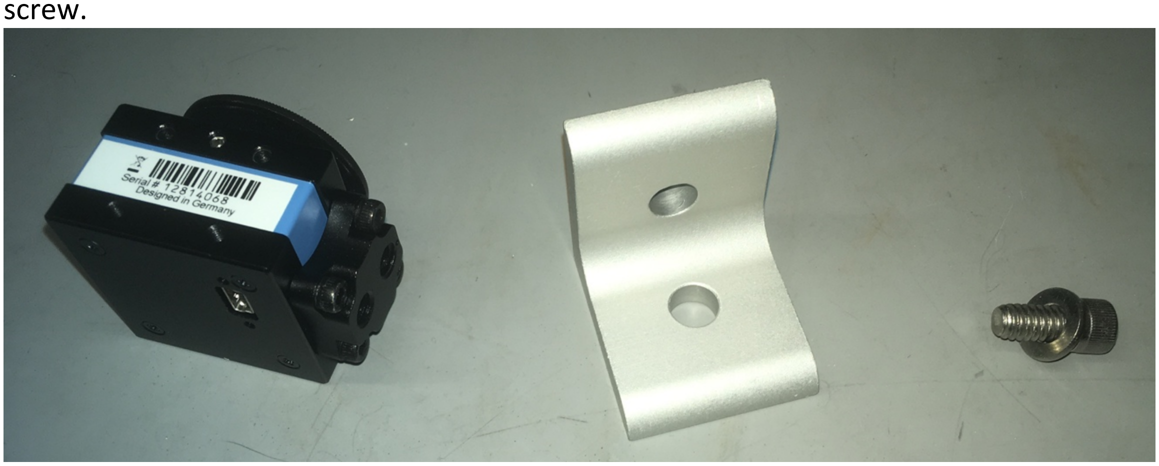


Next, slide the heat sink onto the two 20 mm extrusion pieces flanking the mirror box. The T-nuts should slide into the extrusion. You may need to adjust the spacing of the extrusion.

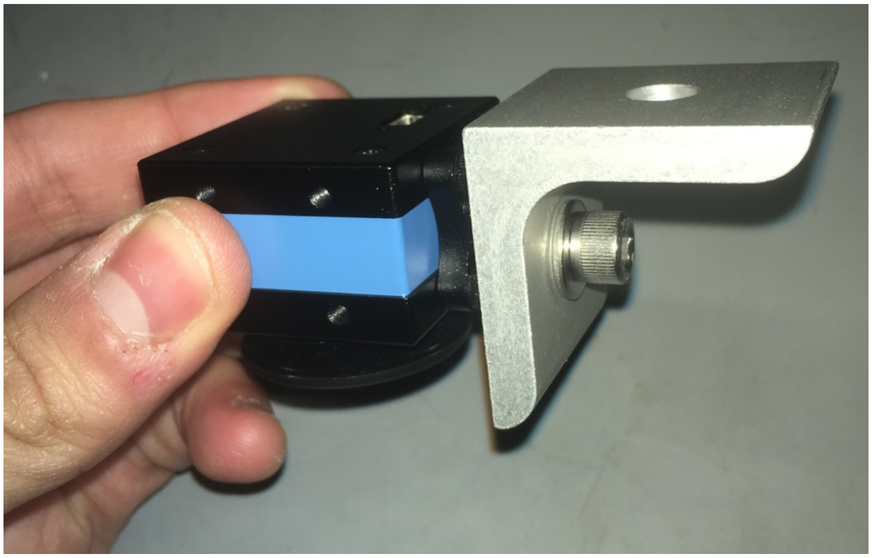


Slide the blue LED heat sink until the LED faces are just above the inside of the mirror box, as shown below.

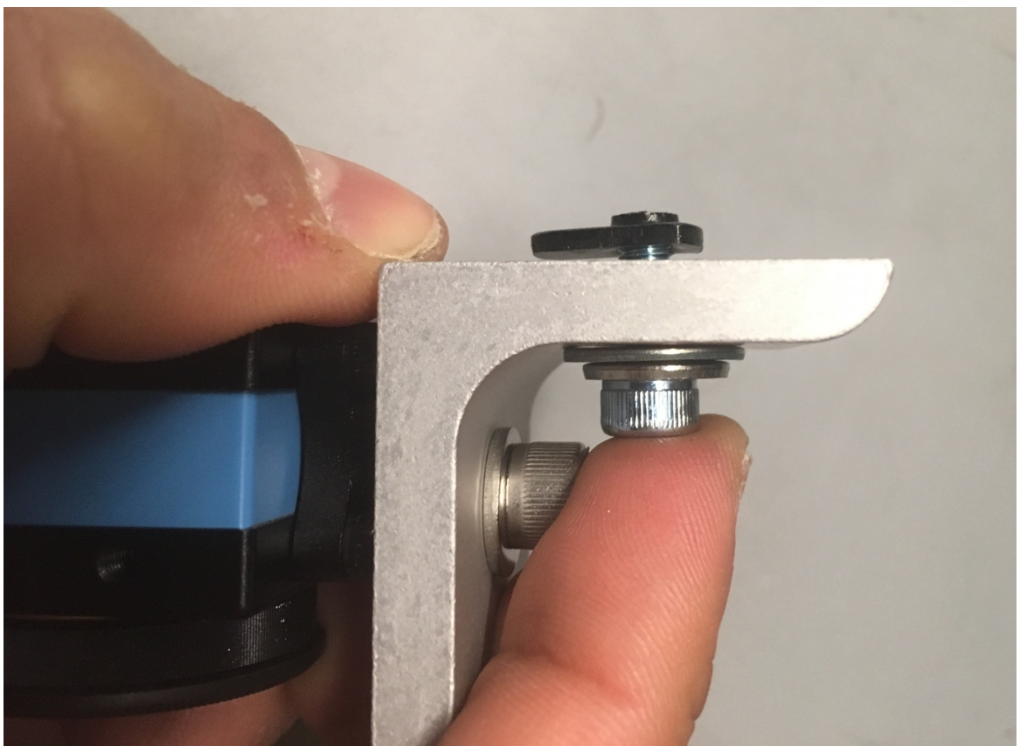
Next, unpack the USB camera, a 1.5"-extrusion corner bracket, a small washer, and a 1/4-20 screw.



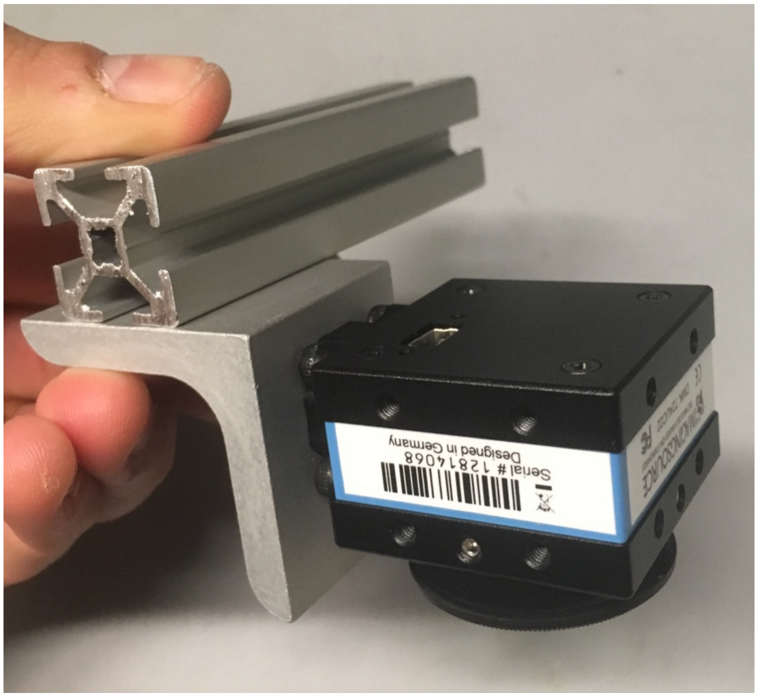
Fasten the corner bracket to the camera using the screw as shown below.



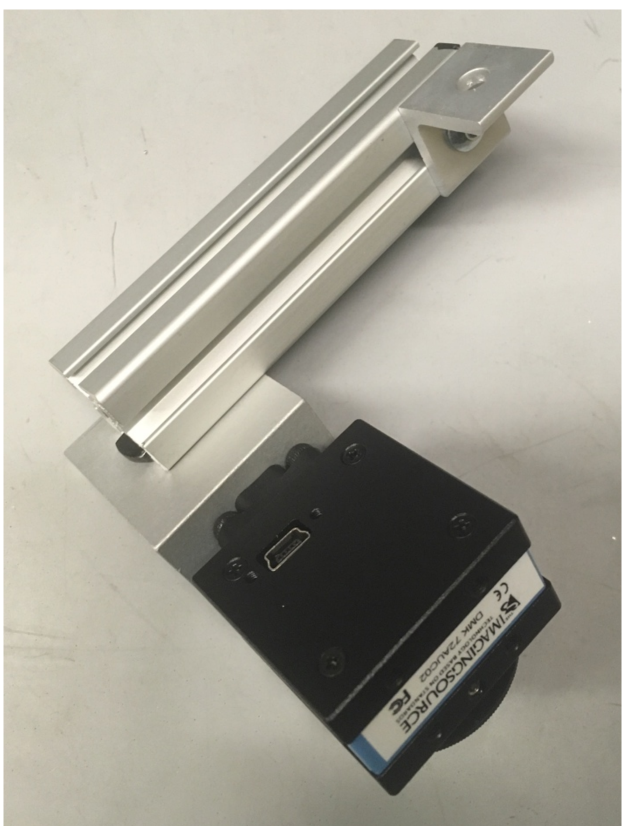
Next, use a 1/2" long M5 screw and small washers and attach a T-nut loosely through the other end of the corner bracket as shown below.



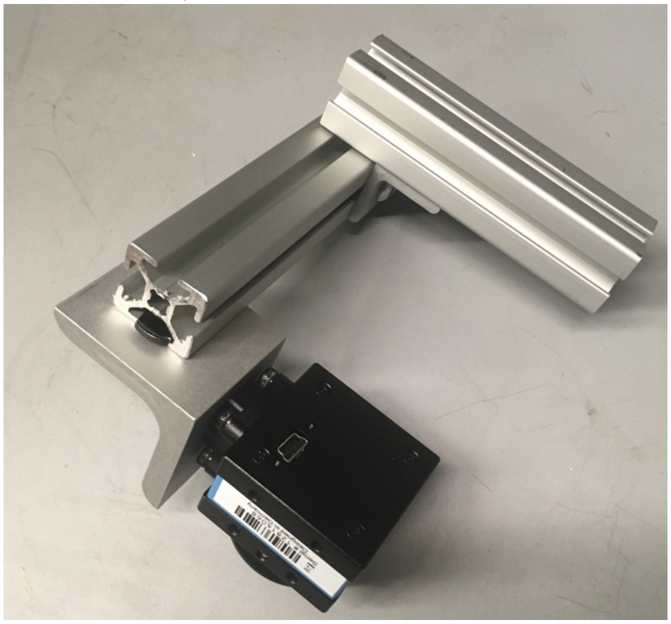
Next, slide the loosely connected T-nut onto a 3" long piece of 20 mm aluminum extrusion.



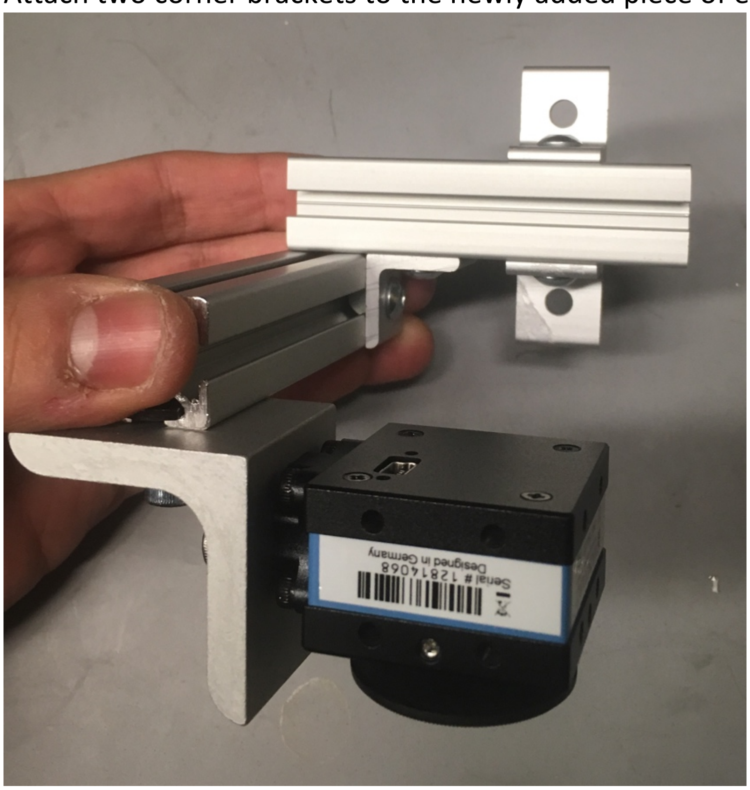
Attach a corner bracket as shown.



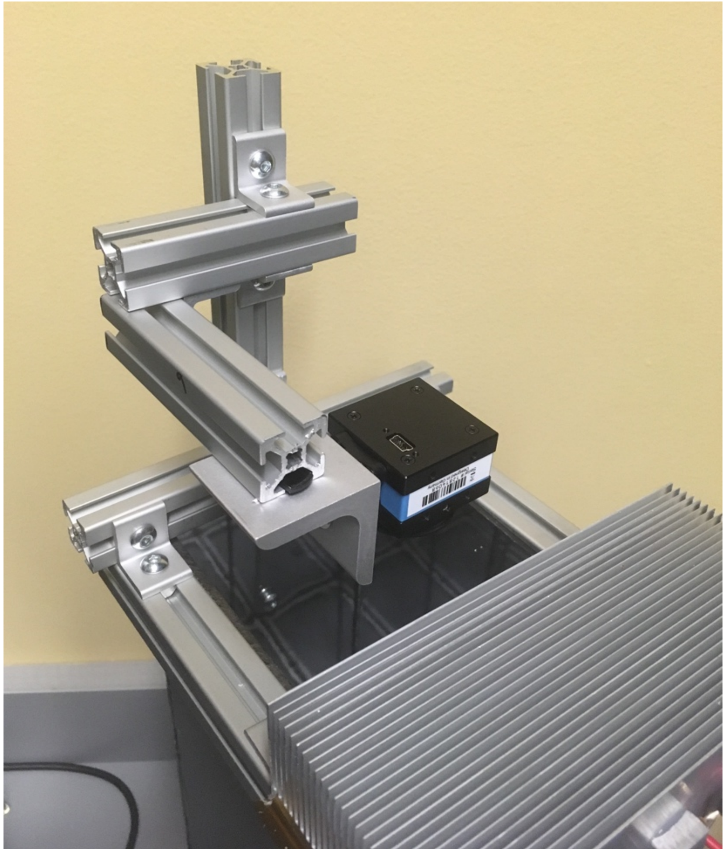
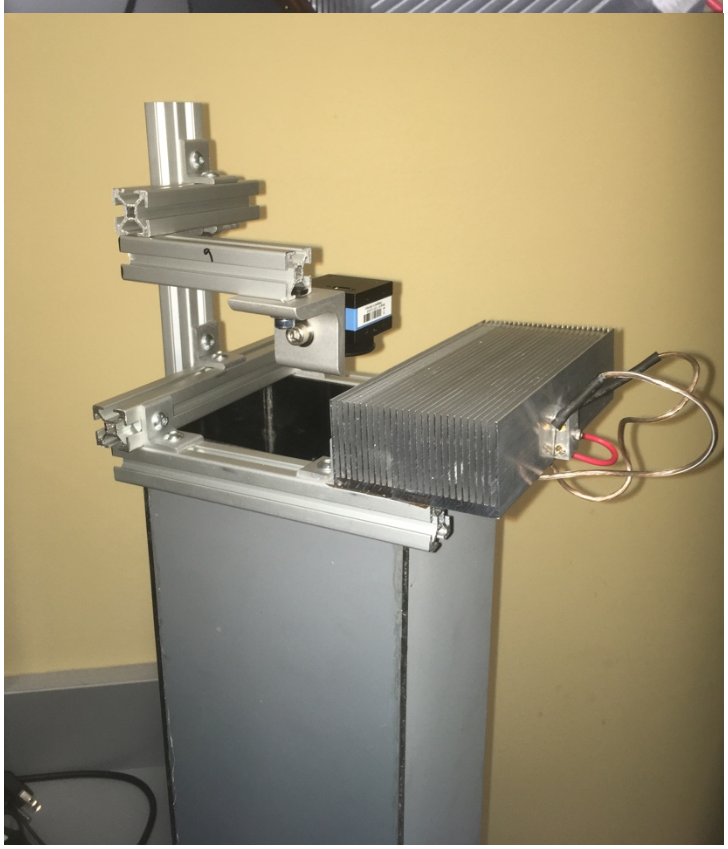
Next, attach another 3" long piece of aluminum extrusion perpendicularly to the one attached to the camera, as shown below.



Attach two corner brackets to the newly added piece of extrusion, as shown below.

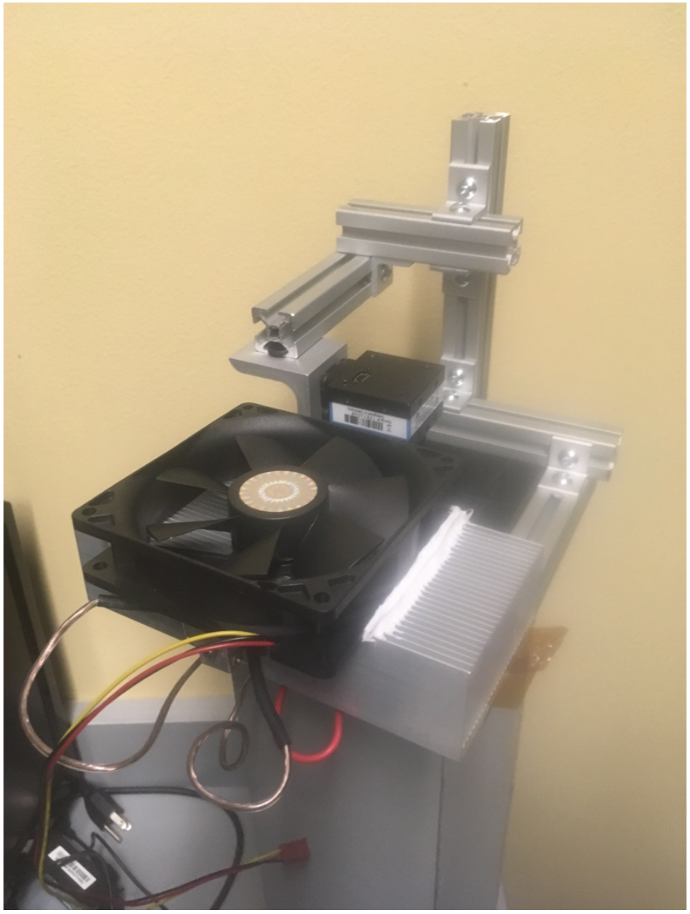


Next, once more loosely attach T-nuts through the holes in the newly attached corner brackets and slide the camera assembly onto the 24" aluminum extrusion backbone fastened to the mirror box.

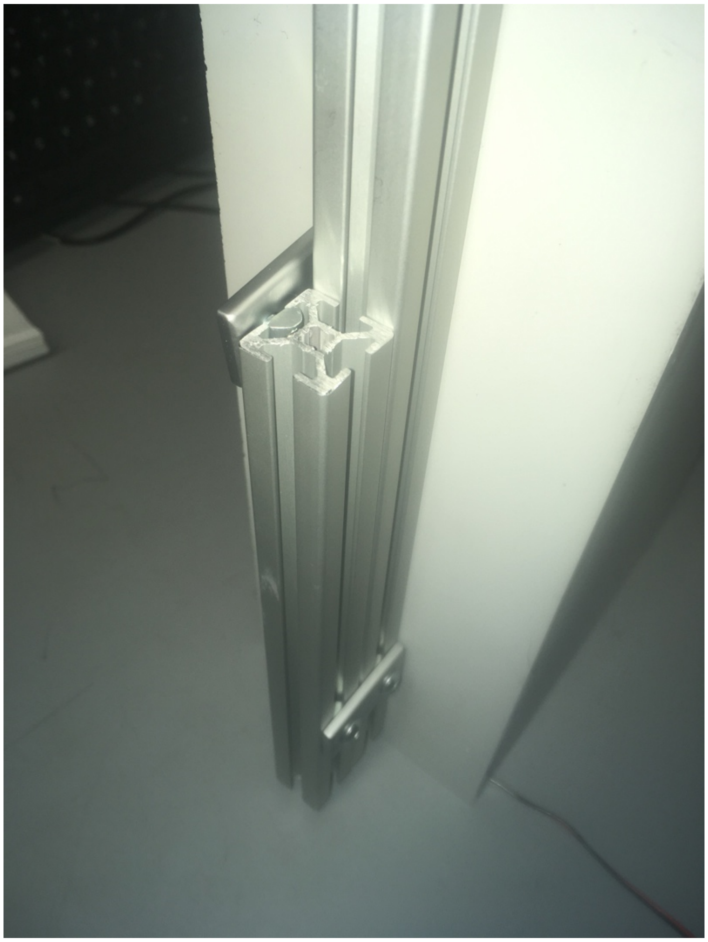
Now place the computer fan on top of the heat sink such that air will blow upward when the fan is powered.

Apply white silicone to the edges of the fan to attach it to the heat sink.



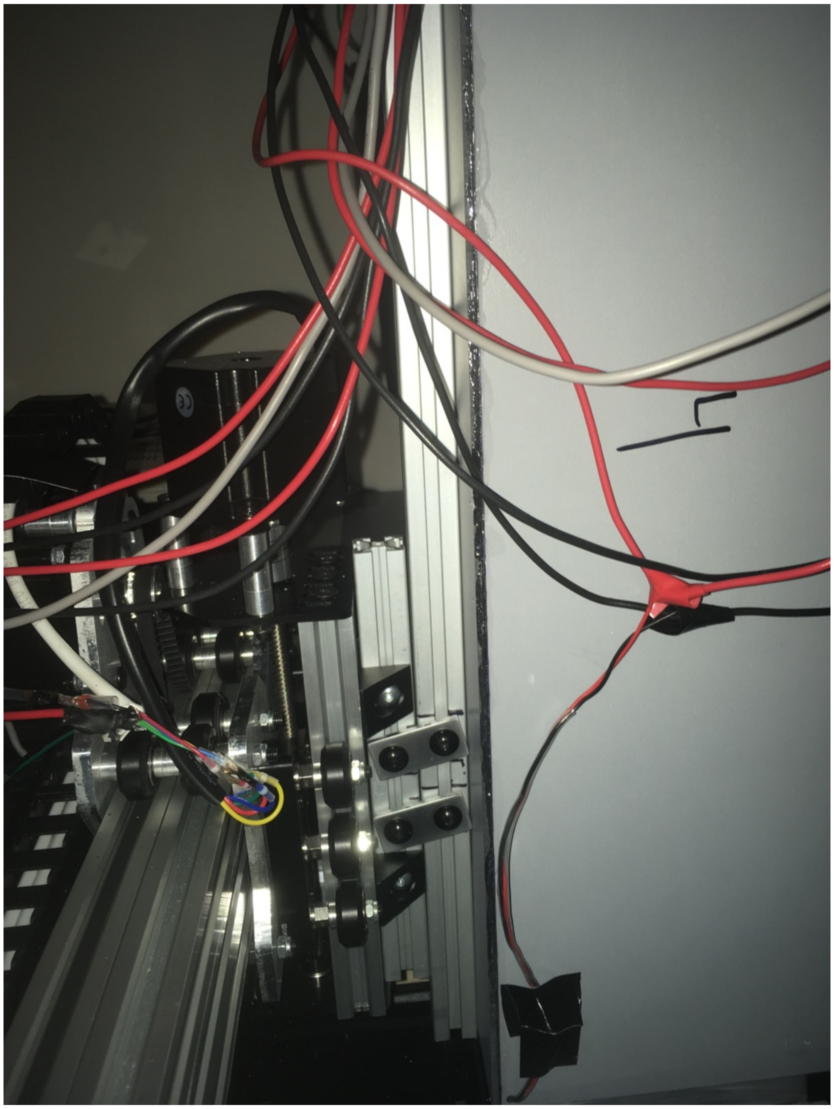
Finally, use 20-mm extrusion straight brackets to attach an 7" long piece of 20 mm aluminum extrusion to the backbone using T-nuts. This 7" piece will be mounted to the CNC robot in lieu of the spindle included with the kit.

Loosely fasten M5 screws to T-nuts through four corner brackets. Then, slide the bracket and T-nuts down along the 24" extrusion backbone of the custom gantry as shown below. The other end of the bracket should fasten to a ~10" piece of extrusion.



Fasten two straight brackets to each side, as shown below.

Once the CNC robot is assembled and in its final location, attach the custom gantry to the robot z-axis as shown below. Four corner brackets (included with CNC robot kit) will attach to the 20 mm extrusion of the custom gantry using T-nuts.

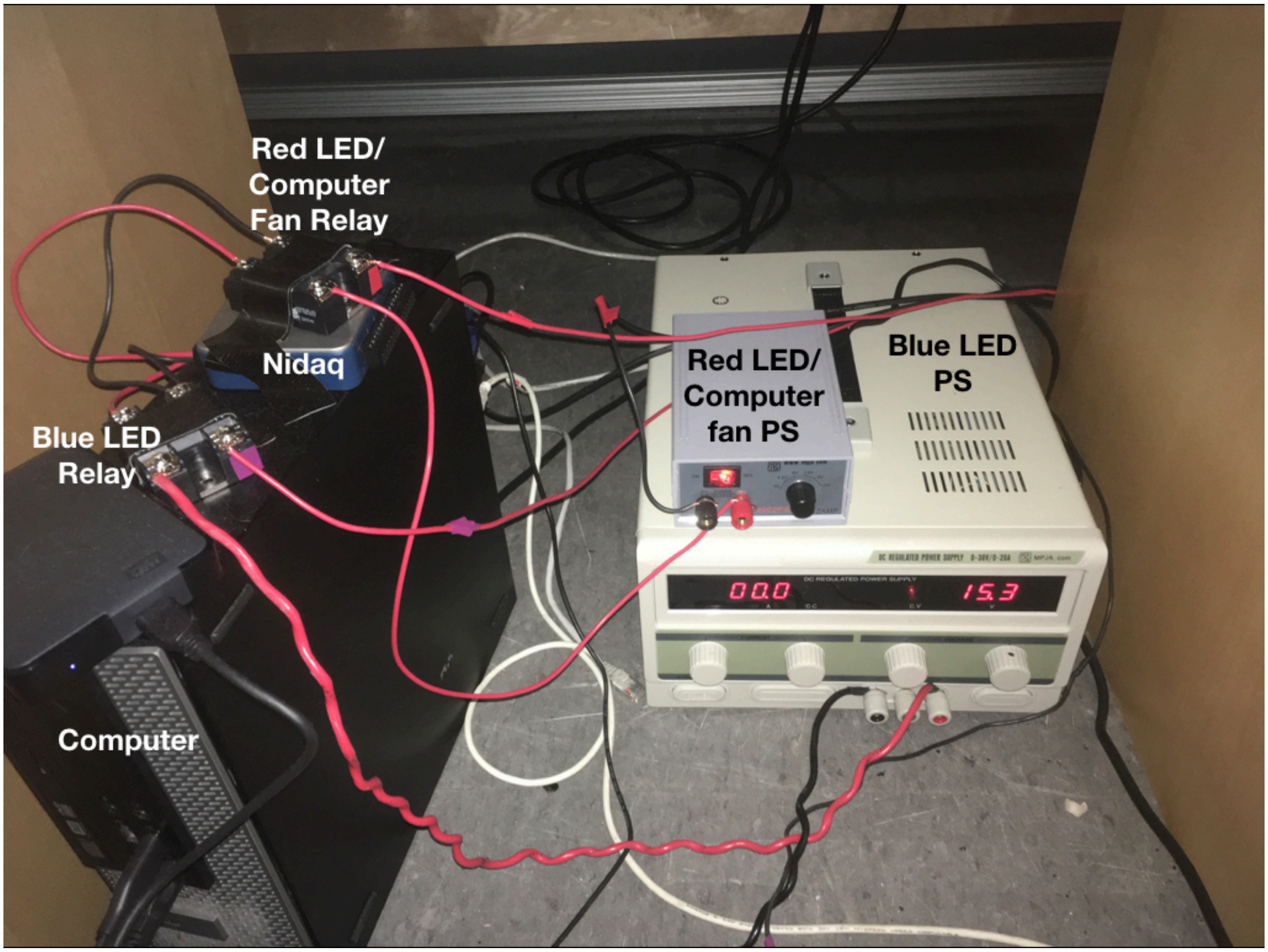


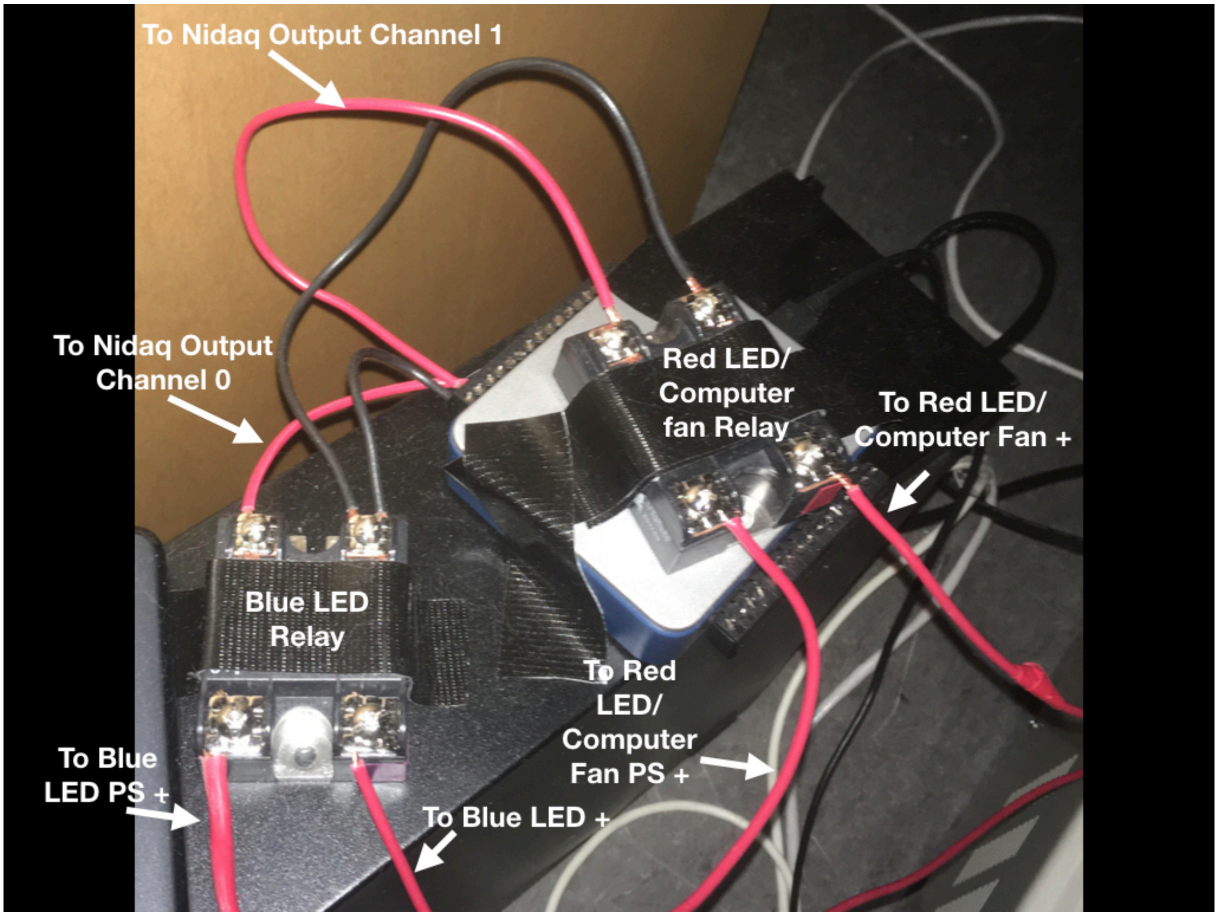
All wires (camera USB cable, red LED and blue LED power) should go through a 2" wide cable chain that also includes the x-axis and z-axis motor cables.

This concludes construction of the custom gantry.

Electronics Setup

Solder together the positive leads of the red LED strip and computer fan. Solder together the negative leads as well. These two elements will receive power from the same 12-V power supply/solid state relay system. The blue LEDs will receive power from a separate 30-V/20-A power supply and solid state relay. Both the blue LED relay and red LED/computer fan relay will receive input from the same Nidaq (input-output interface with Matlab) using channels 0 and 1, respectively.

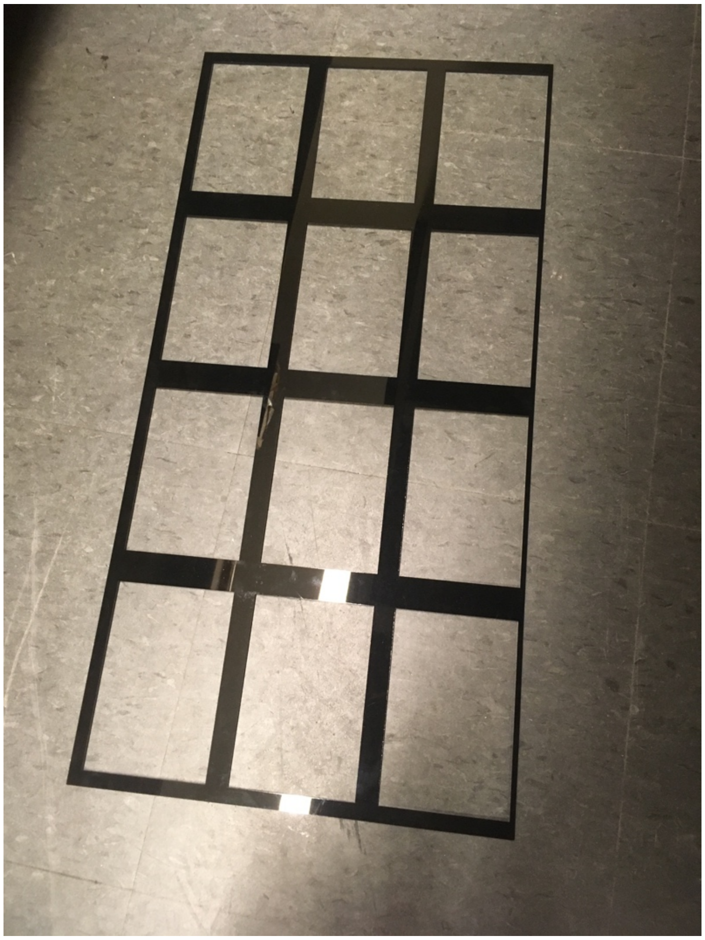




To Nidaq Output Channel 1
To Nidaq Output
Channel 0
Red LED/
Computer
fan Relay
To Red LED/
Computer Fan +
Blue LED
Relay
To Red
LED/
Computer
Fan PS +
To Blue
LED PS +
To Blue LED +

Plate Holder Array and Base

The plate holder array tiles should laser cut from 12"x24" black acrylic, 1/8" thick. There are two files for the plate holder array, "Plateholder_12.dwg" and "Plateholder_4.dwg". The first file produces a 3 by 4 array tile using an entire 12"x24" acrylic sheet (see photo below). The second file produces a 1 by 4 array tile using only a third of a 12"x24" acrylic sheet. Order the correct number of each file to fill your entire base, depending on the number of plate spaces desired.



For example, a plate array with 91 usable spaces (plus 13 unusable spaces due to CNC travel limit), order (or cut yourself with an available laser cutter) 8 copies of the file named "Plateholder_12.dwg" and 2 copies of the file named "Plateholder_4.dwg". Once cut, there should be 10 tiles in total, 8 of size 12"x24" and 2 of size approximately 4"x24".

Once the plate holder tiles have been cut, acrylic cement should then be used to permanently fasten the plate holder tiles to the 1/4" thick acrylic base that the plates will rest on.

The acrylic base should be secured to the CNC machine by drilling through-holes (drill bit 7/32") on at least two corners and fastened to the CNC machine base using 20 mm corner brackets and M5 screws.

Light and Temperature Enclosure

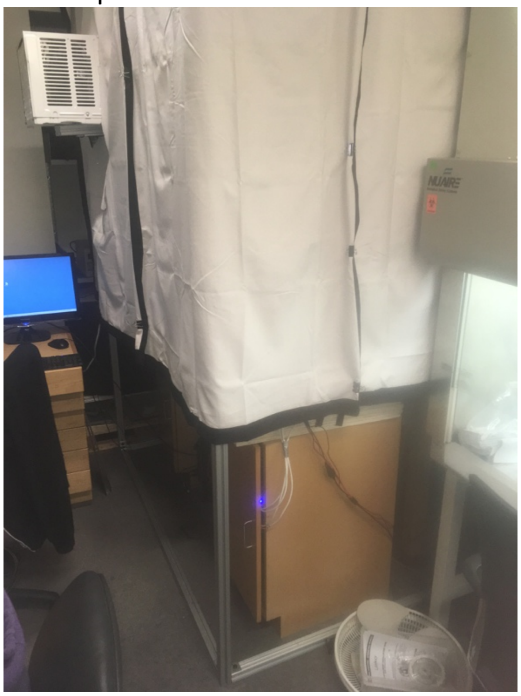
The robot should be placed inside a light-blocking enclosure. We designed a metal cage approximately 8' tall x 6' x 6' constructed out of 1.5" aluminum extrusion. The exact width and length do not need to be exact. We used blackout curtains as the walls of the enclosure and 1/8" plywood as the roof.

All 1.5" aluminum extrusion should be fastened together with simple corner brackets (sized appropriately for use with 1.5" extrusion). Where appropriate (e.g. A/C mounting), corner brackets should be placed both above and below.

The enclosure will consist of 4 vertical pieces (~96" each), 4 base pieces (~72" each), and 4 roof pieces (~72" each), creating a metal cage.

Two additional pieces (~72" each) will be required to mount an air conditioning unit to the enclosure. The A/C should rest on a ~72" long piece of 1.5" extrusion. Then, a second ~72" piece of extrusion should be placed directly above the A/C to stabilize it. Large straight brackets and ~8" pieces of 1.5" extrusion should be place below the A/C for support. These pieces should be fastened to the 1.5" extrusion supporting the A/C, as shown below.

Finally, 6 additional pieces of extrusion (~72" each) can be ordered to form a platform on which the robot can rest inside the enclosure. Two of these pieces should be fastened horizontally to the outside of the enclosure, with the remaining four pieces fasted perpendicularly inside the cage to create cross-struts on which the CNC robot will rest. Alternatively, a lab bench or table can be placed inside the enclosure for the robot to rest on, as shown below.



The roof of the enclosure can be made of 1/8" plywood or blackout curtain. The roof serves no structural role, it only serves to block external light. If using plywood, holes should be drilled in the plywood so it can be fastened to the 1.5" extrusion roof. 1.5" extrusion-appropriate corner brackets should first be fastened to the extrusion roof. Finally, the plywood can be fastened to the roof with 1/4-20 screws and nuts (and washers, if necessary), by securing the wood to the corner brackets (four per piece of wood).

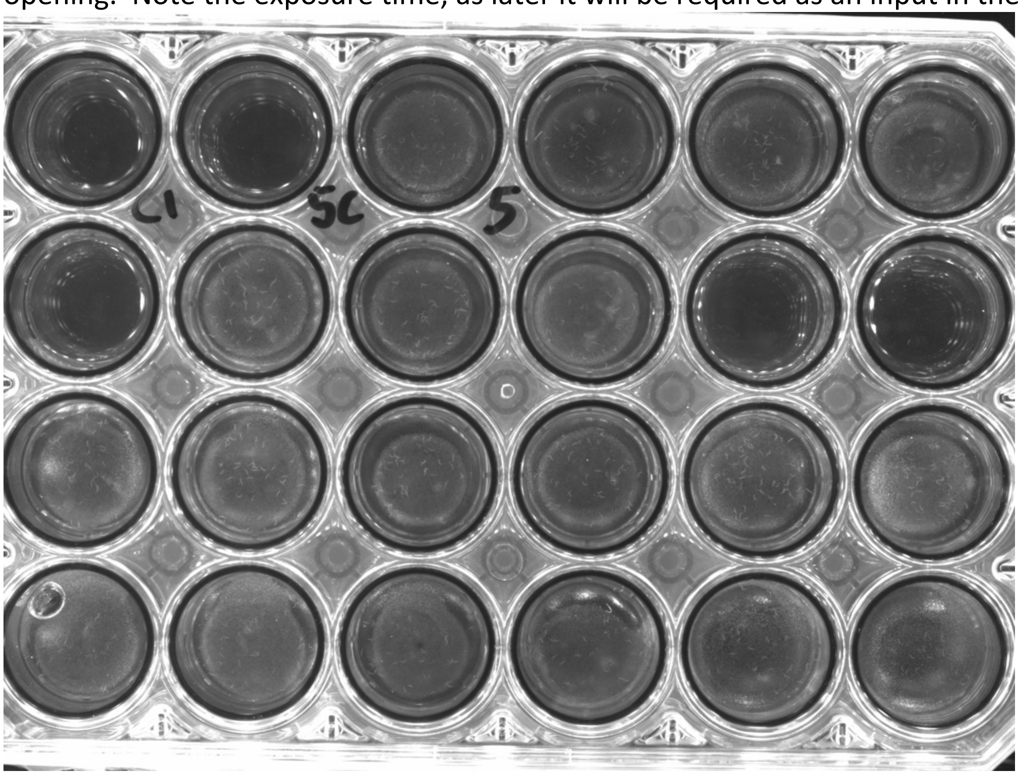
Finally, blackout curtains can be fastened by cutting a hole in the fabric and using 1.5" extrusion T-nuts with a washer to hang the curtains to the extrusion forming the enclosure roof, as shown below.

Camera Alignment and Image Quality

Once the camera and LEDs have been installed, the robot has been wired successfully, and all hardware is working properly, you may align the camera. Turn on the red LEDs either using the CHARISMA GUI or by directly connecting them to a 12-V power supply. Plug the camera in and ensure the 25-mm lens is attached. Open IC Capture (which can be downloaded from The Imaging Source Website). You should see a live video feed captured by the camera.

Manually move or jog the robot over a 24-well plate. Adjust the location of the camera relative to the mirror box such that the all 24-well plate wells are clearly visible and centered on the image. You should use a plate filled with agar and worms and ensure that all 24 wells are in focus simultaneously. If all wells are not simultaneously in focus, you can close the aperture slightly, which will increase the depth of focus.

Adjust the exposure time such that very little part of the image is saturated. At the same time, the image should not be too dark, otherwise the worm contrast will be low, which will limit the worm activity that can be picked up by the software. An exposure time in the range of 0.1 - 1 second is typically fine. The exposure time will depend on the red LED brightness and aperture opening. Note the exposure time, as later it will be required as an input in the CHARISMA GUI.



Note that it is important for automated image processing that the interstitial spaces between the wells be filled with agar, as shown above (see 24-well plate protocol). The software automatically finds the 24 wells by searching for dark circles on a bright background. If agar is not present between the wells, the software will fail to find the 24 wells.

Choice of Camera

The CHARISMA hardware and GUI is designed to work with a 2592x1944 pixel USB camera manufactured by the Imaging Source. However, all downstream image and data analysis is compatible with images acquired from other cameras. Using other cameras may require adjustments to the distance of the camera from the image plane or additional lens spacers. Furthermore, using a different camera will require adjustment to the cameraConnect_Callback function in the CHARISMA GUI. This will entail adjusting the image adaptor and setting camera properties.

Hardware Troubleshooting

Problem: The blue LEDs do not turn on (or not all the LEDs turn on) when current is applied.
1. Ensure that the LEDs are not in electrical contact with the aluminum heat sink. If necessary, remove the LEDs and add another strip of super-thin insulating tape.
2. Check wiring of the thermal fuse box and blue LEDs. Ensure any soldered components are electrically continuous.
3. Ensure each blue LED works on its own.

Problem: The robot is making a strange or jagged noises while moving.
Potential solution: First identify which motor is causing a problem.

1. Check wiring! Ensure that the motor leads are continuous with those at the control board.
2. Check the lock collar on the motor and ensure it is secured.
3. Ensure the GT2 pulley is making contact with the motor-mounted gear teeth.
4. Check wiring again. Faulty wiring (poor soldering) is the most likely cause of hardware errors.

Problem: The robot fails to home.
Potential solution: The limit switches are incorrectly wired or a connection is faulty.

1. Ensure all limit switches (X, Y, and Z) are properly connected to the GRBL board and that the capacitor included with the CNC kit is applied in parallel with each limit switch positive and negative terminal. Homing will likely fail if the capacitors are not included.
2. Ensure all motors are wired properly and there is little resistance when you physically move the robot gantry around.

Part 2: Software

Here we describe use of a Matlab graphical user interface (GUI) to control the CHARISMA robot movements, plate information, image acquisition, and data analysis.

Before You Begin

Test Each Component Independently

Before attempting to connect the robot with the GUI, it is a good idea to ensure that each component works on its own (e.g. all drivers are installed and functioning properly and everything is wired properly). Below is a list of each component of CHARISMA and the software it should be tested in before trying to run CHARISMA software.

- GRBL Board (Control board for CNC stepper motors): Test and set parameters (see next section) with Universal G-code sender (https://winder.github.io/ugs_website/)
- NIDAQ (Controls blue and red LEDs and blue LED heat sink fan): Test with Matlab using BlueLEDNidaqTest.m file (included in CNC_Dependents)
- Camera: Test with appropriate manufacturer-provided software (If using an Imaging Source Camera use IC Capture: https://www.theimagingsource.com/support/downloads-for-windows/end-user-software/iccapture/). NOTE: Please ensure only one camera is connected to the computer you are using. The software assumes only one camera device is connected and automatically connects. An error will result if more than one camera is connected to the computer.

Determine the COM ports to which the GRBL and Nidaq are connected to, as this information is required to initialize the robot in the software.

Set GRBL Parameters with Universal G-code Sender

GRBL is the open-source software that controls the machine by sending G-code to instruct the motors how to move. You can read more about GRBL here: https://github.com/gnea/grbl/wiki. Before opening GRBL with Matlab, the parameters of your particular machine must be set up. The easiest way to do this is with Universal G-code Sender (UGS), which can be downloaded from this website: https://winder.github.io/ugs_website/

Open UGS, select a Baud rate of 115200, and open the Com port connected to the GRBL board. If UGS does not recognize the GRBL board or no Com port is available, ensure that the drivers are installed. Drivers are included in the "CNC_Dependents" folder. Next, type $$ in the UGS command window. This will display the settings for your particular board. You can change a given parameter by typing "$0 = 10" in the command window. Set all the parameters so they match those shown below, but note that you may need to adjust some parameters (highlighted) depending on the footprint of your specific machine:

$0 = 10 (Step pulse time, microseconds)
$1 = 250 (Step idle delay, milliseconds)
$2 = 0 (Step pulse invert, mask)
$3 = 3 (Step direction invert, mask)
$4 = 0 (Invert step enable pin, boolean)
$5 = 0 (Invert limit pins, boolean)
$6 = 0 (Invert probe pin, boolean)
$10 = 3 (Status report options, mask)
$11 = 0.010 (Junction deviation, millimeters)
$12 = 0.002 (Arc tolerance, millimeters)
$13 = 0 (Report in inches, boolean)
$20 = 1 (Soft limits enable, boolean)
$21 = 0 (Hard limits enable, boolean)
$22 = 1 (Homing cycle enable, boolean)
$23 = 0 (Homing direction invert, mask)
$24 = 50.000 (Homing locate feed rate, mm/min)
$25 = 1500.000 (Homing search seek rate, mm/min)
$26 = 25 (Homing switch debounce delay, milliseconds)
$27 = 5.000 (Homing switch pull-off distance, millimeters)
$30 = 12000 (Maximum spindle speed, RPM)
$31 = 4000 (Minimum spindle speed, RPM)
$32 = 0 (Laser-mode enable, boolean)
$100 = 54.000 (X-axis travel resolution, step/mm)
$101 = 55.000 (Y-axis travel resolution, step/mm)
$102 = 400.000 (Z-axis travel resolution, step/mm)
$110 = 7500.000 (X-axis maximum rate, mm/min)
$111 = 7500.000 (Y-axis maximum rate, mm/min)
$112 = 500.000 (Z-axis maximum rate, mm/min)
$120 = 100.000 (X-axis acceleration, mm/sec^2)
$121 = 100.000 (Y-axis acceleration, mm/sec^2)
$122 = 10.000 (Z-axis acceleration, mm/sec^2)
$130 = 1372.000 (X-axis maximum travel, millimeters)
$131 = 915.000 (Y-axis maximum travel, millimeters)
$132 = 40.000 (Z-axis maximum travel, millimeters)

Once these parameters have been set, ensure the robot moves smoothly and can be successfully homed and jogged around the entire footprint of the machine. Make note of the Com port your GRBL is connected to, as the Matlab GUI requires this information to connect to the robot.

Software Dependencies

Please ensure the folder "CNC_Dependents" is located in a specified directory on your computer. This will be the "home directory" you will set when you open the GUI. This directory will eventually contain two additional important files, a parameter file (which contains information about plates currently in the system) and a calibration file (which contains information about plate locations). Therefore, it is best to create a folder dedicated to being the home directory for the robot which will not be heavily accessed or edited.

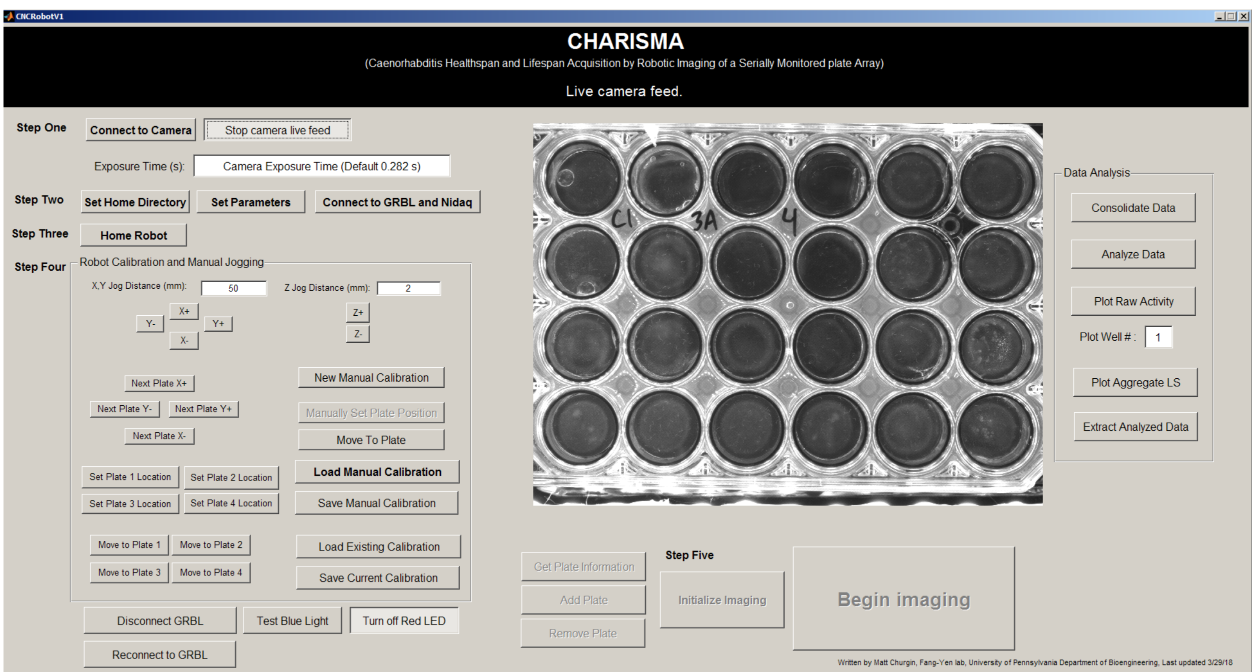
For the camera, ensure the Imaging Source Matlab adaptor is installed and working properly: https://www.theimagingsource.com/support/downloads-for-windows/extensions/icmatlabr2013b/

Overview: Using the GUI

To open a GUI in Matlab, type "guide" in the command window and press enter. You may then select the .fig file corresponding to the GUI you wish to open. Once open, press the "Run Figure" button (right-pointing green triangle) and choose "Add to Path" if prompted to do so. The GUI is now running and you can proceed to step one.

In general, buttons which are mandatory every time the GUI begins are shown in **bold typeface**. The text on non-mandatory buttons or buttons which are only sometimes necessary is shown in normal weight typeface.

Below is a screenshot of the GUI. Steps One through Five are labelled near the buttons required to complete each step. In general, when opening the GUI, these steps should be performed in order. Note that some button labels are gray and other are black. Certain actions cannot be performed while other buttons are active. For example, you may not "Begin Imaging" until you press "Initialize Imaging." This ensures that the camera is positioned over the correct plate in the imaging queue in the event that imaging is interrupted, for example if plates are added while the system is in the imaging loop phase.



Required Steps Every Time the GUI Is Opened

Step One: Click "Connect to Camera".

Step Two: Click "Set Home Directory". This is the directory that contains all functions required to run the GUI. These functions are located in the folder "CNC_Dependents". Please ensure that you set the home directory as the folder that includes the folder "CNC_Dependents."

Next, click "Set Parameters." See the "Setting Experiment Parameters" section for more details on setting parameters for the first time and loading an existing set of parameters.

Next, click "Connect to GRBL and Nidaq". You will be asked to enter the Com ports for both devices.

Step Three: Click "Home Robot". The robot will move to the home position. Once the robot has successfully homed, you may jog it manually or set a new or existing calibration (step four).

Step Four: In this step you will either calibrate the robot or load a previous calibration. You may jog the robot manually to move the robot to a desired location and set plate locations. See the "Robot Calibration" section for more details on setting up a new calibration and loading an existing calibration.

Step Five: After steps one through four have been successfully completed, you may begin imaging if there are plates in the system. To begin imaging, first press "Initialize Imaging". This button moves the camera to the first plate to be imaged. Next, press "Begin Imaging". The system will now image all plates available according to the parameters you have entered.

For more details on how to add plates, remove plates, or get existing plate information, see the "Plate Maintenance" section.

Setting Experiment Parameters

Ensure that the Home Directory has been set before attempting to set parameters.

Setting System Parameters for the First Time

1. Click "Set Parameters"
2. When asked whether you would like to load a previous robot state, type "N" and click OK.
3. Enter parameters for the experiment as desired and press OK.
4. Enter a unique name for the system parameters save file. This will be the file you will load in the future should you need to restart the GUI.

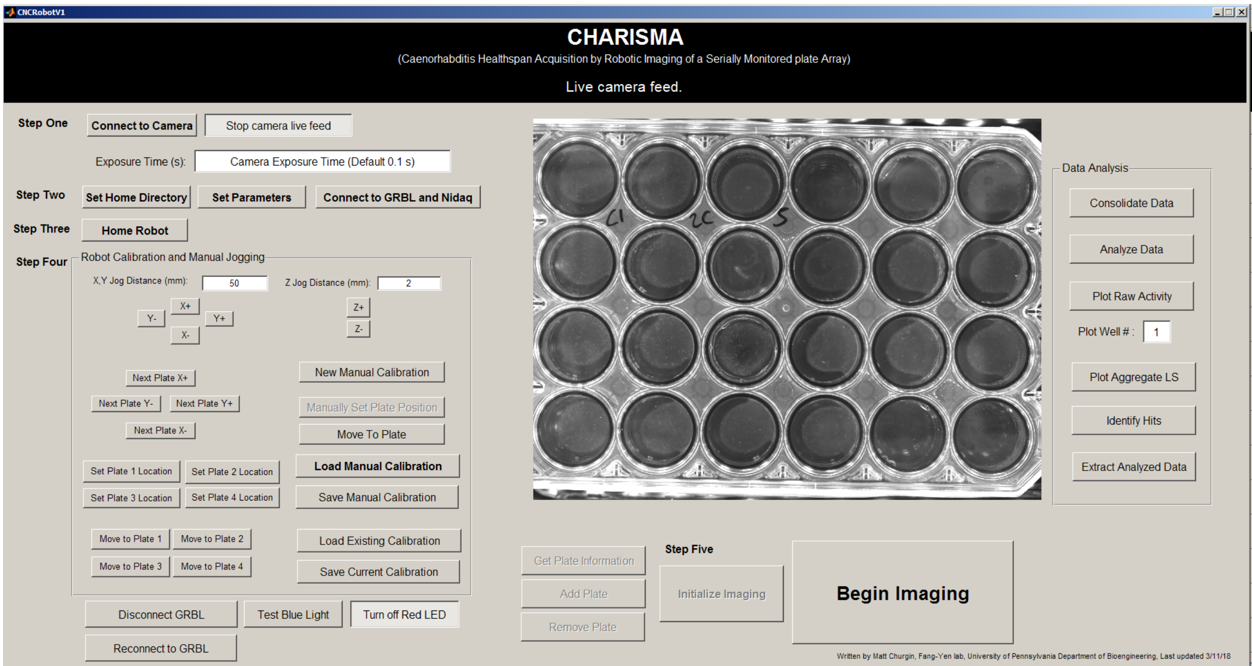
Loading a Previously Initialized Set of Parameters

1. Click "Set Parameters"
2. When asked whether you would like to load a previous robot state, type "Y" and click OK.
3. Select the parameter file (it will be a .mat file) that you previously initialized and press Open.
4. Enter a new name for the save file. Re-entering the original name will result in an overwrite of the robot state if any changes are made (such as plate additions, removal, etc.). Typically this is fine. If desired, however, a unique name can be created each time parameters are set.

Note: On Matlab 2015 or later, loading a previous parameter file causes a duplicate copy of the GUI interface to open. If this occurs, simply close any additional windows that open when loading the previous parameter file and continue. It is important to close the new window(s) that open and continue working in the original window, otherwise errors may result.

Manual Camera Control

Once the camera has been successfully connected, you may want to test the camera in order to focus the image or just calibrate the robot positions. However, if you have connected your red illumination LEDs to the NIDaq, you first need to complete step two which consists of setting the home directory, setting parameters, and connecting to GRBL and NIdaq. Once this step has been completed, you may press the "Turn on Red LED" button at the bottom of the GUI. Now illumination will be present when the camera is in live mode.



Note that the red LEDs and camera live mode should both be turned off before attempting to perform other functions in the GUI, such as calibrating robot positions, adding/removing plates, initializing imaging, or beginning imaging. An error will likely result if either red LEDs are on or camera is in live mode when you attempt any of these other actions.

Robot Calibration

Please ensure that steps one through three (see "Overview" section) have been successfully completed before attempting to jog the robot or set up or load a calibration.

Note: A sample manual calibration file is included in CNC_Dependents as "ManualCalibration_Official.mat". This file contains a sample calibration for a 91 plate CNC machine (13 x 7 plates). If you are using that size CNC footprint, you may load "ManualCalibration_Official.mat" as a starting point for your calibration. It is likely necessary to adjust each plate location to ensure the best alignment.

Manual Jogging

To manually jog the robot in X or Y (lateral) directions, first enter a number in the text boy labelled "X,Y Jog Distance (mm): " and press enter. You may now click the directional buttons X+, X-, Y-, and Y+ to jog the robot in the specified direction. You may change the jog distance at any time by typing and entering a new number in the text box. Vertical jogging is performed in the same way using the Z text box and directional movement buttons. The robot can also be jogged to the next plate in the X or Y direction (approximately) with the buttons labelled "Next Plate" just below the X+, X-, Y+, Y- directional buttons. These are convenient during the calibration process, but small manual adjustments will likely be necessary due to imprecisions in laser cutting or acrylic tile sizes.

Saving and Loading Manual Calibrations

To use the robot, plate positions must be calibrated. This is done manually. To start a new calibration, click "New Manual Calibration". Each plate location must now be set (see section "Manually Setting and Adjusting Plate Positions"). Once all plate positions have been set, click "Save Manual Calibration." Enter a name for the calibration file (this is the file you will load in the future when the GUI is restarted) and press OK.

To load a previously created manual calibration, click "Load Manual Calibration." Select the calibration file created previously and press OK. The plate positions have now been loaded and you may continue to step five.

Manually Setting and Adjusting Plate Positions

To set a plate position, jog the robot to the desired position. You may choose a unique Z-height for each plate position, allowing plates with different heights (and therefore requiring different distances from the fixed focal length camera lens) to be imaged on the same robot. If you have multiple types of plates on the same robot (e.g. 24-Well plate and WorMotel), you will want to ensure that the correct Z-height is calibrated depending on the plate you intend to place in a given position.

Once the robot has been jogged to the plate position you want to calibrate, press "Manually Set Plate Position." The mouse pointer will turn into a crosshair cursor. Next, select the square in the grid corresponding to the current robot position. Once a plate position has been set, a pink star will appear on that square in the grid. If a plate's position has not yet been set, no pink star will be present.

In practice, it is helpful to have a an empty 24-well plate handy during the calibration process. For each plate location, place the 24-well plate in that location, turn on the red LED ("Turn on Red LED") and camera ("Test Camera" or "Start Live Camera Feed") and jog the robot so that the plate is center on the image. Once the plate is centered, be sure to turn off the camera and red LED, then press "Manually Set Plate Position" to lock in the position. **The plate position cannot be set while either the red LED or camera are turned on.**

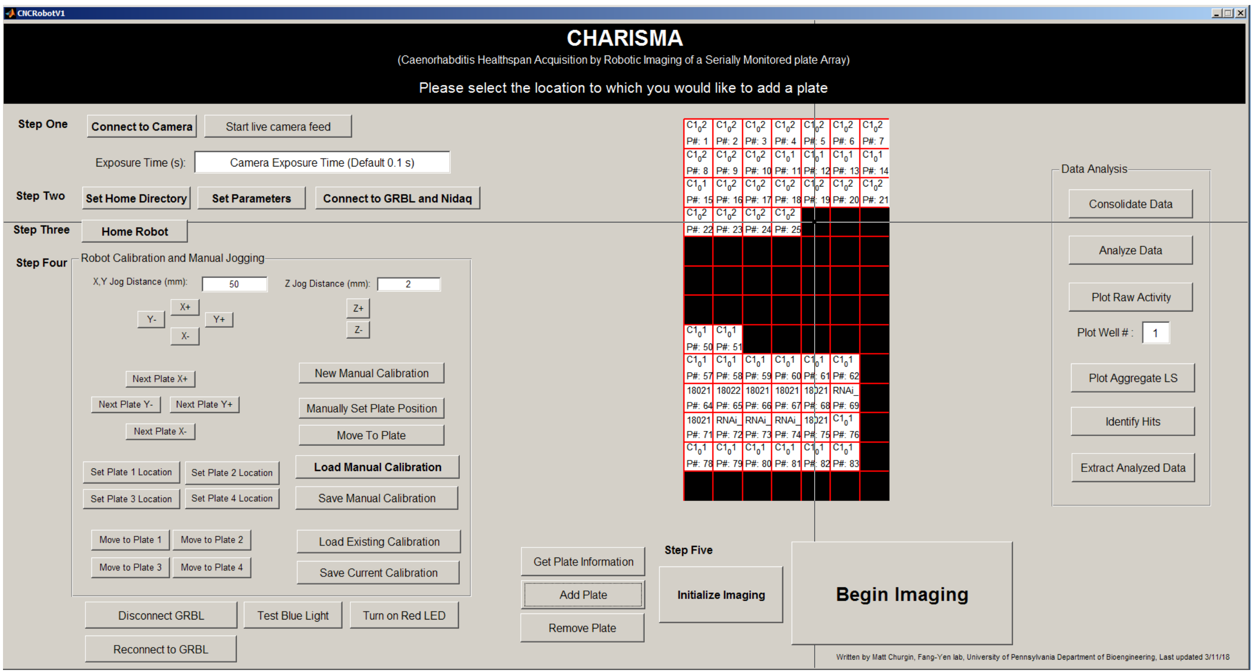
After setting a plate position, you can check that the correct position was recorded by clicking "Move To Plate" and selecting the plate whose location you would like to check. The robot gantry will move to the selected position, allowing you to check that position is calibrated as desired.

Once all plate positions have been set, be sure to save the calibration (see "Saving and Loading Manual Calibrations").

Plate Maintenance

Adding a Plate

1. Click "Add Plate"
2. The mouse pointer will turn into a crosshair. Select the square on the grid to which you would like to add a plate.



3. Enter "Y" and press OK if you have selected the desired location. Type "N" and press OK if you selected the wrong position.
4. Navigate to the directory in which you would like to save the images for the new plate. Click "Select Folder."
5. Enter a unique name for the plate and press OK.
6. If the plate is a 24-well plate that you wish to be processed as a *C. elegans* population aging experiment, type "1" and press OK. If not, type "2" and press OK. 24-well plates will have images from each day saved in a unique subfolder for daily processing by the GUI. Non-24-well plates will have all their images saved in a single directory to be processed by the user off-line as desired.
7. Enter the name of the person adding the plate and press OK.
8. The square on the grid will turn white and display the first few characters of the plate's name, as well as the plate's number in the system.

Removing a Plate

1. Click "Remove Plate"

2. The mouse pointer will turn into a crosshair. Select the square on the grid to which you would like to remove a plate.
3. Enter "Y" and press OK if you have selected the desired location. Type "N" and press OK if you selected the wrong position.
4. The square on the grid will turn black and the plate has been successfully removed.

If a plate has been accidentally removed prior to completion of the experiment, it cannot be re-added under its previous name. When re-adding a plate in this manner, you will have to give it a unique name and later combine images from the individual folders.

Getting Plate Information

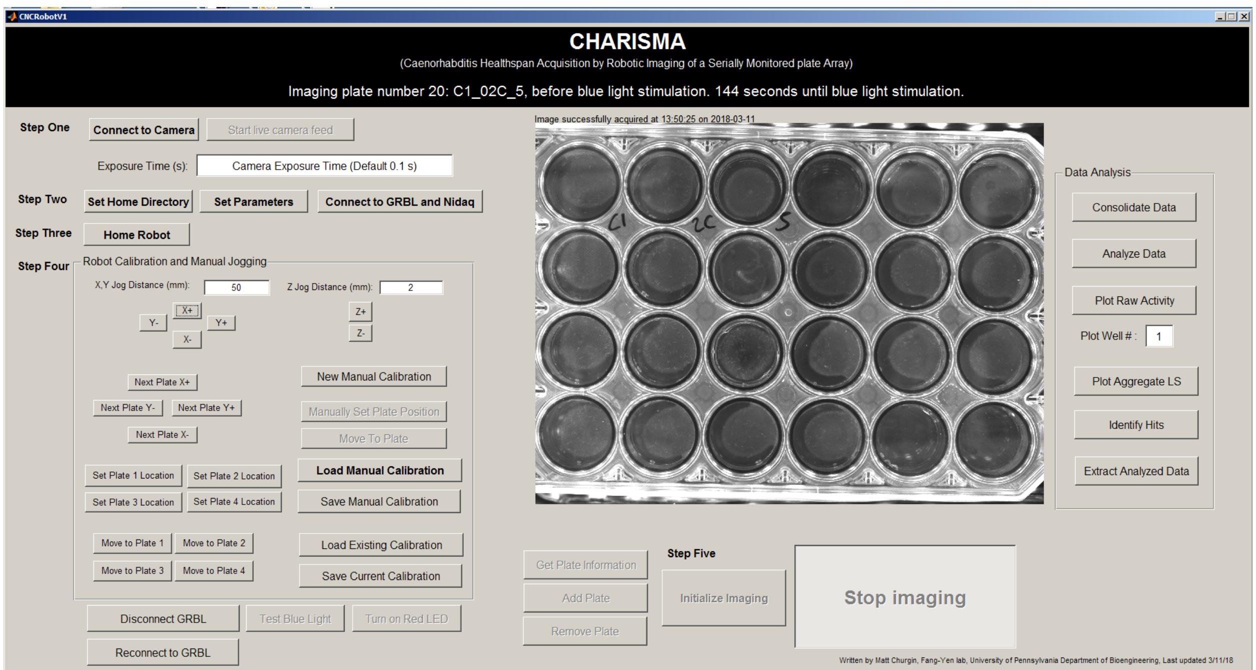
1. Click "Get Plate Information"
2. The mouse pointer will turn into a crosshair. Select the square on the grid to which you would like to get information about.
3. Plate information will be displayed at the top of the GUI.

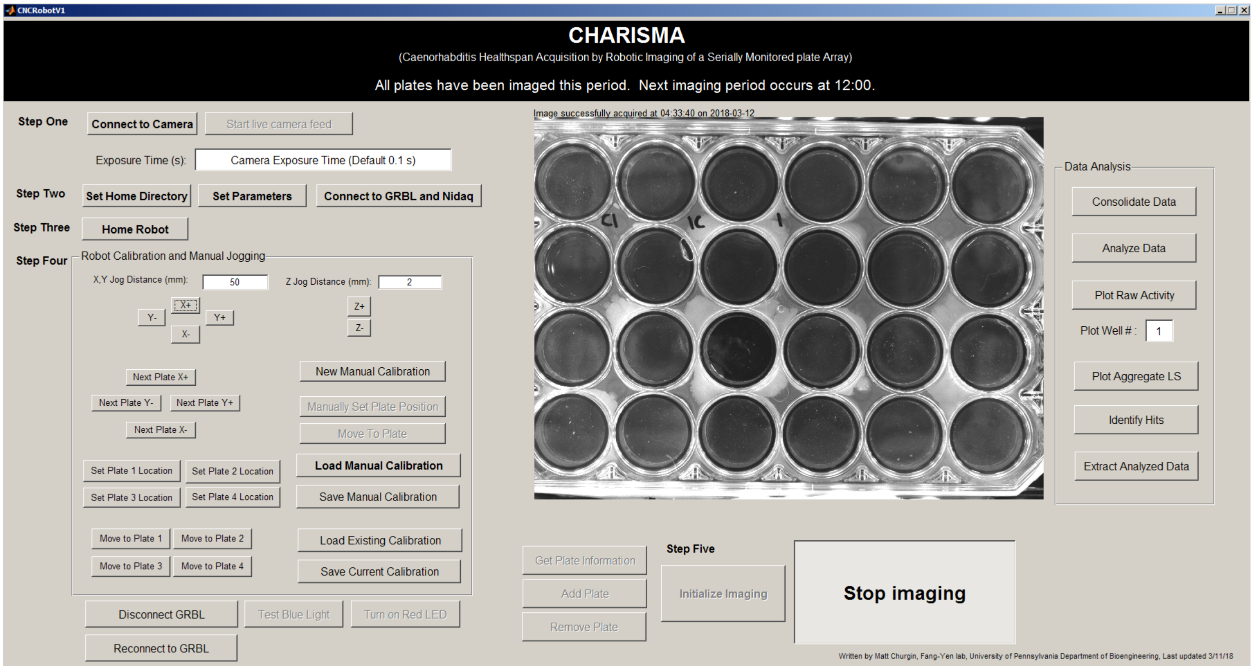
Imaging

Once Steps 1-4 have been successfully completed and any new plates have been added or removed, press "Initialize Imaging" to move the robot to the next plate position to image. Press "Begin Imaging" to begin the imaging loop.

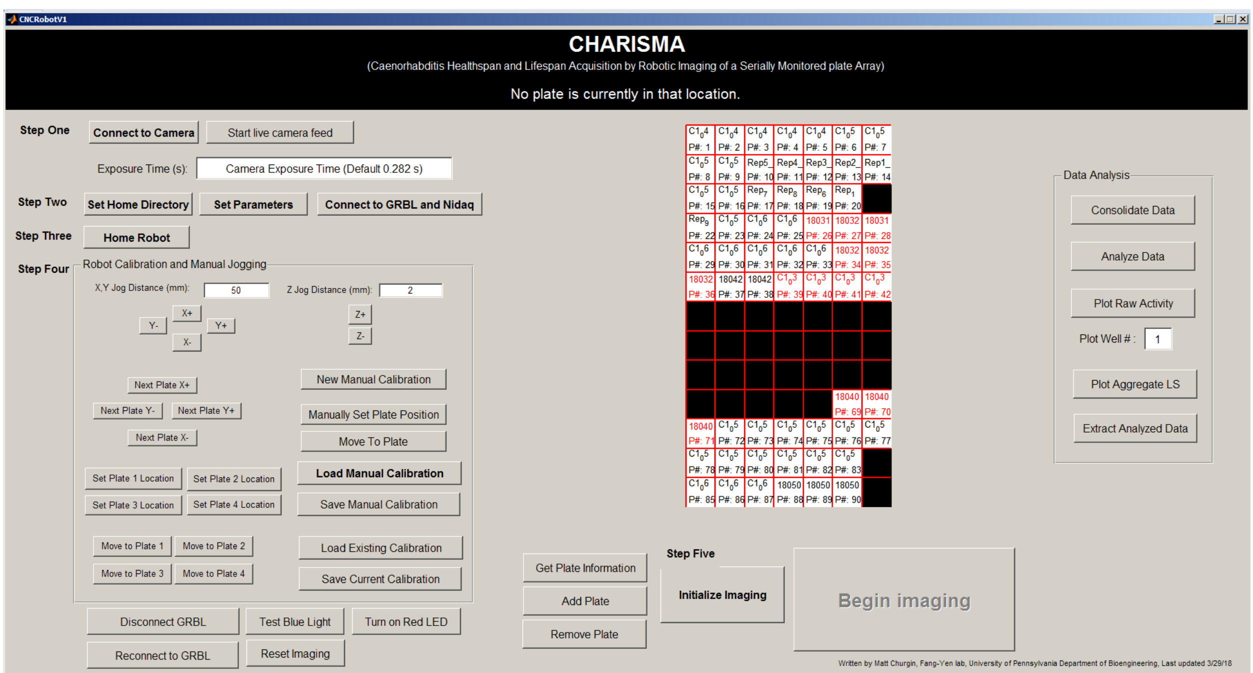
Now the robot will image each plate sequentially according to the parameters defined during initialization.

Imaging can be stopped during two times: 1) while the robot is between imaging plates or 2) when the robot is idle. Imaging cannot be stopped during imaging. A timer at the top of the GUI indicates when the robot will move to the next plate. It is during this time that imaging can be stopped.





Occasionally, the camera may disconnect while imaging. This will cause progress through the imaging loop to freeze. Unfortunately, this is a bug that has not been solved. When the camera disconnects, you should interrupt the Matlab control window (ctrl-x, ctrl-c) and press the "Reset Imaging" button (see below). If the disconnection occurred over night, it is usually best to reset the next plate to image to 1 (first plate in the system). If the disconnection occurs during the day, you may choose to not reset the plate to image, and the system will instead begin imaging the next plate in the queue.



Closing the GUI

If the GUI needs to be closed for any reason, it is advisable to first click "Stop Imaging" and "Disconnect GRBL".   If this step is not taken, the GRBL board will not recognize that the connection with Matlab has been lost and will require being restarted (power cycled) in order to establish connection with a new instance of Matlab.

Accessing Data

Images for each plate are located in the directory and folder name indicated when "Get Plate Information" is clicked.

Non-24-well plates are not processed in any fashion. Images for these plates can be processed offline by the user as desired. The only additional file present will be a "PLATENAME_plateInfo.mat" file which contains information about the plate, such as save directory, date added, person who added the plate, and plate type (24-well plate or not 24-well plate).

24-well plates are processed daily by the GUI. "PLATENAME_Consolidated.mat" contains full activity data for the plate for each day that images are present. "PLATENAME_Analyzed.mat" contains more processed activity data: it contains only the average activity value for each well before and after blue light stimulation. This file also contains AggLS and AggHS, which are the estimated lifespan and healthspan of the well.

To save the processed data from all plates saved in a single directory, click "Extract Analyzed Data." Navigate to the directory containing the plate data you want to save. Click "Select Folder." Next, enter a name for the new folder in which you want to save all the analyzed data for plates contained in the folder you selected and press OK. All consolidated and analyzed .mat files from the plates contained within the selected directory will now be saved together, enabling easy transfer of data.

Analyzing Data

Post-processing analysis scripts are included in the folder "CNC_postprocessing."

"analyzeSinglePlate.m" allows the user to visualize and save lifespan and healthspan data for a single" _Analyzed.mat" file that has been extracted from the GUI using the procedures described in the "Accessing Data" section of this manual.

To analyze data for multiple plates together, use "extractedDataPostprocess.m". This script will analyze data for all "_Analyzed.mat" files located in a single folder. This script will produce and save heatmaps of each plate for visual inspection of data quality. It can also produce histograms of lifespan and healthspan. This script is intended to work with data from 24-well plates filled according to the template described below in accordance with the Ahringer RNAi library. Some functions of the script may not work properly or may need slight modifications if not using this template.

Automatic Gene Name Labelling

If plates contain genes from the Ahringer RNAi library, the gene names can be automatically annotated. In that case, plate names (as entered into the GUI when first added) should be named in the format "C1_01A_1", where C1 indicates "Chromosome 1", "01A" indicates the 96-well plate number of the Ahringer library, and the final "1" digit indicates 1-5, the 24-well plate number that is explained below.

The function "lookUpRNAiClone.m" will read the plate name from the "_Analyzed.mat" file and automatically assign gene names according to the template outlined below for the 5 24-Well plates filled from 1 96-well plate of the Ahringer library.

If automatic gene naming is not necessary or applicable for your experiment, set the variable "genelookup" in the beginning of the script to 0.

Template for 24-Well Plates Filled from One 96-well Plate of the Ahringer RNAi Library

Plate 1

A1	A2	A3	A4	A5	A6
B1	B2	B3	B4	B5	B6
C1	C2	C3	C4	C5	C6
EV	EV	EV	EV	H7	H8

Plate 2

A7	A8	A9	A10	A11	A12
B7	B8	B9	B10	B11	B12
C7	C8	C9	C10	C11	C12
EV	EV	EV	EV	H9	H10

Plate 3

D1	D2	D3	D4	D5	D6
E1	E2	E3	E4	E5	E6
F1	F2	F3	F4	F5	F6
EV	EV	EV	EV	H11	H12

Plate 4

D7	D8	D9	D10	D11	D12
E7	E8	E9	E10	E11	E12
F7	F8	F9	F10	F11	F12
EV	EV	EV	EV	Empty	Empty

Plate 5

G1	G2	G3	G4	G5	G6
H1	H2	H3	H4	H5	H6
G7	G8	G9	G10	G11	G12
EV	EV	EV	EV	Empty	Empty

Software Troubleshooting

Problem: I tried to "Connect to Camera" but it says it failed.
Potential Solutions:

1. Ensure the Imaging Source Matlab adaptor is installed.
2. Ensure that only one camera device is connected to the computer. The software automatically connects to the first device available, and an error may result if multiple cameras are connected to the computer.
3. Close Matlab and restart. Try again. You may need to repeat this process a few times. This is a known bug with the Imaging Source Matlab adaptor that it sometimes fails when running in a new instance of Matlab.

Problem: Robot is not moving when prompted to do so.
Potential solutions:

1. Ensure robot is plugged in to power, USB is connected, and connected in the GUI. If so, disconnect GRBL and click "Reconnect to GRBL."
2. Click "Disconnect GRBL", close Matlab and restart GUI.

Problem: Software not acting as expected.
Potential Solution: In general, if anything unexpected occurs, it is best to close the GUI (after stopping imaging and disconnecting GRBL) and reopen.

Problem: Images are not processed or certain analysis files are missing.
First, ensure that ROIs (regions of interest) are successfully created for the plate(s) in question. The ROI file should be located in the plate folder. Load the ROI file in Matlab and ensure that the variable maskSorted is not equal to 0. It should be equal to a 3-dimensional matrix consisting of the automatically identified well locations. If the ROI file has not been properly created, analysis will fail. The primary reason for ROI creation failure is that the 24-wells are not visible by the software. Ensure that agar is present between the wells of the 24-well plate.