Manual: Head fixed Setup

Contents

Boxes	3
Spout controller Box	3
Breakout Box	4
Tools	4
List of components	4
Lid preparation	4
PCB Preparation	5
Photo Diode	6
Platform	9
Rotary encoder and wheel:	10
Motors and Spouts	10
Spout preparing	11
For each lick detection system, 3 stereo cables are needed as follows:	12
Cable preparation:	12
Wiring of the signal wires:	12
Wiring of the zero-position wires:	12
Grounding:	13
Test your signal:	13
Tactile stimuli system	14
Visual stimuli system	16
List of components	16
Auditory stimuli system	16
List of components	16
Water providing system	17
Camera and recoding system	18
For video recording in each experimental session, we use 2 cameras per setup. Since LEDs within the setup, we need to remove the IR filter of all cameras in the follows:	
First step: step: IR filter removing	18
Second step: Mounting the camera	19
Third step: Lens mounting	20
Fourth step: Mounting the IR LED	20
Ontional step: Mounting the IR Mirror	21

Bpod system	.21
Labcams installation	.22

To find 3D designs and print them, please refer to the part folder in the directory.

Our head-fixed setup is composed of different parts which are illustrated one by one in this manual.

All files that you may need during preparation steps are included over the repository.

Boxes

In the proposed setup we designed two customized PCBs (Printed Circuit Board).

- 1- Spout controller PCB
- 2- Breakout PCB

These two PCBs are housed within custom enclosures equipped with appropriate sockets and connected via Ethernet cables.

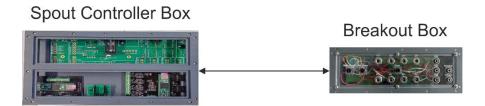


Figure 1 The boxes schematic and their connection.

In order to prepare each box, follow these instructions:

Spout controller Box

- Spout controller PCB
- Bpod teensy shield
- Bpod input module
- Bpod rotary encoder module
- Box (assemble the box based on the location of modules and prints over the panels of the box)

After you assemble the box put all components in the box and then the box is ready to be connected to the breakout box using ethernet connections.

Breakout Box

Tools

- Soldering iron
- Crimping Tool
- Side cutter
- Screw drivers
- "Helping hand"

List of components

- Custom Breakout PCB
- Custom Surrounding Box
- 8x Power Jacks RND (<u>link</u>)
- 5x Phone Audio connector, 3 Contacts, Jack, 3.5 mm (link)
- 3x Plug Housing, Dual Row, with Panel Mount Ears, 4-Circuits (link)
- 26x Oval-Head M3x8 SS
- 12x Crimp Terminal, Male, with Select Gold (Au) Plated Tin/Brass Alloy (link)
- 4x Modular Telephone Jack, 8P8C Right Angle, PCB (link)
- Solder
- 26 AWG Wire (different colors) (link)

Inside this box you just have the breakout PCB (Figure 2)

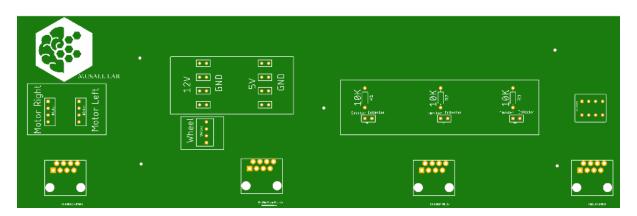


Figure 2 Breakout PCB

The Box consists of 3 main parts.

- 1- Motors
- 2- Wheel and power sources
- 3- Photo diodes

Lid preparation

Fasten all audio connectors and Power Jacks to the dedicated holes in the lid using the enclosed screw nuts.

To prepare the power source connections, the pins must be soldered to ~10 cm long 26 AWG wires as follows:

Long pin → GND holes

Short pin → Positive holes

Therefore deisolate ~2 mm at the end of each wire and put some solder on the ends.

Fill the connections of the Power Jacks with solder as well. Now melt the solder on the Power Jack connections again and insert one end of a wire each. Wait until hardened and use a shrinking tube to isolate the connection.

Repeat this for the audio connections.

For the plug housing start preparing the wires as before. Then use a crimp tool to attach the wires to the male crimp terminals. To ensure a proper connection merge the wire and the crimp terminal with a small amount of solder. Ensure that no stripped wire is outside of the crimp terminals and that it is only in the crimped area (not in the tip of the crimp terminal).

Now push the prepared crimp terminals into the Plug housing. Afterwards push the housings into the dedicated spots in the lid.

PCB Preparation

Push the Rj45 ethernet jacks into the dedicated spot of the PCB and solder the pins to the contacts of the PCB (Figure 3).



Figure 3 Soldered Telephone Jack

Connect the other end of the wires that you soldered to the plugs to the associated holes in the PCB (prepare the connections as seen in Figure 4) and fixate them by soldering them to the contacts.

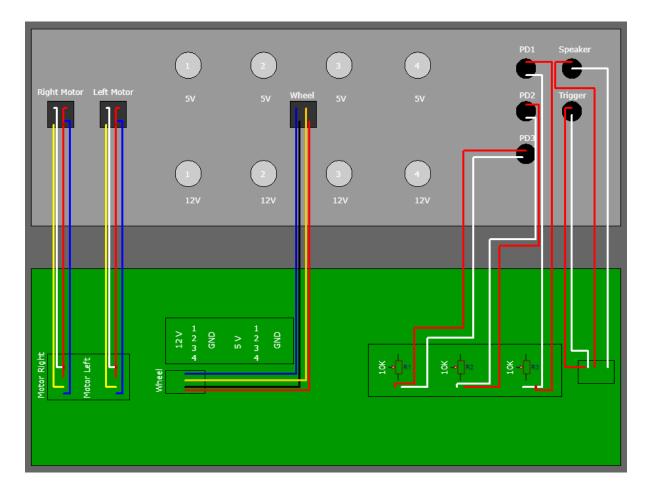


Figure 4 Schematic of the connections between lid and PCB (exemplary color code)

Put everything in the box and glue the telephone jacks to the panel. When the glue dried screw all panels to the box.

Photo Diode

The following picture shows the general configuration of a photo diode.

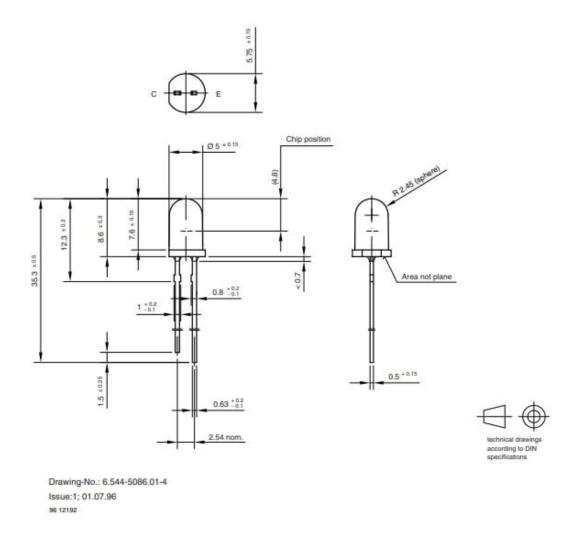


Figure 5 General scheme of a photo diode

First step: Wire up the photo diode

To wire up the photo diode, start by removing the cable jacket of the stereo cable. Next, solder the longer pin of the photo diode to the white wire and the shorter pin to the red wire. It's important to notice that the shielding (small surrounding wires) should be left unconnected in this specific case.

In summary:

White wire: Anode/ Longer pin/ Emitter.

Red Wire: Cathode/ Shorter pin/ Collector

The following pictures show the process and final result of the process.







Figure 6 Preperation steps to connect the photo diode

Second step: Preparing the breakout PCB section

There is a section on the breakout PCB that refers to the photo diode over which you need to solder the components to. On the breakout PCB there is a section related to the photo diode to which you need to solder the components.

First component is 10k resistors, which must be soldered as seen in Figure 7

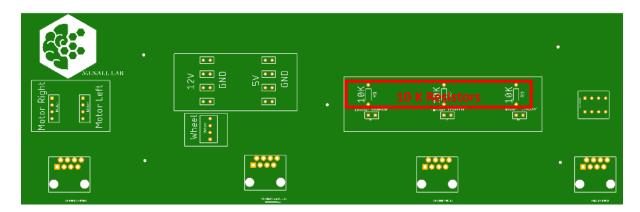


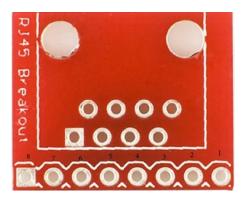
Figure 7 Breakout PCB with attached Hardware

There are three stereo sockets in the lid of the breakout box that are dedicated to the PDs. Solder the respective stereo pins of the plug to the emitter/collector holes in the PCB via a cable.

Third step: Preparing Touchshaker box

As we mentioned before, there is a dedicated ethernet connection for PDs between breakout box and touchshaker box.

As final step you need to prepare the ethernet socket in the touchshaker side. If you consider the ethernet breakout board as bellow:



In order to prepare the PD ethernet socket in the touchshaker box, you need to solder three wires to pins 8 / 7 / 6 and connect them to the positive pins over the Bpod input module inside the touschshaker box.

Alternatively, you can solder the cables directly to an Ethernet socket in the box.

In summary

Pin 8 ethernet breakout board ----- 1⁺ input channels in Bpod input module

Pin 7 ethernet breakout board ----- 2⁺ input channels in Bpod input module

Pin 6 ethernet breakout board ----- 3⁺ input channels in Bpod input module

Pin 2 ethernet breakout board ----- Pins 1⁻,2⁻,3⁻ input channels in Bpod input module

Platform

AND

- Thorlabs platform
- Thorlabs poles
- Thorlabs base

Assemble poles on the platform for each component of your setup that you want to fix to the platform. You may need to adjust their positions later to ensure that everything fits properly and you get proper videos of your test subjects.

Rotary encoder and wheel:

- Wheel (3D-print)
- Wheel Holder (3D print)
- Rotary encoder
- Rotary encoder holder (3D print)
- 1. Attach the wheel to the wheel holder.
- 2. Attach the Rotary encoder to the rotary encoder holder.
- 3. Attach the wheel to the rotary encoder using the wheel holder.
- 4. Attach a ThorLabs pole to the rotary encoder holder.
- 5. Place everything on the platform
- 6. Connect the rotary encoder to the Breakout Box using the "Wheel" 4-pin plug on the Breakout box. (Therefore, you need to prepare the rotary encoder cable with a male 4-pin jack)

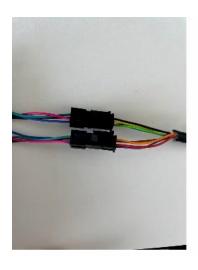
Motors and Spouts

- Stepper motors
- Spouts
- Spout holders (3D design)
- Motor holder (3D design)
- Motor wires (depends on the motor type)
- Shrinking tubes
- Stereo cables
- 4-pole crimp connectors + housings (Female and male)
- Optional: you may use specific core wires with multiple small wires in it for improved cable management (link)

First step: Prepare your motors wiring

Remove the cable jacket to access 8 small wires inside it. Connect these 8 small wires to two different male-4-port connection sockets.

Connect 4 wires of each motor to a female-4-port connection socket. You can see the final result in Figure 8.



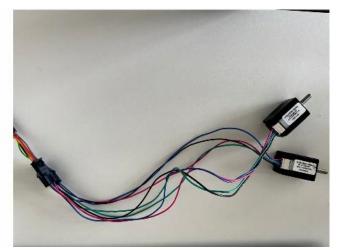


Figure 8

Second step: Connect the free end to the breakout PCB using the following order.

Be aware that the color coding of motors is important in this step. Based on the motor that you are using you may have 4 different colors of wire in each motor (Black/green/blue/red OR blue/orange/red/yellow)

The way that you can replace different types of motors together is:

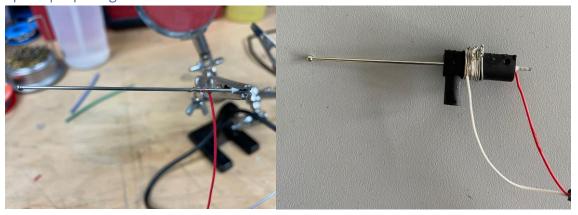
German version: red/blue/yellow/orange

US version: black/green/blue/red

Finally, after checking all color codes, solder free end to the PCB as bellow:

On the PCB side from top: Black / Green / Blue / Red





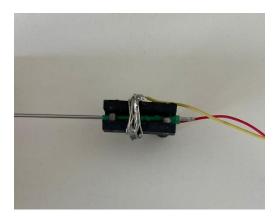


Figure 9 Spout preparing

For each lick detection system, 3 stereo cables are needed as follows:

Cable 1 ---- Signal 1

Cable 2 ----- Signal 2

Cable 3 ---- Zero position and grounding

Cable preparation:

Take off the plastic shielding on all three stereo cables. As a result, each cable will reveal two wires:

red/white or yellow. The concept is to utilize the red wire for transmitting the desired (Licking) signal.

Wiring of the signal wires:

Select the red cable from the stereo cables and solder them to the end of the metal body of the spout (you can wrap it around the spout first and then solder it on). Ensure that the soldered part fits securely inside the spout holder, as depicted in the provided picture. Finally, use a shrinking tube to secure and stabilize the connection between these two components.

Now connect your stereo cable to the stereo sockets labeled "Spout Left" and "Spout Right" on the Touchshaker box.

Wiring of the zero-position wires:

Insert the spout into the holder and fasten it by screwing it tightly. Then, wrap a metal wire around the spout holder to provide additional stability. Proceed by soldering the white wire from cable 3 to the wrapped wire of spout 1 and soldering the red wire from cable 3 to the wrapped wire of spout 2.

Grounding:

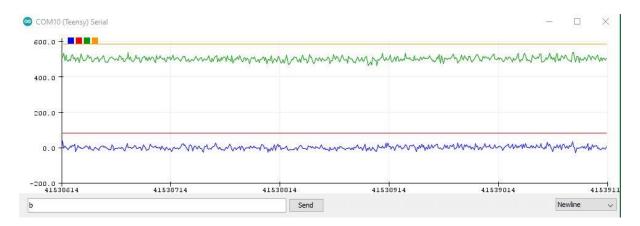
On each motor holder, there are designated spots where screws need to be inserted. Next, take the shielding cables from the third stereo cable and solder it to both screws on the motor holder.

Connect the third cable to the "Motor Zero" socket over the touchshaker box.

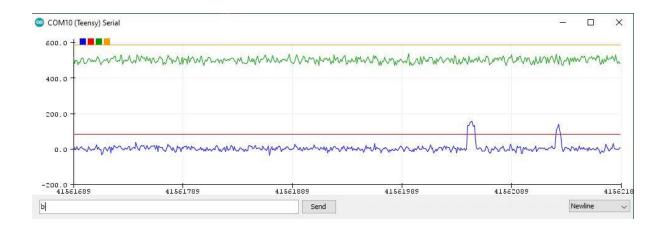
Congratulations! Your spout is now successfully assembled and ready to be used.

Test your signal:

Attach the spouts on top of the motor holder carefully since holders are fragile. To test the spouts, run the paradigm using Bpod and change the position of the spout by using the GUI. Furthermore, to verify the adequacy of the licking signal, open the Arduino IDE, run the Touchshaker.ino and access the serial monitor. Signals without any licks should resemble the example depicted in the provided picture. If the signals appear noisy or distorted, it is advisable to double-check the connections and soldered spots meticulously to ensure a proper and stable connection.



Next, gently touch the top of each spout with a slightly wet finger. You should observe a diagram similar to the one depicted below.



Tactile stimuli system

- Spouts
- Spouts holder (3D design)
- Bpod port interface
- Tubes
- T-connector
- Valves
- Air Compressor System
- Air Pressure Regulator

To set up the pneumatic tactile feedback system, follow these detailed steps:

- 1. Connect the Pressure Regulator to the Compressor System:
 - Take a suitable air tube and connect one end to the output port of the air compressor.
- Attach the other end of the tube to the input port of the pressure regulator. Ensure the connection is secure to prevent air leaks.
- 2. Mount the Valve to the Bpod Port Interface:
 - Locate the Bpod port interface on your system.
- Securely mount the solenoid valve to the designated port on the Bpod interface. Make sure the valve is properly aligned and firmly attached to ensure reliable operation.

3. Connect the Tubing:

- Attach one end of a tube to the output port of the pressure regulator.
- Connect the other end of this tube to the first input port of the valve.
- From the second output port of the valve, connect a soft, flexible tube. Ensure the tube is long enough to reach your desired location but not so long that it causes delays or pressure drops.
 - Attach the free end of this soft tube to the air puff connection on the camera holder.

Detailed Steps with Additional Considerations:

- 1. Connecting the Pressure Regulator:
 - Ensure the air compressor is turned off before making any connections.
 - Check that the pressure regulator is rated for the output pressure of your compressor.
- Use hose clamps if necessary to ensure airtight connections between the compressor and the pressure regulator.

2. Mounting the Valve:

- The Bpod port interface typically has specific slots or connectors for mounting peripherals. Refer to the Bpod documentation for exact mounting procedures.
- 3. Connecting the Tubing:
- Cut the tubing to the required lengths using a sharp utility knife to avoid any uneven edges which could cause leaks.
 - Secure all tubing connections with appropriate fittings or hose clamps.
- Double-check all connections for tightness. Loose connections can lead to loss of pressure and ineffective tactile feedback.

Final Checks and Testing

- **System Integrity**: After all connections are made, ensure the entire system is securely connected and there are no potential points for air leakage.
- **Pressure Adjustment**: Adjust the pressure regulator to the desired level suitable for your tactile feedback requirements.
- **Operational Test**: Turn on the compressor and check for any leaks. Then, operate the system to ensure that the valve correctly modulates air flow to the air puff connection on the camera holder.
- Calibration: If necessary, calibrate the system by adjusting the regulator and valve settings to achieve the desired tactile stimulus.

By following these steps carefully, you can set up a tactile stimuli system that is effective and reliable for your intended applications. You may see the connections bellow:

Visual stimuli system

List of components

- Monitor
- Monitor holder (3D design)
- Cables

Two/one monitor(s) must be mounted in front of the wheel/platform. Then they only need to be connected to the PC and supplied with power. The Python code can now execute the visual stimuli. Some adjustments may have to be made to the code so that the stimuli are executed correctly and on the correct screens.

Auditory stimuli system

List of components

- Stereo Speakers
- BNC sockets
- BNC cables

To enable auditory stimulation, a BNC connection to the Bpod output module is required. Follow these steps to connect both cables to the unsoldered BNC socket and then prepare and solder the BNC sockets to a stereo socket as illustrated in the diagram:

- 1. For each BNC socket, attach a red wire to the central pin (these will be referred to as main wires 1 and 2).
- 2. Connect a shared wire to the smaller pins of both BNC sockets.
- 3. Stereo sockets have 4 pins; the top and bottom ones are the main ones. Connect main wire 1 and main wire 2 from the BNC sockets to these pins (Figure 10).
- 4. Attach the shared wire to one of the remaining pins in the stereo sockets (Figure 10).
- 5. Plug the stereo cable from the speakers into the stereo socket.

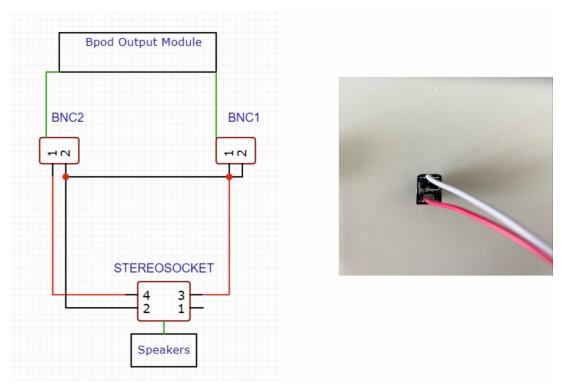


Figure 10 Illustration of the Auditory-Bpod conncetions

Water providing system

- Tubes
- Syringe
- Syringe holder (3D design)
- Three-way valve

- Valves
- T-connector

Steps:

- 1. Attach the Bpod port interfaces with the valves to the T-shaped part of the syringe holder with glue
- 2. Attach the Three-way valve to the syringe and insert it into the holder
- 3. Connect the Three-way valve to the T-connector with a tube
- 4. Connect both other ends of the T-connector to middle input of a valve each using tubes
- 5. Close the input closest to the metal piece of the valves using a tied-up piece of tube
- 6. Connect the output of the valves to the spouts using tubes
- 7. Connect the BPod port interfaces to the state machine with an Ethernet cable

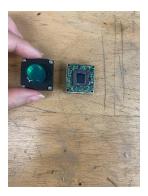
Camera and recoding system

- Camera (We use FLIR Chameleon 3.0 or FLIR Firefly)
- Camera cable
- Custom camera Mount
- IR LED
- IR LED holder (print)
- IR mirror
- IR mirror holder (print)

For video recording in each experimental session, we use 2 cameras per setup. Since we use IR LEDs within the setup, we need to remove the IR filter of all cameras in the following order:

First step: step: IR filter removing

Open the camera carefully. Usually, the CMOS (Complementary Metal-Oxide-Semiconductor) inside the camera is extremely sensitive.



Use the hot gun to lose the glue that is used to fix the filter.





Remove the filter and close the camera.



Second step: Mounting the camera

To securely mount the lens onto the camera, please follow these steps: Install the camera on top of the mounting piece same as the picture (for this step you need very small screws)



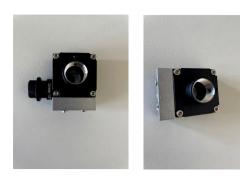


Third step: Lens mounting

Each lens has a fixation ring which needs to be installed first.



There is a small screw on top of the camera lens holder which you need to unscrew first to fit the lens.



Screw the lens into the camera. Finally, it should look like shown below.



Now connect the camera to the PC and adjust the field of view and sharpness to the platform were the animal will sit later (Advice: use a dummy for this). The application that you may use for this purpose is SpinView[®]. The installation instructions can be found in the corresponding section.

Fourth step: Mounting the IR LED

After printing the IR LED-mount, you just need to mount the LED on it and fix it using small screws. For each camera you need one IR LED. Afterwards you can check if the lightning of your

video is properly and adjust it in an appropriate direction by checking the camera view on SpinView.

Optional step: Mounting the IR Mirror

Based on your setup, in some cases you can use a IR mirror to have a better view of the facial movements. To this aim you need to print out the IR mirror holder and stick an IR mirror on top of it and adjust the direction using SpinView.

Bpod system

- Bpod state machine (Here we use Bpod State Machine r2.5)
- Bpod input module
- Bpod output module
- Bpod port interface
- Bpod teensy shield
- Bpod ambient module
- Bpod rotary encoder module

The first step is to connect the various components of Bpod. Figure 11 illustrates the connections between different Bpod components and modules.

After you have connected all the components based on Figure 11 you need to set up Bpod on your PC by using Matlab. The following steps are required for this purpose:

- 1- Clone the Bpod_Gen2 repository from here.
- 2- Add/Bpod Gen2/to the MATLAB path.
- 3- Run Bpod() at the MATLAB command prompt.

You are all set to run Bpod.

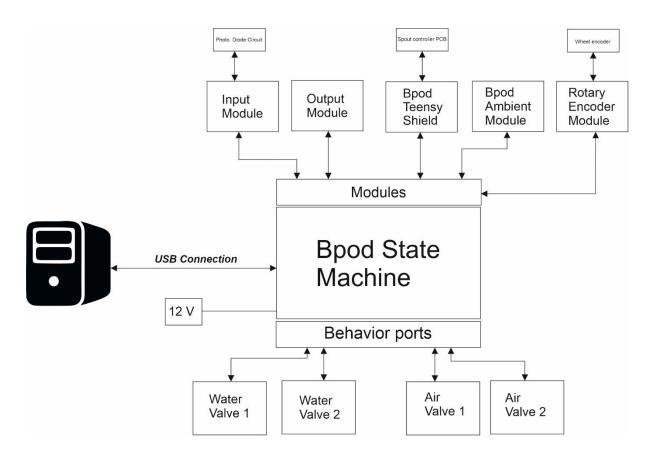


Figure 11 Illustration of the necessary Bpod connections

Labcams installation

In order to install Labcams you may easily follow the instruction over the <u>link</u> or follow these steps:

- 1. Install <u>anaconda</u> if not already done and add conda to system PATH when asked during installation.
- 2. In LABCAMS folder, import the following files onto your desktop from the "labcams installation stuff" folder:
 - a. Repository: Zipped labcams-main folder (save on desktop and extract all)
 - b. FLIR Camera Driver Python file: *spinnaker_python-2.5.0.80-cp38-cp38-win_amd64.whl* (save in labcams-main folder on desktop)
 - c. FLIR Camera Driver: SpinnakerSDK FULL 2.5.0.80 x64.exe
 - d. default.json file
- 3. Download Notepad++.

4. In the anaconda prompt, create a new virtual environment called labcams with a downgraded 3.8.12 version of python. Type the following into the prompt:

conda create -n labcams python=3.8 anaconda conda activate labcams

- 5. Open the labcams-main repository folder in anaconda, type: cd labcams-main
- 6. Install downgraded version of PyQT package, type: pip install PyQT5
- 7. Install the remaining labcams requirements, type: pip install -r requirements.txt
- 8. Install labcams software, type: python setup.py develop
- 9. Install SpinnakerSDK onto the desktop.
- 10. Install camera driver, type:

pip install spinnaker_python-2.5.0.80-cp38-cp38-win_amd64.whl

11. Generate default.json file, type:

labcams

- 12. Edit the default.json file (i.e. add camera serial number) or copy and paste the default.json file. *Note: Labcams only uses the filename default.json!*
- 13. Install ffmpeg, type: conda install -c conda-forge ffmpeg (only do so if you are capturing video)

To run labcams in the future, you must activate the labcams virtual environment first in the anaconda prompt, type: conda activate labcams and then labcams.

Or you may add c:/users/YOURUSER/Anaconda3/envs/Scripts and bin to the environment path on windows.