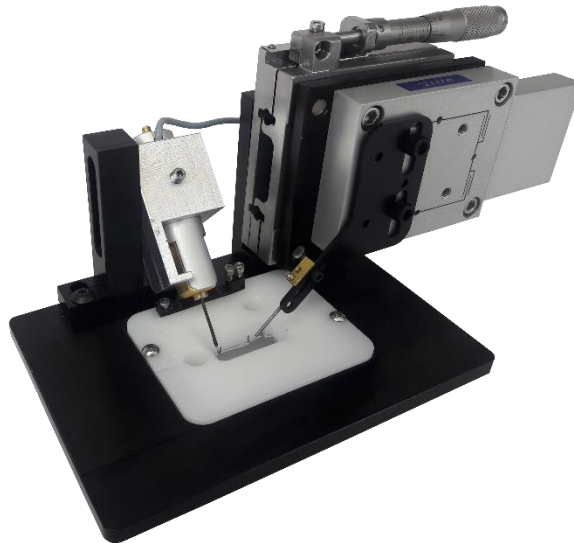


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USER'S GUIDE

# Intact Muscle Chamber



**Manual Revision 1.0**  
**June 2017**



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Disclaimer: This is an electrical device. There is an inherent risk of electrical shock if used improperly. Please take the necessary precautionary measures that are common place for any electrical device. It is solely intended for the applications outlined in this document. The manufacturer is not liable for any misuse, or any injury incurred because of misuse.

**Always unplug device before checking fuse!**

Disclaimer: This product is intended for research purposes only. It is not certified for clinical applications (including diagnostic purposes). Use of this product in uncertified applications is in violation of FDA regulations.

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# 1 Introduction

This equipment is useful for simultaneous recording of contractile force and  $\text{Ca}^{2+}$  handling characteristics in intact muscle strips with dimensions mm-cm. To call the muscle 'intact' means that its membrane is excitable, and electrical stimulation will elicit  $\text{Ca}^{2+}$  release into the cytosol of the muscle cells thus leading to force production and muscle contraction.

The most notable components are (i) the force transducer, (ii) the length controller and (iii) the chamber, which allows for submersion of the muscle in a  $\text{Ca}^{2+}$ -rich solution.

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## 2 Intact Muscle Chamber - Unit Description

### 2.1 Features

#### 2.1.1 Muscle types

This unit is designed to be used for intact, excitable muscles up to ~2 cm in length:

- Cardiac papillary muscle of many species (mouse, rat, rabbit, cat, dog, pig)
- Cardiac endocardial strips or trabeculae (mouse, rat, rabbit, cat, dog, pig, human)
- Cardiac epicardial strips (mouse, rat, rabbit, cat, dog, pig, human)
- Skeletal muscles, e.g., soleus, extensor digitorum longus (EDL), gastrocnemius, cremaster (mouse, rat)

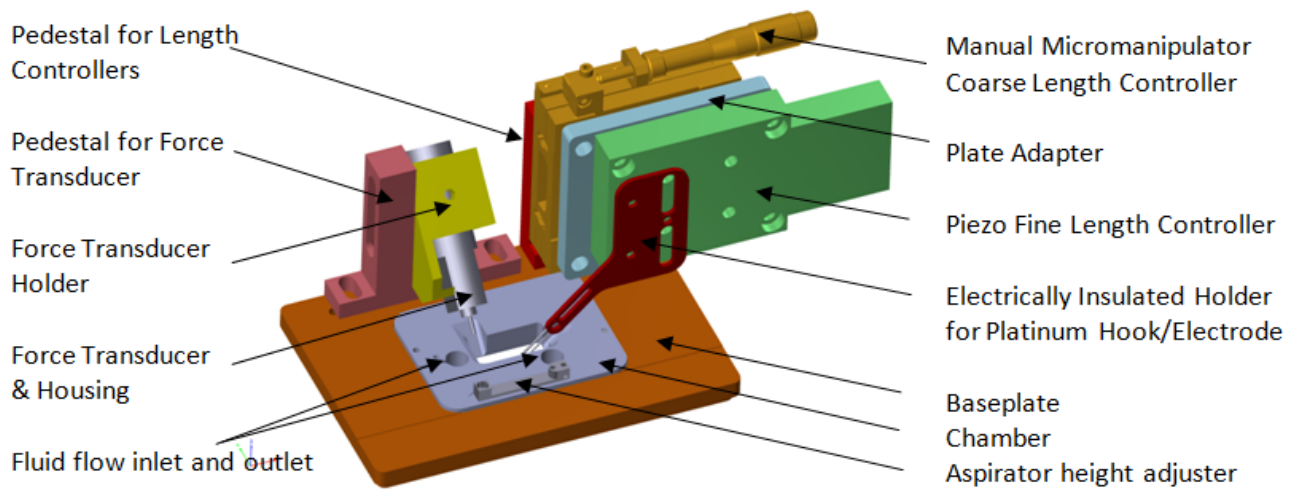
#### 2.1.2 Stimulation through platinum clips/electrodes

Stimulation of the muscle sample is elicited by electrical current passing directly through the muscle sample, therefore obviating the possibility of any reduced stimulation as occurs with bath electrodes. This is accomplished using platinum clips to connect the muscle to platinum electrodes that have been incorporated as part of the force transducer and length controller.

#### 2.1.3 Simultaneous force and $\text{Ca}^{2+}$ recordings

A clear cover slip forms the bottom of the chamber and, if placed on an inverted microscope fitted with a long working distance objective, allows fluorescence light detection. It should be noted that the muscle will be placed 1-2 mm above the surface of the cover slip, therefore the objective should have a working distance of greater than ~1.5 mm.

## 2.2 Diagram



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## **3 Intact Muscle Chamber - Components**

### **3.1 Baseplate, Pedestals, and Housings**

#### **3.1.1 Pieces**

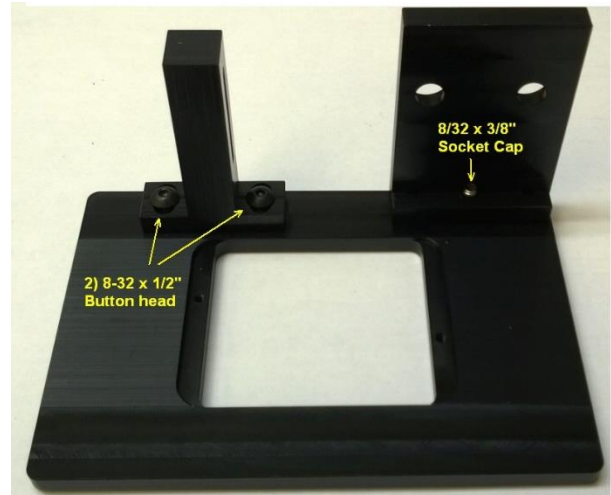
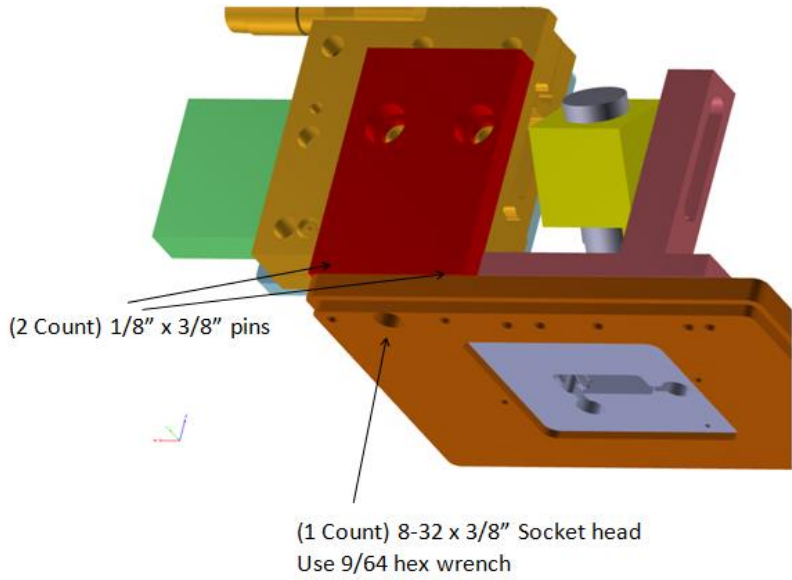
The baseplate is a thin (<1 cm) plate made of aluminum. The baseplate holds all the equipment useful for experimentation (piezo fine-length controller, force transducer, specimen chamber) and a large rectangular cut-out in the plate where the specimen chamber fits. The pedestal and housing for force transducer simply holds and positions the force transducer. The pedestal for length controller is a large post structure that holds the micromanipulator and length motor.

#### **3.1.2 Rationales and options**

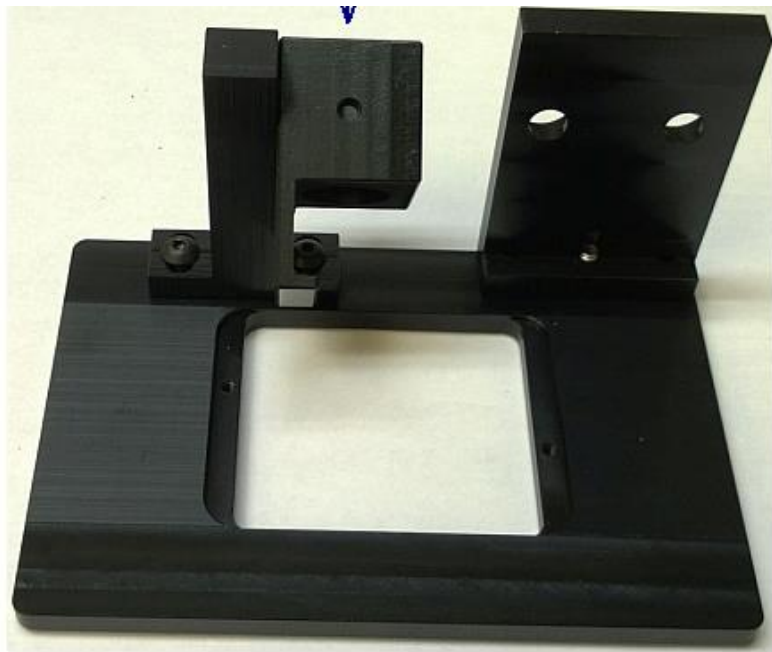
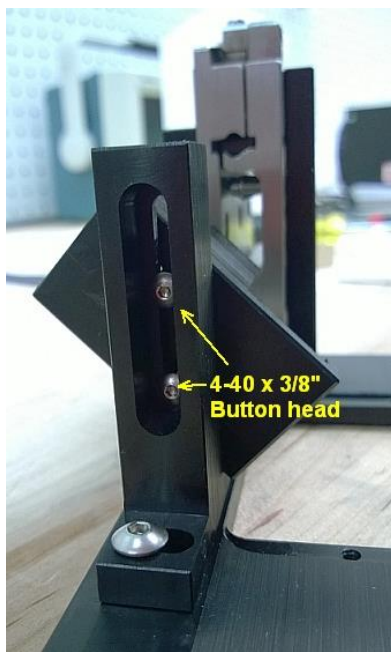
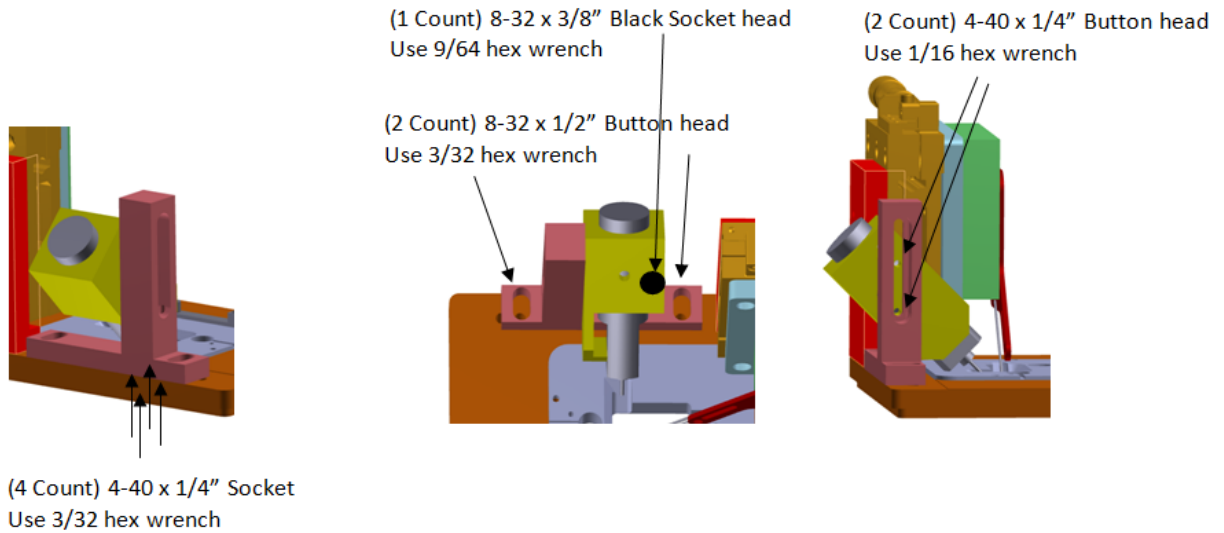
The dimensions of the baseplate allow it to fit into a standard 6-well plate holder, which is a common accessory for biological microscopes. The bottom of the chamber insert is recessed to prevent any surface hitting the coverglass if the baseplate is placed on a table or countertop.

### 3.1.3 Diagrams and Assembly

#### 3.1.3.1 Baseplate, pins and pedestal for length controller:



### 3.1.3.2 Assembly of pedestal and housing for force transducer (only Güth-style force transducers)





## 3.2 Micromanipulator and Adapter Plate

### 3.2.1 Pieces

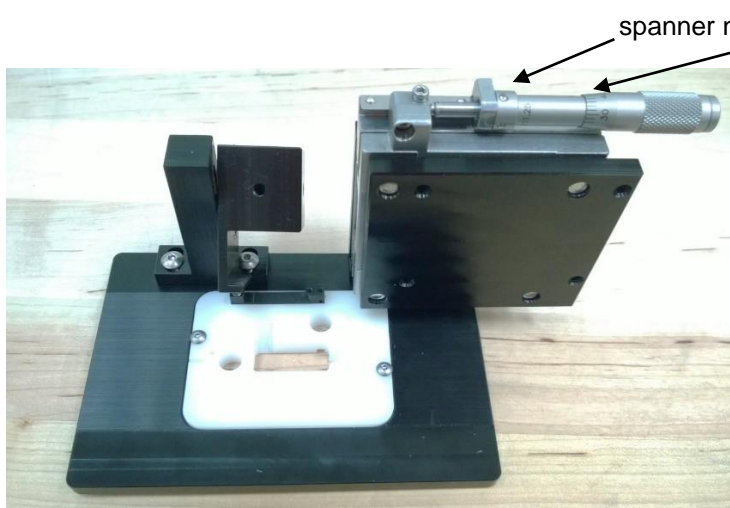
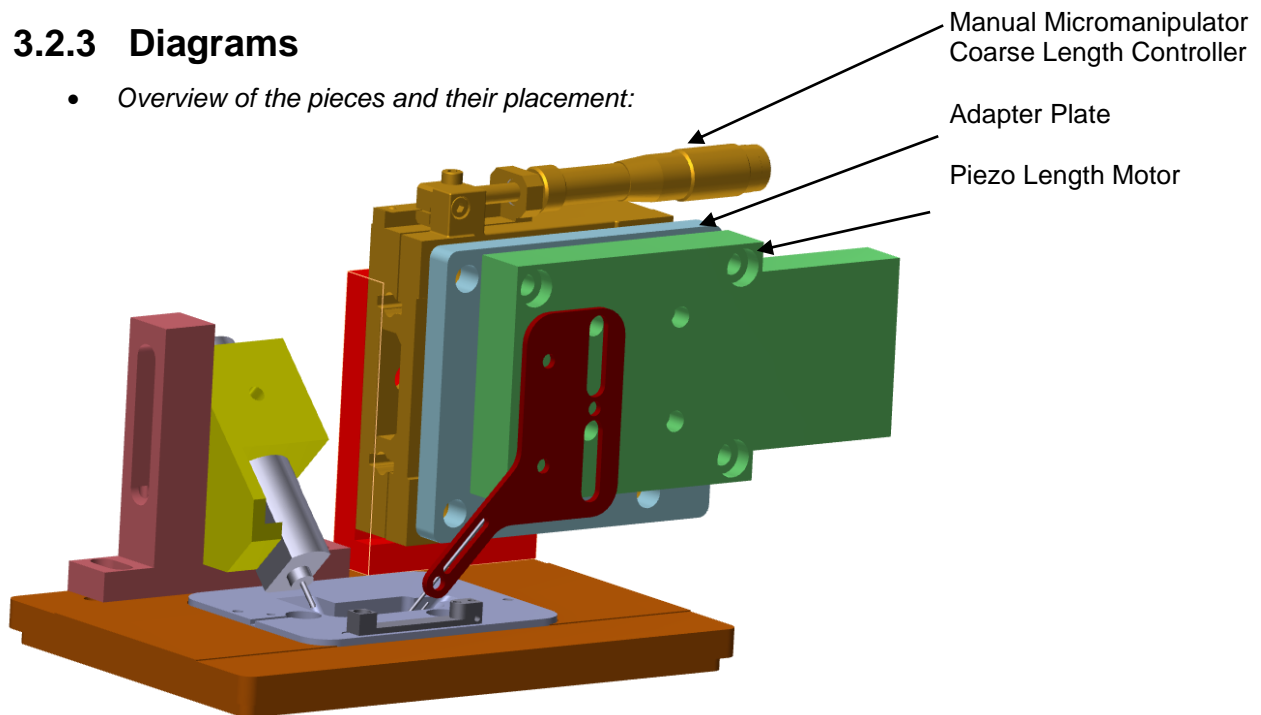
The manual micromanipulator is used for coarse movement of the length control with a range of 25 mm or ~1 inch. This coarse micromanipulator is attached to a pedestal anchored to the baseplate. Fine length control is achieved with a piezo motor with a range of 50 microns.

### 3.2.2 Rationales and options

As muscle lengths widely vary, a manual micromanipulator is important to position the hooks to accommodate muscle sample length.

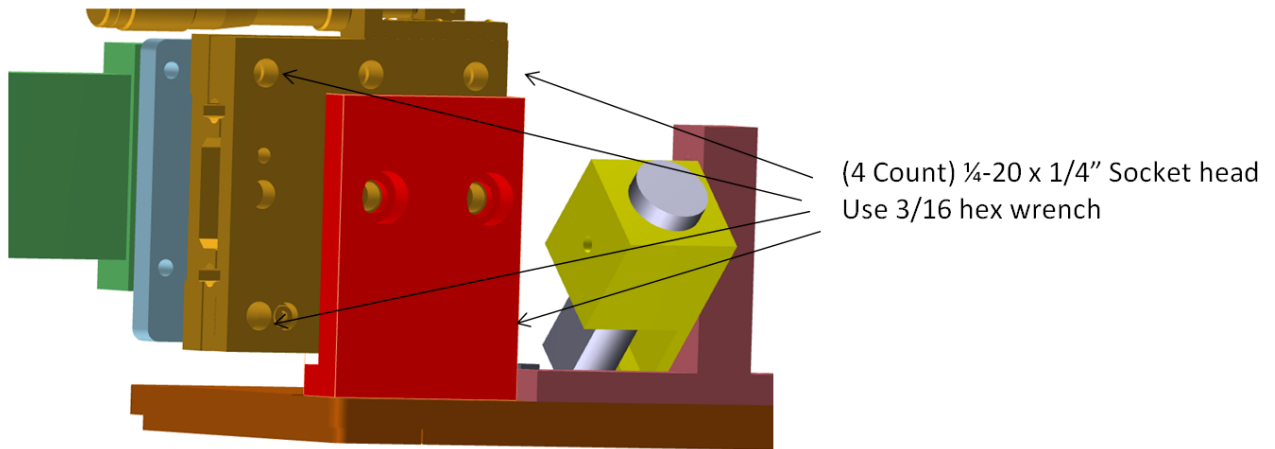
### 3.2.3 Diagrams

- Overview of the pieces and their placement:



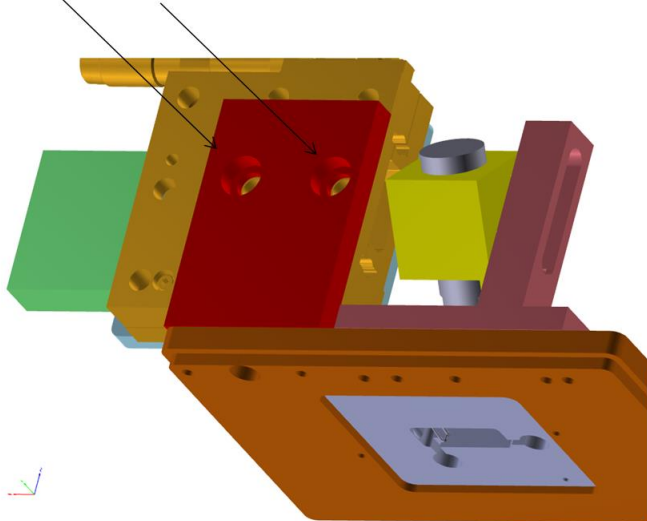
*Note: If the hashmarks are not visible here, as demonstrated in this photo, then you will need to loosen the spanner nut and turn the sleeve so the hashmarks are showing toward the user, i.e., toward the front of the apparatus. You should then tighten the spanner nut when done.*

- *First put the Adapter Plate on the Micromanipulator:*



- *Then put the Micromanipulator onto the Pedestal:*

(2 Count) M6 x 6 mm (or 1/4" - 20 x 1/4")  
Use 3/16 hex wrench



### 3.3 Piezo Motor and Hook Extension (aka Beak)

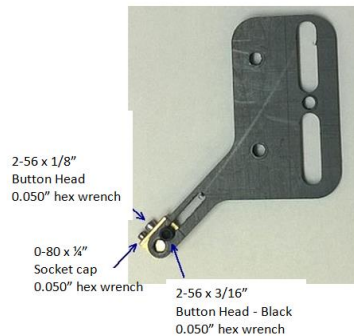
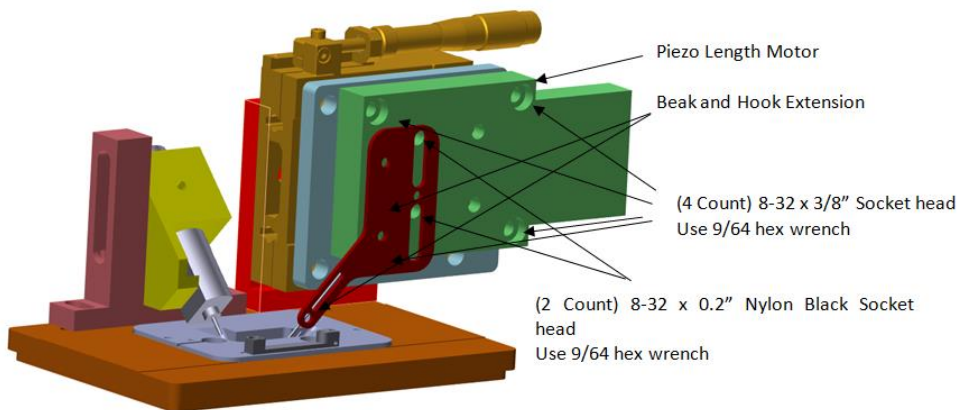
#### 3.3.1 Pieces

The manual micromanipulator is used for coarse movement of the length control with a range of 25 mm or ~1 inch. This coarse micromanipulator is attached to a pedestal anchored to the baseplate. Fine length control is achieved with a piezo motor with a range of 50 microns.

#### 3.3.2 Rationales and options

When it is necessary to change the muscle length with high precision, a piezo motor is needed. A standard beak holder can substitute the piezo motor if high precision length control is not necessary.

#### 3.3.3 Diagrams



*This is what the beak looks like with the tubing/hook attached. The red electrode from the MyoPacer should be attached to the button head screw, which is in electrical conductance with the platinum hook.*

## 3.4 Force Transducers

### 3.4.1 Pieces and Procedure for Assembly

The force transducers (TR6C – carbon rod lever and TR6S – stainless steel lever) are already assembled except for any extension of the lever and the platinum hooks. The force transducer slips into its holder shown in the diagram below.

**IMPORTANT:** Make sure the hook will be oriented such that a pull on the hook will be recorded as a positive deflection by the force transducer!

Note also that the sheath around the force transducer is electrically insulated! No metal portion of the force transducer should be in contact with the aluminum holder.

### 3.4.2 Rationales and options

The force transducers (TR6C – carbon rod lever and TR6S – stainless steel lever) we use for this unit are made by Dr. Konrad Güth at Myotronic, Heidelberg, Germany. Dr. Güth is a bit of legend in the muscle physiology world. He is a brilliant experimentalist and developed many of the techniques and instruments used in the field.

His force transducer is especially impressive for two reasons. First, it is based on detecting the deflection of a mechanical lever using an LED light source and light sensor. It is simple, and also is electrically isolated from the lever, which can now be used to pass current for stimulation of the muscle sample. Second, this design is robust, meaning the force transducer is difficult to physically break. That is an important feature after you consider that the experimentalist must load the muscle sample delicately.

### 3.4.3 Diagrams



## 3.5 Stimulation Wires

### 3.5.1 Pieces

The stimulation wires are simple wires that connect the MyoPacer to the platinum hooks. The connections at the hooks, however, are different on purpose. One is connected to the hook on the length motor by a simple Y-terminal. The other is connected to the force transducer by a modified alligator clip.

### 3.5.2 Rationales and options

It is important that the ground wire be connected to the force transducer to prevent ground loops. That is why the ground wire is terminated with an alligator clip that is shaped to fit the extension out the back of the Güth transducer. The alligator clip allows for fast disconnect. The Y-terminal is used on the other end also allows for relatively fast disconnect once the set screw has been loosened.

### 3.5.3 Diagrams



*Please note that the black wire should be connected to the ground electrode of the MyoPacer.*

## 3.6 Platinum Hooks

### 3.6.1 Pieces and Procedure for Assembly

Platinum rods can be easily cut and bent to shape. We use two different diameters of Pt rod: 0.020" (508 micron) and 0.023" (584 micron) depending on the receptacle. The rod is cut to ~15 mm long and bent at 135° angle so that a 2 mm portion of the rod will point straight up when placed in its receptacle. Looks like a shepherd's crook or a check mark (✓).

**IMPORTANT:** If preparing a force transducer, make sure the hook will be oriented such that a pull on the hook will be recorded as a positive deflection by the force transducer!

The heat shrink tubing covers the boundary between platinum rod and stainless steel tubing.

### 3.6.2 Rationales and options

Platinum is highly conductive, resistant to corrosion, resistant to electrolysis, and is biologically inert. Considering that Pt is still rather soft compared to other very hard metals, like Tungsten (W) or stainless steel, the Pt hook is relatively thick, usually 500-600 microns in diameter, to maintain a stiff connection.

### 3.6.3 Diagrams



This rod is used as the hook extension of the length motor and also used as the lever arm in the TR6 S.

## **3.7 Platinum Omega-clips**

### **3.7.1 Pieces and Procedure for Assembly**

Platinum foil can be easily cut and bent to shape. The Pt foil is only 0.001"-0.002" (25-50 micron) thick and soft enough that it can be cut with ordinary scissors or with a scalpel blade. The dimension of the rectangularly cut piece is approximately 0.5-1 mm wide and 5-8 mm long, depending on the muscle width expected (thicker muscle, wider cut). This foil is then bent in half and shaped to look something like the Greek letter omega,  $\Omega$ , including the feet at the bottom. These Pt clips are relatively expensive and are meant to be reused.

One end of the muscle sample will be placed between the feet of the omega, and ~7-O or similarly sized suture is then used to hold the muscle in place relative to the clip. The loop at the top of the omega clip will slip over the Pt hooks attached to the force transducer and length controller.

Customers should receive two pre-made clips and a strip of Pt foil 100 mm long. That should be enough for at least 20 additional omega clips. See Packaged section.

### **3.7.2 Rationales and options**

Platinum is highly conductive, resistant to corrosion, resistant to electrolysis, and biologically inert. Therefore, Pt will provide electrical contact while not interfering with the bathing solution or muscle preparation.

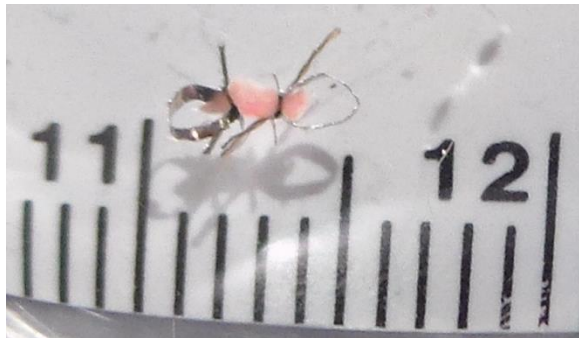
Copper (Cu), on the other hand, is highly toxic to muscle and cannot be used. Other metals, such as Al, Fe (or stainless steel), Zn, Ag, etc, do not possess all four necessary properties. Gold (Au) may be the only metal that is close to Pt in property performance. However, Pt is about 2x stiffer than Au, and therefore works better than Au to provide a stiff mechanical connection between muscle and instrument.



### 3.7.3 Diagrams

Prepared Omega Clips, 0.001" thick, ~0.7 mm wide, ~4 mm long.

Curved to form omega shape,  $\Omega$ , including feet. Now ~1.5 mm long, see photo below.



3.8



## 3.8 Chamber and Fluid flow

### 3.8.1 Pieces and Procedure for Assembly

Chamber insert is made of delrin and fits into the opening of the baseplate. The chamber features one main rectangular chamber, where the sample will be examined, and two circular side chambers, which enable smooth fluid flow.

A no. 1 thickness coverslip (43 mm x 50 mm) is glued (silicon glue) onto the bottom of the chamber as shown below. Aspirator height adjuster is screwed onto the ??

Hypodermic stainless steel tubing (20 G, 1 inch long) can be bent to dip into the side chambers and allow for fluid flow.

Tygon tubing, 1/32" ID, 3/32" OD, 1/32 wall is good for fitting the 18-20 G tubing into a fluid flow circuit.

Blunt hypodermic stainless steel needle (18-20 G, 1 inch long) can be used to allow fluid flow with lure fitting.

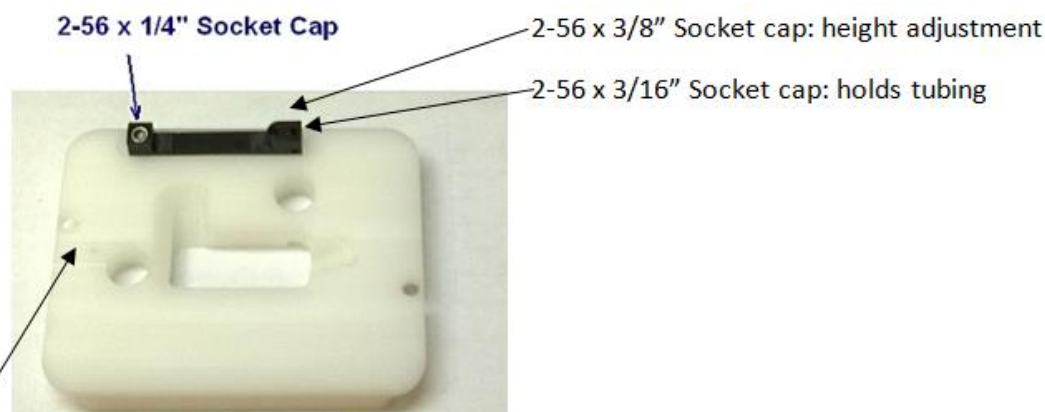
### 3.8.2 Rationales and options

The nice thing about having this plastic insert is this: it can be easily replaced. We don't expect it to break, but we do expect the coverslip to possibly break and/or there be a need for a different shape chamber. Therefore, having an insert makes for easy redesign and fast chamber replacement. Skinned muscle experiments, for example, do not require the side circular chamber for fluid flow. So, a version without the side chambers can fit into the same baseplate.

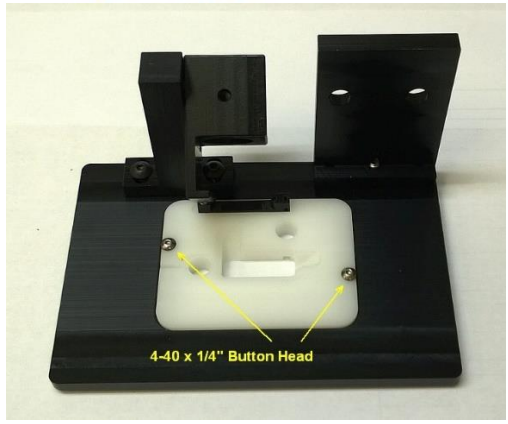
### 3.8.3 Diagrams



Caption?



Nylon 2-56 x 3/16"  
+ #4 plastic washer

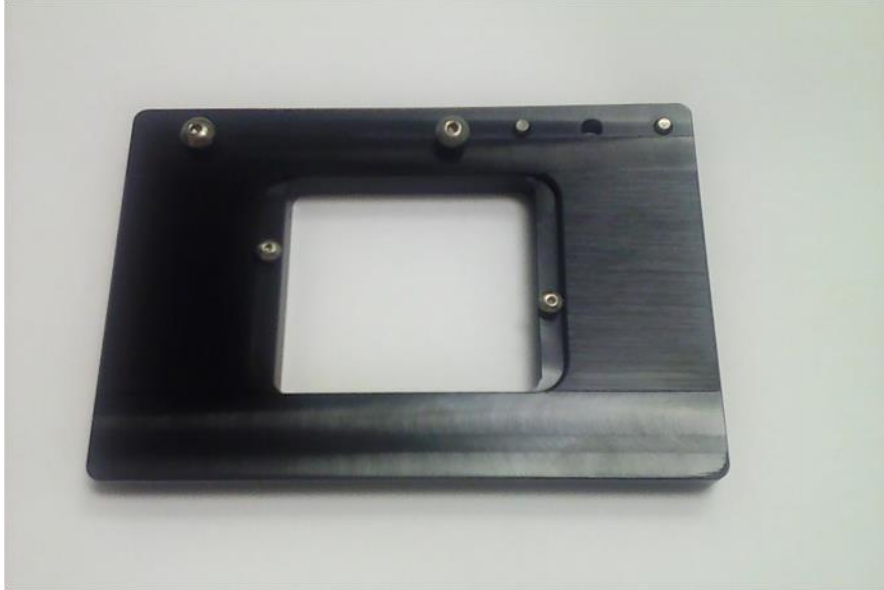


1/4" Button head  
Use 1/16 hex wrench

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## 4 Packaged and Shipped

### 4.1 Baseplate



*Pedestal and holder for Force Transducer*



*Pedestal, micromanipulator, and adapter plate for Length Motor*

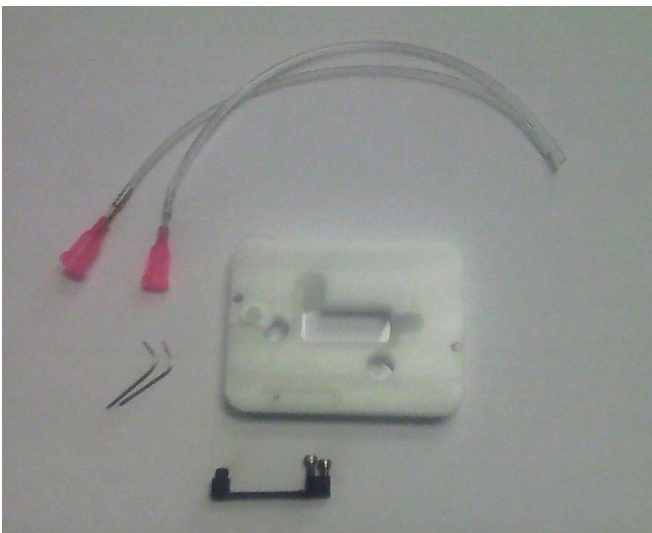
## 4.2 Beak and accessories.

Note that the platinum hook is shipped in the cardboard marked 'fragile'.



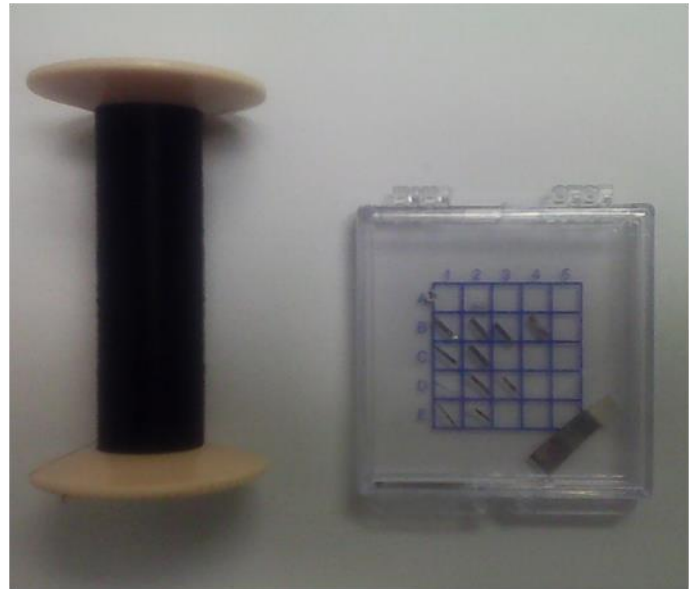
## 4.3 Chamber and fluid control.

Note, during assembly put the bent tubes in place before attaching to the tygon tubing.



#### 4.4 Cable for electrical stimulation.

Note that the thumb screw and cable tie allow for creating a cable stand off as part of the length motor assembly



## 5 Required Accessories

- Magnifying glasses for loading muscle (see below)
- Bathing solutions for intact muscle
- Inline heater
- Flexible polystyrene tubing for delivery of solutions and gases
- Bubbling stone, gases, and vacuum grease
- Peristaltic pump
- Small dishes for dissection and storage of tissue
- Cooled dissection plate
- Peltier circulator for cooled dissection plate
- Dissection tools
- Fluorescent dyes

### 5.1 Magnifying glasses for microdissection and loading muscle

Recommend Standard 1.75X magnification with 14" focal length.

Illumination on magnifier is not necessary if there is sufficient lighting in the working area.

**McMASTER-CARR.**


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**Narrow By** [Clear All](#)

**Style**  
Glasses

**Magnification**



1.25x to 2.25x



2.5x to 5x

**Illumination**  
With  
Without

35 Products How

#### Headband Flip-Up Magnifiers

Designed to fit over prescription and safety glasses, use these magnifiers to read fine print and inspect precision parts.

**Economy** magnifiers are lightweight and come with a 1 1/2" Lg. x 4" Wd. acrylic lens.

**Standard** magnifiers come with a 1 1/2" Lg. x 4" Wd. glass lens.

**Antistatic** magnifiers are the best choice when working with electronics and in clean-room environments. They come with a 1 3/16" Lg. x 3 5/16" Wd. acrylic lens.

**Illuminated** magnifiers have a xenon bulb to improve your view by shining a bright, white light on your work. They come with a 1 1/8" Lg. x 3 1/8" Wd. acrylic lens. Batteries are included.

**LED-illuminated** magnifier is preferred for its long life; it will shine for up to 50,000 hours. It comes with four 1 7/8" Lg. x 4" Wd. acrylic lenses. Requires three AAA batteries.

**Optional 2.5X fine-detail magnifier** serves as a bifocal. Also known as a loupe, it attaches to either side of the standard magnifiers and has a glass lens.

Magnification	Working Distance	Magnifiers		Replacement Lens	
		Each	Each	Each	Each
<b>Economy</b>					
1.75X	14"	11555T3	\$21.37	11555T31	\$10.67
2.25X	8"	11555T1	21.37	11555T11	10.67
2.75X	6"	11555T5	21.37	11555T51	10.67
<b>Standard</b>					
1.5X	20"	1490T1	56.42	1509T31	35.08
1.75X	14"	1490T2	56.42	1509T32	35.08
2X	10"	1490T6	56.42	1509T36	35.08
2.5X	8"	1490T3	56.42	1509T33	35.08
2.75X	6"	1490T4	61.12	1509T34	39.92
3.5X	4"	1490T5	61.98	1509T35	40.52
<b>Antistatic</b>					
1.6X	12"-14"	1485T11	29.81	1485T21	9.58
2.25X	7"-10"	1485T13	33.65	1485T23	13.42
2.75X	4"-7"	1485T14	33.65	1485T24	13.42
<b>Illuminated</b>					
1.75X	14"	1500T2	73.50	1500T12	11.00
2.25X	8"	1500T4	73.50	1500T14	11.00
2.75X	6"	1500T5	73.50	1500T15	11.00
<b>LED Illuminated</b>					
1.5X, 2X, 2.5X, 3X	6.5"-20"	1500T1	40.89	1500T11	20.00
Optional 2.5X Fine-Detail Magnifier for Standard Magnifier		1509T14	Each		\$10.70
Replacement Headband and Visor for Standard Magnifier		1490T8	Each		27.26
Replacement Xenon Bulb		65135T65	Each		6.24

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## **5.2 Accessories required prior to any training visit**

### **5.2.1 Three operational work stations**

#### **5.2.1.1 Macrodissection (excise heart, gross dissection, removal of muscle from animal)**

Depending on the institutions, this work station is often in or close to an animal facility.

This work station also contains all required equipment for euthanasia, such as isoflurane or injectable analgesics and/or anesthetics, and possibly also heparin.

#### **5.2.1.2 Microdissection (trimming muscle to small dimensions, attaching $\Omega$ -clips).**

This is usually a well-lit area with a dissection microscope (e.g., ~2-20 X magnification)

#### **5.2.1.3 Data acquisition area holding intact muscle rig and data acquisition hardware/software.**

### **5.2.2 Other necessities**

- Distilled deionized water
- Bathing solutions for intact muscle (i.e., Krebs-Ringer with bicarbonate buffering system)
- Bubbling stone and tubing for delivering gas to the stone
- Gases and regulator: 95% O<sub>2</sub> – 5% CO<sub>2</sub> gas is common. Have your institution get this for you. Individual institutions often have preferred vendors and contracts. Regulator is for low pressure, low flow.
- Specialized dishes for dissection and storage of tissue
- Transfer pipettes
- Cooled dissection plate (optional). Station with ice will work too.
- Dissection tools including fine forceps and fine scissors
- Disposable gloves
- Fluorescent dyes, e.g. Fluo-4 AM, Fura-2 AM
- DMSO, at least 1 mL volume

---

## 6 Appendix: Helpful Experimental Protocol

### 6.1 Loading a muscle sample (size ~mouse papillary muscle) with AM-form of fluorescent dye

#### 6.1.1 Items:

- 50µg of the AM-form of fluorescent dye such as Fluo-4AM or Fura-2AM.
- DMSO
- Pluronic Acid 20% solution (e.g., TEFLabs #2510 Pluronic F127 20% wt.v in DMSO)
- Krebs-Ringer + (10 - 50 mM BDM) solution bubbled with 95%O<sub>2</sub>-5% CO<sub>2</sub> to maintain pH 7.40 (±0.05)

#### 6.1.2 Method:

1. Dissolve 50µg of the dye into 50µl of DMSO, wait at least 20 min. This is usually done by adding DMSO to a tube.
2. Add 2.5µl of Pluronic Acid 20% and vortex.
3. In a third light-proof tube with cap, prepare 948 µl of bubbled Krebs-Ringer-BDM solution.
4. Add dye and pluronic dissolved in DMSO into that veil, vortex again; final volume is ~1ml.
5. Gently place the muscle (usually clipped is better) into the vial and cap it.
6. Loading time varied between 1 and 2 hours at room temperature.
  - a. Shorter times are better for muscle viability, because the solution cannot be bubbled during this time.
  - b. Longer times are better for dye uptake and fluorescence signal.