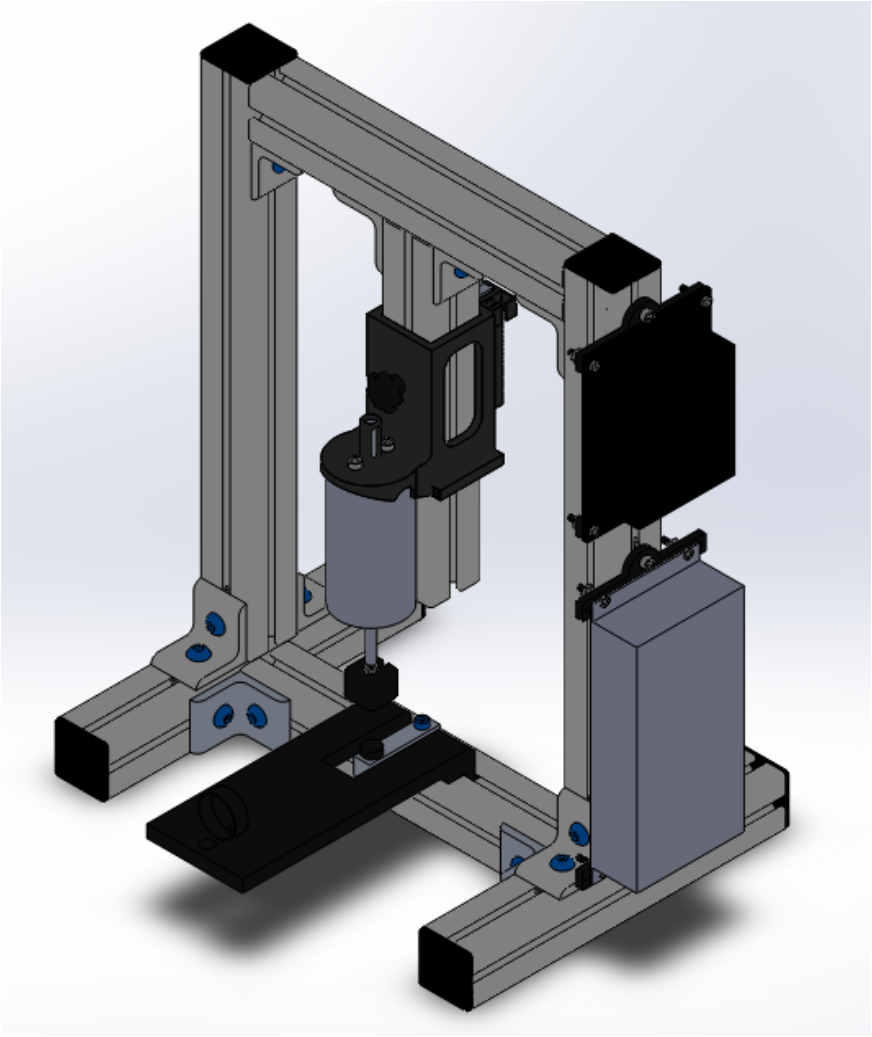
Tibial Compressive Overload Testing Apparatus

Ordering and Assembly Instructions



Index

cc) **Metal Crimps**
dd) **Red Plastic Crimps**
ee) **Jumpers (wires)**
ff) **44-Pin Cable**
gg) **End Caps**
hh) **Peripheral Mount**
ii) **Bolt Carriage**
jj) **M4 Load Cell Screw**
kk) **Wing Nut**
ll) **Knee Cup**

4. Frame

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b) **Stand**
c) **Connecting the Stand to the Base**
d) **Tube End Caps and Silicone Rubber Strips**

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b) **Adjustment Knob**
c) **Ankle Holster**

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b) **Wall Plug—Power Supply**
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d) **Load Cell—Amplifier**
e) **Amplifier—44-Pin Adapter**
f) **Motion Controller and Power Supply—Frame**
g) **Load Cell, Amplifier, and 44-Pin Adapter—Frame**
h) **VCA—Motion Controller**
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b) **Load Cell Calibration**
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c) **Data Acquisition**
d) **Sterilization**
e) **Customization**
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    6) **Device/Rupture Speed**

1. Introduction

The mouse knee injury experimental setup is intended for labs across the country to easily reproduce the tibial compressive overload test in mice in order to rupture ACL's and induce osteoarthritis for study. The testing apparatus is designed to require no machining and few tools. All parts will be ordered from online manufacturers (McMaster-Carr, Amazon, Galil Motion Control, Moticont, and 80/20 Inc.) or 3D printed using an online service (Shapeways). Please refer to section 2 for all ordering information, section 3 for images of all the parts and their names, sections 4-9 for detailed assembly instructions, and section 10 for operating instructions, safety considerations and cleaning. See a step by step video **found at** for the complete assembly of the testing apparatus (sections 4-9).

Useful notation examples:

2x40cm = 2 separate pieces each 40cm in length.

Hex head screw (3q) = the image for the Hex head screw is listed as part q in section 3.

2. Ordering

a) 80/20 Inc.

- Order the following parts from 80/20 Inc. (https://www.8020.net/)

Name/Part #	Quantity	Link
40 mm Framing/ 40-4040 (specify 400mm length)	2	https://8020.net/40-4040.html
40 mm Framing/ 40-4040 (specify 250mm length)	5	https://8020.net/40-4040.html
40 Series 2 Hole- Inside Corner Bracket, single/ 40-4302	10	https://8020.net/40-4302.html
M8x16mm Blue with Slide-In Economy T-Nut/ 75-3422	20	https://8020.net/75-3422.html
M5 Short Self-Aligning Roll-in T-Nut with Spring Leaf/ 13091	4	https://8020.net/shop/13091.html
M5 Roll-In T-Nut/ 13090	2	https://8020.net/13090.html
End Cap/ 12260	6	https://8020.net/12260.htm

b) Amazon

- Order the following parts from Amazon **(**https://www.amazon.com/**)**

Name	Quantity	Link
Power Supply (Singpad newstyle 240W DC 24V 10A)	1	https://www.amazon.com/transformer-Universal-Regulated-Switching-110-240V/dp/B00QCJ9FAY/ref=sr_1_1?ie=UTF8&qid=1517273135&sr=8-1&keywords=singpad+new+style
Power Cord	1	https://www.amazon.com/eDragon-5-15P-Standard-Conductor-ED71132/dp/B00J4Z9AXK/ref=sr_1_14?srs=9032328011&ie=UTF8&qid=1517273389&sr=8-14&keywords=power%2Bcord%2B6ft&th=1
4 Pin Molex Mini Fit Jr	1	https://www.amazon.com/Molex-Connector-Matched-4-Circuits-18-24/dp/B075431J2Z/ref=sr_1_1?s=hi&ie=UTF8&qid=1517273156&sr=1-1&keywords=4+Pin+Molex+Mini+Fit+Jr#feature-bullets-btf
22 AWG Wire (10')	1	https://www.amazon.com/Electrical-Gauge-Silicone-Cable-Black/dp/B0746HG158/ref=sr_1_5?s=hi&ie=UTF8&qid=1522783388&sr=1-5&keywords=22+gauge+wire&dpID=31kv48E-7aL&preST=_SY300_QL70_&dpSrc=srch
Crimps	1	https://www.amazon.com/Ginsco-Insulated-Connector-Electrical-Connectors/dp/B01D4Q3LD4/ref=sr_1_4?s=hi&ie=UTF8&qid=1522783775&sr=1-4&keywords=wire+crimps&dpID=51cg58qKI7L&preST=_SX342_QL70_&dpSrc=srch

D-SUB 15 Cable M/F	1	https://www.amazon.com/Amphenol-CS-DSDHD15MF0-002-5-15-Pin-Deluxe-Shielded/dp/B00O072KHU/ref=sr_1_1?ie=UTF8&qid=1522871611&sr=8-1&keywords=HD15+D+Sub+2.5%27&dpID=41zzTBlc4PL&preST=_SX342_QL70_&dpSrc=srch
D-SUB 44 Cable M/F	1	https://www.amazon.com/Amphenol-CS-DSDHD44MF0-005-44-Pin-Deluxe-Shielded/dp/B00O072X3Q/ref=sr_1_1?s=industrial&ie=UTF8&qid=1522871330&sr=1-1&keywords=HD44+male%2Ffemale+5%27
Ethernet Cable	1	https://www.amazon.com/AmazonBasics-RJ45-Cat-6-Ethernet-Patch-Cable-5-Feet-1-5-Meters/dp/B00N2VILDM/ref=sr_1_4?s=pc&ie=UTF8&qid=1522787645&sr=1-4&keywords=ethernet+cable&dpID=413S0B5HVxL&preST=_SX300_QL70_&dpSrc=srch

c) RobotShop

Name/Part #	Quantity	Link
Load Cell Amplifier / RB-Onl-38	1	https://www.robotshop.com/en/strain-gauge-load-cell-amplifier-shield-2ch.html#reviewBox
5kg Strain gauge load cell/ RB-Phi-118	1	https://www.robotshop.com/en/micro-load-cell-5-kg.html
12" 4-Pin Jumper Wire/ RB-Spa-1107	1	https://www.robotshop.com/en/12-4-pin-jumper-wire.html
65 x 22 gauge assorted jumper wires (Pack of 65)/ RB-Cix-01	1	https://www.robotshop.com/en/65-22-gauge-assorted-jumper-wires.html

d) Galil

- Order the following parts from Galil Motion Control **(**http://www.galilmc.com/**)**

Name/Part #	Quantity	Link
Motion Controller/ DMC-30012-BOX	1	http://www.galilmc.com/motion-controllers/single-axis/dmc-3x01x
15 Pin Adapter/ ICS-48015-M	1	http://www.galilmc.com/accessory/cables#ICS-48015-M
44 Pin Adapter/ ICS-48044-M	1	http://www.galilmc.com/accessory/cables#ICS-48044-M
GDK Software/ GDK SOFTWARE	1	http://www.galilmc.com/downloads/software/gdk

e) Shapeways

- Download the file and unzip the files of the parts from **"LINK HERE"**.
- To unzip the part files, right click on the Zip folder and select "Extract All". Then, the individual part files can be placed into a normal folder as desired.
- Create a Shapeways account and click on the "Make a Product" tab and upload each file individually to Shapeways.
- Choose HP Nylon Plastic as the material for each part.
- Order the following parts after the above steps have been completed. This can be done in one bulk order **(**https://www.shapeways.com/**)**

Name	Quantity	File Name
Anesthesia Pivot	1	
Knee Cup	1	
Platform	1	
Ankle Holster	1	
Collar	1	
Measuring Tool	1	
Universal Mounts	4	
Peripheral Mount	1	
Bolt Carriage	1	

f) McMaster-Carr

- Order the following parts from McMaster-Carr **(**https://www.mcmaster.com/**)**
- Or, obtain these parts from a local hardware store to prevent purchasing in bulk.

Name/Part #	Quantity	Link
Silicon Rubber Strips/ 1466N12	1	https://www.mcmaster.com/#1466n12/=1bce72y
Adjustment Knob/ 2907T52	2	https://www.mcmaster.com/#2907t52/=1bce79j
Steel 6-32 Hex Nut (pack of 100)/ 90480A007	1	https://www.mcmaster.com/#90480a007/=1bcyl87
Steel 6-32 Thread Size 3/4" Hex Head Screw (pack of 25)/ 92620A406	1	https://www.mcmaster.com/#92620a406/=1bcx5h2
10-32 3/8" long Screws for VCA Mount (pack of 50)/ 97763A231	1	https://www.mcmaster.com/#catalog/124/3077/=1c9ubdz
M5 15mm long, 4mm hex drive (pack of 100)/ 91290A231	1	https://www.mcmaster.com/#standard-socket-head-screws/=1c98m9l

M4 30mm long (pack of 100)/ 91280A148	1	https://www.mcmaster.com/#91280a148/=1cgmvd3
M4 "Thumb" Nut /96115A440	1	https://www.mcmaster.com/#thumb-screw-nuts/=1cgmxoo
Oversize M4 Washer/ 98040A102	1	https://www.mcmaster.com/#98040a102/=1cgn0d3

g) Moticont

- Order the following part from Moticont **(**http://www.moticont.com/**)**

Name/Part #	Quantity	Link
Voice Coil Actuator/ SDLM-051-095-01-01	1	http://www.moticont.com/SDLM-051-095-01-01.htm

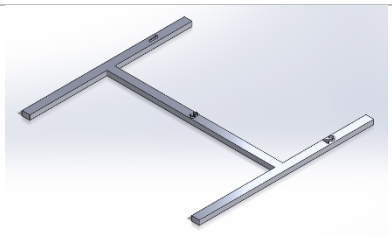
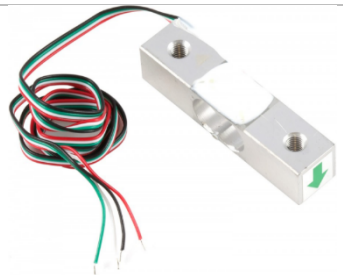
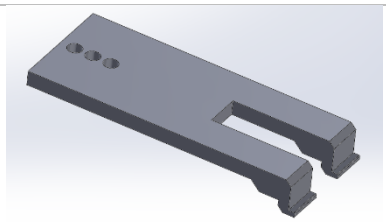
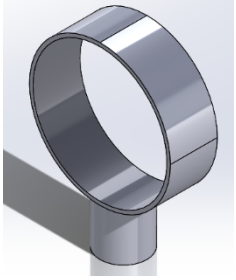
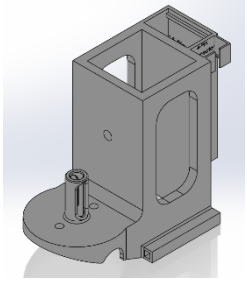
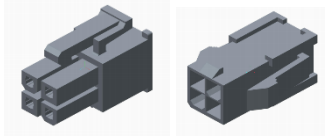
h) Optional (all fulfilled by Amazon)

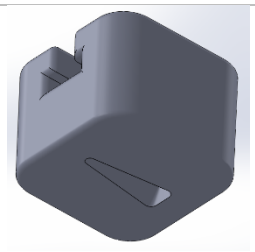
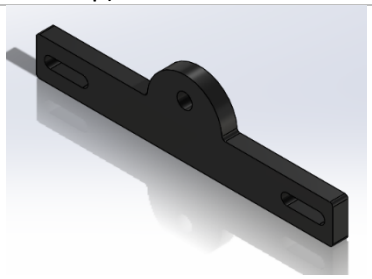
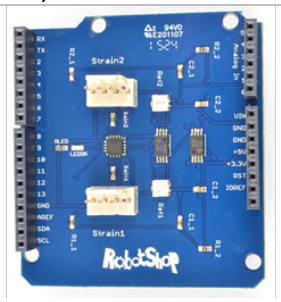
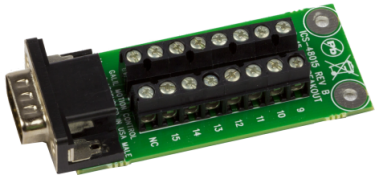
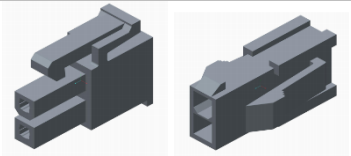
Name	Quantity	Link
Wire Strippers-Crimpers	1	https://www.amazon.com/WGGE-Professional-crimping-Multi-Tool-Multi-Function/dp/B073YG65N2/ref=sr_1_11?s=power-hand-tools&ie=UTF8&qid=1522790032&sr=1-11&keywords=wire+stripper
Micro Screwdriver Set	1	https://www.amazon.com/Stanley-66-039-Jewelers-Precision-Screwdriver/dp/B00002X29G/ref=sr_1_4?ie=UTF8&qid=1522789753&sr=8-4&keywords=small+screwdriver&dpID=21KW8GCN2ZL&preST=_QL70_&dpSrc=srch
Hex Set (SAE and Metric)	1	https://www.amazon.com/TEKTON-Wrench-Metric-30-Piece-25253/dp/B00I5TH074/ref=sr_1_3?ie=UTF8&qid=1522789871&sr=8-3&keywords=hex+wrench+set&dpID=51DxY719GTL&preST=_SX300_QL70_&dpSrc=srch
Ethernet to USB Adapter	1	https://www.amazon.com/llano-Portable-Ethernet-Multi-port-Aluminum/dp/B073PVB9MM/ref=sr_1_12?s=hi&ie=UTF8&qid=1522790291&sr=1-12&keywords=ethernet+to+usb

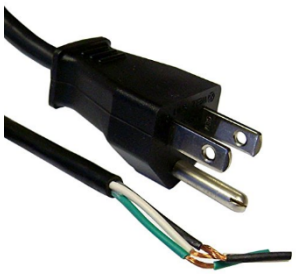
3. Parts Index

These parts will be indicated in the text with a number and a letter in parentheses referencing the section number and the location in the table.

Examples: Tubing (3a), Measuring Tool (3b), 44-Pin Cable (3ff), etc.

 a) Tubes (or Tubing)	 b) Measuring Tool	 c) Slider Nut
 d) 90° Elbows	 e) Button Head Socket Cap Screw	 f) Self-aligning roll-in T-nut with Leaf Spring
 g) Load Cell	 h) Platform	 i) Anesthesia Pivot
 j) Socket Head Cap Screw	 k.) Collar	 l) 4-Pin Connector Male (Left) Female (Right)

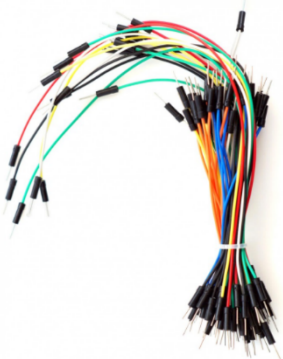
 m) Adjustment Knob	 n) Voice Coil Actuator (VCA)	 o) VCA Screws
 p) Ankle Holster	 q) Hex Head Screw	 r) Hex Nut
 s) Universal Mount	 t) Power Supply	 u) Motion Controller
 v) Amplifier	 w) 15-Pin Adapter	 x) 15-Pin Cable
 y) Regular Roll-In T-Nut	 z) 2-Pin Connector Male (Left) Female (Right)	 aa) 4-Wire Harness



bb) Power Wire

cc) Metal Crimps

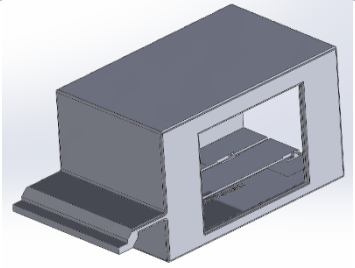
dd) Red Plastic Crimps



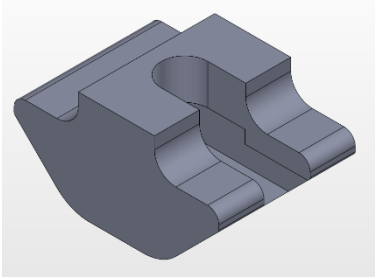
ee) Jumpers (wires)

ff) 44-Pin Cable

gg) End Caps



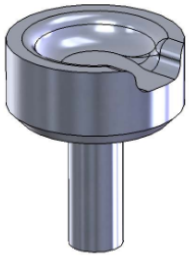
hh) Peripheral Mount



ii) Bolt Carriage

jj) M4 Load Cell Screw

kk) Wing Nut



ll) Knee Cup

4. Frame Assembly

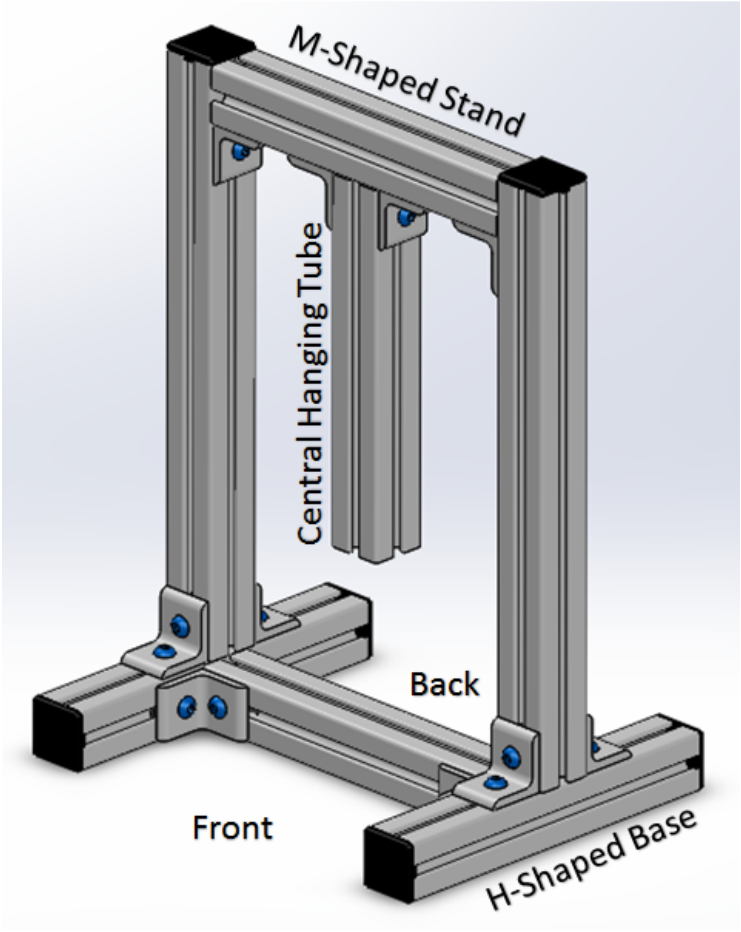


Figure 1. Front view of assembled Frame

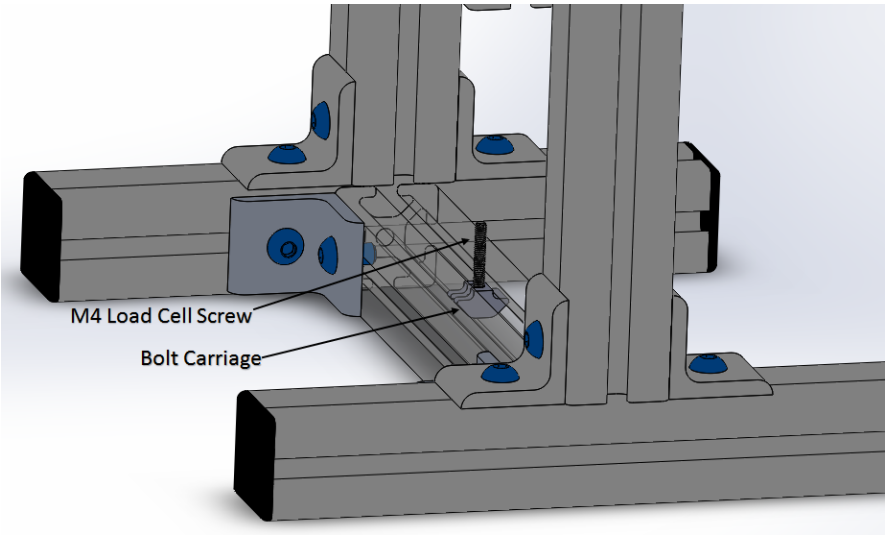


Figure 2. Detail of Bolt Carriage and M4 Load Cell Screw

To assemble the frame, begin by separating the 7 tubes (3a) into their respective lengths: 40 cm and 25 cm. There will be (5) 25 cm tubes and (2) 40 cm tubes.

a) Start by making the base with an H-shape, using a 25 cm tube horizontally and (2) 25 cm tubes as the vertical ends.

1. Slide the M4 Load Cell Screw (3jj) into the Bolt Carriage (3ii) and slide this into the top groove on the horizontal tube, placing it near the center for later use (Figure 2).
2. Insert 2 Slider Nuts (3c) into the same groove on one of the sides on the horizontal bar with the Slider Nuts resting at opposite ends. The protrusions of the Slider Nuts should face towards the center of the tube.
3. Run 1 Slider Nut into each of the side grooves of the side tubes on the H-shape base that are adjacent to the 2 Slider Nuts just placed on the center tube.
4. Place a 90**°** Elbow (3d) at each tube connection and line up the Slider Nuts so the holes match with the holes of the elbows.
5. Place 2 Button Head Socket Cap Screws (3e) in each elbow.
6. Use the Measuring Tool (3D printed and delivered by Shapeways) to align the horizontal tube to its specific height relative to the ends of the vertical tubes. This dimension uses side 1 of the Measuring Tool (Figure 3).
7. With the tubes for the base lined up, tighten all 4 screws with an Allen wrench (see Figure 3 for the schematic of a 90° Elbow connection).
8. The side of the H-Shaped Base that has the 90° Elbows will be the front of the apparatus.

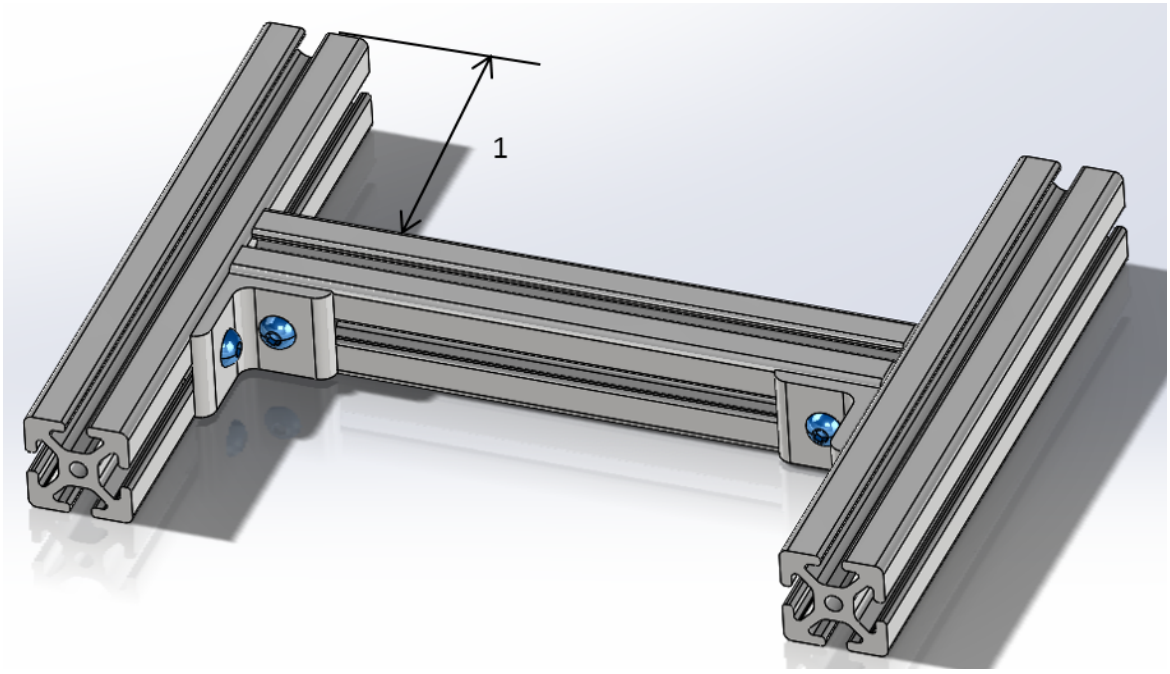


Figure 3. Assembled base of the frame

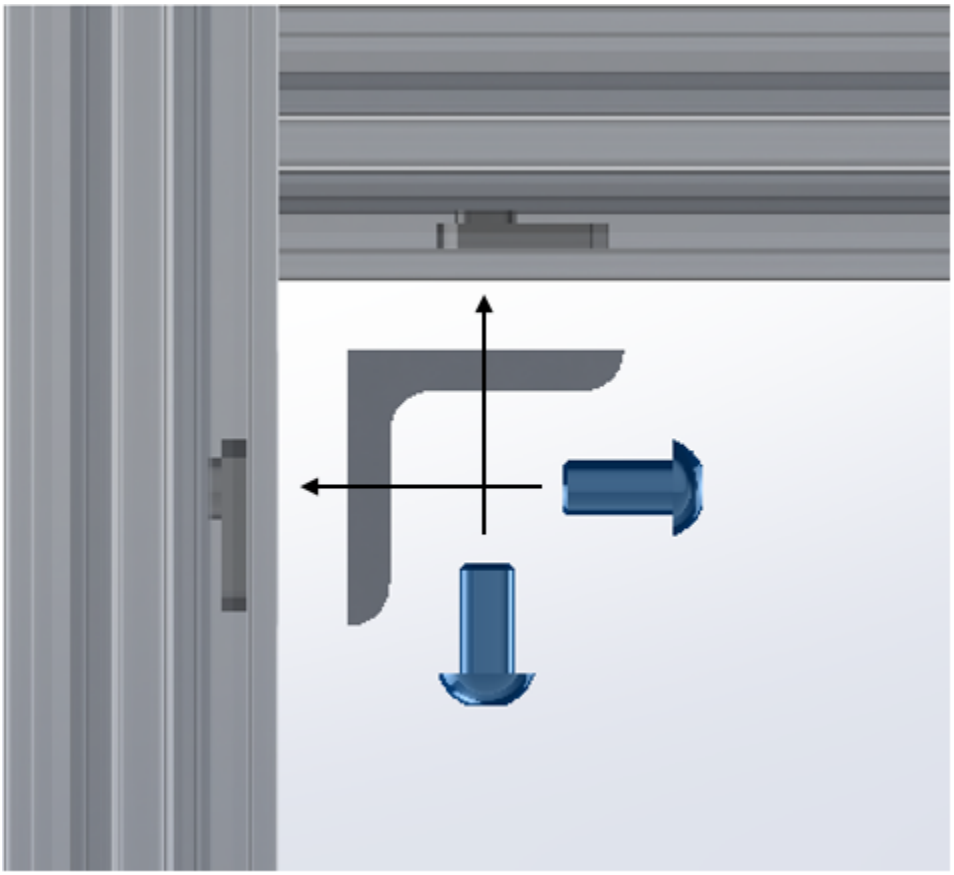


Figure 4. Exploded view of 90° Elbow connection

b) Now, assemble the M-shaped frame stand by using 40 cm tubes for the 2 vertical sides and a 25 cm tube for the horizontal top (with the horizontal top fitting between the vertical sides) and a 25 cm tube hanging down from the center of the horizontal top.

1. Insert 4 Slider Nuts into the same groove (2 at either end) of the horizontal tube.
2. Next, run a slider nut through a groove in each of the 45 cm vertical tubes so that one slider nut lies near each end of the horizontal tube. Next, insert 2 Slider Nuts in opposite sides of the hanging 25 cm tube (1 on either side).
3. Now, place a 90**°** Elbow at each tube connection (4 total, see Figure 4) and line up the slider nuts so the holes match with the holes of the elbows. Place 2 Button Head Socket Cap Screws in each elbow and use the Measuring tool to align the hanging tube to the center of the horizontal piece. This centerline dimension uses side 1 of the measuring tool (Figure 4).
4. With the tubes for the frame stand lined up, tighten all 8 screws with an Allen wrench (see Figure 3 for the schematic of a 90° elbow connection).

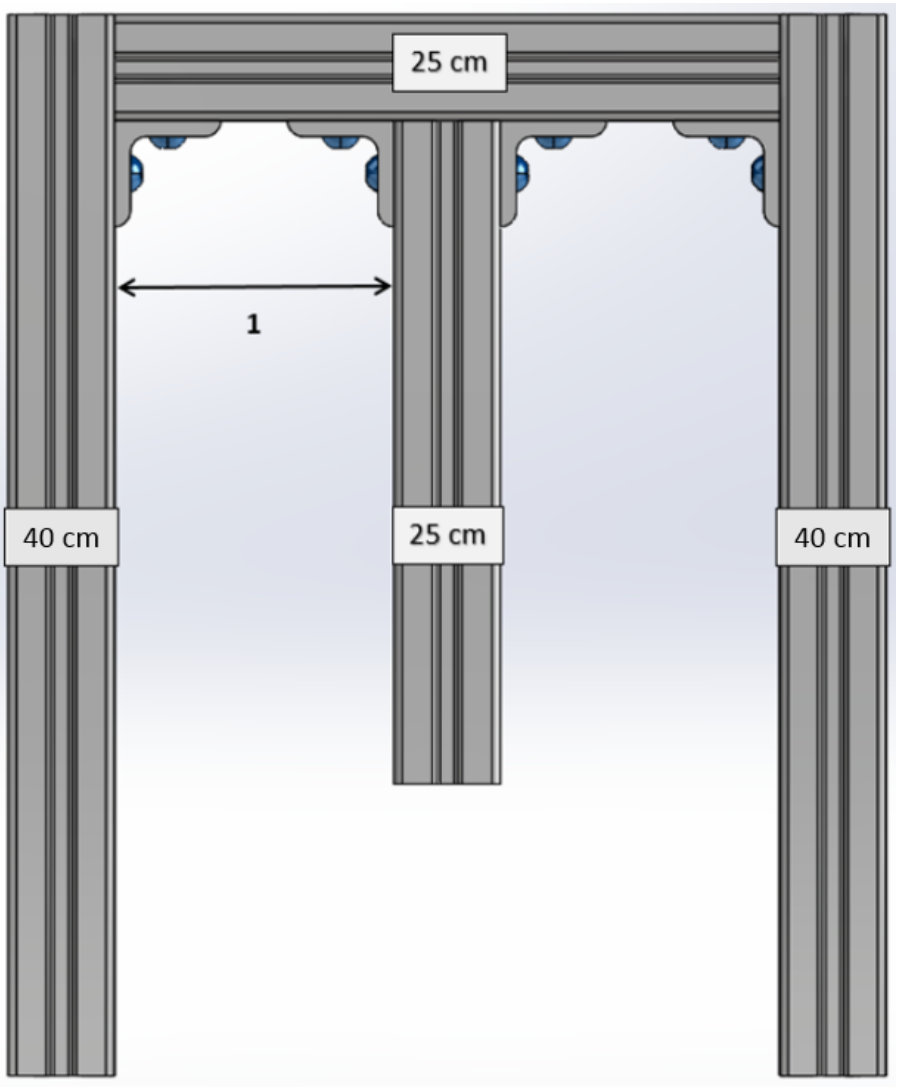


Figure 5. Assembled frame stand

c) Next, place the frame stand onto the frame base. The frame stand should lie slightly off center of the H-shaped base.

1. Use side 2 of the Measuring Tool to measure the offset of the M-Shaped Stand. *Note that this measurement should be done on the side with the 90° elbows attached to the center bar of the H-shaped base* (see Figure 6).
2. Affix the frame stand to the frame base using the same slider nut, elbow, bolt technique as described in Section 3.

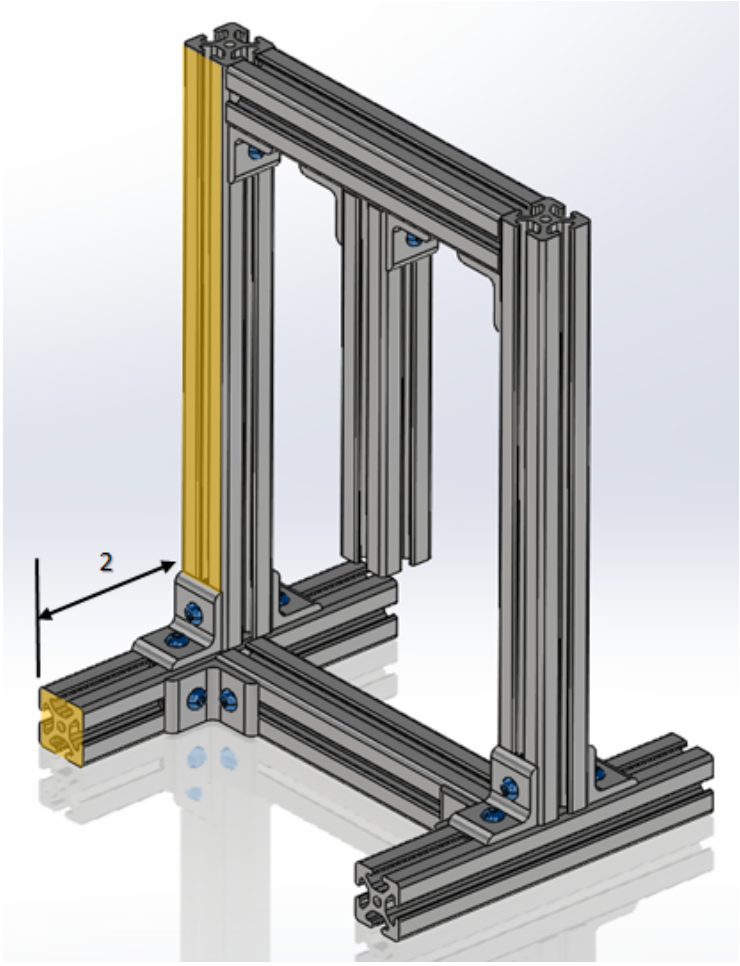


Figure 6. Placement of frame stand relative to frame base

d) Finally, place end caps and silicon rubber strips onto the frame

1. Tap in end caps on all of the exposed ends of the tubes except for the base of the hanging tube with a rubber mallet (Figure 7).
2. Cut and apply (2) 25cm silicon rubber strips to the underside of the base to prevent sliding (Figure 8).
3. Double check that all screws at the elbow connections are properly tightened and double check final placements with the measuring tool. Now, the frame is completely assembled and the other components can be placed onto it.

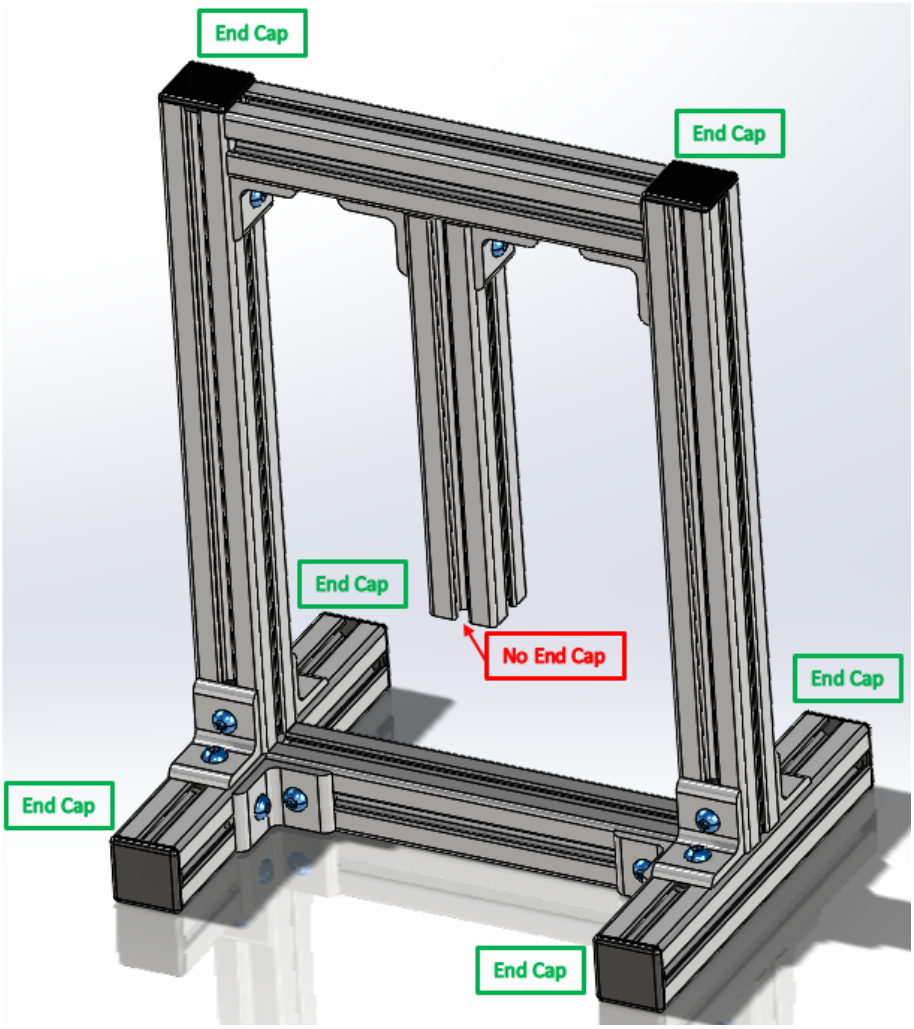


Figure 7. End Cap placement on the assembled frame

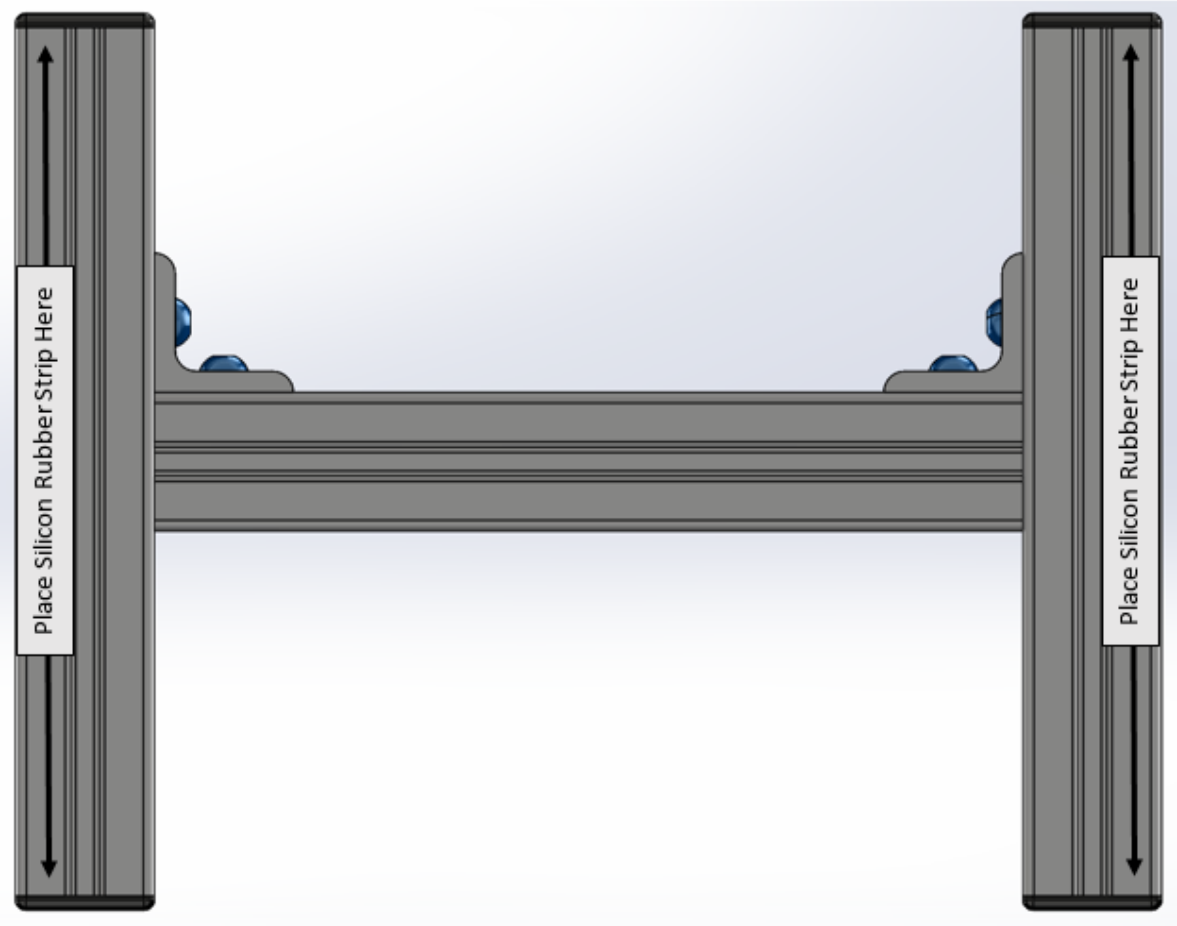


Figure 8. Placement of silicon rubber strips

5. VCA

1. Attach the VCA to the collar using two of the VCA screws (3o). It is extremely important that the end of the VCA with the wires exiting is the end that is screwed into the VCA collar. Ensure that the wires exit the same side of the collar that the connector chip slot is on (Figure 9). There will be a cutaway in the collar where the wires can fit through.
2. Thread the wires from the VCA through the wire corner (Figure 9).
3. Insert a Hex Head Screw (3q) into the Upside-Down T-slot of the Ankle Holster (3p) so that the head of the screw sits in the center of the Ankle Holster and the threaded part protrudes from the top of the Ankle Holster. This may be a tight fit. It is okay to press hard to get the screw fully into the slot on the Ankle Holster (Figure 10).
4. Thread a Hex Nut (3r) onto the Hex Head Screw and tighten it down onto the Ankle Holster (Figure 10).
5. Thread a second Hex Nut onto the Hex Head Screw and place it close to the other Hex Nut but do not tighten it (Figure 10).
6. Thread the Hex Head Screw into the threaded hole in the VCA piston as far as possible. Then, unthread slightly it until the Ankle Holster is oriented so that when facing the front of the apparatus, the Upside-Down T-slot is not visible.
7. Once the Ankle Holster is oriented correctly, tighten the top Hex Nut up against the VCA piston to ensure a tight fit.
8. Attach the Collar to the hanging tube of the frame stand using a Regular Roll-In T-nut (3y) and an Adjustment Knob (3m), tightening the Adjustment Knob to hold the Collar in place.

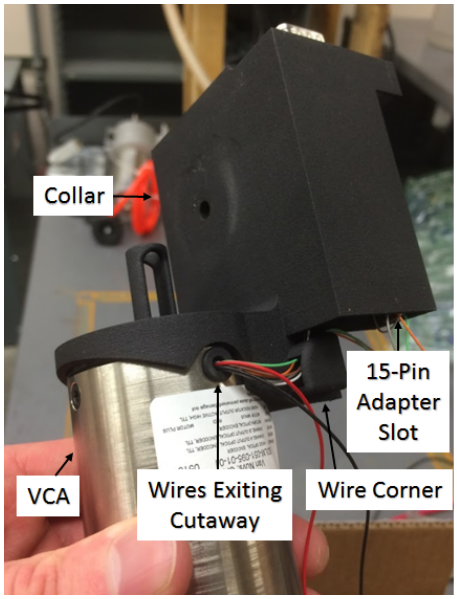


Figure 9. VCA wires exiting cutaway in collar

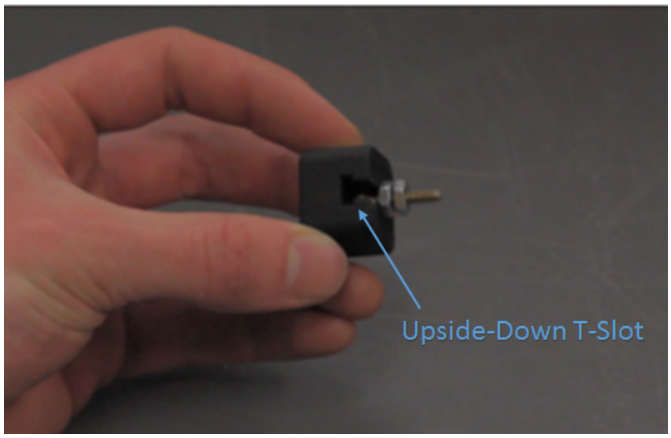


Figure 10. Ankle Holster with Hex Head Screw placed into Upside-Down T-Slot and 2 Hex Nuts threaded onto screw

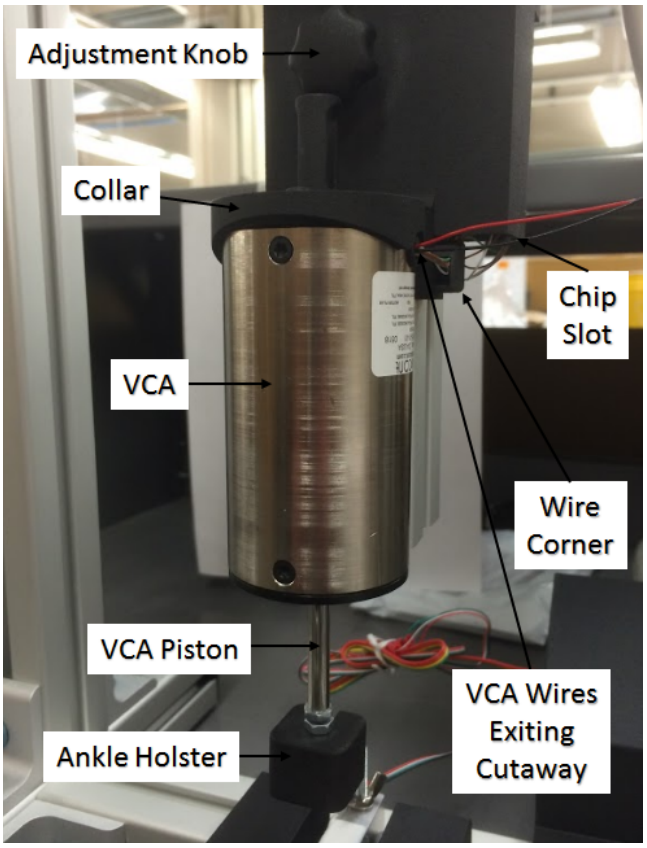


Figure 11. VCA Collar assembly with Ankle Holster properly oriented. Front faces of the Ankle Holster and Collar are parallel and Upside-Down T-Slot of the Ankle Holster faces backwards.

6. Electronics & Wiring

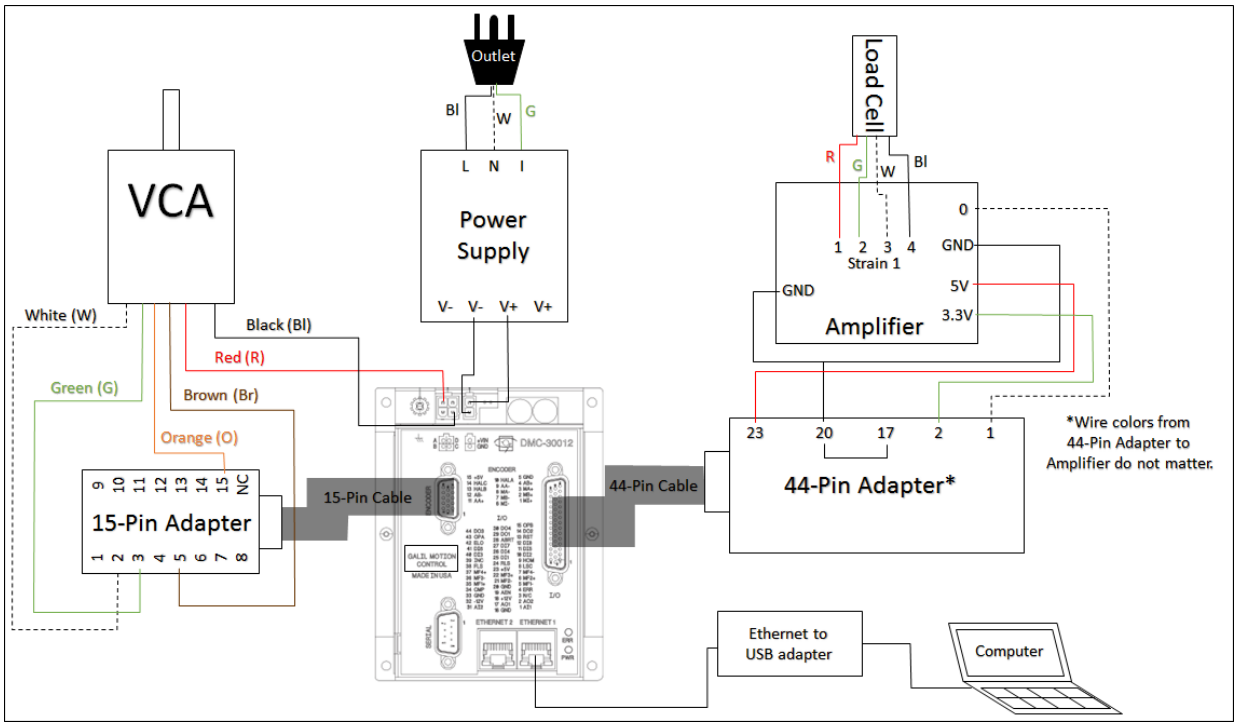


Figure 12. Wiring diagram

a) Important Note: *How to Use Metal Crimps (3cc).*

To use Metal Crimps, insert a stripped wire into the tube of the Metal Crimp until it has reached all the way to the end. Fold over the large metal wings at the end of the crimp so that it holds the insulated part of the wire. Then, fold over the smaller metal wings next to the large wings so that they clamp down onto the exposed part of the wire (Figure 13). The crimped wire can then be placed into the necessary connections.

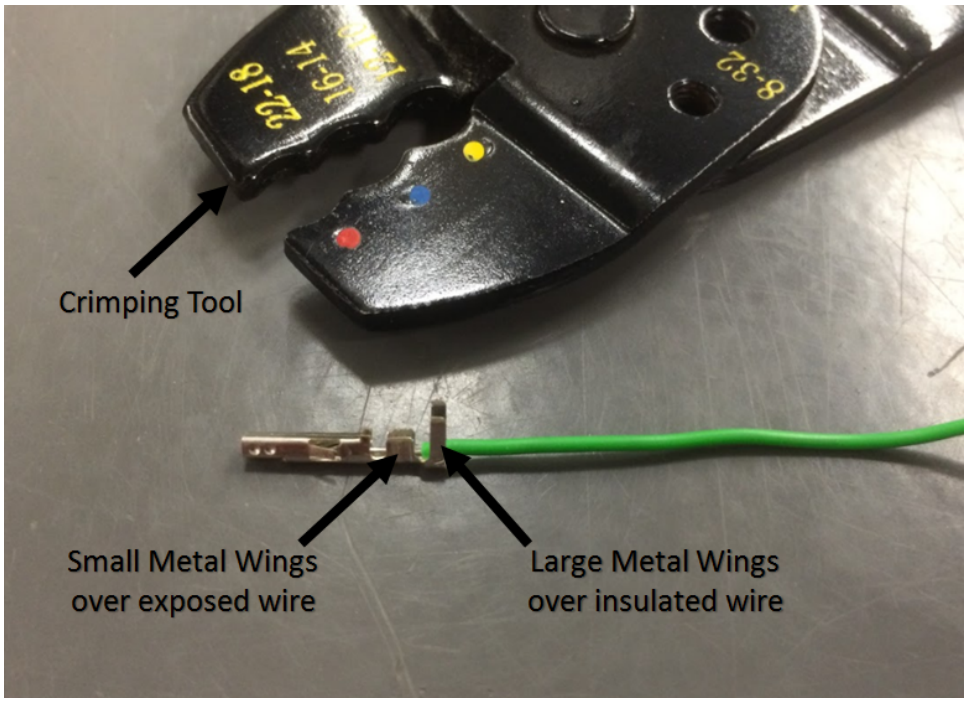


Figure 13. Preparing a Metal Crimp. Use the crimping tool to press the metal wings down onto the respective wire parts.

b) Wall Plug ⟶ Power Supply

Connect the following wires from the Wall Plug to the corresponding terminals on the Power Supply as detailed in the table below.

From Wall Plug	Start Connection	To Power Supply	End Connection
Black Wire	N/A (wires are pre-attached)	Terminal L	Strip wire, loosen screw terminal, insert wire, tighten
White Wire	N/A (wires are pre-attached)	Terminal N	Strip wire, loosen screw terminal, insert wire, tighten
Green Wire	N/A (wires are pre-attached)	Terminal ±	Strip wire, loosen screw terminal, insert wire, tighten

c) VCA ⟶ 15-Pin Adapter

1. Connect the following wires form the VCA to the corresponding terminals on the 15-Pin Adapter as detailed in the table below.

From VCA	Start Connection	To 15 Pin Adapter	End Connection
Brown Wire	N/A (wires are pre-attached)	Terminal 5	Strip wire, insert bare metal into the terminal
White Wire	N/A (wires are pre-attached)	Terminal 2	Strip wire, insert bare metal into the terminal
Green Wire	N/A (wires are pre-attached)	Terminal 3	Strip wire, insert bare metal into the terminal
Orange Wire	N/A (wires are pre-attached)	Terminal 15	Strip wire, insert bare metal into the terminal

2. Slide the 15-Pin Adapter into the corresponding slot on the Collar (Figure 14).

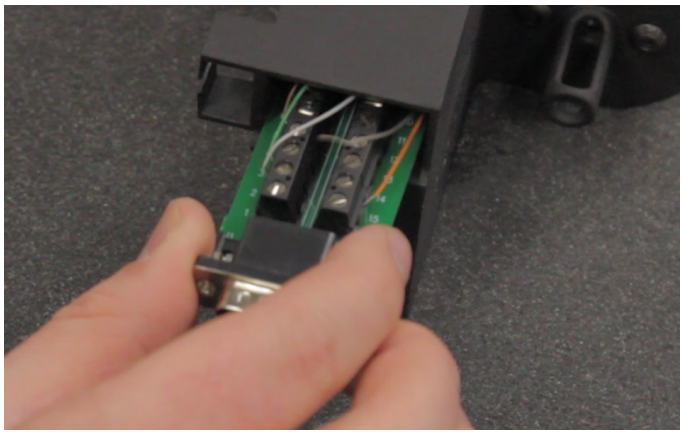


Figure 14. Inserting the 15-Pin Adapter into the corresponding slot on the Collar

d) Load Cell➡Amplifier

Connect the following wires from the Load Cell to the corresponding terminals on the Amplifier via the 4 Wire Harness and crimps as detailed below.

1. Assign a wire color on either side of the 4 Wire Harness to correspond with terminal 1 on the amplifier.
2. Assign the wire color on the opposite side of the 4 Wire Harness to correspond with terminal 4 of the amplifier.
3. Assign the remaining wire colors to correspond in order with terminals 2 and 3 of the amplifier (i.e. 1,2,3,4 (Figure 15)).
4. Cut off the plastic connector and strip all 4 wires at one end of the 4 Wire Harness.
5. Strip the free ends of the wires leaving the load cell and crimp each wire to the correct wire in the 4 Wire Harness (i.e. the wire that corresponds with the correct terminal on the Amplifier (see table below)). To crimp, place the stripped wires into the Red Plastic Crimps and press down near the ends of the Red Plastic Crimps with a crimping tool so that the wires are held tightly inside.

From Load Cell	**To Amplifier**
Red Wire	Terminal 1
Green Wire	Terminal 2
White Wire	Terminal 3
Black Wire	Terminal 4

6. Plug the free end of the 4 Wire Harness (the end that still has the plastic connector) into the strain1 port of the amplifier. Make sure to align the wire color assigned to correspond with terminal 1 with terminal 1. The terminals are numbered in ascending order from left to right (Figure 15).

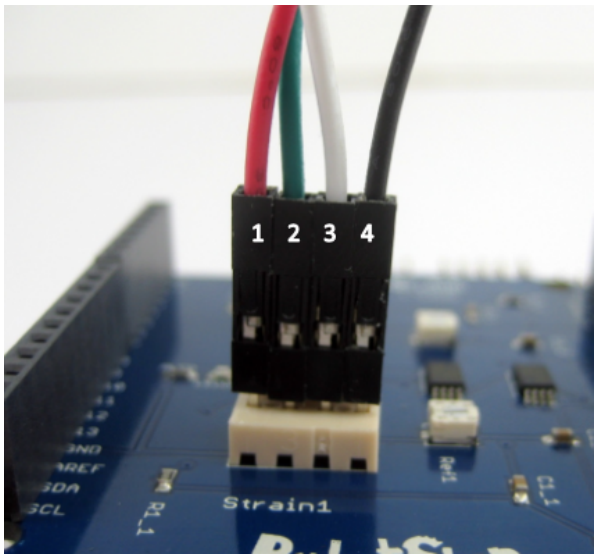


Figure 15. Close up of the plastic connector of the 4 Wire Harness plugged into the strain1 port of the Amplifier

e) Amplifier ➡ 44-Pin Adapter

1. Connect the following terminals from the Amplifier terminals to the corresponding terminals on the 44-Pin Adapter as detailed in the table below. The connections will be made using the Jumper Wires. These wires come pre-stripped and the colors do not matter. Simply insert the stripped end into the desired terminal (Figure 16). *Note; 2 grounds will be utilized on the amplifier chip. It is important that one ground terminal (GND) is on the right-hand side and the other is on the left-hand side (see Figure 16).*

From Amplifier	**To 44 Pin Adapter**
Terminal GND Left	Terminal 20
Terminal GND Right	Terminal 20
Terminal 0	Terminal 1
Terminal +5V	Terminal 23
Terminal +3.3V	Terminal 2

2. Connect terminals 20 and 17 together on the 44-Pin Adapter using a Jumper Wire.

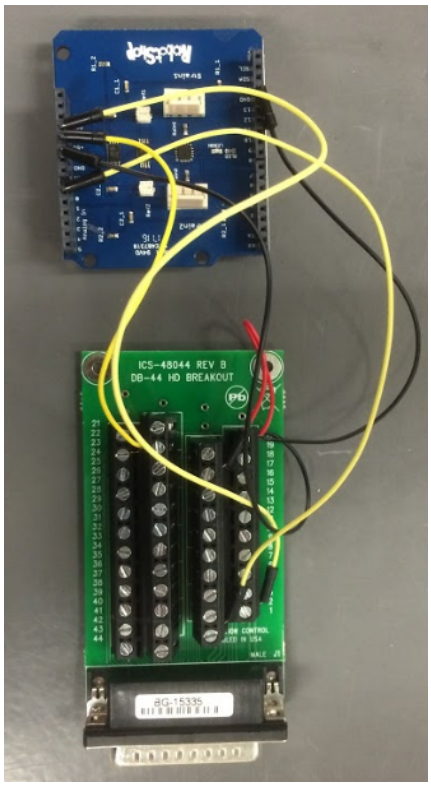


Figure 16. Closeup of Amplifier (Top) to 44-Pin Adapter (Bottom) connections

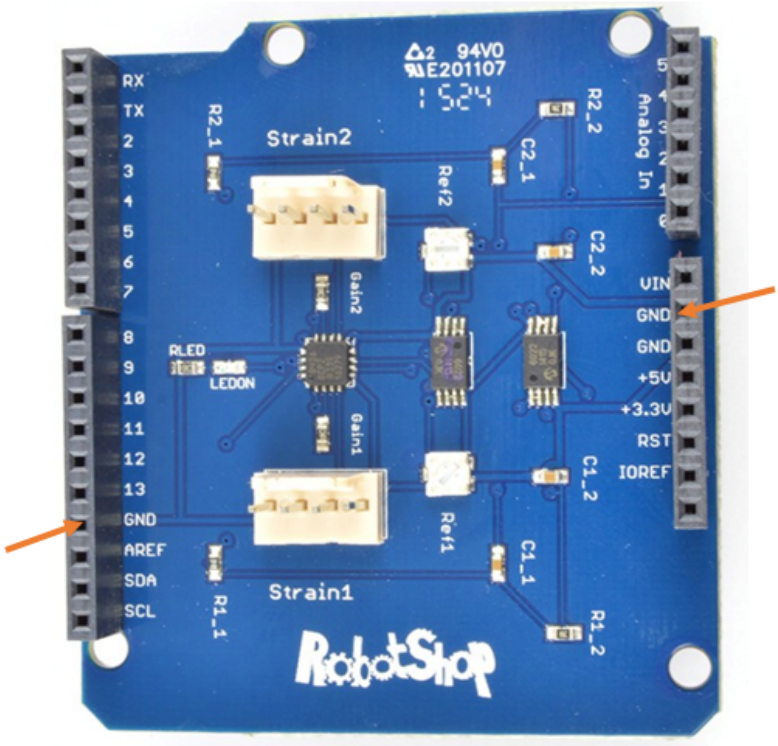


Figure 17. Schematic of the grounds (GND) on the left and right sides of the Amplifier

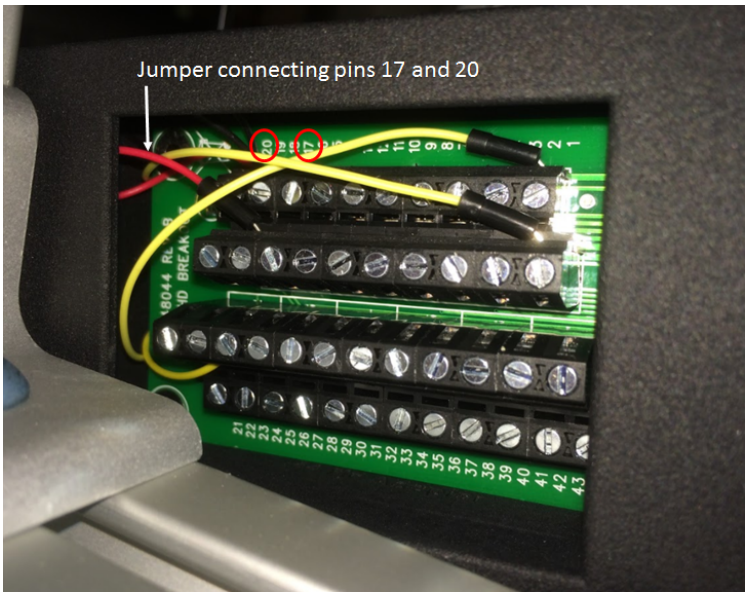


Figure 18. Jumper connection between ports 17 and 20 on the 44-pin Adapter

f) Motion Controller and Power Supply ⟶ Frame

1. Place a universal Mount (3s) at the top and the bottom of the Motion Controller and the Power Supply. Attach using the 2 Hex Head Bolts (3q) and 2 Hex Nuts (3r) at each end.
2. Place a Socket Head Cap Screw into the center hole on the Universal Mounts.
3. Insert 4 Self-aligning roll-in T-nuts with Leaf Springs (3f) into the right groove on the right vertical tube of the frame stand.
4. Thread the Socket Head Cap Screws into the Self-Aligning roll-in T-nuts with Leaf Springs and tighten to affix the Motion Controller and the Power Supply to the frame.

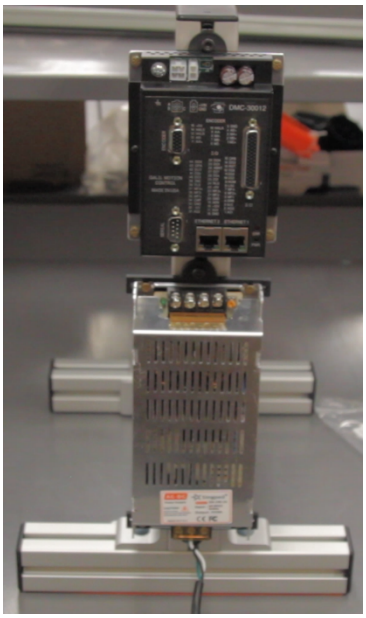


Figure 19. Motion Controller and Power Supply affixed to frame via Universal Mounts

g) Load Cell, Amplifier, and 44-Pin Adapter ⟶ Frame

1. Insert the tab of the Peripheral Mount into the top groove of the center bar on the H-shape base so that it extends over the back of the frame. Slide the Amplifier and the 44-Pin Adapter into the internal slots of the Peripheral Mount as indicated by the labels.

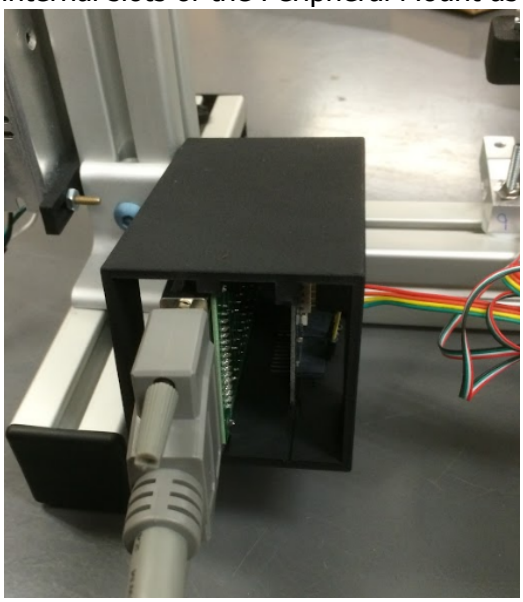


Figure 20. Peripheral Mount hanging from the center bar and containing Amplifier and 44-Pin Adapter. Note that it hangs on the backside of the frame, away from the load cell

h) VCA ⟶ Motion Controller

1. Cut off the excess red and black VCA wires, leaving about 1 inch exiting from the top of the 4-Pin Connecter Holder on the collar. Save this excess for step 2. Strip the free ends and connect the following wires from the VCA to the corresponding terminals of the 4-Pin connector as detailed in the table below (see Figure 21 for the port number scheme of the 4 Pin Connector).

Figure 21. Port numbering scheme for the 4-Pin Connector

From VCA	Start Connection	To 4-Pin Connector	End Connection
Black Wire	N/A (wires are pre-attached)	Port 1	Metal Crimps -see section a) *How to use metal crimps*
Red Wire	N/A (wires are pre-attached)	Port 4	Metal Crimps -see section a) *How to use metal crimps*

2. Strip both ends of the excess red and black wires saved from step 1. As in step 1, add metal crimps to the ends of the wires. Then attach a 4-Pin Male Connecter to each end of the wires (Figure 22), again following the table above for the corresponding ports in the 4-Pin Connectors.
3. Plug one of the Male Connectors into the 4-Pin Female Connector in the Collar and the other Male Connector into the 4-pin female port on the Motion Controller (Figure 23).

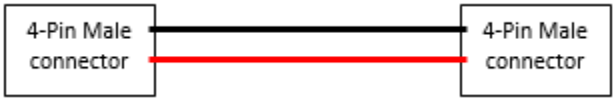


Figure 22. Schematic for step 2 which shows that there will be 2 male connectors joined by the red and the black wires

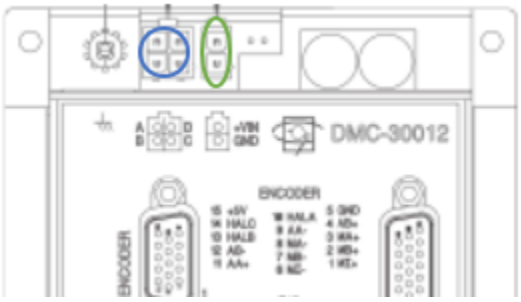


Figure 23. Location of the female 4-pin (blue) and 2-pin (green) ports on the Motion Controller

i) Power Supply ⟶ Motion Controller

1. Connect the following terminals from the Power Supply to the corresponding terminals on the 2-Pin Connector as detailed in the table below (see Figure 24 for the port number scheme of the 4 Pin Connector).

Figure 24. Port numbering scheme for the 2-Pin Connector

From Power Supply	Start Connection	To 2-Pin Connector	End Connection
Terminal V-	6 inches of Power Wire, strip wire, loosen screw terminal, insert wire, tighten	Port 1	Metal Crimps (see *How to use metal crimps)*
Terminal V+	6 inches of Power Wire, strip wire, loosen screw terminal, insert wire, tighten	Port 2	Metal Crimps (see *How to use metal crimps)*

2. Plug the 2-Pin Male Connector into the female 2-pin port on the Motion Controller (Figure 23).

j) Motion Controller➡Computer

1. Connect the Motion Controller (ethernet 1) to the Computer using the Ethernet Cable. If the Computer does not have an ethernet port, an ethernet to USB converted may be used. One is specified in Ordering (section 2, part h).

k) 44-Pin Adapter➡Motion Controller

1. Connect the 44-Pin Adapter to the Motion Controller using the 44-Pin Cable (3ff), (Figure 25).

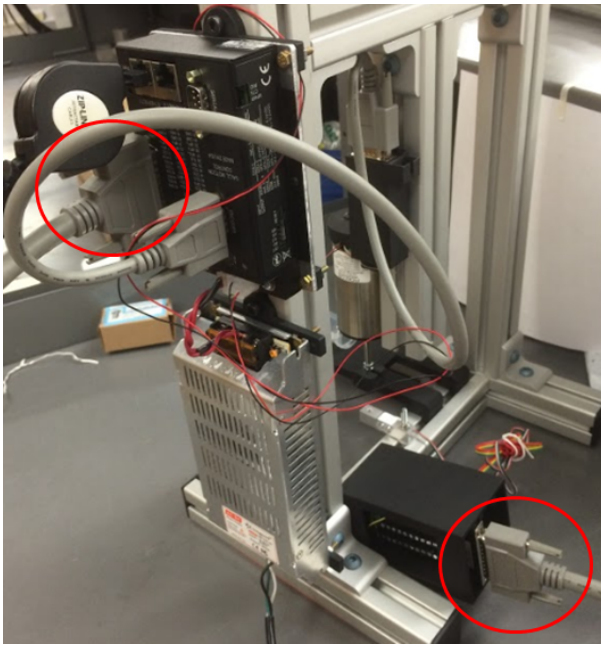


Figure 25. 44-Pin Cable going from 44-Pin Adapter to Motion Controller

l) 15-Pin Adapter➡Motion Controller

1. Connect the 15-Pin Adapter to the Motion Controller using the 15-Pin Cable (3x), (Figure 26).

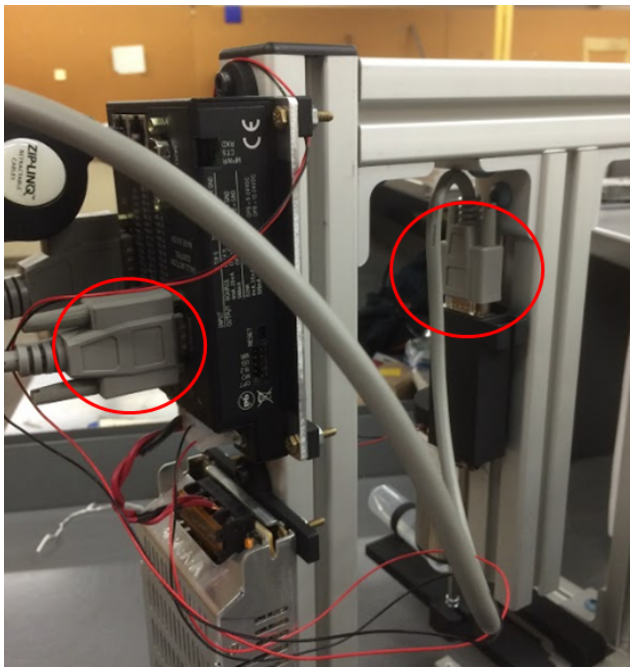


Figure 26. 15-Pin Cable going from 15-Pin Adapter to Motion Controller

7. Platform

a)The Platform (3h) will support the mouse during injury and will house the anesthesia interface. The Platform should extend out on the same side as the 90° Elbows holding the H-shaped base together (Figure 27).

1. Place the Platform onto the frame by placing the Rocker Arms of the Platform into the top groove of the center bar of the H-Shaped Base. This is the groove onto which the Load Cell was installed in section 4 (the load cell should fit in the center of the gap between the two arms of the Platform).
2. Insert the platform rocker arm vertically into the groove and roll it into the groove by pushing gently down on the Platform until it rests flat and horizontally on the tubing.

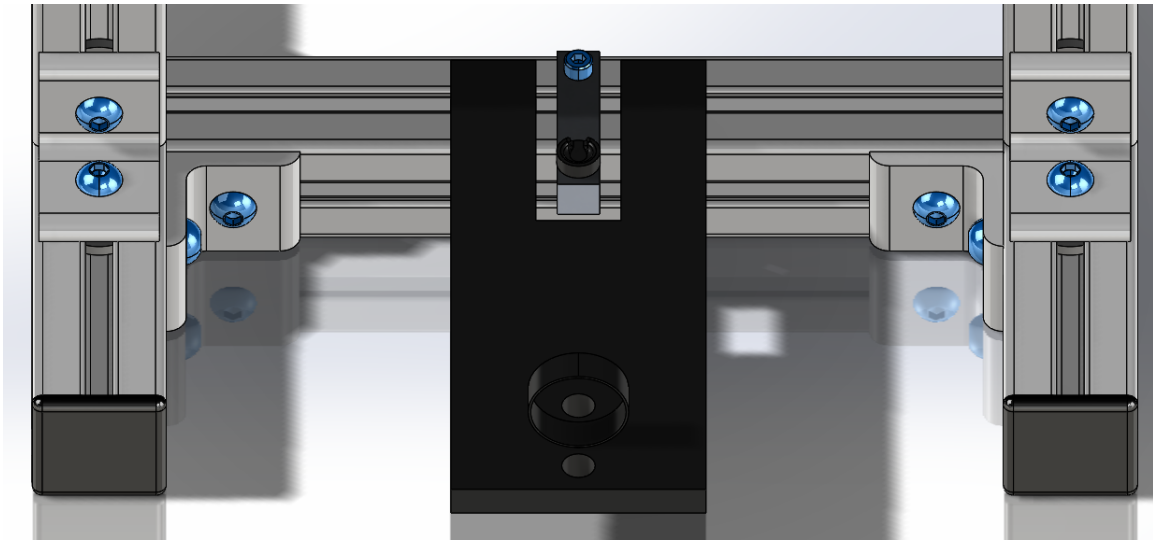


Figure 27. Location of mouse platform

b) Insert the post of the Anesthesia Pivot into one of the three holes at the end of the platform. These holes allow for adjustment of the distance between the mouse and the anesthesia. The Anesthesia Pivot can be moved as needed. This includes sliding the anesthesia tube through the Anesthesia Pivot and rotating the pivot to arrange the anesthesia tube exactly as desired.

c) Insert the post of the Knee Cup (3ll) into the hole at the free end of the Load Cell (the side which has not been bolted to the framing).

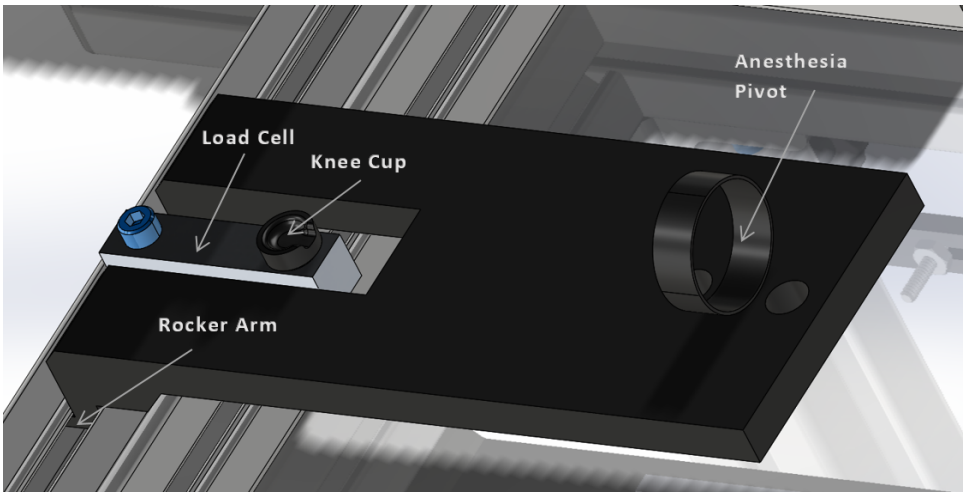


Figure 28. Schematic of components placed on the mouse platform

8. Code and Load Cell Calibration

a.) Installation of Software and Code

1.) Download ACLR.dmc file from the ACLR device downloads location (subject to change).

2.) Purchase a GDK Pro Key License by contacting Galil Motion Controllers at

email: support@galil.com

phone: 1 (800) 377-6329

3.) Download the Galil Design Kit (GDK) from www.galilmc.com/downloads/software/gdk for windows or Linux.

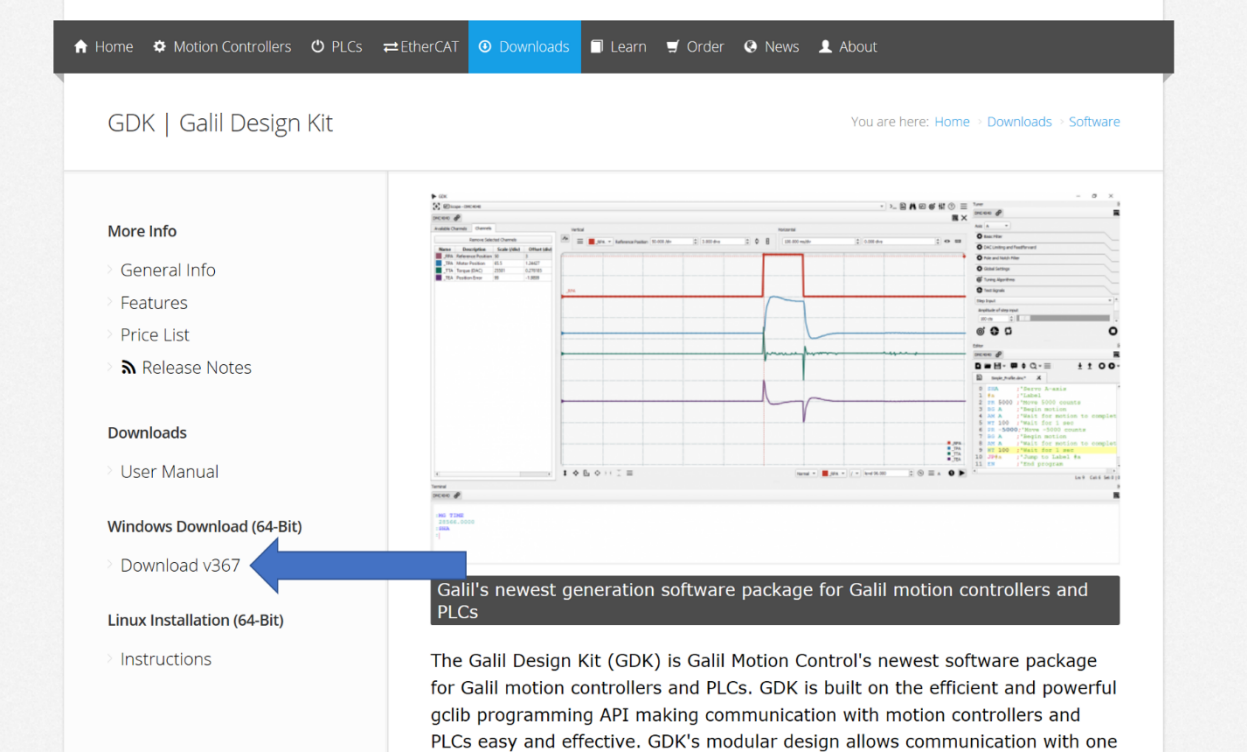


Figure 28. Download page on galilmc's website for GDK software

4.) After downloading and running the installer, click "Next", then "Install" with the default options ticked.

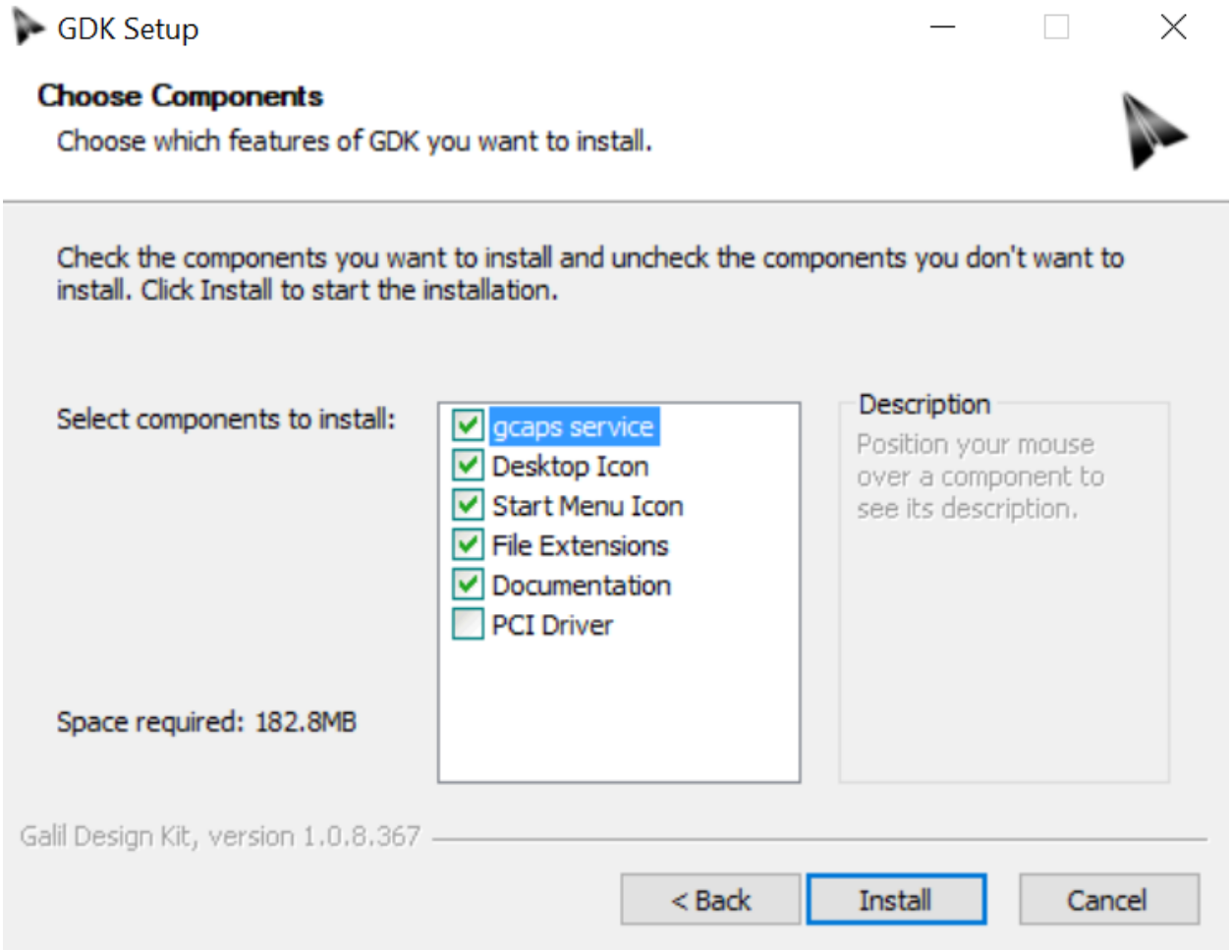


Figure 29. Installation options for Galilmc's GDK software (Windows)

5.) After the Installation has finished, Open Galil Design Kit software and open scope. Click the lock icon in the upper left and enter the pro key that was received via email.

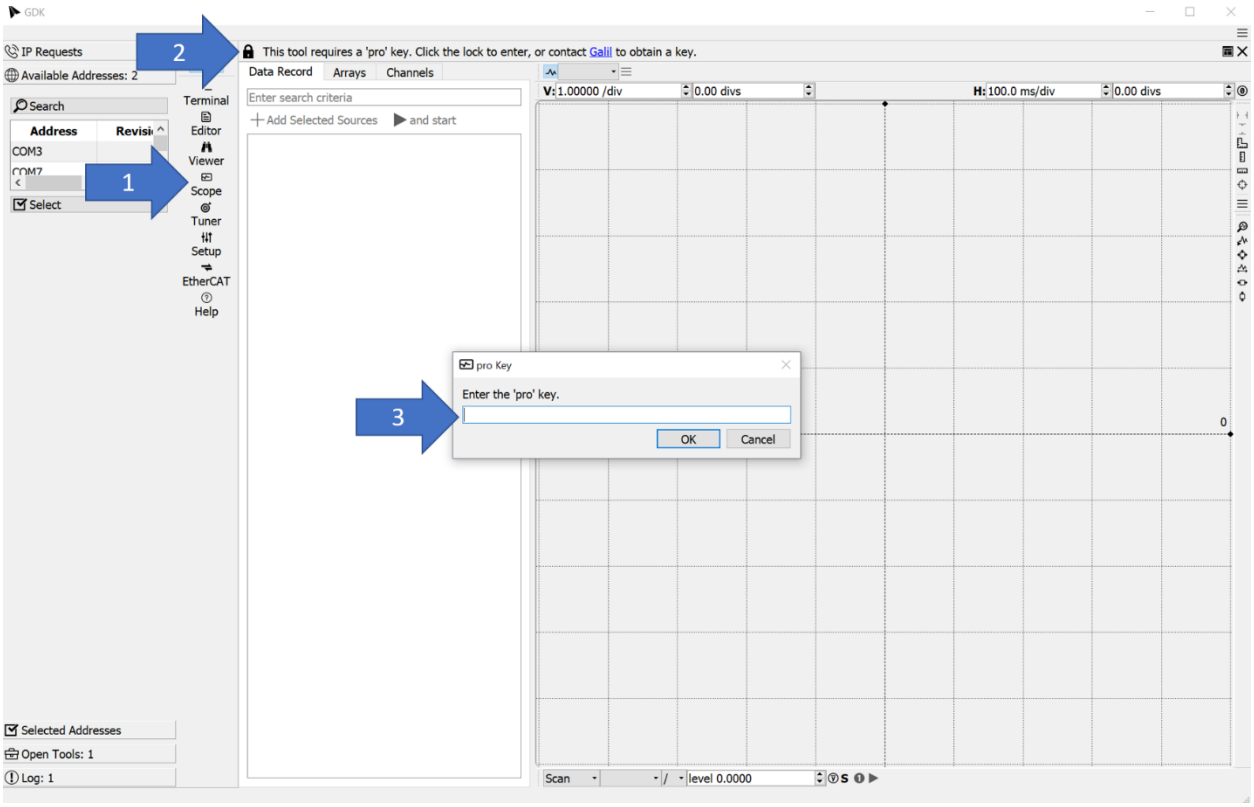


Figure 30. In the Scope (1) of the GDK software, the lock icon (2) opens the location to enter the pro key (3)

6.) connect to the motion controller by attaching the ethernet cord from the ethernet port on the computer to either ethernet port on the motion controller.

7.)Disconnect the VCA's power cords from the motion controller (this can be done by unplugging the clip-on 4-pin Molex connector used in wiring step 6-h-3). The motion controller can now be powered on by plugging the power supply's wall plug into a standard outlet (120V).

8.) Ensure connection between GDK and the motion controller by:

i.) "Unhide" the manager tools by clicking the paper airplane icon in the upper left part of the screen.

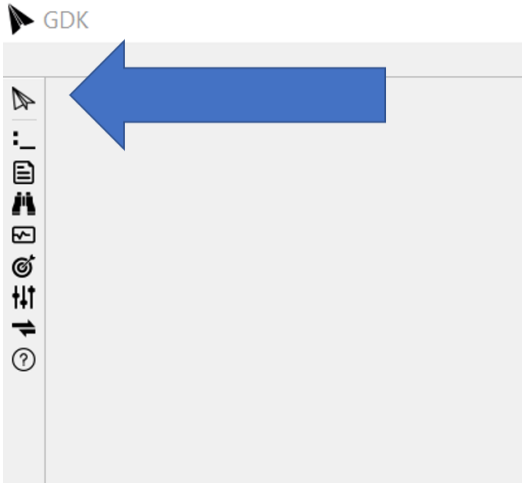


Figure 31. "Unhide" the GDK Manager drawer.

ii.) Click "Listen" to get a new IP address for the motion controller.

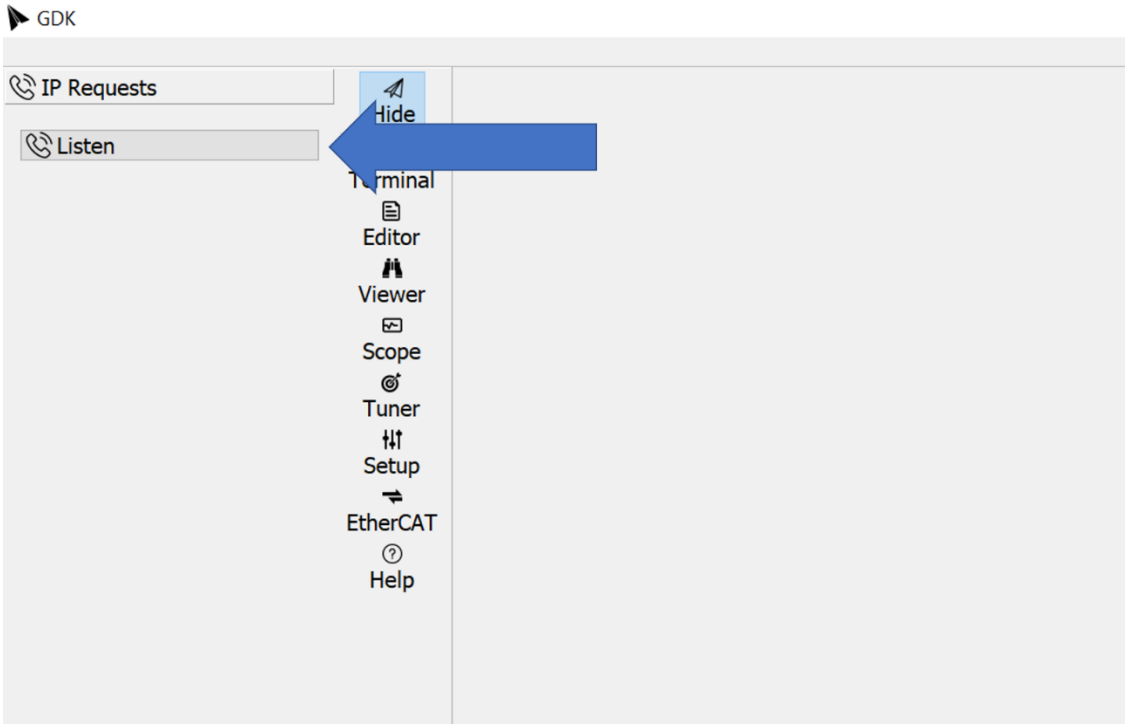


Figure 32. "Listen" for a new IP address

iii.) Select your device (name will start with "DMC") by double clicking on it from the list of available addresses. If your device does not appear, click "Search."

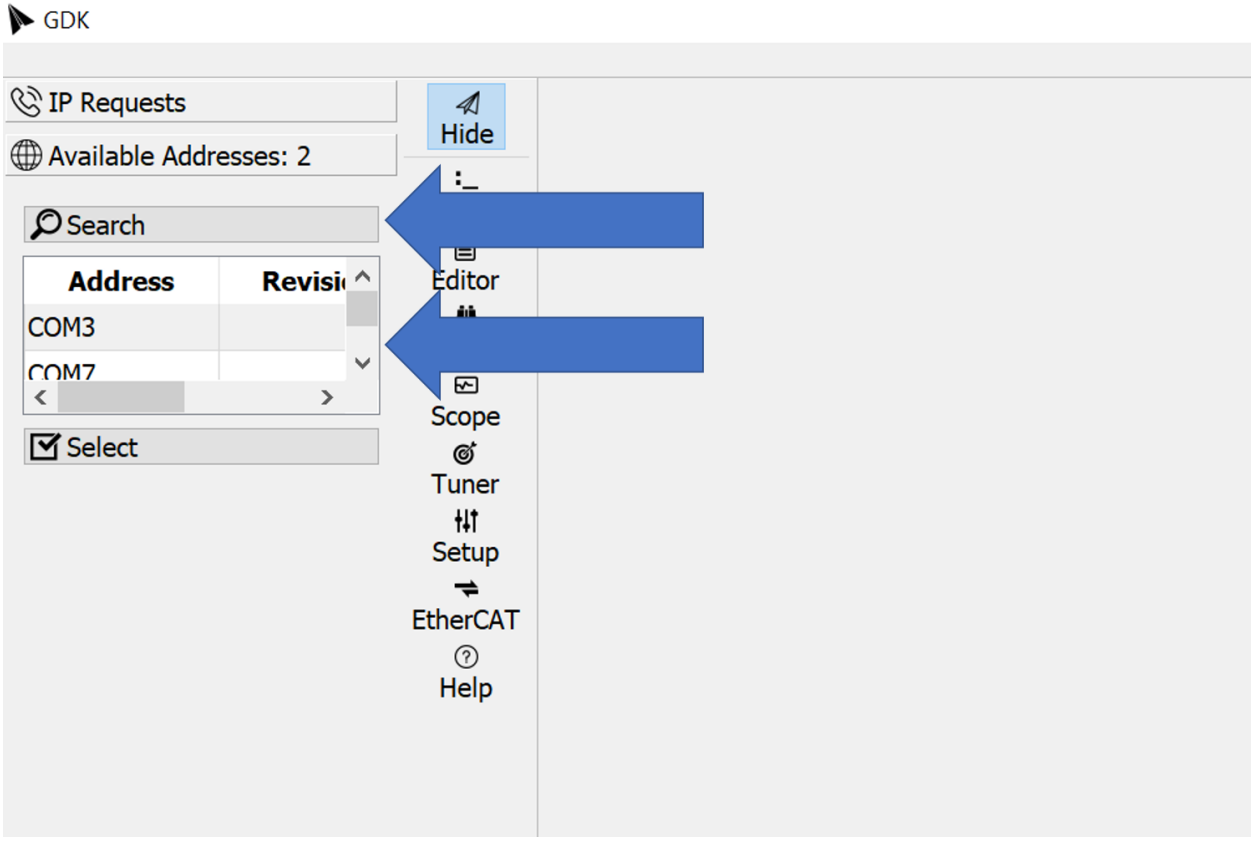


Figure 33. List of available addresses (bottom arrow), or "Search" button (top arrow) which should be used if the device's address does not appear.

9.) Open the "Editor" then open the ACLR.dmc program. Next, click "Download and Execute Program," or "Download Program to Hardware."

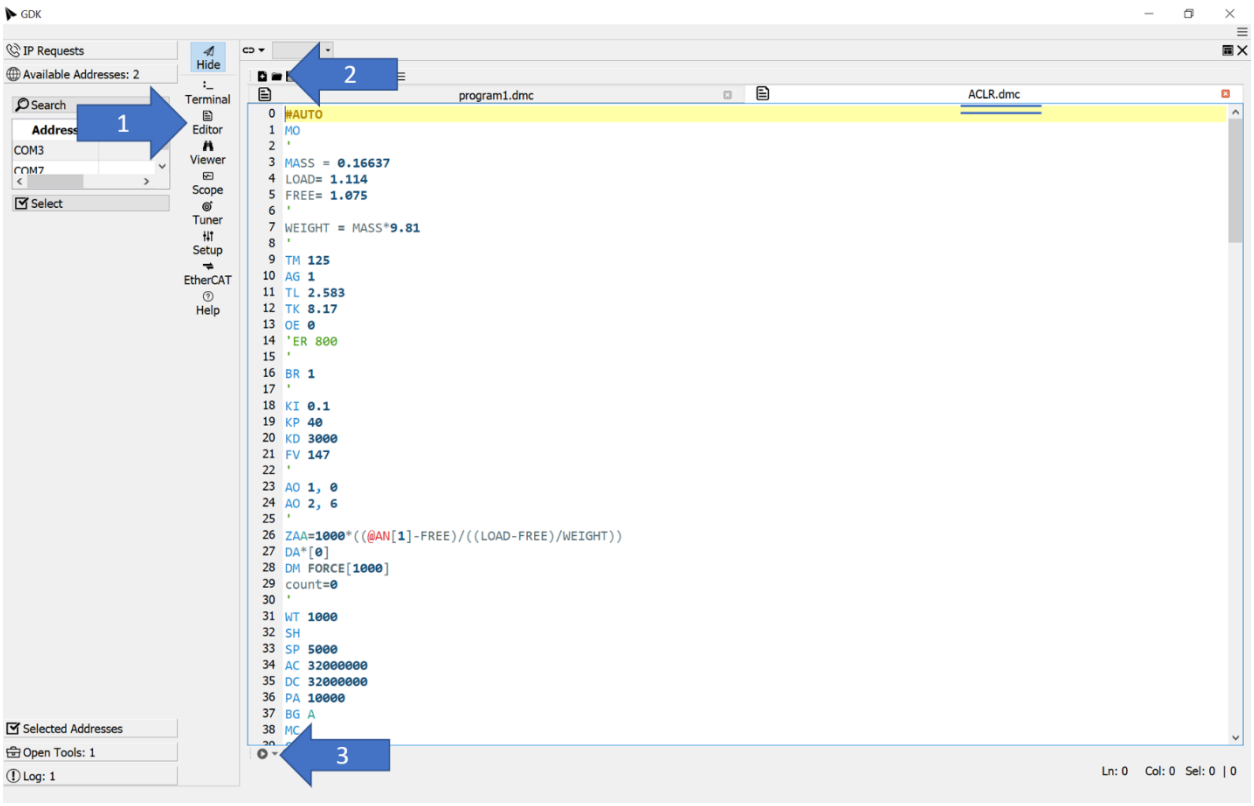


Figure 34. Open the "Editor" (1). Open the "ACLR.dmc" file that was saved in step 'a' (2). Click "Download and Execute Program" (3).

10.) When the program has successfully downloaded to the motion controller, power down the motion controller by unplugging the power supply from the wall outlet. Reconnect the VCA power to the power supply as in Wiring step 6-h-3.

11.) Power on the device by plugging in the power supply's wall plug into a standard outlet. (NOTE: 2 seconds after plugging the power supply into power, the VCA should slowly move up to its mid-stroke position and stop. If any other behavior is observed, Power should be unplugged immediately. Wiring between the VCA and motion controller should be double checked meticulously. If no errors are found, additional tuning may be required. Tuning information and guides can be found on galil's website at www.galilmc.com).

12.) Open the Scope and, while holding Ctrl on the keyboard, select _TPA, _ZAA, and @OUT[1] from the Data Record Source options. Click the play and start button.

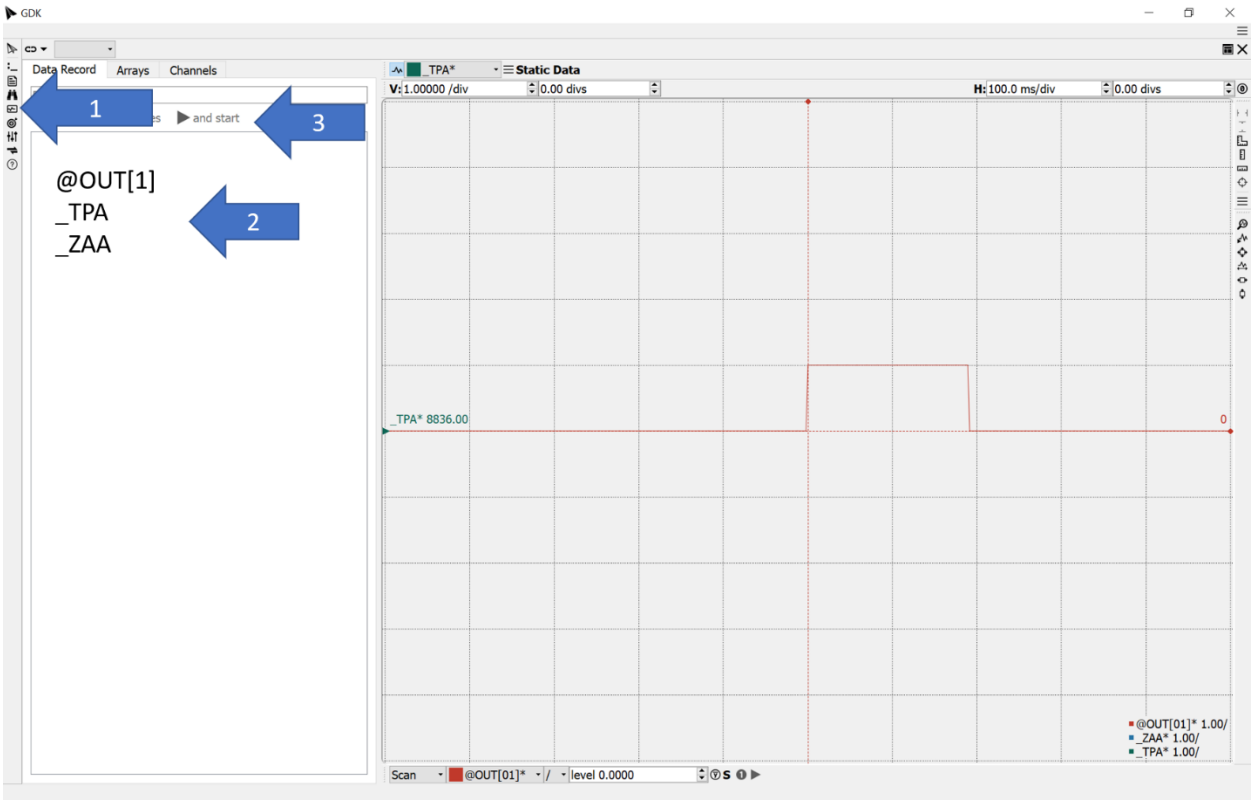


Figure 35. Select the desired data from the Scope

13.) Select Channels and adjust the Scale and Offset as shown in Figure 36 (below). Adjust the scope trigger settings to trigger normally on @OUT[1] on a rising edge at a level of 0.5 as shown in Figure 36 (below). Clicking the Run/Stop button should now change the trigger state from S (stopped) to A (armed).

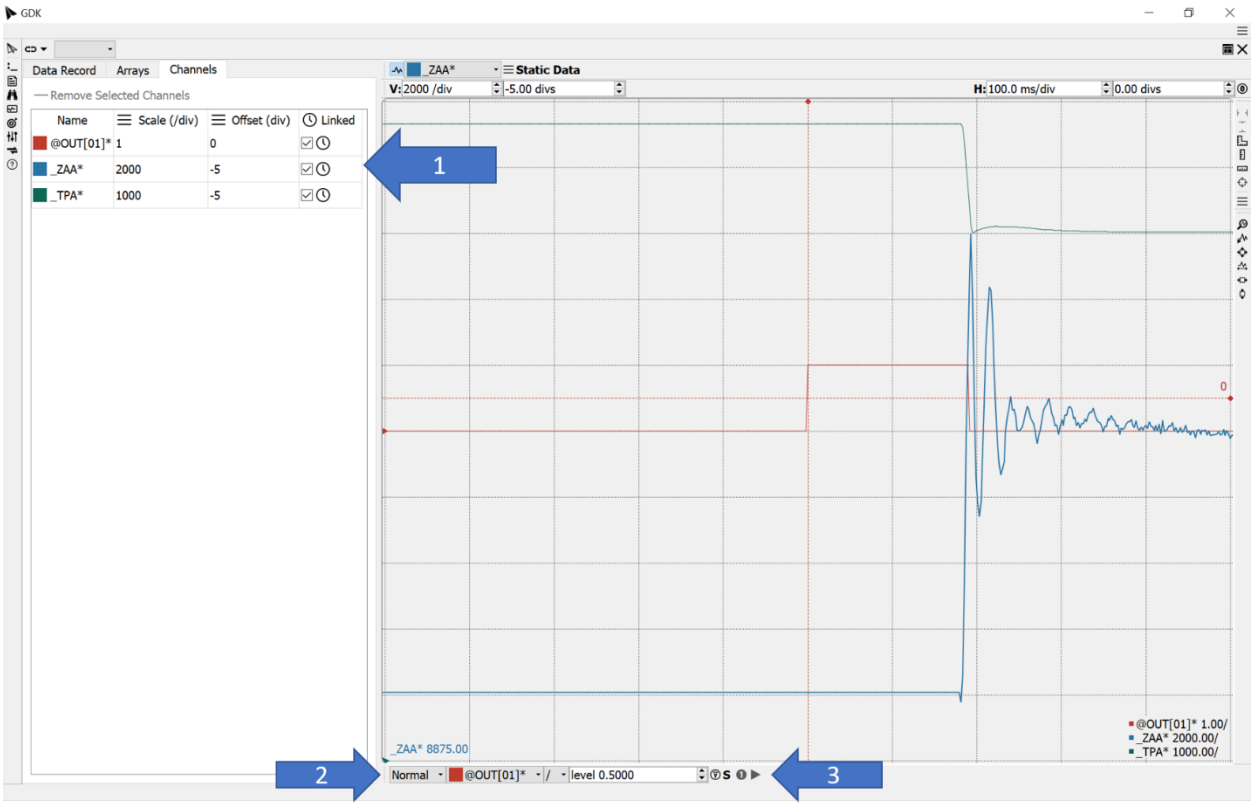


Figure 36. Scale and Offset Data (1). Adjust Trigger State (2). Click Play to arm the scope (3)

14.) Seen on the Scope is the blue line (_ZAA) which shows the force on the load cell in millinewtons. Each horizontal line represents a 2N change in force, with 0N at the bottom of the Scope. The green line (_TPA) shows the position of the VCA in counts (800 counts/mm). Each horizontal line represents a 1000 count change in position, with zero position at the bottom of the Scope. The redline (@OUT[01]) is a digital output that is used in the ACLR.dmc program to trigger the scope on and off to ensure that only useful data is being recorded.

b.) Load Cell Calibration

1.) After the code has been installed on the motion controller and the device has been powered on with the VCA behaving normally and moving to its mid-stroke position, Open the terminal, and dock it to the bottom of the screen.

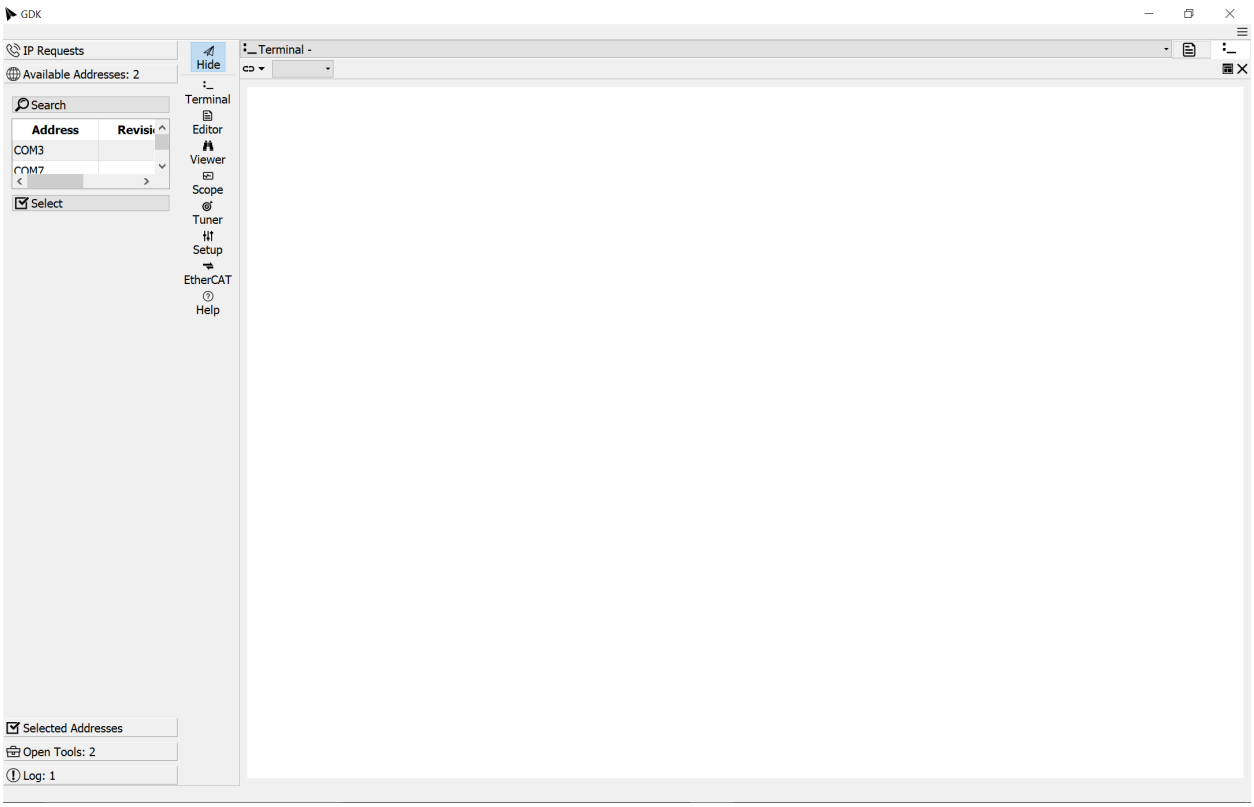


Figure 37. Open the terminal and dock to the bottom of the screen

2.) Use a mass equivalent to 1-2kg, and get an exact scale measurement of the mass in kilograms. Enter this mass (in kilograms) as the quantity initialized as "MASS" in line 3 of the ACLR.dmc code in the Editor.

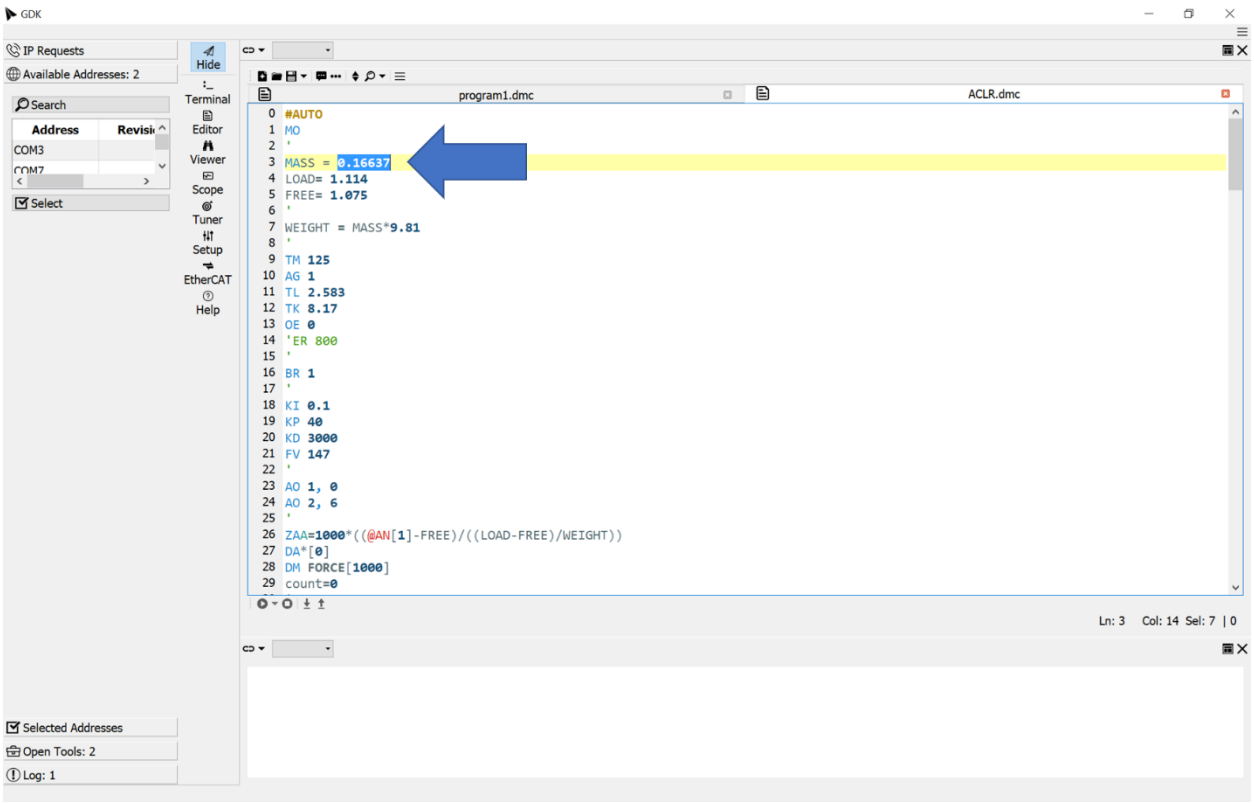


Figure 38. Line 3 in ACLR.dmc holds the quantity for the mass used in the load cell calibration IN KILOGRAMS.

3.) With nothing on the load cell, type the following command into the terminal and hit Enter:

MG @AN[1]

This command will read and display the data coming to analog input 1 which is connected to the load cell. Enter the command repeatedly by pressing the up-arrow key to bring up the last command, then hitting the Enter key. The data displayed will slightly vary due to slight electrical noise in the wiring for the load cell. Average 10+ of these data readings and enter this number in "FREE" (line 5) of the ACLR code in the editor.

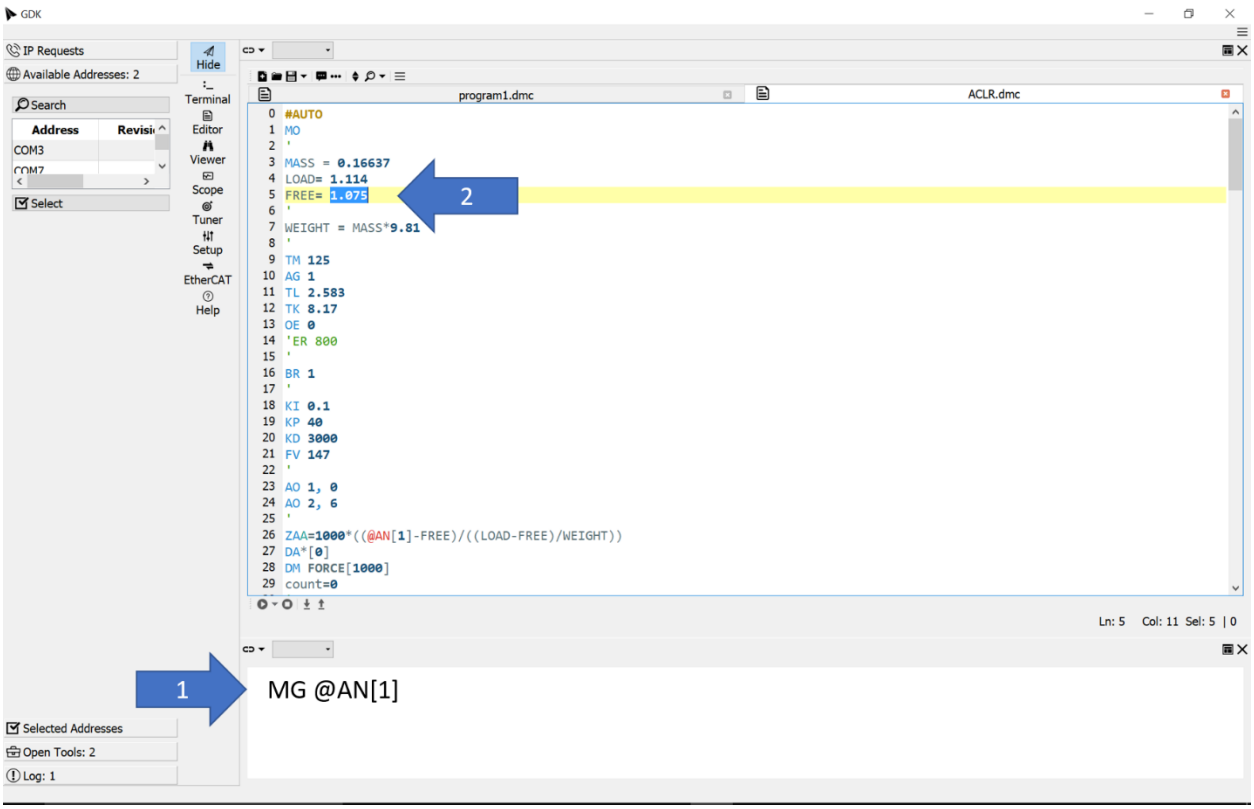


Figure 39. An average value from multiple "MG @AN[1]" calls (1) is the quantity to be used as "FREE," in line 5 of the ACLR.dmc code (2).

4.) With the measured mass on the load cell, enter the same command "MG @AN[1]" multiple times and enter the average of these data outputs in "LOAD," (line 4) of the ACLR code in the editor. Download and Execute the program. Finally, save the calibrated ACLR.dmc file.

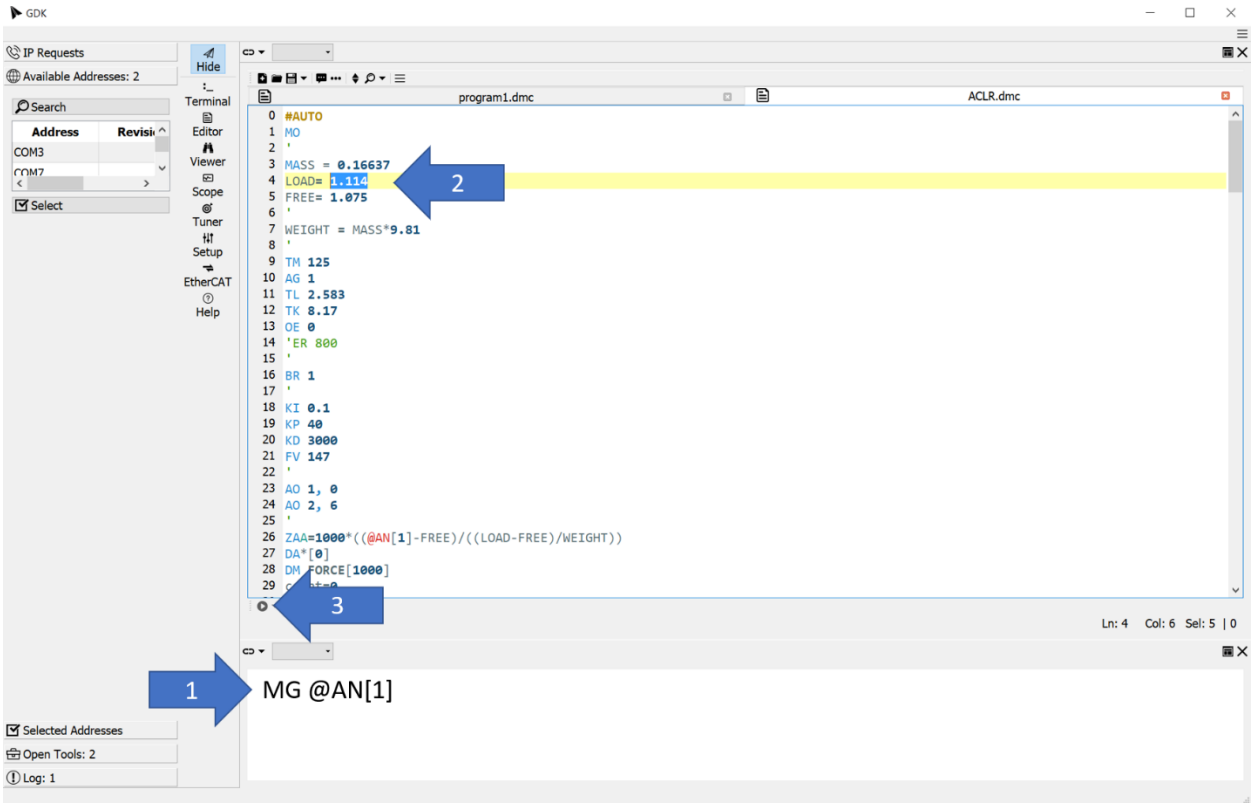


Figure 40. With the scaled mass on the load cell, an average value from multiple "MG @AN[1]" calls (1) is the quantity to be used as "LOAD," in line 4 of the ACLR.dmc code (2). The program can now be downloaded to the motion controller and executed (3).

c.) Additional code information

A complete, searchable manual of all operands used in GDK can be found at:

www.galilmc.com/download/comref/com3xx1x/index.html#cover.html+DMC30010

Additionally, the following operands are commonly used and define many of the settings for adequate ACL rupture:

ST – sends a stop command to the motion controller and VCA. This should be used in the terminal whenever erroneous motion occurs, or if the motion controller is un-wantedly working in a subroutine.

MO – sends a Motor Off command to the VCA. This should be used in the terminal whenever an immediate stop of the motion controller is necessary. Download and Execute of the ACLR program from the Editor may be necessary to begin use of the device after this command is called in the terminal.

SP – sets the speed of the VCA in counts/second. There are 800 counts (on the position) encoder) per mm, so 160000 ct/s is equivalent to 200 mm/s.

AC, DC – set the acceleration and deceleration respectively in counts/second^2. There are 800 counts (on the position) encoder) per mm.

ZAA – a user defined variable that can be monitored on the scope for data collection. ZAA is using the analog input from the load cell to measure force acting on the load cell in millinewtons.

PA – sets the desired absolute position in counts. The VCA should read a 0 position when it is fully extended and a position of ~20000 when it is fully raised. There are 800 counts (on the position) encoder) per mm.

PR – sets the relative position in counts. There are 800 counts (on the position) encoder) per mm.

BG – Begin Motion.

MC – Do not continue program execution until prescribed Motion is Complete.

JG – set Jog speed of the VCA in counts/second without a prescribed ending position. There are 800 counts (on the position) encoder) per mm, so 160000 ct/s is equivalent to 200 mm/s.

9. Operating Instructions/Safety/Sterilization

a.) Mouse Placement

Once the apparatus is completely set up tests can begin. Start by loading the mouse onto the platform, placing its knee into the knee holster on the end of the load cell and placing its nose into the nose cone. Turn on the anesthesia. Once the mouse is unconscious, the mouse leg can be preloaded. To do this, loosen the Adjustment Knob and lower the VCA, making sure that the mouse foot goes into the groove on the bottom of the ankle holster. Lock the VCA knob in place once the mouse leg cannot fall out but refrain from compressing the mouse leg manually.

b.) Experimentation

The mouse is now unconscious and in place, so the next step is making sure that your fingers are away from the VCA and the mouse. Activate the VCA through GDK software by entering the following command in the terminal and hitting Enter:

XQ#ACLR

The electronic preload will activate followed by the full compression. The preload is set to gradually increase force until the leg is preloaded at 2N so this step can take a few seconds (see part d, Customization, to adjust these settings) . Once the preload is complete the VCA will run at full speed and then stop moving once ACL rupture has been detected, or the maximum displacement limit is reached, or the maximum force has been applied. From here it is okay to stabilize the mouse leg and loosen the VCA knob to pull the VCA up and lock it in place away from the mouse. The mouse can then be removed. Be sure to turn off the anesthesia. Next, in the GDK Software, enter the following command into the terminal and hit Enter:

XQ#MOVE

This will return the VCA to its mid-stroke position so that it is ready for use on the next mouse. These steps can be repeated to as many mice as needed.

c.) Data Acquisition

Data can be saved from the GDK scope in a .csv, .png, .pdf, .gcs, or .svg file. .gcs files can be opened in GDK for data analysis in the Scope, and .csv files can be opened in excel, MatLab, and other data analysis software.

To export data from the Scope, open the plot options, and select export plot to file. Then be sure to select the desired file type before saving the file in a desired file location and with an appropriate file-name.

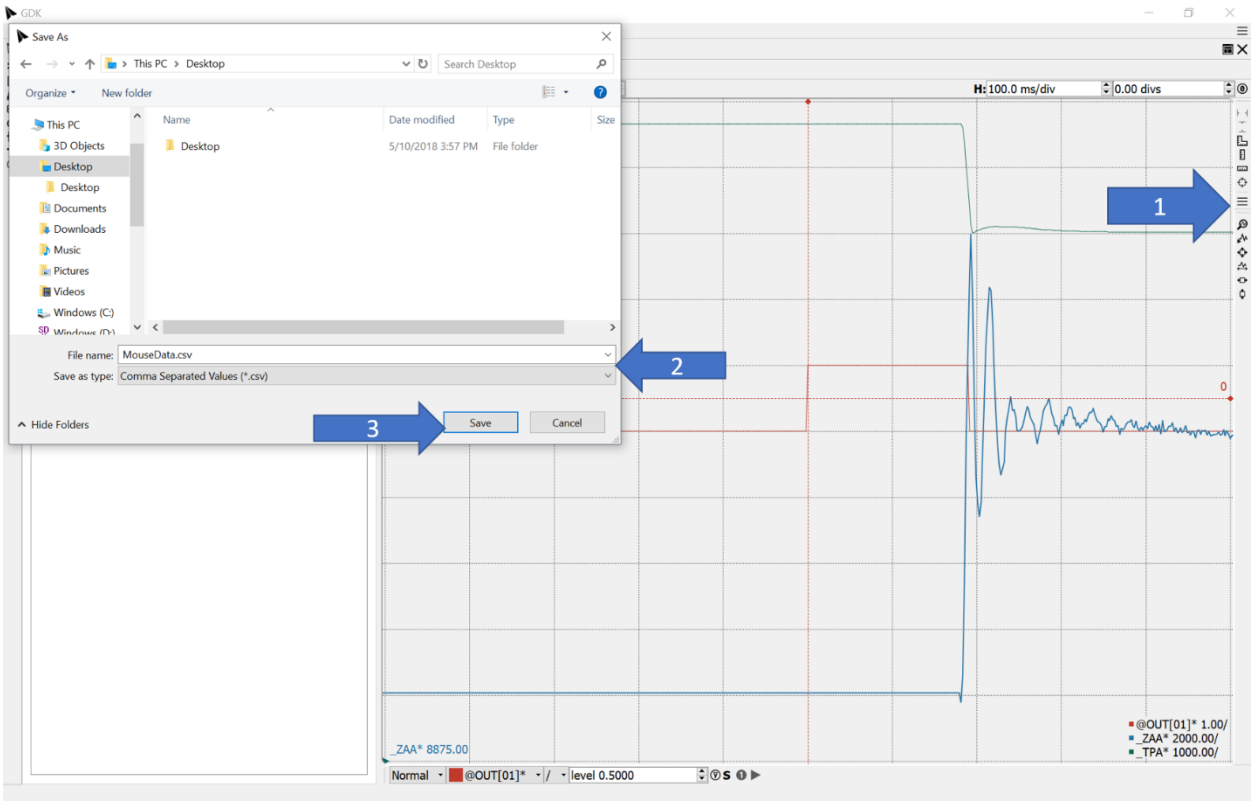


Figure 41. "Select Export Plot to File" from Plot Options (1). Select the desired filetype, filename, and file location (2). Save (3).

In the CSV file, the first, third and fifth columns of data show the time that the data point was taken in milliseconds. The second column of data shows the output of @OUT[01] that was used as a trigger for the scope, all data of interest will be found when this value is 1. The fourth column shows _ZAA; the force on the load cell in millinewtons. The sixth column of data shows the position of the VCA in counts (there are 800 counts/mm).

d.) Sterilization

Once done, unplug the apparatus from the wall outlet and the laptop. Now, it is important to sterilize the components that came into contact with the mice. These include the mouse platform, the knee holster, the ankle holster, the nose cone, and the load cell. All of these components can be easily removed, (the ankle holster will require a wrench if it is to be completely removed) for sterilization or sterilized where they are, just be sure not to get ethanol onto the electronics. The sterilization should be done by rubbing down the components with ethanol. Once sterilized, the components can be placed back onto the apparatus and the apparatus will be ready for the next experiment.

e.) Customization

Many settings and limits are used to ensure ACL rupture, while preventing collateral damage to the mouse. These settings may need to be adjusted depending on the particular strain of mouse used.

1.) Preload Force
   a. To adjust the prescribed force of the preload, First adjust the desired preload force (in millinewtons) by adjusting the quantity in line 146:

      IF (_ZAA<2000) *2000 refers to a 2000millinewton preload*

   b. Next adjust the bounds of acceptable preloads in lines 156 and 158:

      JP#PRELOA,(_ZAA<1900)|(_ZAA>2100) *boundaries 1900mN and*
      WT 1000 *2000mN are shown here*
      JP#PRELOA,(_ZAA<1900)|(_ZAA>2100)

2.) Preload Speed
   a. If the preload takes an excessive amount of time, the prescribed motion may be adjusted. The preload function operates by moving the VCA some prescribed distance, then checking if the force on the load cell is within some margin on the preload, if not, it repeats. Making the prescribed distance for the VCA to move too large, could result in consistently moving the VCA back and forth, without reaching a preload within the margin of accessible force.

   b. To adjust this distance, change the numerical values in lines 147 and 149 (be sure to avoid changing the sign of these numbers and doing so *will* result in damaging the mouse. Small changes are recommended.

      PR -16 *-16 and 16 refer to 16 counts (0.02mm) of displacement in*
      ELSE *either direction, depending on current force*
      PR 16 *measurement.*

3.) Maximum Force
   a. A maximum force of 14 Newtons is in place and was chosen from empirical tests on C57BL/6 mice whose ACLs ruptured around 7-10 Newtons. Should a mouse with a stronger ACL be used, this maximum may need to be adjusted or removed.
   b. The 14000 millinewton force can be adjusted in line 181. Or the content of the line can be deleted to remove this force catch.

      JP#END,(_ZAA>14000)

4.) Maximum Displacement
    a. A maximum displacement of 2.25 mm is in place to avoid fracture of the femoral condyles. Should adjustment of this displacement be necessary, it can be changed in line 179, which defines this displacement in counts (800 counts/mm). It should be noted that the motion controller's clock speed is not fast enough to catch an exact number of counts. The displacement catch after 1250 counts was found empirically to bring the VCA to a complete stop at just over 1600 counts (2mm).

```
JP#END,(pos-_TPA>1250)
```

5.) Rupture Detection
    a. The motion controller detects ACL rupture by checking for a sudden drop in force on the load cell under the mouse's knee. Rupture is only detected if that drop is 0.25 Newtons or greater. To adjust this value, adjust the number in line 180 which defines the force drop in millinewtons.

```
JP#DCHECK2,(_ZAA-FORCE1>-250)
```

6.) Device/Rupture Speed
    a. The VCA moves at a prescribed speed if 200mm/s to ensure ACL rupture without avulsion of the ligament. Should adjustment of this speed be necessary, it can be changed by adjusting the number in line 173, which defines the speed (jog speed) in counts per second. (there are 800 counts/mm, so 160000 ct/s is equivalent to 200mm/s). This value must be negative to ensure downward (compressive) motion of the VCA.

```
JG -160000
```