INSTRUCTION MANUAL

Models 801C-400, 801C-1900, 801C-300

Small Intact Fiber Test Apparatus

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1.0 Introduction

The 801C small intact fiber test apparatus was designed to enable physiology researchers to easily test small intact muscle tissue with an ASI model 322C high-speed length controller and an ASI series 400A force transducer. The 801C can also be used with an ASI model 300C or 300C-LR dual-mode lever system.

The 801C consists of the main apparatus, a model 825A Thermocouple Thermometer/TEC Controller, a model 826A Water Cooler, a 1.0 liter Radnoti temperature jacketed reservoir for the perfusate and all fittings, tubes, cables, screws, tools, etc. required to connect the various parts together (see Figure 1). The main 801C apparatus consists of an aluminum bath plate with a glass bottom, perfusion inlet and outlet, 2 Peltier (TEC) heater/coolers, water-cooled heat sink plates, a T-type thermocouple, micrometer drive XYZ translation stages for motor and force transducer positioning, and mounts for the 322C motor and the 400A series force transducer. As an option an XYZ stage and motor mount for a 300C or 300C-LR lever system can be substituted for the 322C motor mount and translation stage. All parts are manufactured from corrosion resistant materials (stainless steel, anodized aluminum and Delrin). Note: the 322C, 400A and 300C instruments must be purchased separately; they are not included with the 801C apparatus.

The 801C apparatus was designed to be used either on the bench top or on an inverted microscope. Please note that the force transducer protrudes below the lower surface of the main plate so if you are using it on a bench top then ensure you leave the main plate on the included stand. If the apparatus is being used on an inverted microscope then ensure that all of the stage inserts are removed prior to placing the apparatus on the microscope stage. When not on the microscope the apparatus should be kept on the stand to ensure that the apparatus and particularly the force transducer are not damaged.

Figure 1 801C Apparatus complete with 825A, 826A and Radnoti Reservoir

The bath plate is manufactured from aluminum that is coated with a hard anodized coating followed by polymer impregnation and polymer coating. The resultant surface has a Rockwell hardness of about 50 with excellent dielectric properties while maintaining high thermal conductivity. The aluminum is completely sealed from the tissue and the perfusate. Field stimulation can be performed within the bath with little or no current flowing to the chamber walls because the surface of the bath plate is insulated.

The bath plate is temperature controlled using Peltier (TEC) modules mounted on either end of the plate. The Peltier modules are controlled by an ASI 825A Thermometer/TEC Controller (see Figure 3). The 825A is capable of controlling the temperature of the bath plate from 1 to 40°C with 0.1°C accuracy. A T-type thermocouple is provided with the 801C apparatus for measuring the liquid temperature in the bath. The thermocouple is plugged into the 825A which provides a digital readout of the bath temperature and also an output voltage proportional to the bath temperature.

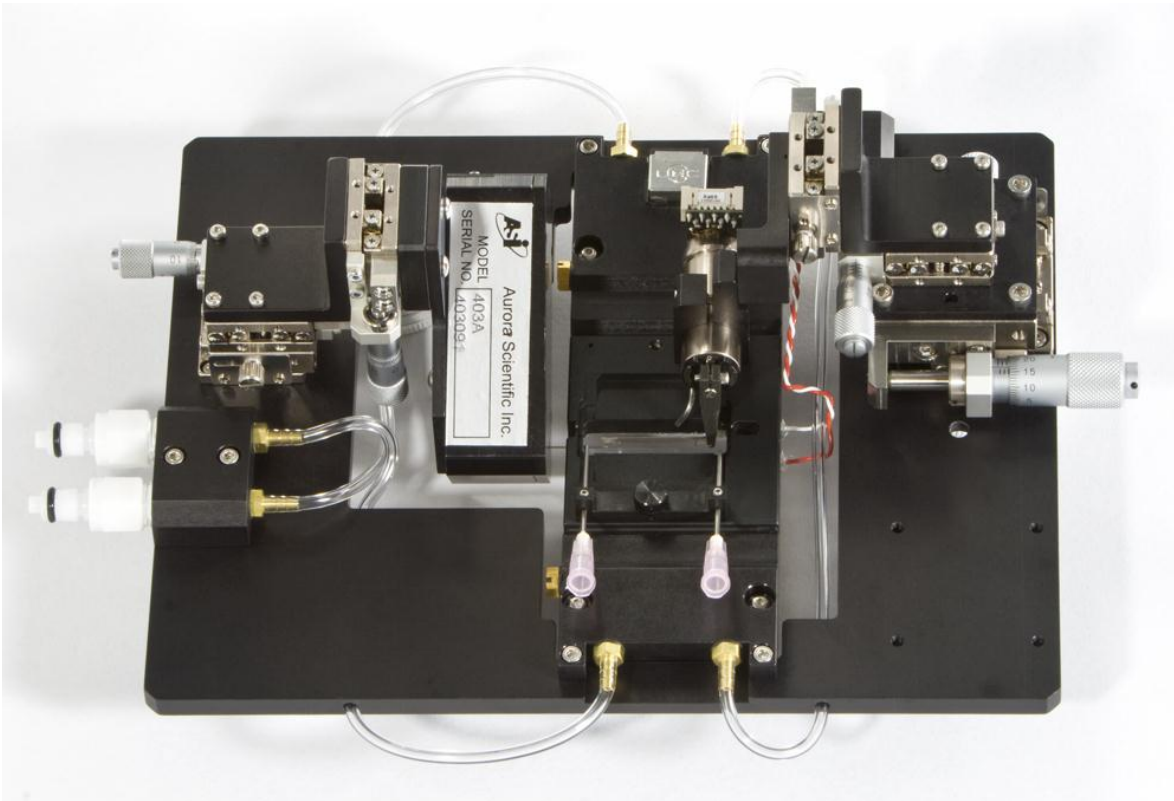


Figure 2 801C-1900 Apparatus with 322C motor and 403A force transducer

The perfusion liquid enters the 801C chamber through an 18AWG hypodermic needle. It is important that the liquid entering the bath is close to the operating temperature of the plate to ensure the most effective temperature control of the liquid (see next paragraph for a discussion of temperature control of the liquid reservoir). The liquid is removed through an 18AWG hypodermic needle located at the force transducer end of the bath. The researcher needs to provide suction to remove the liquid. Please see chapter 3.0 for more information about temperature control and liquid flow through the bath.

The perfusion liquid stored in the Radnoti reservoir is maintained at the appropriate temperature by use of a laboratory water circulator that is supplied by the researcher. The water jacketed glass reservoir provides for liquid storage, oxygenation, and temperature control. The researcher will also need to provide a source of oxygen, usually in a high pressure gas cylinder, and the appropriate regulator, flow controls and tubing to attach the oxygen source to the bubbler apparatus in the reservoir.

It is recommended that the apparatus be used on a vibration isolation table. A vibration isolation table is not included with the apparatus. Please contact Aurora Scientific Inc. for suggested vibration isolation tables and ancillary equipment.

Both the high-speed length controller and the force transducer are mounted on XYZ micrometer translation stages to allow them to be positioned relative to the bath plate.

An AD590 temperature sensor is attached to the bath plate and provides temperature feedback to the model 825A TEC controller. Further details of the controller can be found in the 825A Instruction Manual.

Figure 3 825A Thermometer/TEC Controller

Peltier modules use electric current to pump heat from one side of the module to the other side. By reversing the current flow the module can be used to either heat or cool an object. A consequence of this heating/cooling is that the opposite side of the module will get hot if the object is being cooled or it will get cold if the object is being heated. For proper operation, heat must be supplied or removed from the opposite side of the Peltier module. This is the purpose of the heat sink plates and the 826A Water Cooler. The heat sink plates are drilled to allow water to circulate through the plate to either supply or remove heat. The water is supplied by the 826A Water Cooler (see Figure 4).

Figure 4 826A Water Circulator

The 826A consists of a liquid reservoir, pump, fan and a radiator. Its purpose is to supply room temperature water to the heat sink plates. Running the 825A without the 826A attached to the apparatus will result in permanent damage to the Peltier modules. For this reason the power for the 826A comes from the back of the 825A and turns on when the 825A is powered. Further information about the 826A Water Cooler and instructions for its setup and use can be found in the 826A Instruction Manual.

1.1 Specifications

Bath Plate	
No. of Baths:	1
Bath Plate Material:	6061-T6 aluminum with Magnaplate Tufram R66 coating
Bath Dimensions:	
Model 801C-400	24mm (0.945") L x 6mm (0.236") W x 3mm (0.118") D
Model 801C-1900	34mm (1.339") L x 10mm (0.394") W x 6mm (0.236") D
Bath Volume:	
Model 801C-400	400 µL
Model 801C-1900	1900 µL
Inlet	
Vinyl Tube Size:	1/16" ID x 1/8" OD
Stainless Tube Size:	18AWG
Outlet	
Vinyl Tube Size:	1/16" ID x 1/8" OD
Stainless Tube Size:	18AWG
Suction Height Range:	
Model 801C-400	3.0-6.0mm (measured from bottom of bath)
Model 801C-1900	6.0-9.0mm (measured from bottom of bath)
Thermoelectric Coolers	
No. of TECs:	2
Power:	33 W each
Voltage:	15 V
Temperature Sensor:	AD590
Thermocouple	
Type:	T
Model:	Physitemp model IPT-18
Stimulation Electrodes	
Number:	2 mounted through end wall of bath plate
Material:	99.9% pure Platinum
Dimensions:	47mm (1.850") L x 2mm (0.018") Wide x 0.25mm (0.010") Thick
Equipment Included	
Reservoir:	Radnoti model: 120142-1, 1 liter volume
TEC Controller:	ASI model 825A Thermometer/TEC Controller
Water Cooler:	ASI model 826A Water Circulator

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the three shipping boxes. The first box contains the 801C apparatus with the XYZ translation stages attached. The second contains the 825A Thermometer/TEC Controller and an accessory kit. The third box contains the 826A Water Cooler and the Radnoti Reservoir with bubbler and mounting clamp. Also included in the third box are 2 - 1/4" ID x 6' Tygon Tubing for cooling water and 2 – 3/16" ID x 6' Tygon tubing for temperature control of Radnoti reservoir.

The accessory kit includes:

- 4 – Nylon Tie Straps
- 2 – Adhesive Tie Downs
- 1 – Male Luer Fitting x 1/16" Hose Barb
- 1 – Male Luer Fitting x 1/8" Hose Barb
- 1 – Male Luer Fitting x Male Luer Adapter
- 1 – Syringe Vacuum Grease
- 1 – Imperial Allen Key Set
- 1 – 1.5mm Allen Key
- 1 – 2mm Allen Key
- 1 – 2.5mm Allen Key
- 1 – 0.89mm Allen Key for the Thermocouple Holder
- 4 – 4-40x3/8" Button Head Screws for the Force Transducer
- 1 – IT-18 Thermocouple Packaging
- 1 – Thermocouple Extension Cable
- 1 – Package 22x50x1 ½ Microscope Cover Glass
- 2 – 1/16" ID x 6' perfusate hoses with Luer fittings attached
- 1- BNC cable
- 1 - BNC to plug adapter for the stimulation electrodes.

The 801C apparatus is shipped assembled however the user must attach the motor, force transducer, water lines, perfusate lines, TEC control cable and the stimulation BNC cable.

2.2 Equipment Location

The layout of the lab space and the positioning of the various components of the 801C system should be considered prior to setup of the equipment. Some considerations to take into account are:

1) Selection of, and location for, the dissection microscope required for observation and attachment of the tissue to the motor and force transducer.
2) Location of an inverted microscope that may be required during testing.
3) Cable lengths and interconnections between the various components to allow ease of apparatus movement and to provide the safest workspace.

4) Location of equipment to minimize electrical noise, vibration and other environmental factors.
5) Location of ancillary equipment such as a laboratory circulator, oxygen supply, and suction.

When selecting a dissection microscope the main feature to check is that the throat distance (distance between the object under observation and the mounting post of the microscope) is at least 110mm (4.375in). A better option is to purchase a boom arm for the dissection microscope that allows the microscope to be positioned in a variety of locations by simply sliding the mount on the boom arm.

Layout is most affected by whether the researcher will require an inverted microscope for tissue observation during testing. If an inverted microscope is to be used then the workspace should be arranged such that the inverted microscope is in the centre of the work area with the dissection microscope located either immediately adjacent to the inverted microscope or mounted above the inverted microscope. Mounting the dissection microscope above the inverted microscope allows the tissue to be attached while the 801C apparatus is mounted on the inverted microscope's XY stage.

If the two microscopes are placed beside each other then the apparatus will need to be moved from under the dissection microscope to the XY stage of the inverted microscope before running a test. Since it is best to have the force transducer and motor turned on during tissue mounting the various electronic control units for the instruments must be located such that there is sufficient cable length to facilitate moving the 801C apparatus between the microscopes. We suggest placing the electronic controllers on a shelf above the inverted microscope and on the side of the inverted microscope closest to the dissection microscope's location. This allows the various cords and lines to be moved between the two locations.

The TEC control cable and the force transducer cable are each 2m (6 ft) long and are thus the shortest cables interconnecting the apparatus and the control electronics. Therefore the layout should be designed to place the electronic controllers for these two instruments within 2m of both the dissection and the inverted microscopes.

In the case where an inverted microscope is not used then the layout is a little simpler in that the apparatus will remain in a fixed location under the dissection microscope. In this case it is still wise to mount the electronic controllers on a shelf above and a little to the side of the microscope. This allows easy reach of the controls while keeping the electronics off the bench and away from the risk of liquid spillage onto the electronics.

Figure 5 Equipment layout at Harris Lab, UC Davis

Figure 5 shows a good equipment layout with the inverted and dissection microscopes located beside each other and the electronic controllers on the shelf above the vibration isolation table. The dissection microscope is mounted on a boom arm for ease of positioning the microscope. Note the lab circulator on the floor beneath the table where vibration from the pump will have the least effect on the experiment.

When positioning equipment and running wires between the various components of a system take care to route signal wires away from power wires. It is also good practice to coil excess signal wires and to attach them to a surface to reduce noise due to cable movement. Plug the power cables from all of the measurement instruments (length controller, force transducer, PC) into a single power bar to prevent ground loops. Plug power cables from lab circulators, heaters or other high current devices into a separate outlet so that the power draw by these components doesn't create unwanted noise on the force transducer, motor or PC data acquisition systems.

2.3 Attaching the Motor

Before attaching the motor it is recommended that the X and Y motor mount translation stages be positioned at about their mid travel location (3 on the micrometer). The Z stage should be raised as high as it will go.

To mount a model 322C motor simply insert the motor into the clamp without the lever arm attached. Rotate the 322C motor so that the connector is at the top and then tighten the ring clamp. Once the motor is clamped in position you can then attach the lever arm.

After attaching the arm you may need to loosen the clamp screw and slide the motor in the clamp to position the tip of the lever arm near the center of the bath. Tighten the clamp ring screw. Once the motor is locked in place the XYZ translation stages can be used to fine-tune the position of the lever arm in the bath. Ensure that when you lower the motor using the Z axis stage that the lever arm doesn't hit the glass cover slip at the bottom of the bath.

The X axis micrometer stage for the motor has a range of motion of 13mm (the X axis being the direction along the length of the tissue) which is not sufficient to cover the entire length of the 801C-1900 bath plate (30mm). In addition since the 801C-400 bath is located at the force transducer end of the bath plate the X axis stage motion is also not sufficient to position the lever arm near the force transducer end of the 801C-400 bath. To allow the motor to be positioned closer to the force transducer a motor spacer is installed between the motor mount and the Z axis stage. Figures 6 and 7 show a model 801C-1900 with the motor mount spacer installed. Four M2x18mm long screws are included with the spacer.

The model 801C-400 is shipped with the motor mount spacer installed. The model 801C-1900 is shipped without the motor spacer installed which allows for the maximum length of tissue to be mounted. If you intend to use short tissue preparations with the 801C-1900 bath then you will need to install the motor spacer.

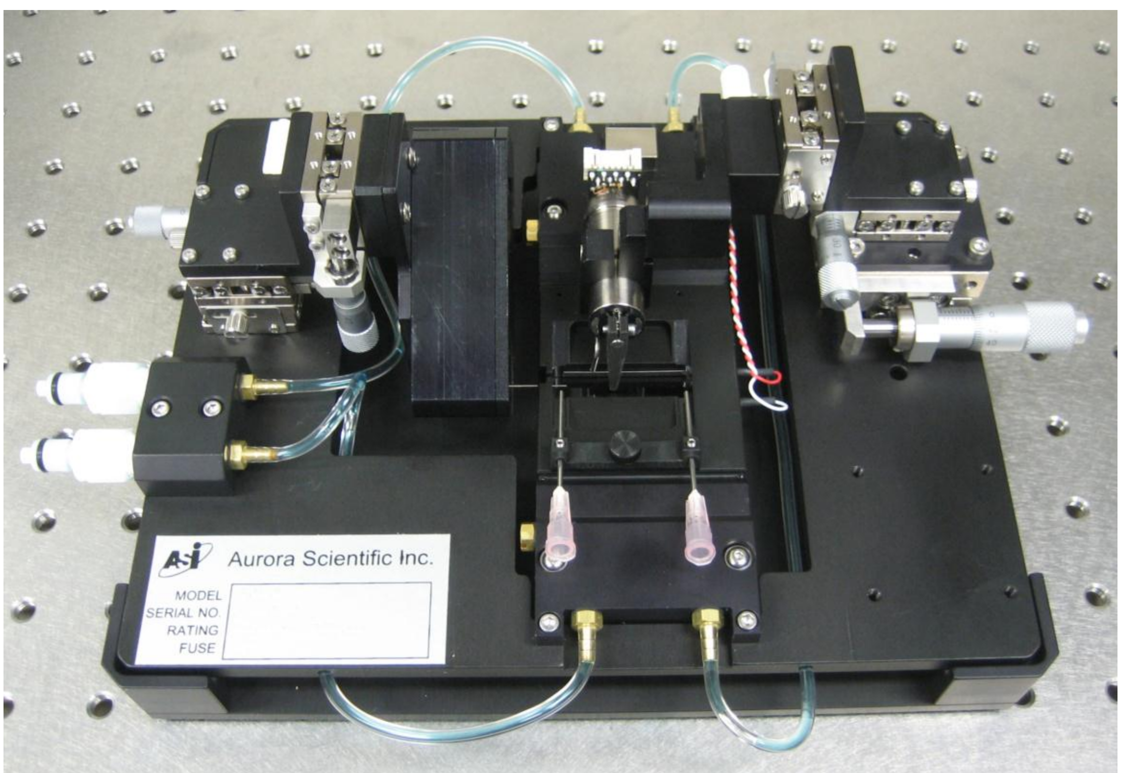


Figure 6 801C-1900 on stand with motor spacer installed

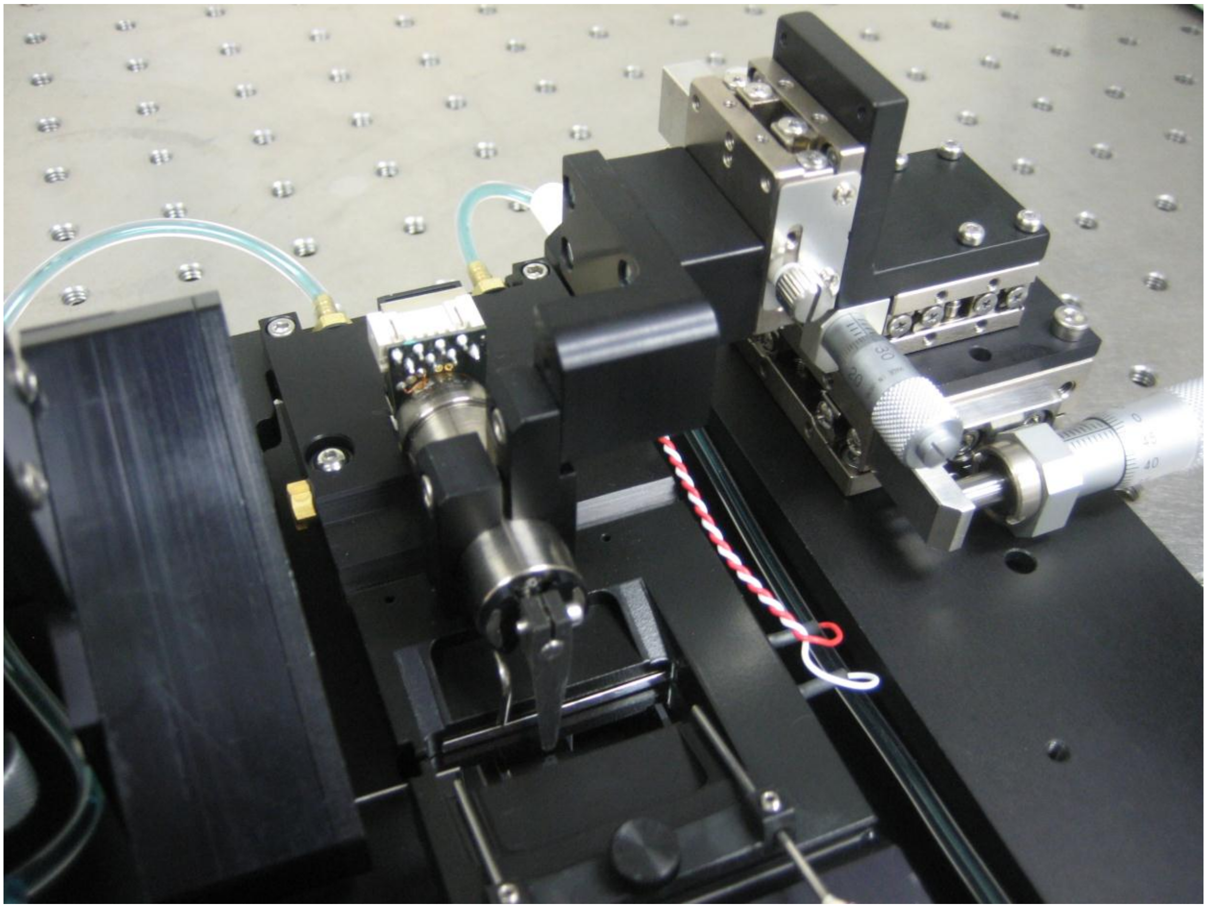


Figure 7 Close-up of 801C-1900 showing 322C motor mount with motor spacer installed

2.3.1 Alternate Mounting Location for the 322C Motor

The 801C apparatus is designed to allow the motor to be mounted in two different locations. The default location positions the motor as seen in Figures 6 and 7 with the motor on the same side of the bath as the force transducer. This default orientation is designated as the normal location.

The other possible mounting location places the motor on the opposite side of the bath from the force transducer and we designate this as the alternate motor location. See Figure 8 for a photo showing the alternate motor location.

The choice of normal or alternate location for the motor should be based on the researcher's preference when attaching the tissue to the force transducer and motor. The normal location is designed for both hands to be on the front of the apparatus (both hands on the same side of the bath). The alternate location is designed for one hand to be on one side of the bath and the other hand to be on the opposite side of the bath. An advantage of the normal motor location is that the cables from the force transducer, the motor and the 801C TEC plate all exit on the same side of the apparatus. This can make it easier when moving the apparatus from the dissection microscope to the inverted microscope.

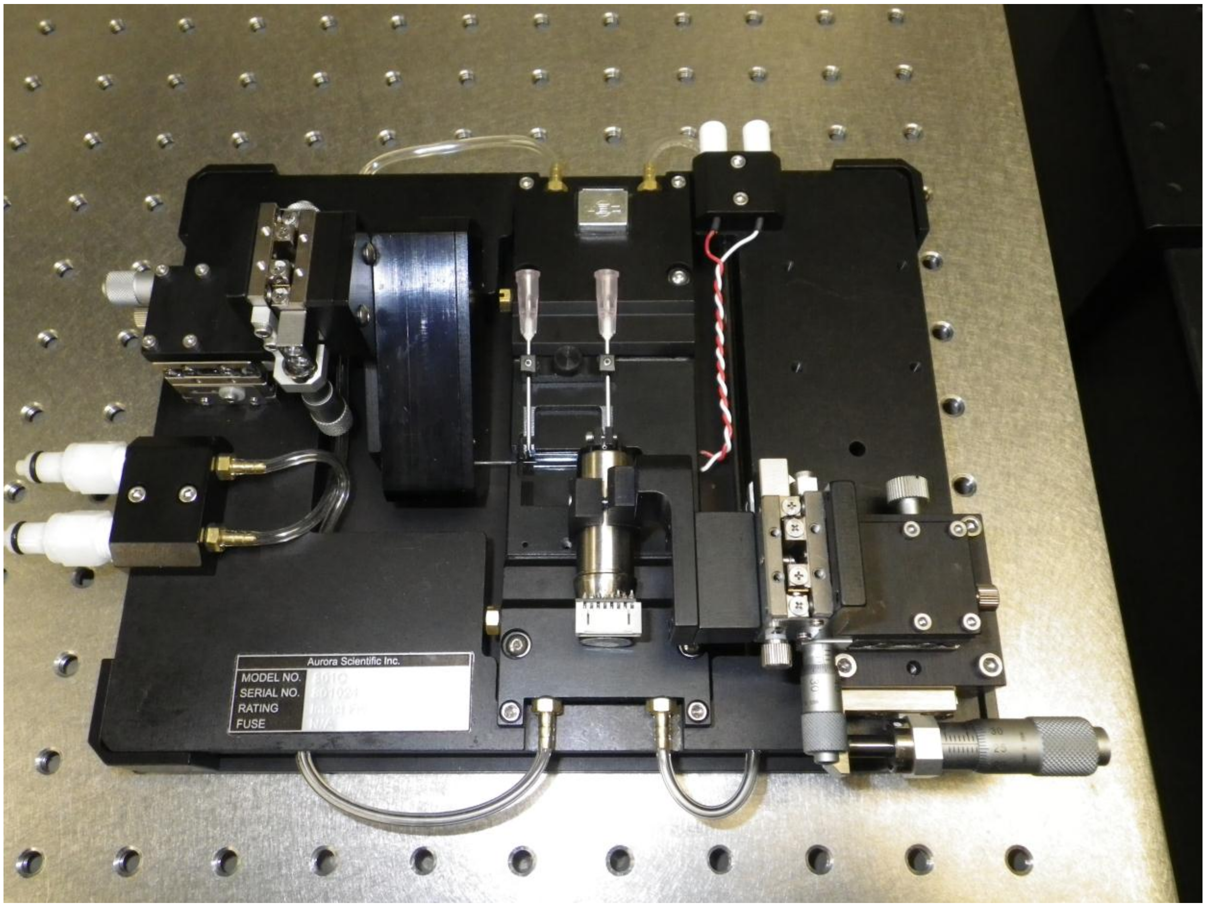


Figure 8 801C-400 with 322C motor mounted in alternate location

Follow the procedure outlined below to switch the motor mounting location from the normal location to the alternate location.

1. Remove the inlet/outlet tube holder by unscrewing the knob located between the inlet and outlet and pulling the holder up and off of the bath plate. The holder will be reattached after the motor has been moved.
2. The Y and Z stages along with a right angle bracket and the motor and its mount are all attached to a black plate that is attached to the top of the X-axis stage. Remove the four M3 socket head screws holding the plate to the X stage. Use a 2.5mm Allen key. Set this entire assembly aside for now.
3. Position the X-axis stage so the dial reads about 12. This should expose two of the four mounting screws that fasten the X stage to the base plate. Remove the screws using the 2.5mm Allen key.
4. Position the X-axis stage so the dial reads about 0. This should expose the other two screws holding the X stage to the base plate. Remove these screws.
5. Re-locate the X stage to the other set of tapped holes which can be seen in the base plate. Re-fasten the X stage using the screws removed in steps 3 and 4.
6. Now return to the Y-Z/motor subassembly and remove the four screws that hold the motor L bracket to the Z stage.
7. Remove the motor clamp ring from the L bracket.

8. Flip the motor L bracket 180 degrees and refasten it to the Z stage using the set of mounting holes at the bottom of the Z stage (in other words mount the L bracket as low as possible on the Z stage).
9. Remove the arm from the motor shaft, loosen the motor clamp screw and remove the motor from the clamp ring.
10. Insert the motor in the other side of the clamp ring and retighten the clamp ring. Re-attached the lever arm to the motor.
11. Loosen the 2-56 set screw that holds the thermocouple tube into the clamp ring.
12. Remove the thermocouple from the stainless tube and then pull the tube from the clamp ring. Reinsert the tube in the opposite face of the clamp ring and then reinsert the thermocouple in the tube.
13. Refasten the motor ring clamp complete with the motor to the motor L bracket. Use the lower tapped hole in the motor L bracket.
14. Now reattach the entire Y-Z/motor assembly to the top of the X stage using the screws you removed in step 2.
15. Loosen the clamp ring and slide the motor to line up the lever arm with the bath. Tighten the screw once the motor is positioned correctly. The apparatus should now appear as is seen in Figure 8.
16. Use the XYZ stages to set the final position of the lever arm in the bath.
17. Attach the inlet/outlet tube holder on the side opposite the motor and refasten it with the screw provided.

2.3.2 Mounting a 300C Motor

The 801C-300 allows a model 300C motor to be mounted on the apparatus, see Figure 9. The 300C motor can only be mounted in the alternate location due to the fact that the 300C motor will not clear the TEC connector if it is placed on the normal side. A larger Y and Z stage is used to hold the 300C motor due to the added size and weight of the motor. The 300C option is only available with the 1900uL bath size. When using the 300C there is an option to mount a 400A series force transducer as seen in Figure 9 or a fixed post as can be seen in Figure 10.

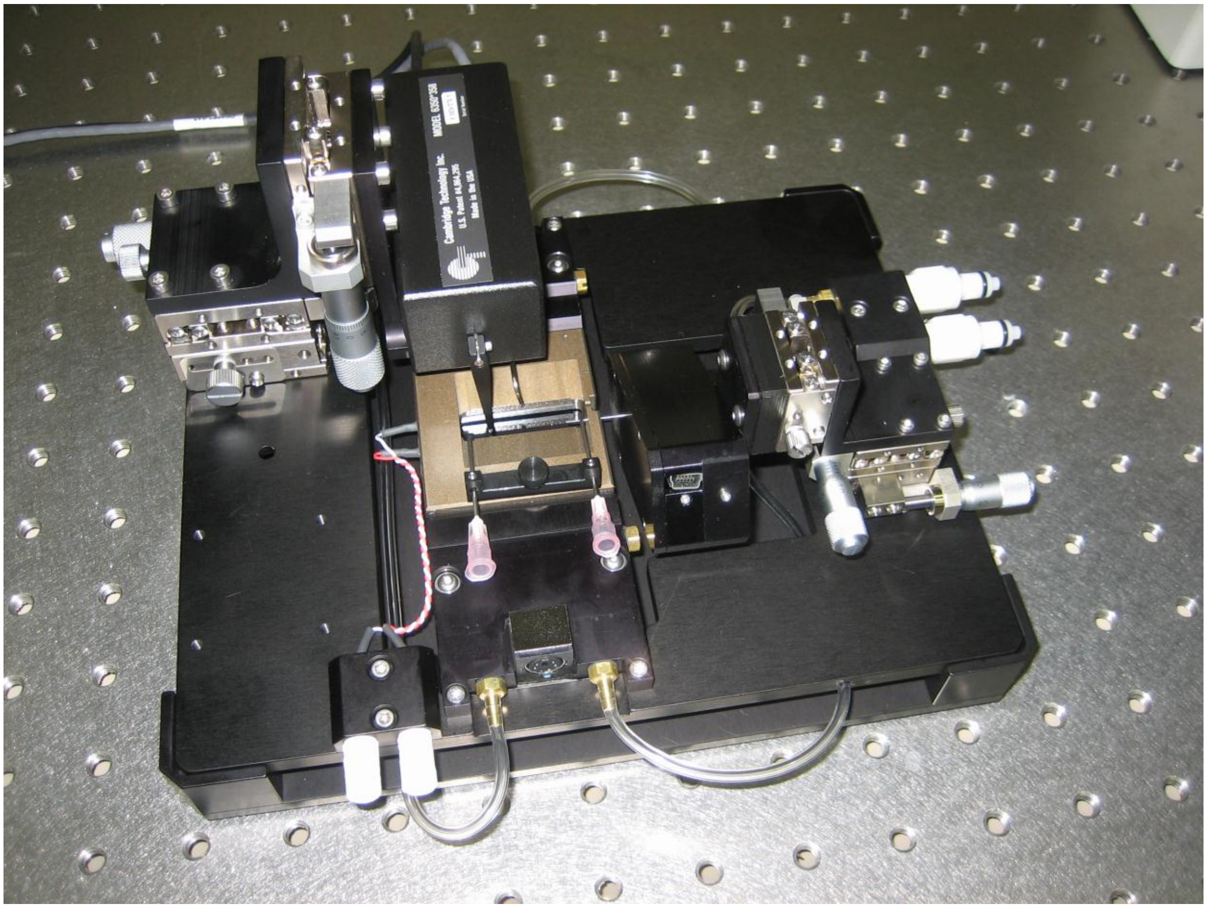


Figure 9 801C-300 with 300C motor and 400A force transducer

Adapter plates are available that allow a 322C motor to be mounted on the larger Y and Z stages that are supplied with the model 801C-300. Figure 11 shows the 801C-300 with a 322C motor mounted. This allows a researcher to tailor the apparatus to higher or lower forces as required by the type of tissue being tested.

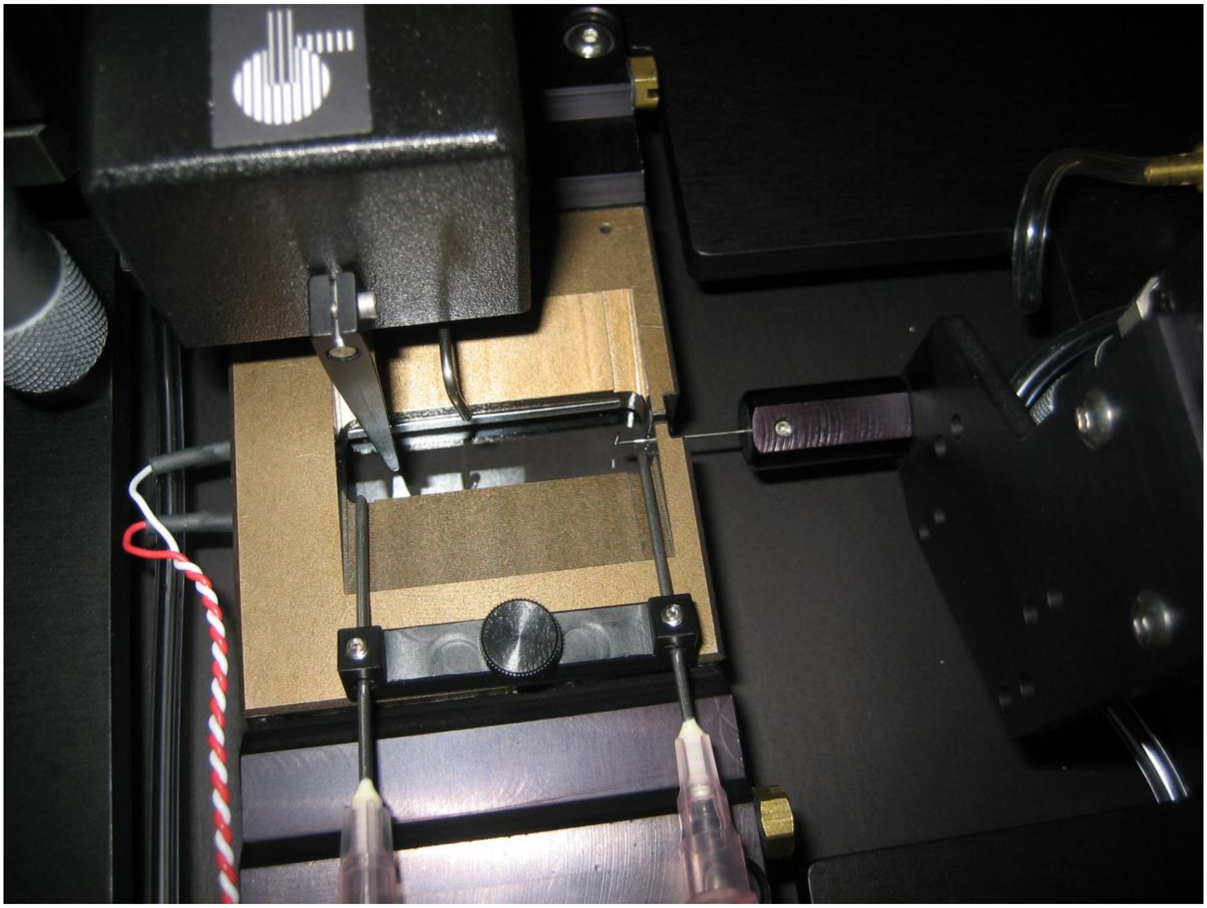


Figure 10 801C-300 with 300C motor and fixed post

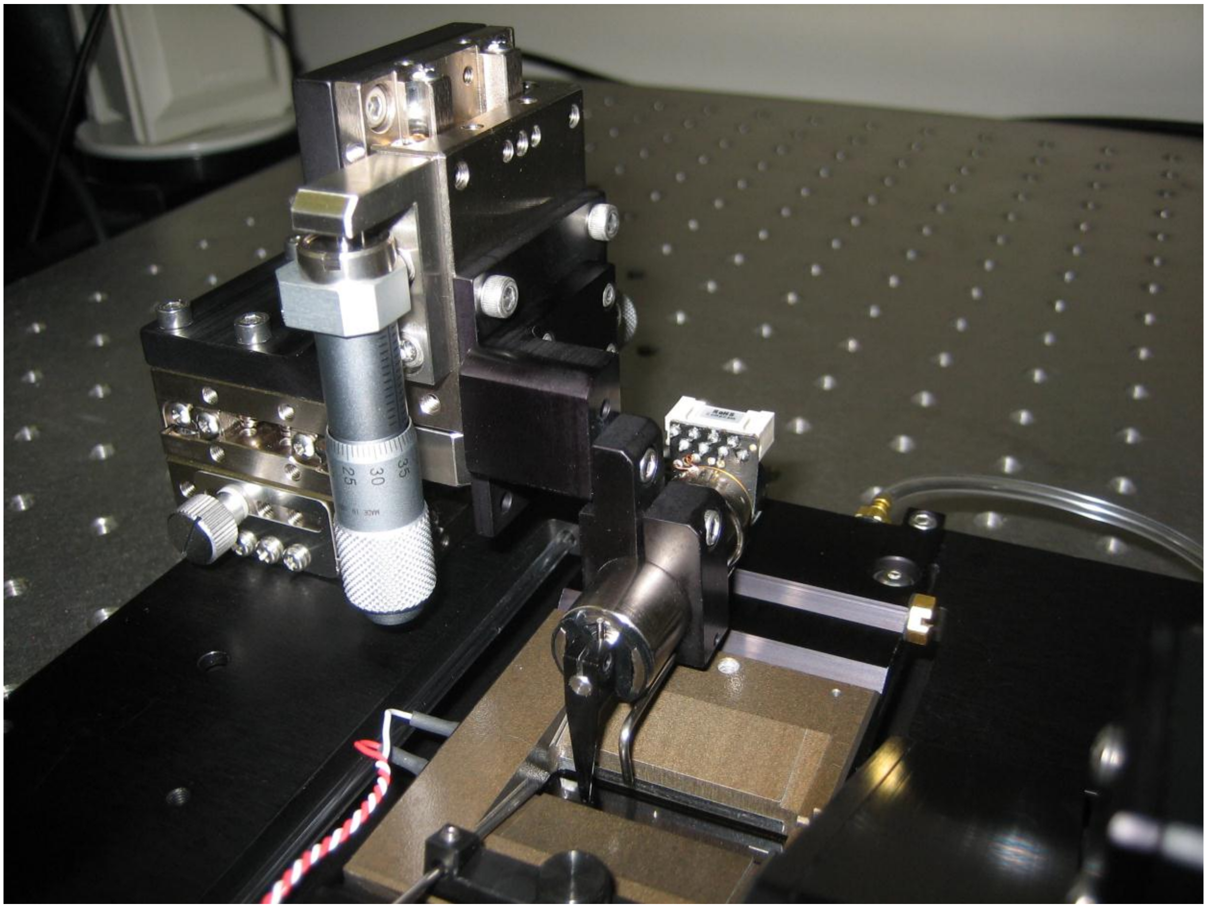


Figure 11 801C-300 with 322C mounted on large XYZ stages

2.4 Attaching the Force Transducer

Refer to Figures 6 through 9 for correct orientation of the force transducer in the 801C apparatus. The transducer is tipped back at a 30-degree angle to the horizontal. This angle provides better access to the bath while allowing the apparatus to be mounted on an inverted microscope.

Before mounting the transducer, position the X-axis translation stage as far from the bath plate as possible. Position the Y-axis stage at the midpoint on the micrometer dial. Raise the Z-axis stage as high as it will go. Remove the transducer mounting plate from the Z stage and then use the four 4-40x1/4" button head screws supplied to attach the force transducer to the mounting plate. Now reattach the transducer mounting plate, along with the transducer, to the Delrin mount plate attached to the Z stage using the two 4-40x3/8" button head screws provided. Take care when attaching the transducer that the output tube doesn’t strike the bath plate as this could break the transducer.

An alternate method of attaching the transducer is to first remove the Z subassembly (Z-axis stage and right-angle bracket) from the Y-axis stage. Attach the transducer to the mount plate and then re-attach the entire Z subassembly.

Normally a length of fine gauge stainless steel tubing is fastened into the end of the force transducer output tube; see Appendix B for details of various attachment methods. This tube then passes through the slot in the end of the bath plate and the muscle tissue is attached to it. Take note that the glass output tube on the force transducer is too large to fit into the slot so if the transducer is located too close to the plate the output tube could be broken when the transducer is moved.

Once the transducer is attached use the X, Y and Z stages to locate the needle that is attached to the transducer output tube in the center of the slot at the end of the bath. Use a microscope to observe the needle and the slot before attempting to position the needle in the slot. Use the Z-axis stage to set the depth of the output tube, and thus the muscle tissue, in the bath. If at any time you observe interference between the bath plate and the transducer simply use the XYZ stages to reposition the transducer to the middle of the slot. We recommend that you connect the force transducer head to the control electronics and turn on the electronics before handling the force transducer. The force transducer electronics will beep if the force transducer goes off scale. This beep can be used to monitor the load on the transducer. If the transducer or the needle comes in contact with the slot the force transducer will beep and warn you that you are about to break the transducer.

Ensure that the transducer cable is routed clear of the bath plate. It is best to strain relieve the transducer and motor cables by attaching them to the 801C base plate. Cable ties and cable mounts are provided for this purpose. If you don't strain relieve the cables then movement can lead to increased noise on the length controller and force transducer signals.

2.5 Attaching the Cooling Water Lines

Cooling water lines are attached to the 801C when it is shipped. These lines are terminated with dry-break quick-connect fittings. The shipping box that contains the 826A Water Cooler has two 6 foot long 1/4"x3/8" clear hoses with mating quick-connectors that plug onto the connectors attached to the 801C. Before using the 826A ensure that you attach the hoses to the outlets on back of the 826A. See the 826A manual for details of these connections. The quick-connect fittings include a valve inside them so they can be disconnected with water in the lines without any spillage. The connectors are disconnected by pressing the metal tab on the side of the connector and then pulling the connection apart.

Connect the hoses to the mating fittings on the back of the 826A Water Cooler and the other end to the mating fitting on the 801C. Please refer to the 826A operating manual for instructions on filling and using the water cooler.

Since the 826A Water Cooler is powered by the 825A TEC Controller you will need to turn on the 825A in order to fill the water lines. The TEC modules can be damaged if they are heated with no cooling water flowing. To prevent TEC damage you should disconnect the TEC cable at the 801C apparatus while setting up the 826A. Operating the thermoelectric coolers (TEC) without water flowing can permanently damage the TECs.

2.6 Thermoelectric Controller

Two thermoelectric coolers (TECs) are built into the 801C. These devices are rated at 15 volts and 33 watts. A model 825A Thermometer/TEC Controller is also included with the 801C. A separate manual for the controller has been provided and therefore only a minimum of information about its operation will be provided here.

The system has been designed to control the temperature between 1 and 40ºC with an accuracy of ±0.1ºC. The 825A measures the bath plate temperature using an AD590 temperature sensor embedded into the bath plate. The sensor temperature can be viewed on the 825A by setting the display switch to Process. When the switch is in the Setpoint location the LED display shows the Setpoint. A Setpoint adjustment knob allows the Setpoint to be set between 0 and 40ºC with an accuracy of 0.1ºC. The Process temperature should match the Setpoint temperature within about 3 minutes. However the temperature of the liquid in the bath will take up to 7 minutes to stabilize after a large Setpoint change. Also there is normally a temperature offset between the AD590 temperature and the liquid temperature. This offset is greater the farther the Setpoint is from room temperature. The 825A includes a thermocouple meter and the apparatus includes a T-type Teflon thermocouple probe that can be used to monitor the temperature of the liquid in the bath.

Ensure that the 826A Water Cooler is operating and that water is flowing through the cooling plates before changing the set point to a temperature other than room temperature (Setpoint dial set to 5). Set the desired temperature on the controller and then monitor the actual temperature to ensure that the temperature is approaching the set point. Adjust the set point as required to achieve the desired bath temperature based on the temperature reported by the thermocouple probe.

2.7 Attaching the Perfusion Inlet and Suction Outlet Lines

There are two 18AWG hypodermic needles attached to the apparatus. Either needle can be used as the inlet or outlet for the perfusion liquid but we normally configure the apparatus so that the needle located at the motor end of the bath is the supply. Then the needle mounted at the force transducer end becomes the suction. Two 2m (6 foot) long 1/8" diameter vinyl tubes are provided for the perfusion liquid inlet and the suction outlet. Both tubes are terminated with a Luer fitting that slides into the hypodermic needle.

Connect the inlet hose to the outlet of the Radnoti reservoir using the stopcock supplied with the reservoir (see Figure 12). The suction needle should be connected to a suction source to drain liquid from the bath. Both the inlet and outlet needles are mounted on a 30° angle and there is a set screw located on the top of needle holder that locks the needle in place. To adjust the level of the liquid in the bath loosen the set screw, slide the needle up or down in the holder and re-fasten the set screw. A 0.89mm Allen key that fits the set screw has been supplied with the 801C.

For proper temperature control you must control the temperature of the perfusate reservoir and in some cases insulate the vinyl supply hose that connects the reservoir to the 801C. The temperature of the reservoir can be controlled using a standard temperature controlled laboratory water circulator. This is not supplied with the 801C. Two lengths of vinyl tubing (3/16"ID x 5/16" OD) have been included with the reservoir to be used to attach the laboratory water circulator to the Radnoti reservoir. Attach the supply line from the circulator to the lower fitting on the reservoir and the return line to the upper fitting. Refer to the Radnoti instruction sheet found on the last page of this manual for details of connecting the water jacket tubing to the reservoir.

The reservoir includes a clamp ring that mounts to standard retort stand clamps. An oxygenating bubbler is also included to allow the perfusion liquid to have gas bubbled through it. Several fittings and valves are also supplied with the reservoir. Attach the valve supplied to the outlet of the reservoir and then attach the 1/16" tubing to the other side of the valve.

See Appendix A to this manual for a tubing connection diagram.

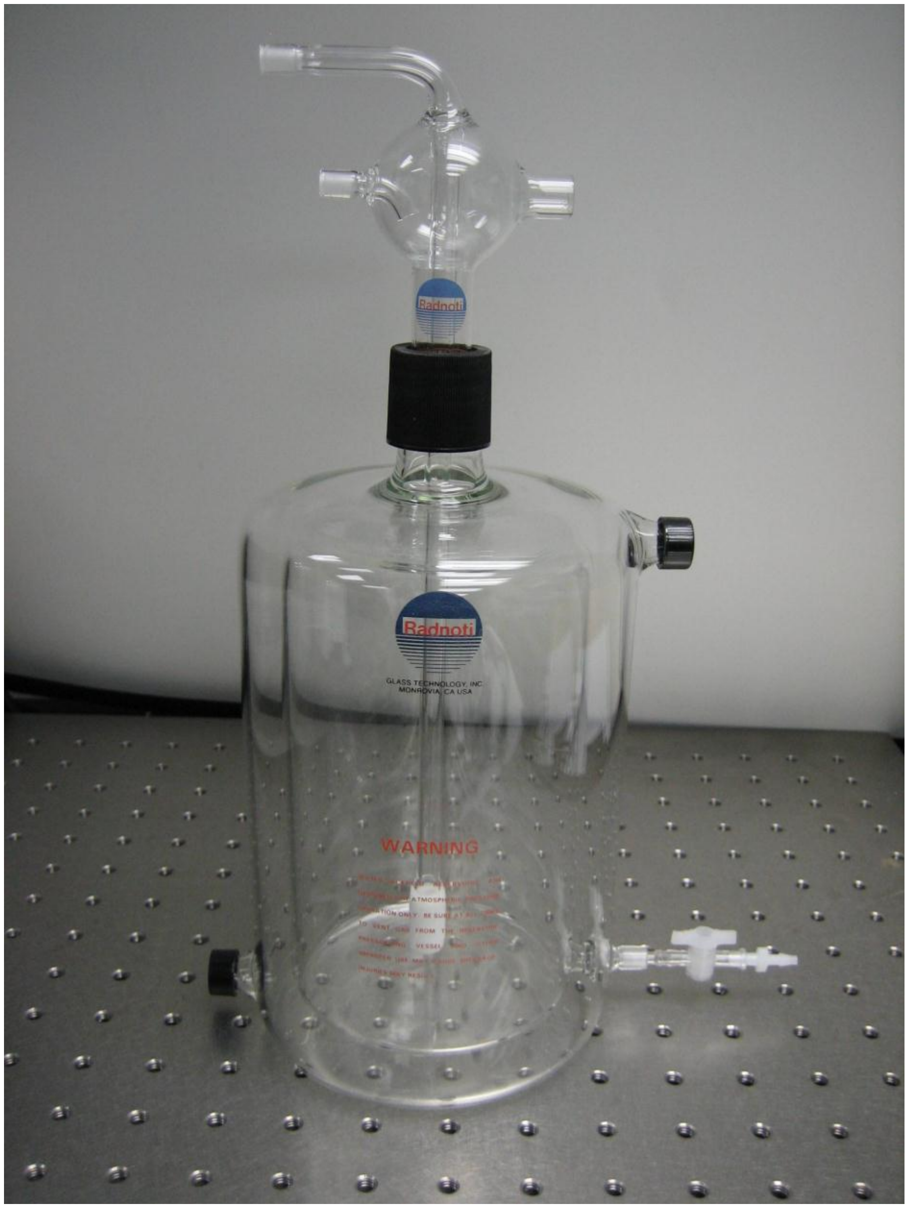


Figure 12 Radnoti 1 liter perfusion reservoir with oxygenating bubbler

2.8 Mounting the 801C to an Inverted Microscope

The 801C has been designed to minimize the distance between the objective and the muscle tissue. As a consequence the force transducer housing protrudes below the level of the bottom of the 801C mounting plate. For this reason care must be taken when mounting the 801C to a microscope stage. Remove any inserts present in the microscope stage. Remove the 801C from its stand; simply lift the apparatus off of its stand. Locate the 801C on the microscope stage with the force transducer protruding downwards into the hole in the stage. The base plate of the 801C has four rubber feet mounted to it. These help to prevent the 801C from sliding when placed on the microscope stage. It is normally not necessary to clamp the 801C to the stage as it doesn't tend to move when in operation. However, if desired, the base plate can be clamped to the stage. Normally the 801C will be used with an

XY microscope platform. This allows the entire 801C to be moved with respect to the objective to allow for tissue observation, see Figure 13 for a photo of the apparatus mounted to a Nikon inverted microscope. As you will note the force transducer mount protrudes into the 100mm diameter cutout on the top of the microscope's XY stage near the right edge of the hole. Figure 14 is a view from underneath the microscope stage showing the relative positions of the apparatus and the objective lens.

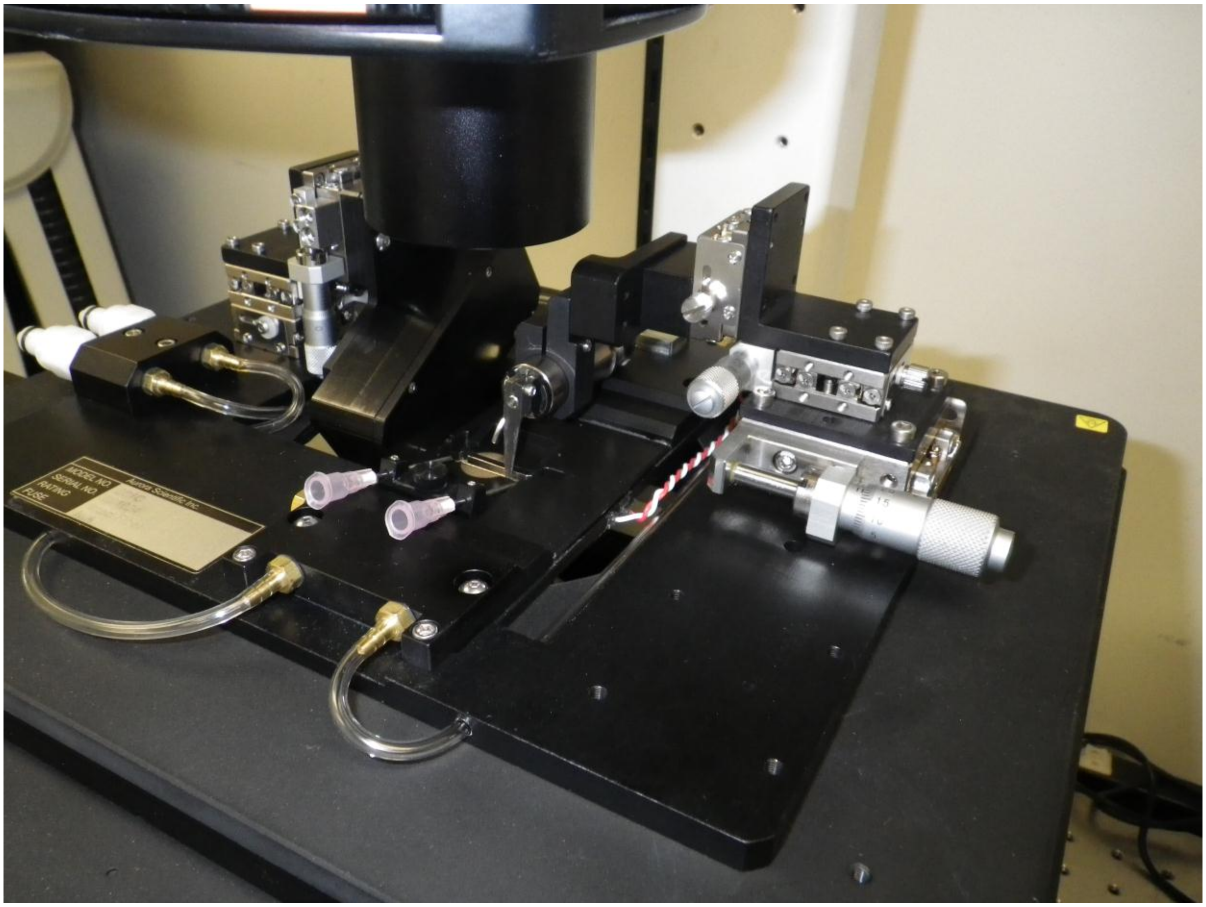


Figure 13 801C-400 mounted on a Nikon inverted microscope

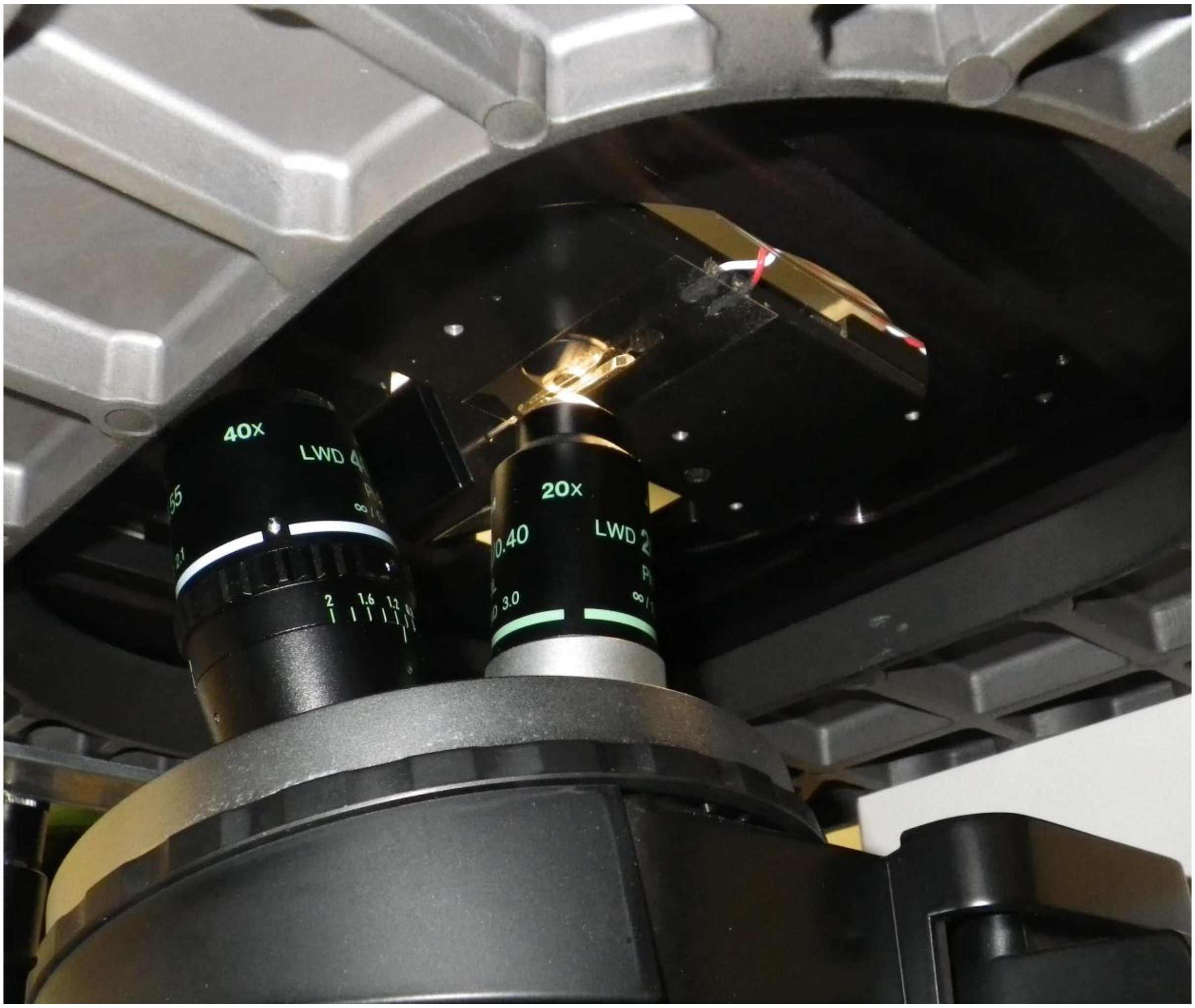


Figure 14 Bottom view of 801C-400 mounted on microscope stage

Note: Force transducer protruding down through 100mm stage cutout on microscope platform.

3.0 Using the 801C

3.1 Adjusting the Location of the Force Transducer

The force transducer location can be adjusted using the XYZ translation stages. It is strongly recommended that the position of the force transducer be adjusted with the aid of a microscope. Lowering the force transducer needle into the bath without first adjusting its position can result in breakage of the force transducer or damage to the bath plate.

3.2 Adjusting the Location of the Lever Arm

The lever system motor and lever arm location should be adjusted prior to mounting tissue. Use a combination of the XYZ translation stage, the angular position of the motor mount clamp ring and the position of motor in the clamp ring to align the lever arm with the bath.

3.3 Controlling the Flow of Perfusate through the Bath

Liquid can be supplied to the bath by a simple gravity feed. Mount the exit of the Radnoti reservoir a minimum of 14” above the bath level to ensure sufficient pressure to drive the liquid into the bath. Use the stopcock supplied with the reservoir to turn on and off and control the flow of liquid.

The suction needle can be connected to a laboratory suction source or to a peristaltic pump if suction is not available. Please note that the pulsing of a peristaltic pump will affect the force readings so it is best to turn off the pump during mechanical testing of the tissue.

For best results we recommend filling the bath with fresh liquid from the reservoir then stopping the flow before performing the mechanical tests on the tissue. We make this suggestion because any flow through the bath will introduce noise into the force readings. A simple method of determining the extent of this noise is to record force data with the liquid flowing and with it stopped. Comparison of the signals will provide a measure of the increased noise due to the flow.

In most cases it is fine to stop the flow during testing since the pH, oxygen content and other characteristics of the perfusion liquid will remain relatively constant for 5 to 10 minutes. Since most testing takes less than this length of time it is reasonable to introduce fresh solution, stop the flow, do the mechanical testing and then introduce more fresh solution before continuing with testing. Having said this it is the responsibility of the researcher to ensure that this method of stopping the flow will work in their circumstances.

If a continuous flow is required then it is important that the flowrate be sufficient to prevent surging in the inlet to the bath. Due to the coating used on the bath plate the liquid contact angle is quite high and this can result in a flow regime where a pool of liquid grows at the inlet needle until it is large enough to drain into the bath and then another large pool forms. This results in a surging type flow which adds far more noise to the force transducer readings. Raising the flow rate by a small amount will usually prevent this surging from happening. Our tests indicate a flowrate of about 0.25ml/sec (14ml/minute) is sufficient to prevent surging. If the stopped flow method is used then the surging is not an issue as no readings are taken while the flow is turned on.

3.4 Attaching a Muscle Fiber to the 801C

Researchers have various methods for attaching muscle fiber to a lever arm and force transducer. Most methods involve attachment of a short length of fine gauge hypodermic tubing to the force transducer and the lever arm. The fiber is then attached to the tubing by tying, clips, glue or a similar technique. Refer to Appendix B for more details of attaching fibers.

The 801C was designed for the lever arm to be in the bath along with the fiber. The 322C lever arm is made from aluminum that is coated with the same material as the bath plate. This electrically insulates the arm while sealing the aluminum from contacting the liquid in the bath. If desired, the lever arm may be coated with a thin layer of epoxy if it is felt that the arm requires additional sealing from the solutions in the bath.

The 801C design calls for the force transducer to be outside of the bath with the fiber mount wire or tube entering the bath chamber through a narrow vertical slot that is 0.7mm (0.028") wide. In most cases the surface tension of the bath liquid will be high enough to easily retain the liquid in the bath. Under no circumstances should you attempt to insert the glass tube that is attached to the force transducer into the slot. It will not fit and the transducer will be broken.

3.5 Adjusting the Resting Tension or Sarcomere Length

Once the fiber is attached the resting tension or initial sarcomere length can be adjusted by several methods. The X-axis translation stages for either the force transducer or the motor mount can be used to adjust the fiber length or resting tension. The lever system can also be used to adjust the fiber length/force. To use the lever system to set resting tension simply turn the front panel Length Offset control to change the position of the lever arm. The digital panel meter on the front of the 322C controller can be used to monitor the amount of movement. For the 322C a 1 micron movement corresponds to a voltage change of 0.003 volts.

3.6 Attaching a Stimulator and Adjusting the Stimulation Electrodes

Two MT type stimulator connectors are located on the side of the 801C apparatus. A BNC to MT adapter cable has been included with the apparatus. Plug the red and black connectors into the mating white connectors located on the side of the 801C. As you will note these connectors are attached to the Platinum stimulation electrodes with a red and white wire. Attach the output of the stimulator to the BNC end of the adapter cable.

The position of the stimulation electrodes may need to be adjusted for optimum tissue stimulation. The electrodes are made from 2mm wide Platinum strip and they can be bent to a new position. In most cases it is advantageous to locate the electrodes within a millimeter of the tissue.

If you elect to bend the electrodes then ensure that neither of the electrodes contacts the lever arm or the force transducer. The thermocouple probe in encased in a Teflon tube and therefore it can rest against the electrodes.

3.7 Adjusting the Thermocouple Position

The position of the thermocouple can be controlled by moving the stainless steel holder tube that the thermocouple is mounted in. A 0.89mm Allen key has been provided that is used to loosen the set screw holding the stainless steel thermocouple holder. In addition to repositioning the stainless tube you can also slide the thermocouple in or out of the tube. The thermocouple is located at the tip of the Teflon tube so the tip should be placed close to the centre of the tissue.

4.0 Warranty

The 801C Small Intact Fiber Apparatus is warranted to be free of defects in materials and workmanship for three years from the date of shipment. Aurora Scientific Inc. will repair or replace, at our option, any part of the system that upon our examination is found to be defective while under warranty. Obligations under this warranty are limited to repair or replacement of the instrument. Aurora Scientific Inc. shall not be liable for any other damages of any kind, including consequential damages, personal injury, or the like. Disassembly of the unit will void this warranty. Damage to the system through misuse will void this warranty.

Aurora Scientific Inc. pursues a policy of continual product development and improvement therefore we reserve the right to change published specifications without prior notice.

Appendix A Drawings

Drawing Number	Title
A801C-A001	801C-400 Normal Motor Location
A801C-A002	801C-400 Alternate Motor Location
A801C-A003	801C-1900 Normal Motor Location
A801C-A004	801C-1900 Alternate Motor Location
A801C-A005	801C-300 300C Motor Location
A801C-A006	801C-300 300C Motor Location with Fixed Test Post
AS1500A-E001	Small Intact Muscle Test System Electrical Interconnection Drawing
AS1500A-P002	Small Intact Muscle Test System Plumbing Interconnection Drawing

Appendix B Fiber Attachment Methods

Three main attachment methods exist. These are:
1) Tying
2) T-clips
3) Glue

1. Tying

Tying requires adding a short length of fine gauge hypodermic needle tubing to the force transducer and length controller. The fiber is then tied to these two needles using fine suture (10-0 monofilament nylon suture). See the attached photo of a small intact muscle tied to a force transducer and lever. Neither the force transducer output tube nor the lever arm are visible in this photo but the researcher was using a model 400A force transducer and a model 322C length controller. In the photo the wire at the bottom is a platinum stimulation electrode and the needle at the top is a thermocouple. The force generated by the fiber will determine the number of suture loops to use. This photo shows four on each end which was required to prevent slippage.

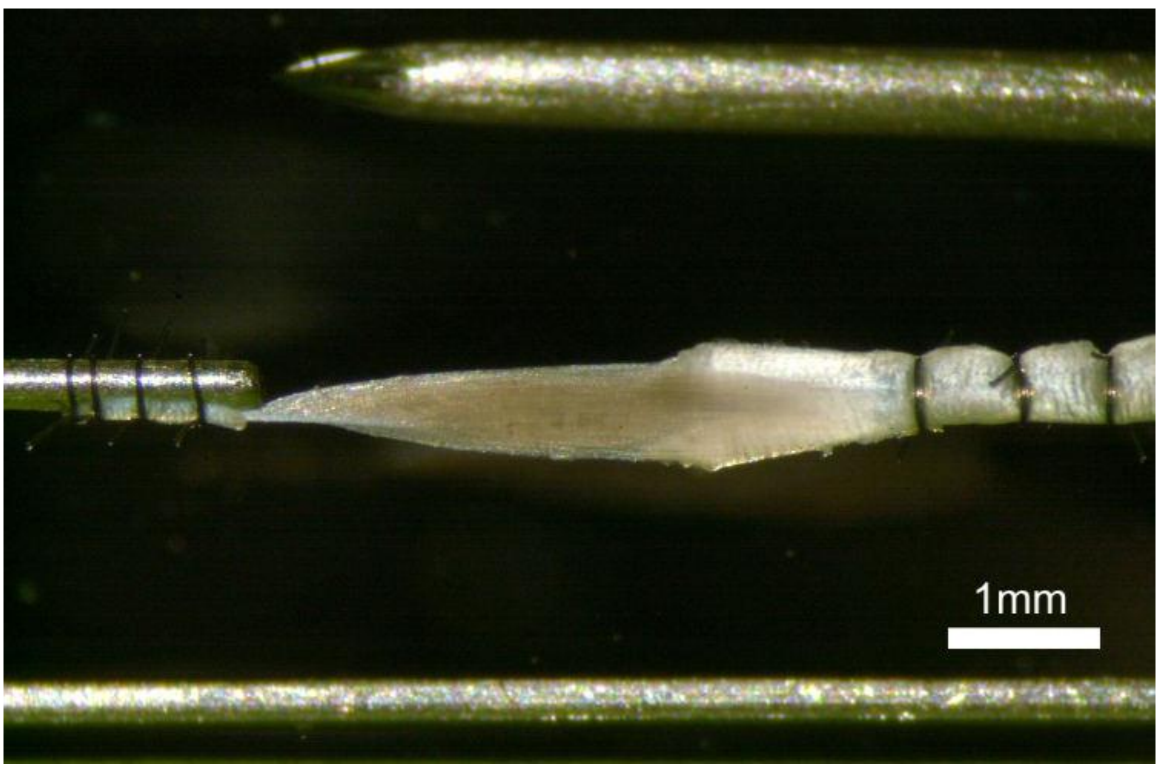


Photo 1 Lumbrical Muscle Tied to 29 AWG Tubes

With permeabilized fibers, or even fiber bundles it is more likely that you would only require 2 or 3 sutures per end. Our force transducer has a hollow glass tube that the load is attached to. Stainless steel tubing, or a 29AWG hypodermic needle, can be attached to the transducer by sliding the needle 5-10mm down inside the glass tube and then applying a small amount of melted paraffin wax to the joint between the needle and tube. The overall length of the tube will be dependent on the bath arrangement you are using but I would think the tube would project out of the force transducer tube about 8-10mm, overall length 13 to 20mm. The wax will "wick" up the glass tube and, when hard, it will easily be able to withstand the forces generated by fibers. Using wax has the added advantage that all you need to do is warm up the wax in order to remove the needle should it require changing. If you are expecting very high forces 10g or more then you may need to epoxy the tube into the glass tube of the transducer as wax may start to slip at these higher forces.

To attach the needle to the lever arm we suggest bending the needle into an "L" shape and then using epoxy to glue the needle to the arm. It is easiest to first "tack" the needle to the arm using superglue and then apply the epoxy. We suggest a 2 hour epoxy as the 5 minute kind often breaks down in water. The vertical part of the L should lie parallel to the length of the arm with the bottom of the L facing perpendicular to the long axis of the arm. The bottom part of the L should be kept as short as practical and the least amount of glue that will do the job should be used. This is because the added mass of the needle and the arm will affect the tuning of the lever system. The L should be about 5mm x 4 mm long. 29AWG stainless tubing works well for this task and if you can't find any then you can cut up a hypodermic needle. Please note that the ends of the tubing should be carefully tapered to prevent metal burrs from cutting into the fiber. A short, abrupt taper seems to work best as the fiber is less likely to slip. Two research groups using the tying method include Prof. John Faulkner's group at the University of Michigan and Prof. Rick Moss's group at the University of Wisconsin-Madison.

2. T-Clips

T-clips are small clips shaped in a "T" shape and cut from aluminum foil. The bottom of the T has a small hole in it. The top of the T is wrapped around the fiber and squashed down onto the fiber. This leaves the vertical part of the T sticking out the end of the fiber. A small hook is created in a similar manner to the tube used in the tying method but this time the tube is bent to form a hook. In operation the hole in the T clip is slipped over the hook and the fiber is then attached. T clips seem to work fine for low force applications, single permeabilized fibers. If you intend to test higher force fibers, for example frog fibers or bundles or strips you may find that the tissue slips inside the T clip. Pieter de Tombe's group in Chicago and Frank Brozovich, at the Mayo, use T clips.

3. Glue

Depending on the size of the tissue preparation I have seen researchers also attach tissue with glue. Typically this seems to be used with very small preps including skinned myocytes. In this method a small amount of glue is placed on the tip of a tube attached to the force transducer and another to the lever arm. The tissue is then brought into contact with the glue and attached. Two main types of glue seem to be used, the first is expanding foam called Great Stuff that is available in the USA. The other is silicone glue. Pieter de Tombe from Chicago and G. Stienen from the VU University Medical Centre in Amsterdam are two researchers using glue.

Appendix C Motor and Force Transducer Attachments

For the motor (315C or 322C) start with a piece of 29 gauge stainless steel tubing that's about 10mm long and then "dress up" the ends by inserting the tubing into a "pin vise" and using a very fine sharpening stone. Aim for a very smooth end with a fairly abrupt taper (long taper encourages slipping of prep). Next put a 90degree bend in the tubing by chucking it in the pin vise 1/2 way down its length and then, starting with the pin vise at a very shallow angle with respect to a flat surface, slowly raise the pin vise while keeping the half of the tubing that extends from the vise in full-length-contact with the flat surface. Continue to raise the vise until it is normal to the flat surface (at which time the tubing ought to have a 90deg bend). Too much bend and the tubing will "crimp" and you will have to start over again. Once you have an L-shaped piece of tubing that you are happy with (the legs of the "L" will be on the order of 4.5mm each), you will epoxy it in such a way that one leg is in line with the long axis of the motor arm and the other leg comes off the end of the motor arm at a 90deg angle. Use 2 hour epoxy (5min isn't typically waterproof). First "tack" the "L" to the arm using a tiny amount of superglue - this is only to hold the "L" in place while the epoxy is curing. The 315C motor is very sensitive to added mass. You may need to make the “L” about 3mm x 3mm when used with a 315C. Also use the minimum amount of epoxy that will get the job done. Even the added mass of the epoxy can have a detrimental effect on motor tuning.

For the transducer, the length of tubing will depend in part on bath configuration. As a rule of thumb use the shortest tube that will do the job (less mass, higher frequency response). Reasonable dimensions are approx. 13mm, 5mm of which are inside the glass capillary "coupling tube" with the remaining 8 outside. Dress the ends of the tubing as described above and then epoxy one end to the coupling tube. Don't try to get it concentric with the coupling tube, this can be very difficult. Instead let its outside surface rest against the inner surface of the coupling tube, this ensures that the stainless steel tubing and the glass output tube are lined up. Getting epoxy in the coupling tube is a bit of a trick. Dip the end of the stainless steel tubing in the epoxy pool and then insert it. It takes very little glue to hold it. You will want to keep an eye on it until the epoxy is cured, making constant minor adjustments to maintain a straight stainless steel tube extension.

Use 29 gauge stainless steel tubing (0.013 inch outside diameter) that is readily available from McMaster-Carr or you can make the attachments from a 29 gauge hypodermic needle. The pin vise and sharpening stones are also available from McMaster-Carr.

Hypodermic Needle Tubing:	McMaster-Carr Catalogue #8988K429
Pin Vise:	McMaster-Carr Catalogue #8455A14
Sharpening stones:	McMaster-Carr Catalogue #4456A17

Radnoti Instruction Sheet

Radnoti

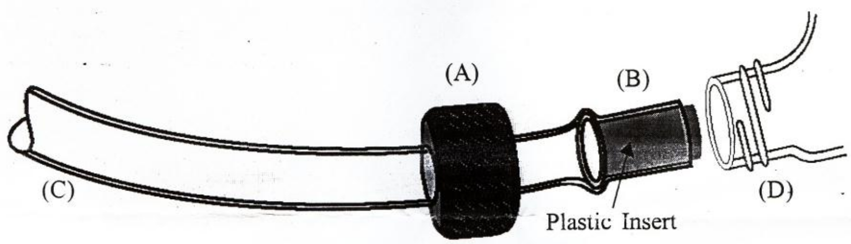
RADNOTI "QUICK DISCONNECT" INSTRUCTIONS

A. Slide CAP-WITH HOLE onto the water line (Tygon tubing 120159).

B. Push the Plastic Sleeve Insert into the end of the Tygon tubing with the flared end first. (Catalog No. 120160).

C. Be sure to use 5/16 x 3/16 inch Tygon tubing (Catalog No.120159).

D. Moisten tip of Tygon tubing before insertion into the threaded glass.
CAUTION: *To Avoid Breakage, Do Not Over-Tighten The Screw Cap.*



Replacement Part List:

Catalog No.	Description
120160	Plastic Sleeve Insert, 25/Pkg
120159	Tygon Tubing 5/16 x 3/16 ID, 50 Ft/Min.
160196	Drilled caps for Water Jacketed QD Connections 12/Pkg

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