-Behavioral Rig Construction Notes

Kwan Lab, Yale
Updated by HA Jan, 2020

Introduction

- This manual has information about **lick detection set-up** as well as **steering wheel set-up.**
    - **Lick detection set-up** is designed for two choices and two outcome paradigm. There are left and right lick-ports which are used for choice detection as well as outcome consumption. This paradigm is used first lick as choice, and the rest of the licks as outcome/reward consummatory behaviour. Animals use the tongue for choice and outcome.
    - **Steering wheel set-up** is designed for two choices and one outcome paradigm. There are one wheel (used for left and right turns for choices) and one lick port for reward consumption. It uses steering wheel responses as choice, and lick responses for reward consumption. Animal use palms for turn/choice, tongue forlicking outcome.
- All necessary components/parts with vendors and prices can be found: https://docs.google.com/spreadsheets/d/1VIZEDlVTupoTt61jeeu2QYp-AVEco69VJNO5CFrp0Lw/edit#gid=0 (if link is not working: Component List excel should be in Kwan Google Drive. For external usage, you should receive a components list as well as this manual. If not, please contact the person that sent you the manual)
- **Section 1 - Hardware**: includes lick port detection, steering wheel and water reward system.
    - For lick detection set-up; you may ignore steering wheel part.
    - For steering wheel set-up; you still need lick detection section, as lick detection is used for reward delivery system. Only differences, we used only one lick port in steering wheel set-up, but two lick ports in lick detection set-up.
- **Section 2 - Software**: Briefly list the software needed for both systems.
- **Section 3 - How to assemble lick detection set-up** : Each step is described in details for assembling the lick detection set-up.
- **Section 4 - How to assemble steering wheel set-up** : Each step is described in details for assembling the steering wheel set-up.
- **Section 5 - Pupillometry:** Pupillometry is an additional set up that can be used with lick detection set-up as well as steering wheel set-up to record pupil images. It requires a different computer which allows adapter card entry with different software/ hardware described in details in section 5.
- **Troubleshooting section** has some common problems we have encountered.

Section 1: Hardware

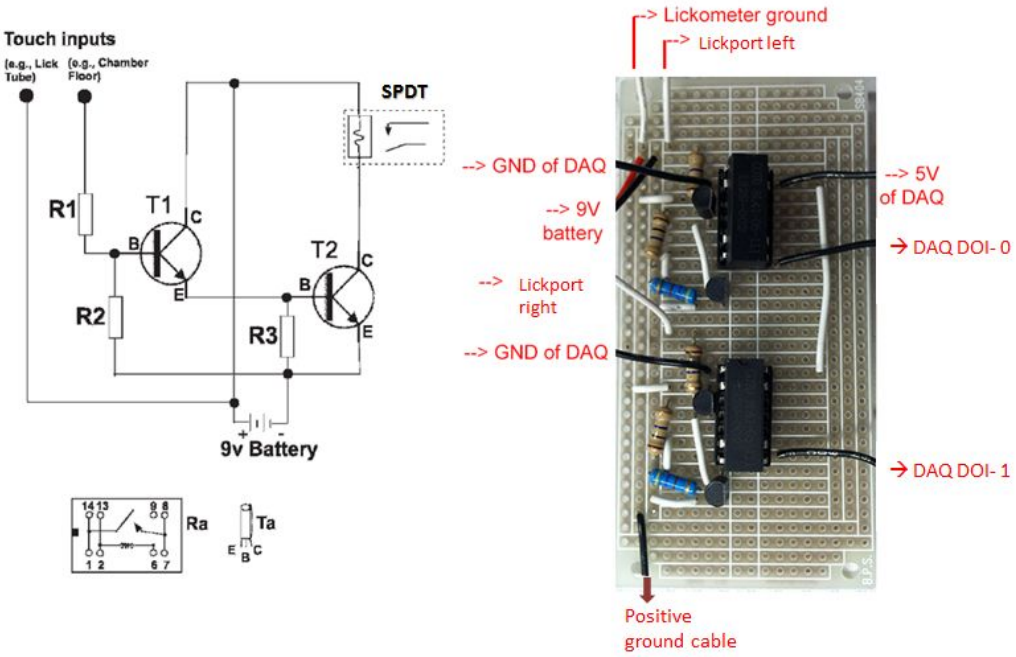
This section includes 3 parts. Lick detection, steering wheel and water delivery system. All necessary components can be found in the Components List.

1. Lick detection

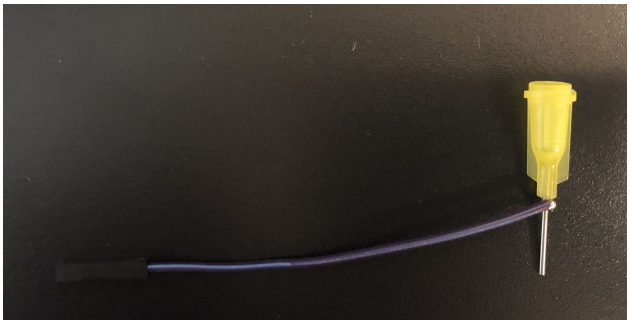
a. Lick detection circuit

Solder all the components for 2 detector circuits onto the PCB. The circuit follows Slotnick's paper (J Experimental Analysis of Behavior, 91 253, 2009,https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2648519/#jeab-91-02-07-Field1), with the following differences:

- Using SPDT instead of SPST relay, so that the output voltage can be pinned to 0 or 5V. In the original design using SPST, when the relay is open, the output voltage is floating, causing error in parallel port reading.
- The SPDT contains a diode within the package, so there is no need for external diode
- For SPDT, connect ground to pin 1, 5V to pin 14, and output voltage to pin 7 or 8.
- Check to see 9V from C to E for both transistors
- R1 and R2, 10 megohm resistors. R3, 47K ohm resistor



b. Lick ports with metal syringe

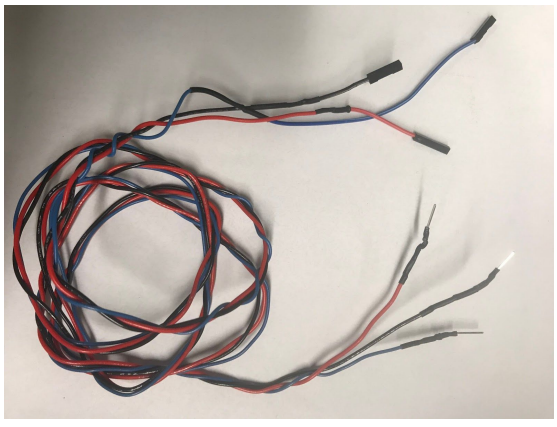


Solder a wire with female connector on to the metal syringe needle as shown in the figure. Female connector will be used to connect to detection extension cable. Use flux pen to increase connectivity between the wire and syringe. We need two metal lick ports with metal syringe for lick detection set-up, one metal lick port with metal syringe for steering wheel set-up.(This metal syringe will be used for lick detection and water delivery)

c. Positive ground cable

This cable will be connected to the headplate on the animal. That's why it needs alligator clip on one side, female connector on the other side.Female connector will be used to connect to detection extension cable.

d. Detection extension wire



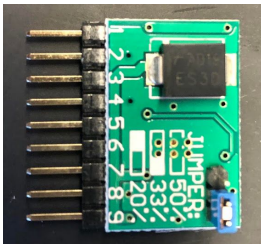
This will connect lick ports and positive ground cable to circuits that sits outside of the box. It should be long enough based on the setup positions. 3 long wires for the lick detection set-up will be needed. It will connect female connectors of left & right lickports with metal syringe and positive ground cable female to lick detection circuits. For steering wheel set-up, two long wires will be needed.

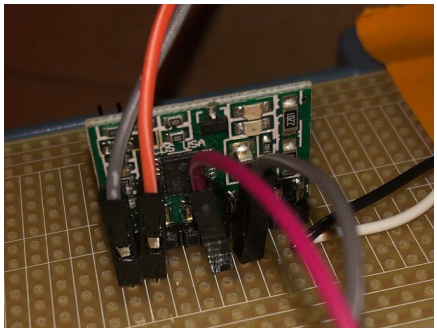
2) Water reward system

a) Water delivery circuit

Use valve driver as shown in the photo (see details of valve)

Take out the blue jumper for faster leaking!



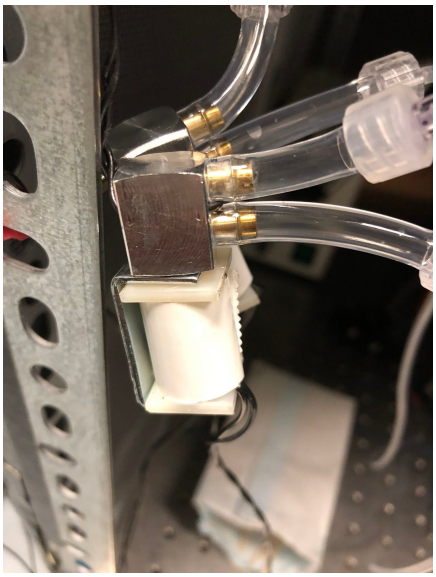


Pin # 1 = Positive Line Voltage (8-24VDC.)
Pin # 2 = Ground (Zero Volts.)
Pin # 3 = Not Connected
Pin # 4 = Ground (DAQ - GND)
Pin # 5 = Trigger (DAQ - DIO*)
Pin # 6 = Not Connected
Pin # 7 = Not Connected
Pin # 8 = Output To Valve**
Pin # 9 = Output to Valve** (In case the valve has one red and one different colour wire, pin# 9 is to be connected to the red wire.)

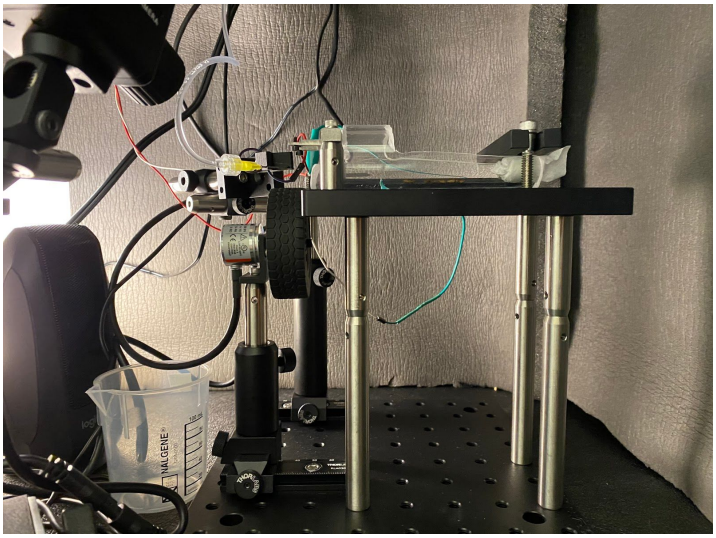
* We used DIO-2 for left valve which will be read as 4, DIO-3 for right valve - will be read as 8 in NBS-Presentation
**There is no polarity for valves, both directions for one valve should work.

b) Water extension tube & valves

Connects main water tank (or 5ml syringe) to stopcock connector, then connect stopcock left/ right end to valves top yellow holes with tubes. Bottom yellow holes in valve should be connected to yellow lickport syringe. Use integral male luer lock rings whenever necessary.



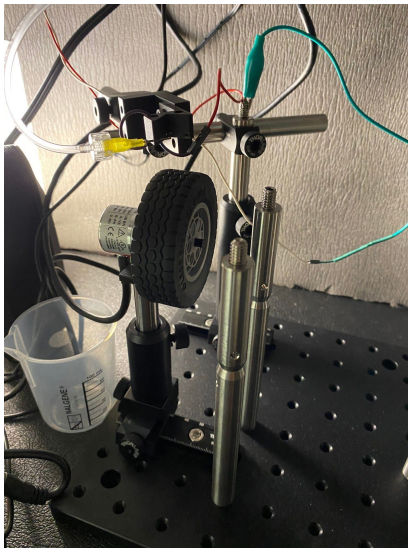
3) Steering wheel system

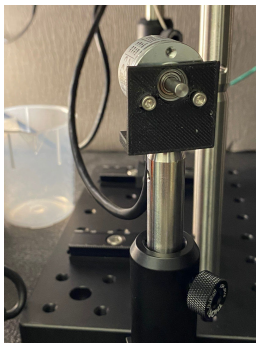


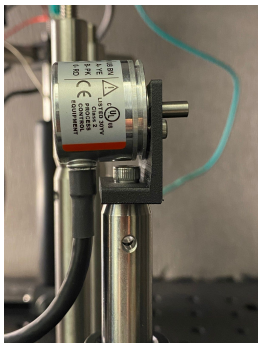
The Kwan Lab steering wheel behavioral setup is loosely adapted from the Carandini Cortical Processing Laboratory (see: https://www.ucl.ac.uk/cortexlab/tools/wheel). Briefly, the setup uses a LEGO Wheel (62.4 x 20 w/ short axle hub) resting on an incremental rotary encoder (Kubler 05.2400.1122.1024), which passes state information to an Arduino Uno R3 (https://store.arduino.cc/usa/arduino-uno-rev3). The Arduino board runs a serial decoding program based on rapidly refreshing encoder state, in turn outputting data to the USB-205 DAQ that NBS Presentation reads to detect clockwise and counterclockwise wheel rotations. **To the right: example completed wheel setup in Kwan Lab behavior rig.**

Rotary encoder apparatus:

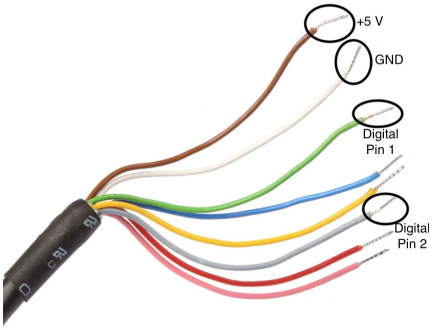
A LEGO wheel is placed on the shaft of a Kubler incremental rotary encoder, which sits fixed to a custom 3D printed holder (Kwan Lab & Yale Neurotechnology Core design; see parts list) by 2-3 screws. The 3D printed holder fastens to an adjustable Thorlabs post sitting on 2D Thorlabs sliders on a behavior box breadboard (**see pictures below of: wheel, rotary encoder fixed to post--front view, rotary encoder fixed to post--side view, wheel setup on behavior box breadboard**).



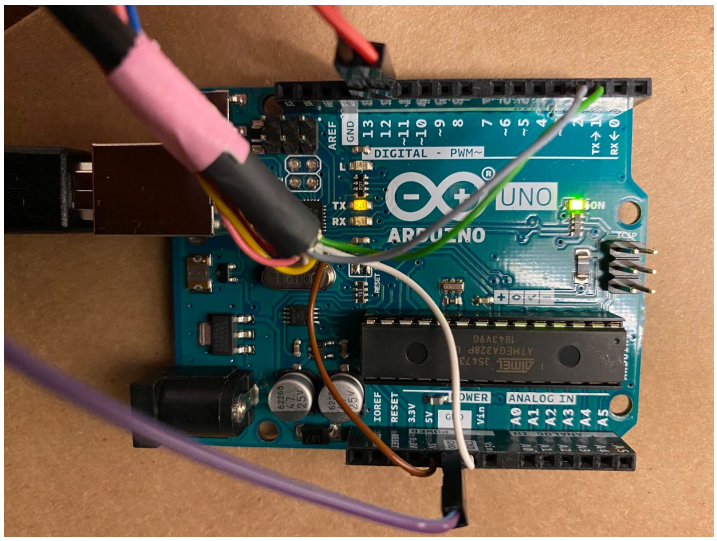




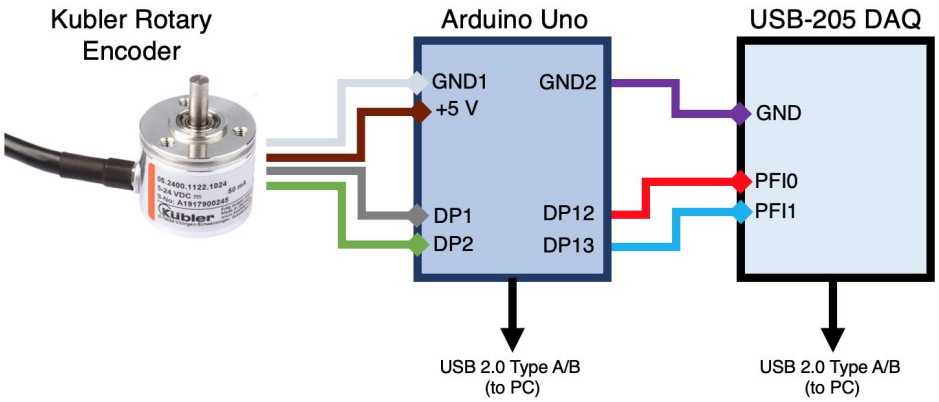
The Kubler incremental rotary encoder (datasheet; **depicted to the right**) comes with 8-terminal output. Only the **WHITE** (GND), **BROWN** (+5 V) , **GREY** (Digital Pin 1), and **GREEN** (Digital Pin 2) are connected as input to the Arduino Uno board in our system (red, yellow, pink, and blue terminals can be taped/secured to the encoder bundle.



The **used encoder terminals are circled and labeled with the associated Arduino socket in black to the left**. A top-down view of the **completed Arduino board is depicted to the right**.



From the Arduino board, a wire connects one of the GND sockets to the USB-205 GND. Then Arduino Digital Pins 12 and 13 are connected to USB-205's DIO0 and DIO1 sockets, respectively. **See circuit mock-up below**. The USB-205 will also usually be outfitted to provide single lickport water delivery for behavioral reward (see water reward system in section above).



The use of digital pins 12 & 13 are hard-coded into the Arduino Uno program used to run the steering wheel setup (needs link?; see Sections 3 & 4 below).

Section 2: Software Setup

1) Install - **Presentation** from Neurobehavioral Systems

2) Install - **Instacal** Measurement Computing software from CD for USB 201 DAQ. It is required for communication of DAQs with NBS- Presentation software. When you click Instacal, you should see your connected DAQs.

3) For Wheel set up, you additionally need to install Arduino IDE software (https://www.arduino.cc/en/Main/Software). Arduino code for the serial encoder is available on the Kwan Lab GitHub (LINK TBD). Additionally, it is recommended you download some serial decoding software (e.g., PuTTy [recommended], CoolTerm; allows for live stream/reading Arduino output) for troubleshooting involving the Arduino board/code. For the arduino/wheel setup to work correctly, please (1) connect all hardware, (2) download all appropriate/most up-to-date scripts (i.e., for Arduino IDE, Presentation), (3) Verify that the COM port being used for the Arduino is reading 115200 bits per second wit 8 data bits (Do this via the following: Open Control Panel -> Device Manager -> Ports (COM & LPT) -> Arduino Uno (usually COM3) -> right click -> Properties-> Port Settings -> Set Bits per second: 115200; Data bits: 8; Parity: None; Stop bits: 1; Flow control: None;

4) For pupillometry, you need another computer - setting for that computer is described in pupillometry section

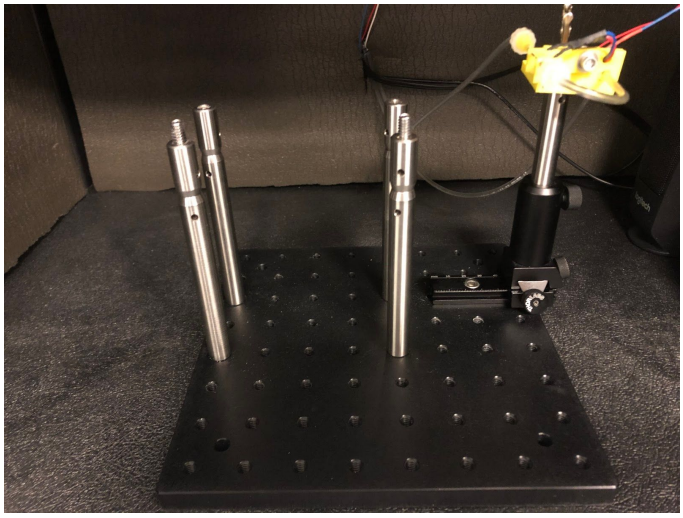
Section 3: How to assemble lick port detection set-up

This set-up requires **lick detection** and **water reward system** as described in the hardware section. Before starting to assemble the pieces make sure you have all components.
Main components needs to be ready before assembling the box;

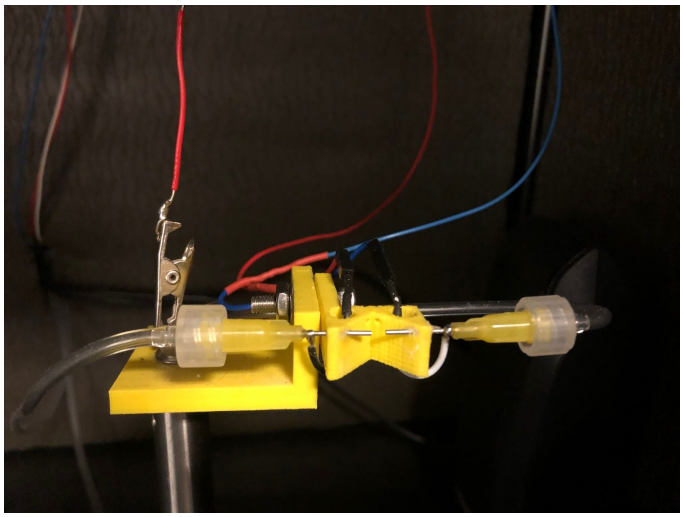
Lick detection circuit
Lick ports with metal syringe
Positive ground cable
Detection extension wire
Water delivery circuit
Water extension tube & valves

Steps for assembling the lick port box:

1) If you are using AV cart with cabinet, assemble it as it is shown in the user guide.
2) Dress the 6 side of the box with foam to isolate the sound from outside.
3) Put valve on the higher level of lick ports at the back of the box, extended tubes go to inside of the box from back hole for lick ports.
4) Assemble the mouse holder frame according to your needs. Picture shows what we have used in Kwan lab. (Mice holder sits on 4 posts as shown in the right picture)



5) Super glue the lick ports with syringe onto the lickometer 3D printed piece, with the two sprout ends separated by ~3 mm as it is seen in the bottom photo.
6) Connect this 3D printed piece to main post. This post needs flexibility in three directions (x,y,z) for positioning the lick ports according to the mice mouth/tongue position. See troubleshoot1 for more details.
7) Use detection extension wires. Connect lick ports female ends and positive ground cable to the lick detection detection circuit.
8) Put a piece of paper or cardboard in the metal enclosure, so the exposed metals on the PCB do not short-circuit on the enclosure.



9) Connect the rest of the wires from detection circuit to the USB-201 DAQ -1.
10) Set up water lines: For each water line, connect tubings so water starts from a 5mL syringe (or water tank) → stopcock connector → IN of clippard valve → OUT of clippard valve → luer tubing adapter → metal syringe needle to be inserted into the 3D printed lickometer
11) Connect valves cables to water reward delivery circuit. There is no polarity for valves, both directions for one valve should work.
12) Then connect water delivery circuit to USB-201 DAQ -2. USe DIO-2 for left valve which will be read as 4, DIO-3 for right valve - will be read as 8 in NBS-Presentation.
13) Water delivery system uses gravity to move water throughout tubes. Make sure the source (5ml syringe/water tank) is placed in a higher position than metal syringes.
14) In Presentation, configure "Settings":

<u>Set these parameters for both the Default and the current .sce</u>

- For lick detection:
    - Add DAQ as an input ports: Setting →Ports → Port Properties You should see two MC Universal Library (Board 0/ Board1). Be careful which board is connected to valve circuit or lick detection circuit.
    - In Port Input Channel, Add USB 201 #0 (data source: AUXPORT, register span: 1, status port, no mask, inversion mask = 0, no interrupt, check box for independent lines)
    - Add responses: Setting → Response →Port device (USB- 201 #0) → Buttons Add 1(start) and 2(start) as active responses. If you don't see the buttons on the input port, then the Input Ports was not configured properly.
    - Click Test, you should see value(s):1 if positive ground cable touches left syringes, and you should see value(s):2 when it touches right syringe. (If that is not the case, system is not working correctly)
    - Add DAQ as an input ports: Setting →Ports: Add USB-201 #0 as port input channel and uncheck Log Code (otherwise it will record response code as well as log code and size of the log files will be bigger)
- For water delivery:
    - Output Ports: Port: USB-201 DAQ-2, data source: AUXPORT, register span: 1, check independent lines, no delay codes, inversion mask: 0
    - Click Test, In Code 4, pulse length 1000 (1sec) - you should see water on left syringe. Code 8 for right syringe. If you don't see any water, flush all tubes - make sure there is no air in the tubes.
- Other Settings:
    - Advanced: Random number generator: Mersenne Twister
    - Logfiles: check Prefix subject name, check Append counter if exists, Subject Name/ID set to Prompt
    - General: uncheck Wait for return to start; check Never show report

Section 4: How to assemble steering wheel set-up

Coming soon

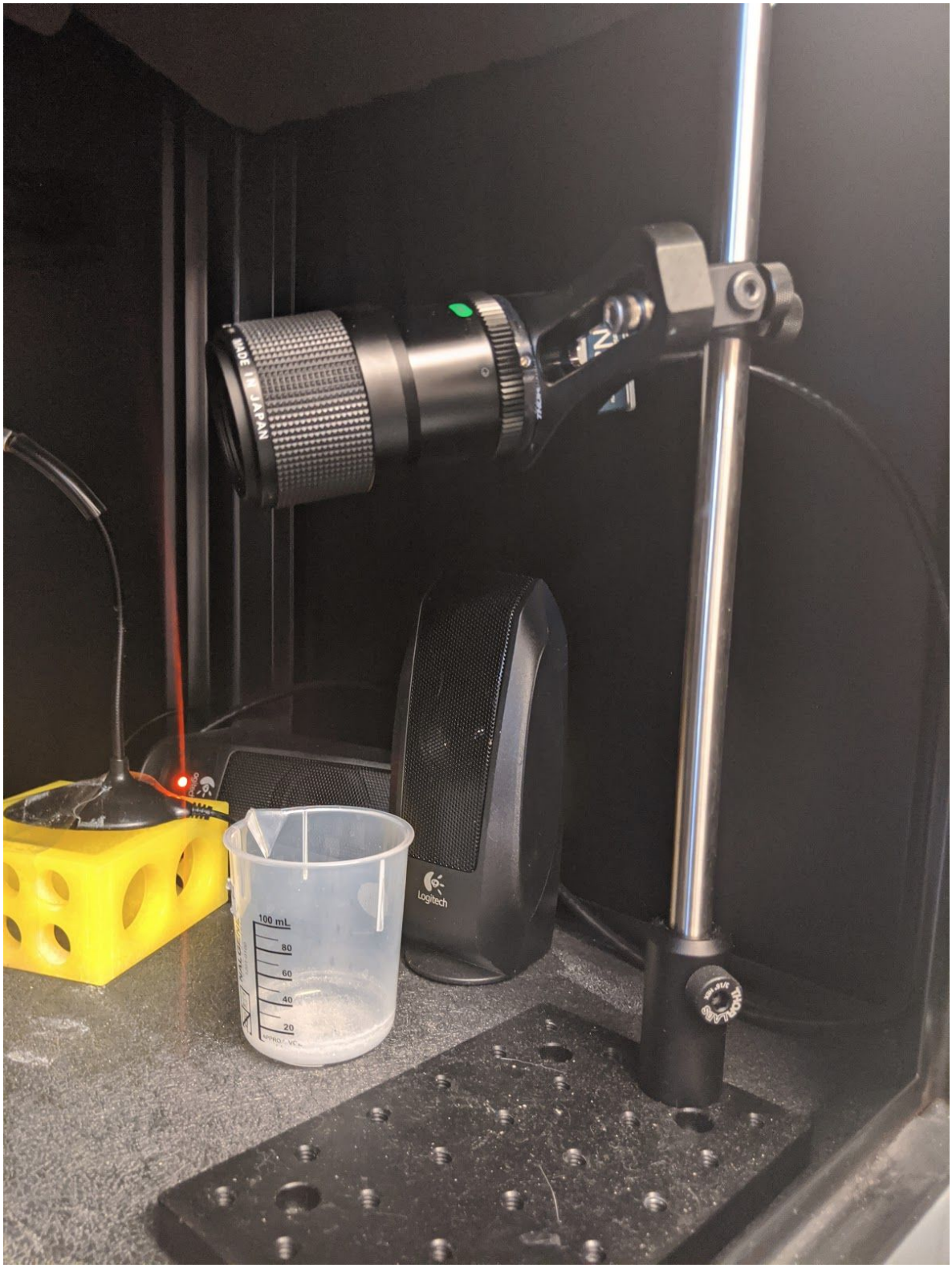
Section 5: Pupillometry

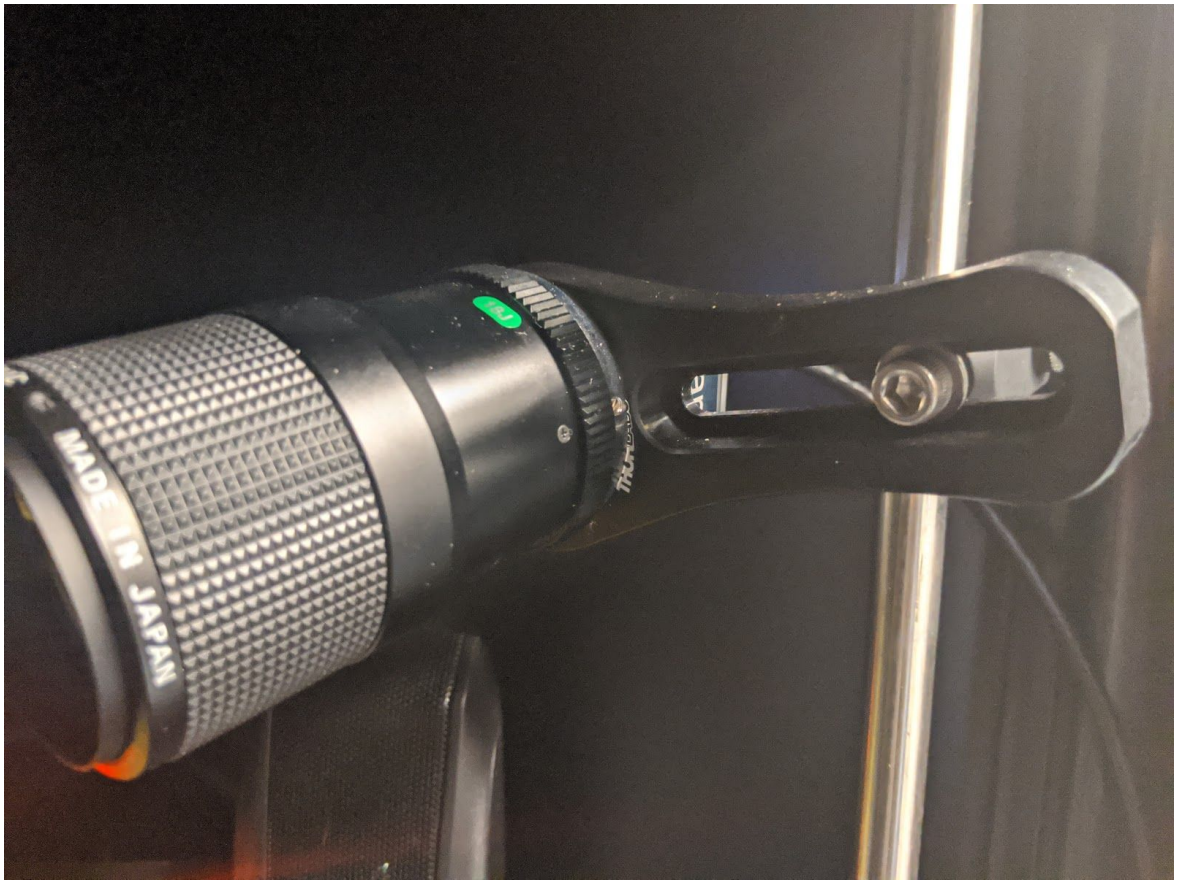
Necessary items can be found in the same excel file:
https://docs.google.com/spreadsheets/d/1VIZEDlVTupoTt61jeeu2QYp-AVEco69VJNO5CFrp0Lw/edit#gid=0

Require a new set of computer, computer needs to have *extra port for adapter card/ driver.*

Set Up:

1. Connect the adapter card to the motherboard of the computer.
2. With power cable, connect the power supply of the card to SATA port inside the computer.
3. Download the Sapera Explorer with SDK (Online or archived)
4. Connect nano-camera to LENS
5. With ethernet cable, connect the camera to the computer. This ethernet cable also provide power to the nano-camera. If it is working correctly, you should see the blue light on the nano camera.
   Figures: camera setup (shown in different viewing angles)


MADE IN JAPAN
Logitech
100 mL
80
60
40
20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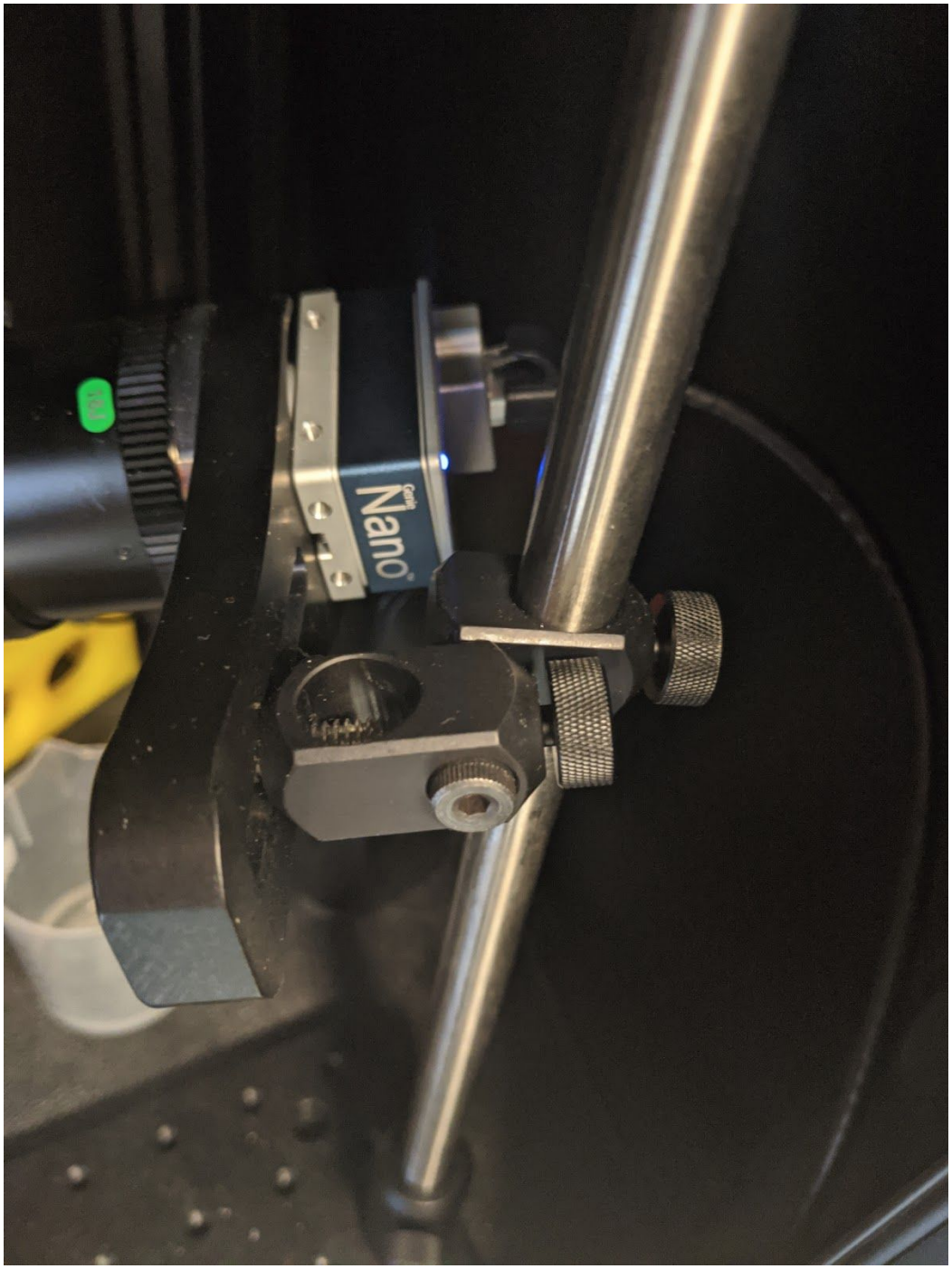
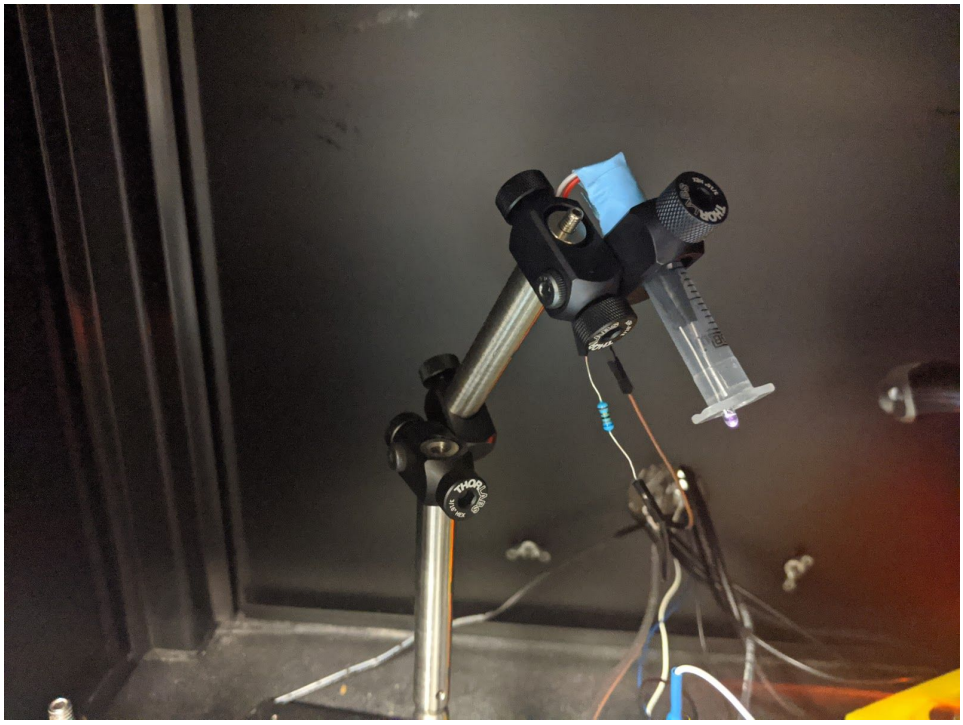


6. Open Sapera Explorer to grab the image. Focus is zoomed, that's why do not expect to see anything at the beginning. After adjusting focus, you will be able to see some sensible images.
7. One infrared LED might be needed for recording based on the setup for lighting up the area around the eye.

   Constant LED: 90 ohm resistors with 9V 1A power adapter

Figure: IR LED set up



8. Ambient Light might be necessary for medium pupil size if the recording area is not bright enough. In the dark, the pupil is large, harder to see the fluctuation in pupil size.
9. USB-201 daq is used to connect behavior and pupil recording computer. Connecting output DAQ device from the behavior computer (DIO, presentation code need to be adjusted accordingly) to the DAQ device (CH0) of the pupil recording computer.

Software: MATLAB (2018b used). The code is written by HA, only works if ;
(There is a ReadMe.txt for details of the code)

- Matlab is 2017b or above
- Download Instacal - USB-201 (for communication between behaviour computer and pupil computer - for time stamps)
- Measurement Computing, MCC toolbox downloaded
- Image Acquisition Toolbox Support Package for GigE Vision Hardware **Follow the steps in this guideline.** There are some parameters to set for GigE! (It will not work, if these parameters are not set correctly.)
  https://www.mathworks.com/matlabcentral/answers/uploaded_files/41167/GigEVisionQuickStart.pdf

Not used in this setup, but might be useful for future setups.
Trigger Cable: PartNumber: **P/N: CC C1677-XXM**
https://www.componentsexpress.com/_Teledyne-Dalsa-Genie_Nano

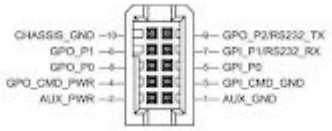
10-pin I/O Connector Pinout Details (Special Order)

Pin Number	Genie Nano	Direction	Definition
1	PWR-GND	--	Camera Power – Ground
2	PWR-VCC	--	Camera Power – DC +10 to +36 Volts
3	GPI-Common	--	General Input Common Ground
4	GPO-Power	--	General Output Common Power
5	GPI 1	In	General External Input 1
6	GPO 1	Out	General External Output 1
7	RS232_RX	In	*Serial Port Input for G3-Gx4 models*
	Reserved	--	*Input not available with G3-Gx2 models*
8	GPO 2	Out	General External Output 2
9	RS232_TX	Out	*Serial Port Output for G3-Gx4 models*
	GPO 3	Out	*General External Output 3 for G3-Gx2* ⚡
10	Chassis		Camera Chassis

⚡ Note: Output 3 only supports Software Controlled logic High or Low signals.

Nano: "G3-GM2.. or G3-GC2.." part numbers denote optional "1 input / 3 output" special order models.

Nano: "G3-GM4.. or G3-GC4.." part numbers denote optional Serial Port special order models.



SAMTEC
TFM-105-02-L-D-WT

TroubleShooting

Better tongue positioning: We tried using XYZ translation stage with differential adjuster from Thorlabs (MT3, ~$1K), it slightly improved and standardised lick positioning unfortunately, not a complete solution. It limits adjusting the angle for left/right ports.