INSTRUCTION MANUAL

Models 801A

Small Intact Fiber Test Apparatus

August 22, 2005, Revision 1

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1.0 Introduction

The 801A small intact fiber test apparatus was designed to enable physiology researchers to easily test small intact muscle tissue with an ASI model 312C or 322C high-speed length controller and an ASI series 400A force transducer.

The 801A consists of an aluminum bath plate with a single perfusion well with a glass bottom, 2 TEC heater/coolers, water-cooled heat sink plate, model 825A TEC controller, micrometer drive XYZ translation stages for motor and force transducer positioning, and mounts for the high-speed length controller and the 400A series force transducer. The system also comes with a 1.0 liter Radnoti temperature jacketed reservoir for the perfusate. Also included are mounting screws for mounting the force transducer and a set of Imperial Allen keys. All parts are manufactured from corrosion resistant materials (stainless steel, anodized aluminum, Plexiglas and Delrin).

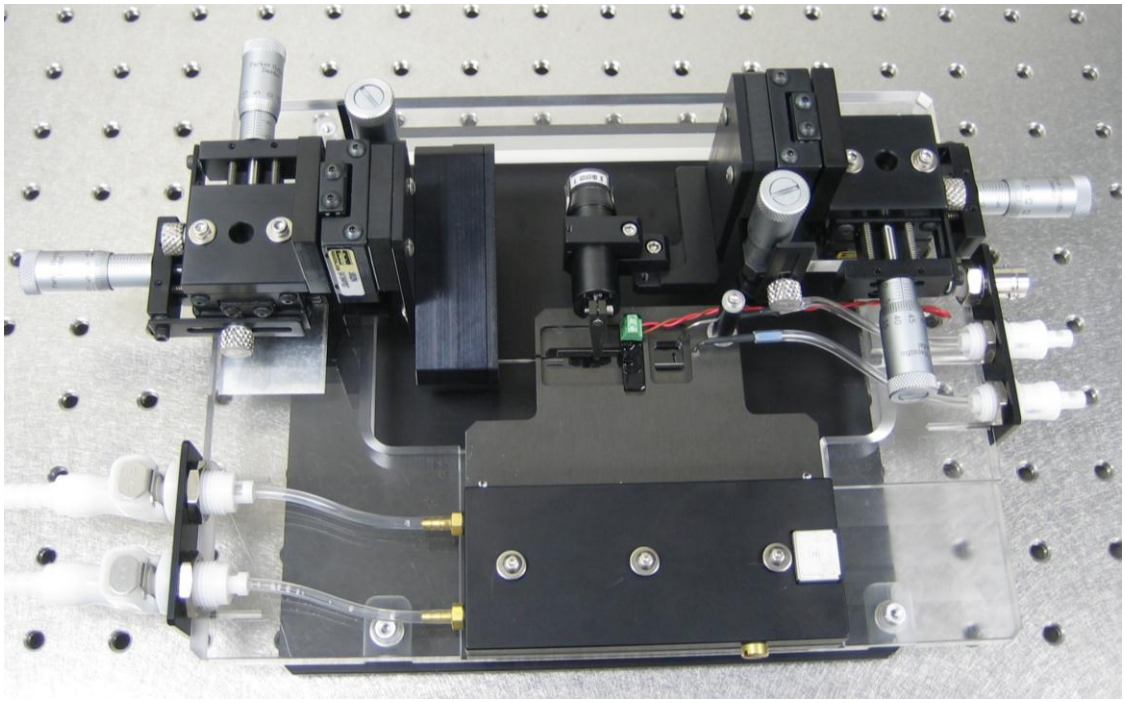


Figure 1 801A Apparatus.

The bath plate is manufactured from aluminum that is coated with Magnaplate HCR 31. This is a hard anodized coating followed by polymer impregnation and polymer coating. The resultant surface has a Rockwell hardness of about 50 while providing excellent dielectric properties while maintaining high thermal conductivity. Thus 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 a dielectric. The perfusion liquid is brought to the temperature of the plate by flowing along the bottom of the plate along the length of the bath. The liquid then enters the chamber via two opposing entrances at the force transducer end of the bath. The liquid is

removed from the chamber at the far end. The combination of opposing entry at the force transducer end and removal at the length controller end assures laminar flow in the bath with good purging.

Figure 2 825A TEC Controller

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 located on the bottom of 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.

1.1 Specifications

Bath Plate

No. of Baths:	1
Bath Plate Material:	6061-T6 aluminum with Magnaplate HCR 31 coating
Bath Dimensions:	27mm (1.063”) L x 6.5mm (0.256”) W x 4mm (0.157”) D
Bath Volume:	702µl
Suction Reservoir Dimensions:	8.9mm (0.350”) L x 10.16mm (0.400”) W x 3.43mm (0.135”) D
Suction Reservoir Volume:	310µl

Inlet

Vinyl Tube Size:	1/16” ID x 1/8” OD
Stainless Tube Size:	19AWG

Outlet

Vinyl Tube Size:	1/16” ID x 1/8” OD
Stainless Tube Size:	19AWG
Suction Height Range:	2.5mm

Thermoelectric Coolers

No. of TECs:	2
Power:	33 W
Voltage:	15 V
Temperature Sensor:	AD590

Stimulation Electrodes

Number:	2 mounted to a circuit board along with a terminal block
Material:	99.9% pure Platinum
Dimensions:	22mm (0.866”) L x 2mm (0.079”) W x 30AWG (0.010”) Thick

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the three shipping boxes. The first box contains the Radnoti Reservoir with bubbler and mounting clamp, mounting screws for the force transducer, water hoses, perfusate hoses, BNC cable for the stimulation electrodes and Allen keys. The second box holds the 801A apparatus with the XYZ translation stages attached. The third box contains a model 825A TEC controller and thermocouple thermometer. The 801A apparatus is shipped assembled however the user must attach the motor, force transducer, water lines, perfusate lines and the stimulation BNC cable.

2.2 Attaching the 801A to an Inverted Microscope

The 801A has been designed to minimize the distance between the objective and the muscle tissue. Part of this is accomplished by having the force transducer housing protrude below the level of the bottom of the 801A mounting plate. For this reason care must be taken when mounting the 801A to a microscope stage. Remove the 801A from its stand; simply lift the Plexiglas plate off of the stand. Remove any inserts present in the microscope stage. Locate the 801A on the microscope stage with the force transducer protruding downwards into the hole in the stage. Try to position the 801A so that the location of the fiber is in the centre of the stage cutout. The Plexiglas base plate of the 801A has four rubber feet mounted to it. These help to prevent the 801A from sliding when placed on the microscope stage. It is normally not necessary to clamp the 801A 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 801A will be used with an XY microscope platform. This allows the entire 801A to be moved with respect to the objective.

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 (7 on the micrometer). The Z stage should be raised (to the 2 mark on the micrometer).

In some cases it is easiest to attach the motor by first removing the motor mount clamp ring. The clamp ring is held on with a single 4-40 socket head cap (SHC) screw. To mount a model 312C motor first remove the clamp screw completely from the clamp ring. Then line up the arm with the slot in the clamp and insert the motor into the motor clamp ring. Now rotate the motor so that the connector is at the top and then gently tighten the motor mount ring clamp screw to hold the motor in position. To mount a model 322C motor simply insert the motor into the clamp without the lever arm attached. It is advantageous to mount the 322C motor with the back of the motor tipped lower than the front. This provides more clearance for access to the bath and the tissue. The motor mount plate has three holes drilled in it to allow the plate to be mounted in either a horizontal or tipped orientation. Rotate the 322C motor so that the connector is at the bottom and then tighten the ring clamp. Once the motor is clamped in position you can then attach the lever arm.

The motor can also slide within the mount. Loosen the clamp ring screw if necessary and slide the motor in the clamp so that the tip of the lever arm is located in the center of the bath. The X and Y stages may also require adjustment to properly locate the motor.

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 the Z axis stage is positioned so that the lever arm doesn't hit the glass cover slip at the bottom of the bath.

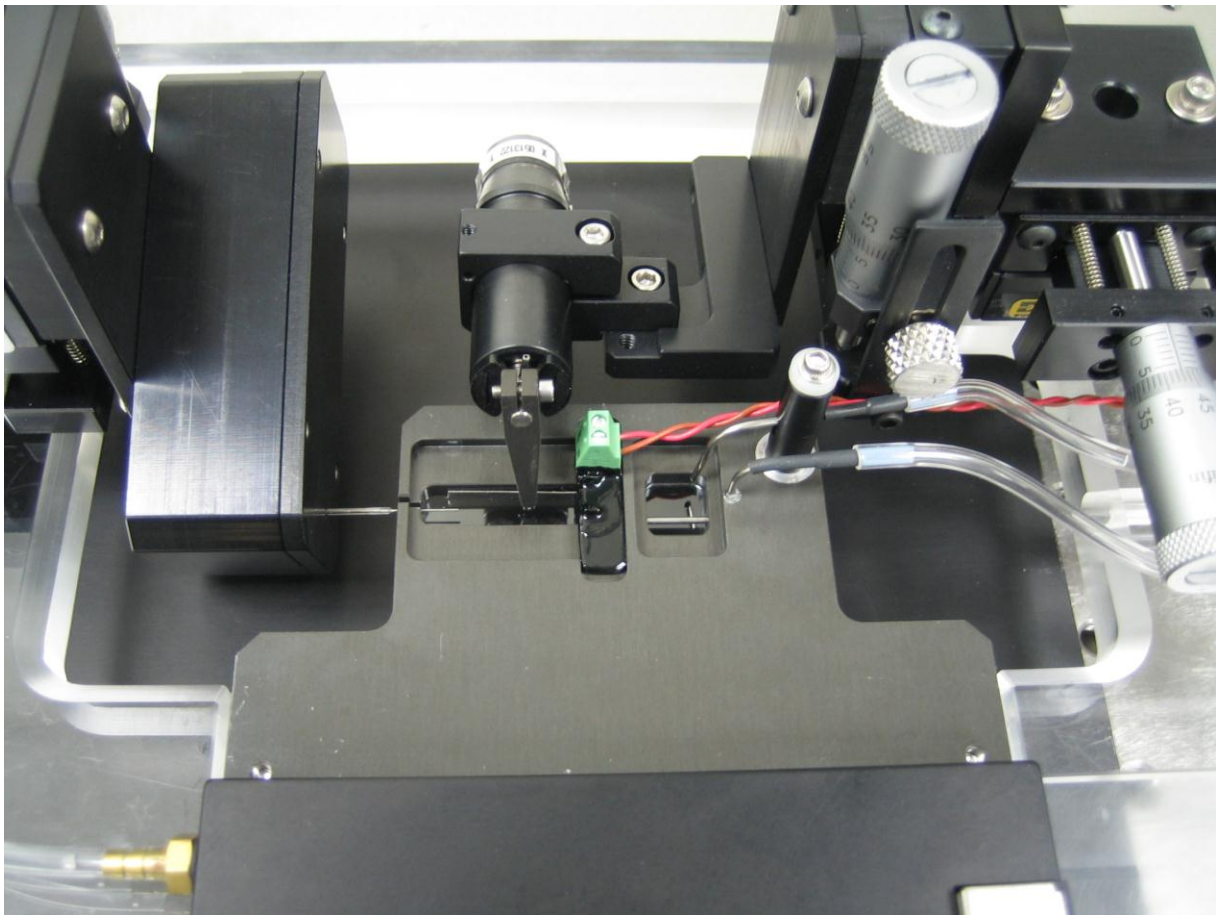


Figure 3 Close-up of Length Controller and Force Transducer in the Bath

2.4 Attaching the Force Transducer

Normally a length of fine gauge stainless steel tubing is fastened into the end of the force transducer output tube. This tube then passes through the slot in the end of the bath plate and the fiber is attached to it. Make sure that the glass output tube on the force transducer is clear of the bath plate. The glass tube is too large to fit into the slot and if the transducer is located too close to the plate the output tube could be broken when the transducer is moved.

Refer to Figure 3 for correct orientation of the force transducer in the 801A apparatus. The transducer is tipped back at a 15-degree angle to the horizontal. This angle provides greater access to the bath. Before mounting the transducer, position the Y-axis translation stage as far from the bath plate as possible. Note the stage movement is limited by the Z-axis stage. Position the X-axis stage at about the 7 mark on the micrometer dial. Raise the Z-axis

stage to near the top (2 on the dial). Remove the transducer mounting plate from the XYZ 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 two of the 4-40x1/4" 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-axis right-angle bracket along with the Z-axis stage from the X-axis stage. Attach the transducer to the mount plate and then re-attach the Z stage.

Once the transducer is attached use the X, Y and Z stages to orient the needle attached to the transducer output tube on the centerline of the slot that is at the end of bath. Use a microscope to observe the needle and slot positions relative to each other. Use the Z-axis stage to set the depth of the output tube, and thus the fiber, 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.

Ensure that the transducer cable is routed clear of the bath plate. It is best to strain relief the transducer and motor cables by attaching them to the 801A base plate. Cable ties and tape backed cable mounts are provided for this purpose. Cable movement can lead to increased noise on the length and force signals.

2.5 Attaching the Cooling Water Lines

On the force transducer end of the 801A you will see two dry-break quick-connect tube fittings. The quick connect fittings can be disconnected by pressing the metal tab on the side of the connector and then pulling the connection apart. These fittings have a hose barb designed for 1/4" ID vinyl tubing. Two 6-foot sections of vinyl hose have been supplied along with the 801A. Connect one hose to a source of cool water and attach the other to a drain. Ensure that you have a flow of water through the cooling plate before turning on the 825A TEC Controller. The flow rate does not have to be very high, there just has to be some water circulating. Also it is best to circulate water that is about 20°C temperature. There is no need to circulate very hot or very cold water.

Note: Operating the thermoelectric coolers (TEC) without water flowing can damage the TECs.

2.6 Thermoelectric Controller

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

The system has been designed to control the temperature between 0 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 20ºC. The 825A includes a thermocouple meter and the apparatus includes a T-type stainless steel thermocouple probe that can be used to monitor the temperature of the liquid in the bath.

Ensure that water is flowing through the cooling plate before changing the set point to a temperature other than room temperature. Set the desired temperature on the controller and then monitor the actual temperature to ensure that the temperature is approaching the set point.

2.7 Attaching the Perfusion Inlet and Suction Outlet Lines

On the motor end of the 801A you will see two quick-connect tube fittings. These fittings are connected to the perfusion liquid inlet and to the suction outlet. The quick connect fittings have a hose barb designed for 1/16" ID vinyl tubing. Two 6-foot sections of vinyl hose have been supplied along with the 801A.

Connect the hose that leads to the inlet fitting to the outlet of the Radnoti temperature jacketed reservoir. The temperature of the reservoir can be controlled using a standard temperature controlled laboratory water circulator. This is not supplied with the 801A. 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.

The suction fitting should be connected to a suction source to drain liquid from the bath. A 6 foot length of 1/16" ID tubing has been supplied for this purpose. The suction assembly mounted to the bath plate includes a screw on the top of the assembly for adjusting the level of the liquid in the bath. Simply turn the screw clockwise to raise the level and counterclockwise to lower the level. An Allen key that fits the screw has been supplied with the 801A.

3.0 Using the 801A

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 Attaching a Muscle Fiber to the 801A

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.

The 801A was designed for the lever arm to be in the bath along with the fiber. The 312C lever arm is made from titanium to minimize corrosion and the possibility of contaminating the bath. 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 801A design calls for the force transducer to be outside of the bath with the fiber mount tubing entering the bath chamber through a narrow vertical slot (700 microns (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 have the glass tube that is attached to the force transducer enter the slot. It will not fit and the transducer will be broken.

3.4 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 Y-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. Use the front panel Length Offset control to change the position of the lever arm. The digital panel meter on the front of the 312C or 322C controller can be used to monitor the amount of movement. For the 312C a 1 micron movement corresponds to a voltage change of 0.007 volts. For the 322C a 1 micron movement corresponds to a voltage change of 0.003 volts.

4.0 Warranty

The 801A 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.

Radnoti Instruction Sheet

Radnoti

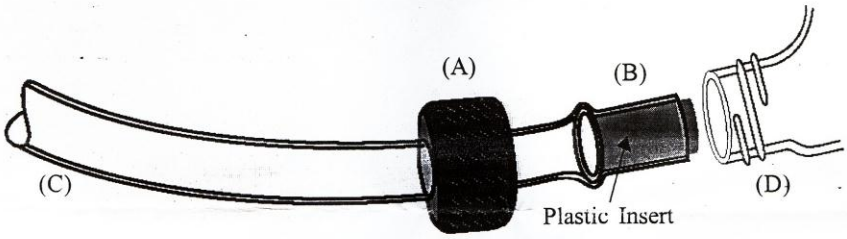
RADNOTI "QUICK DISCONNECT" INSTRUCTIONS

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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