INSTRUCTION MANUAL

Models 803B

Myocyte Test Apparatus

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1.0 Introduction

The 803B Myocyte test apparatus was designed to enable physiology researchers to easily test myocytes with an ASI series 312C motor and a 400A series force transducer.

The 803B consists of a 6-well aluminum bath plate with a glass bottom on all 6 wells, 2 TEC heater/coolers, water-cooled heat sink and an ASI model 825A Thermometer/TEC controller (see separate 825A manual for details on operating the temperature controller). All parts are manufactured from corrosion resistant materials (stainless steel, anodized aluminum and Delrin).

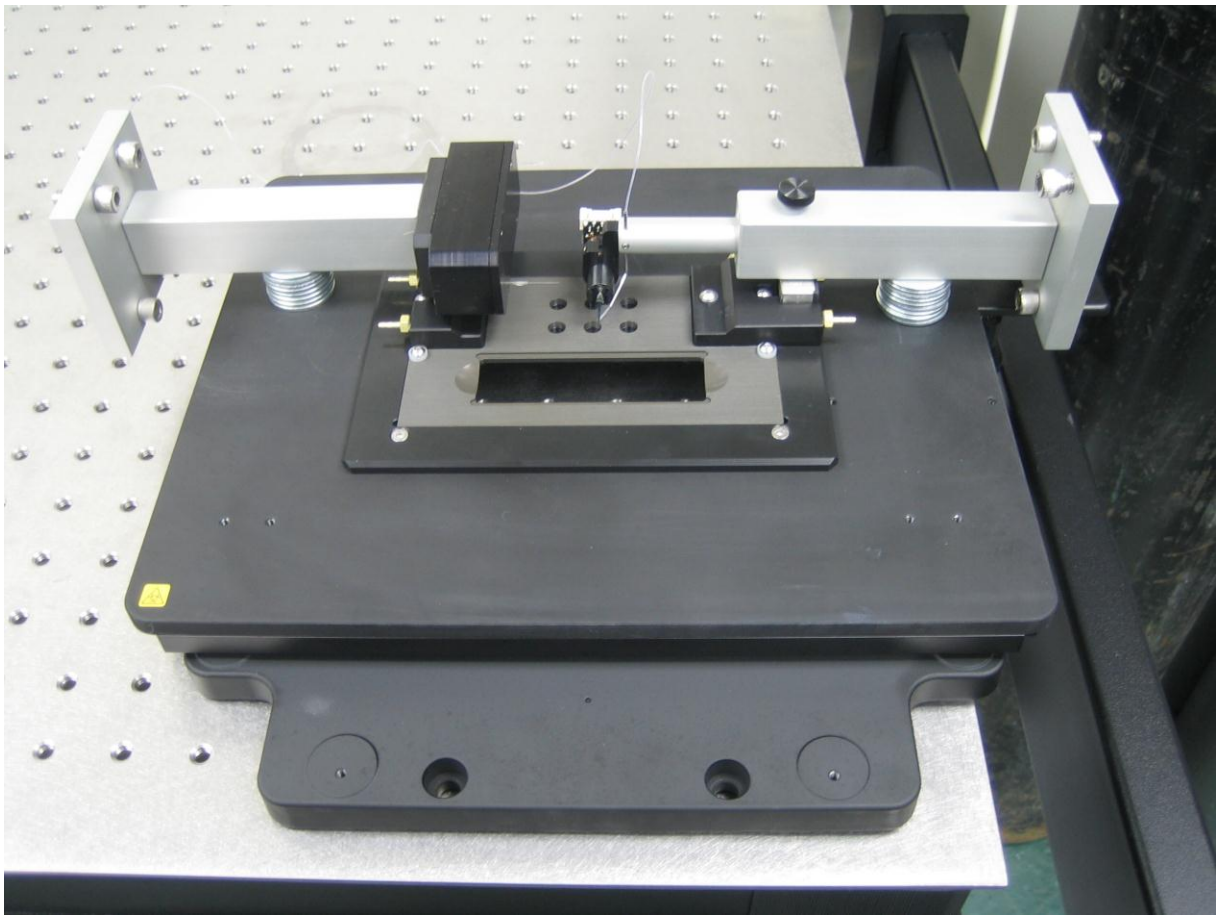

Photo 1 803B Apparatus Mounted to Nikon Microscope XY Stage

The 803B also includes a large cell mounting area located in front of the 6 working wells. The cell mounting area accepts a standard 3”x1”x1mm microscope slide. The upper surface of the microscope slide is flush with the top surface of the plate to allow easy transfer of mounted cells from the mounting slide to the working wells. The cell is normally glued between the output tube of the force transducer and the motor arm of the 312C Length Controller. The apparatus mounts on the surface of a microscope XY translation stage and is held in place with four 3mm flat head screws. In operation the desired bath is selected by

moving the 803B bath plate relative to the length controller and force transducer using the microscope's XY translation stage.

The bath plate is manufactured from anodized aluminum and has six round wells machined in its upper surface. This number allows for test protocols involving multiple solutions without the need to empty and re-fill the baths. All baths have a glass bottom to allow for microscope observation of the cell or for sarcomere length measurement. The glass on the bottom of the plate is a standard 1 ½ microscope cover slip that measures 22x40mm. The aluminum plate is coated with a special hard anodized coating with polymer impregnated in the surface. This ensures that the surface is inert as well as corrosion resistant.

Both the length controller and the force transducer are mounted on XYZ micrometer translation stages that are attached to a vibration isolation table. This allows them to be positioned relative to the bath plate.

The 825A Thermometer/TEC Controller features a T-type thermocouple readout that can be used to measure the solution temperature in the bath being used. The 825A also includes a PID TEC controller. This controller maintains the temperature of the bath plate to within ±0.1°C. The controller measures the plate temperature using an AD590 temperature sensor that is mounted in the main bath plate.

Photo 2 825A Thermometer/TEC Controller

The length controller mount boom has been drilled to accept an 18AWG hypodermic needle tube that holds a small diameter T-type thermocouple encased in a Teflon jacket. This allows the researcher to monitor the temperature of the bath in use.

1.1 Specifications

Bath Plate	
No. of Baths:	6
Bath Plate Material:	6061-T6 aluminum with Magnaplate Tufram HCR 31 coating
Bath Dimensions:	6.35mm (0.250”) diameter x 2.95mm (0.116”) D
Bath Volume:	94µl
Centerline Distance X-axis:	6.35 mm (0.500”)
Centerline Distance Y-axis:	6.35 mm (0.500”)
Cover Slip on Bottom:	22 x 40mm x 1 ½ (0.17mm) Thick
Mounting Slide:	76.2 mm (3”) L x 25.4 mm (1”) W x 1 mm Thick
Thermoelectric Coolers	
No. of TECs:	2
Power:	16 W
Voltage:	8 V
Power Requirements:	120VAC±10%, 60Hz, 2 amps max.

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the two shipping boxes. One box contains the 825A TEC Controller along with the power cord and interconnection cables. The other holds the 803B apparatus, the Force Transducer boom mount, the 312C boom mount, cooling water manifold, tubing, screws and tools. The 803B myocyte plate is shipped assembled however the user must attach the apparatus to the microscope stage, install the boom mounts on the XYZ translation stages, install the motor and force transducer on the boom mounts and finally install the cooling water manifold and lines.

2.2 Attaching the 803B to a Nikon Microscope Stage

The Delrin base plate of the 803B has four countersunk holes in the top surface. There are four 3mm flat-head screws provided that are used to screw the apparatus to the stage. Remove the round stage insert before attaching the apparatus. Position the apparatus so that the rectangular cut out for the microscope slide is closest to the front of the microscope (see Photo 1). Align the mounting holes with the tapped holes in the stage and then simply screw the plate to the stage.

2.3 Attaching the Cooling Water Lines

Four tubes are connected to the 803B, two supply tubes and two drain tubes (see Photo 3). All of these tubes include a dry-break quick connector. These connectors are different for the supply tubes and the drain tubes. Connect the two supply tubes to the cooling water manifold at the locations marked “To 803B”. Likewise connect the two drain tubes to the cooling water manifold at the locations marked “From 803B”. The quick connect fittings can be disconnected by pushing the metal tab and pulling the two halves of the connector apart. Organize the 1/16” ID vinyl lines from the 803B so that the tubes are clear of the rest of the equipment and allow free movement of the microscope XY stage. The cooling water manifold includes a hole in it that can be used to screw the manifold to the vibration isolation table in a convenient location. Two screws have been provide to mount the manifold, use the appropriate one based on the type of holes in your table. The two screws are a ¼-20 screw and an M6 screw.

There are two other fittings on the manifold marked IN and OUT. These are for attaching a supply of cooling water and a drain for the water. Two 1/4” ID vinyl tubes with quick connectors have also been supplied for the water. Connect the water source to the IN connector and attach the OUT connector to the drain. Ensure that you have a steady flow of water to the manifold and in turn to the heat sinks located on the 803B apparatus **before** turning on the 825A thermoelectric controller. Operating the thermoelectric coolers (TEC) without water flowing can damage them.

The 803B does not require a large flow of water but water should circulate any time the TEC controller is powered. Also use water that is about 20°C temperature, very cold or hot water will adversely affect the ability of the 825A and the TECs to control the plate temperature.

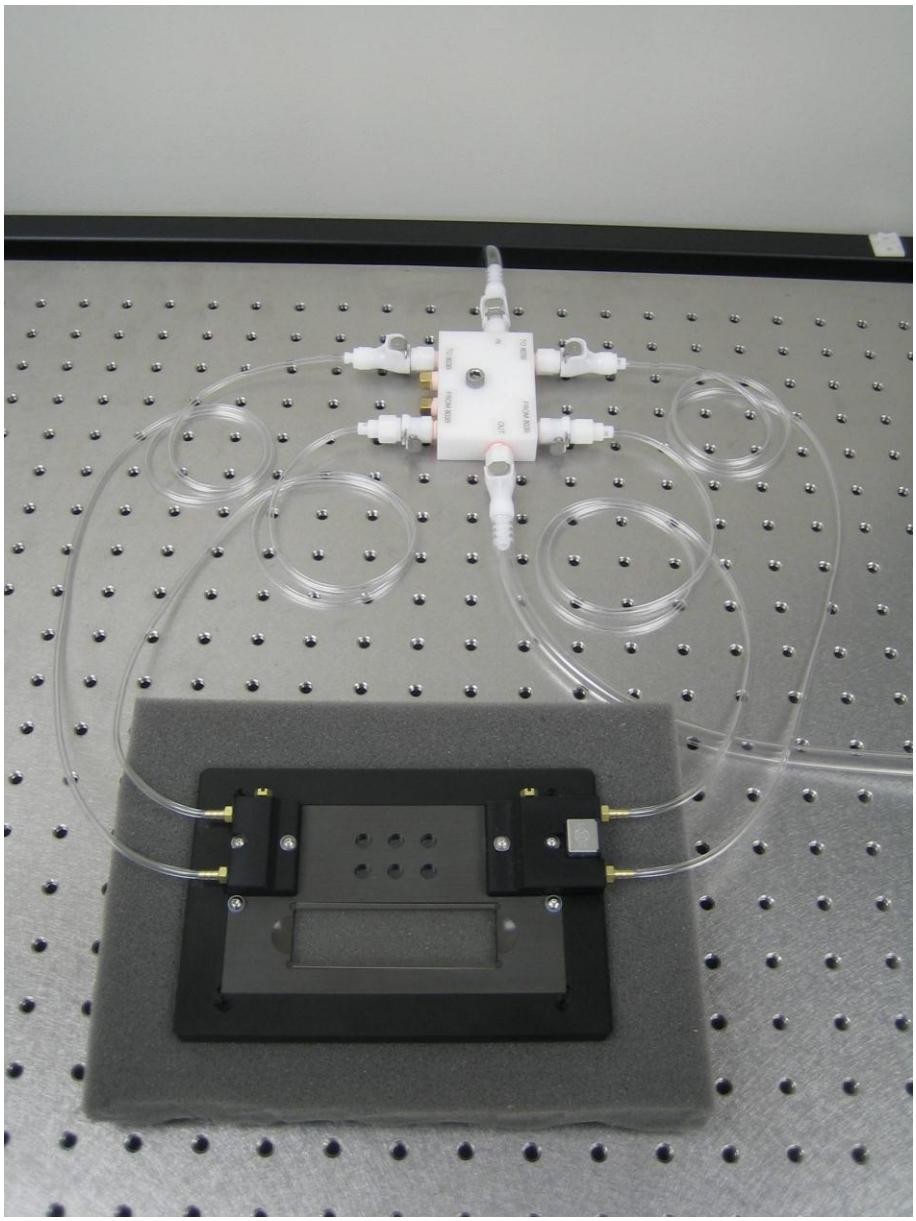

Photo 3 Cooling Water Manifold and Tube Attachment

2.4 Attaching the Thermoelectric Controller

Please refer to the 825A Instruction Manual for correct setup and operation of the TEC controller. Two thermoelectric coolers (TECs) are included with the 803B. These devices are rated at 8 volts and 16 watts. An external TEC controller (model 825A) is included. The plate should reach the set point temperature in less than five minutes.

Using the supplied PS2 cable, (6-pin mini-DIN cable similar to that used for a PC keyboard) connect the 825A to the 6-pin connector located on the right side of the 802B.

Ensure that water is flowing through the cooling plate and then turn on the 825A thermoelectric controller. Set the desired temperature on the controller and then monitor the actual temperature to ensure that the temperature is approaching the set point. It is normal for there to be a small temperature difference between the Setpoint temperature and the

temperature measured by the thermocouple located in any of the 6 wells. This is due to the fact that the process temperature is measured very close to the TEC while the thermocouple is measuring the liquid temperature in the centre of the plate. To obtain a precise temperature in the well adjust the Setpoint temperature until the desired temperature is reached.

2.5 Attaching the Force Transducer and Length Controller Boom Mounts

The apparatus includes two boom mounts that must be mounted to XYZ translation stages that are mounted to the vibration isolation table on either side of the microscope.

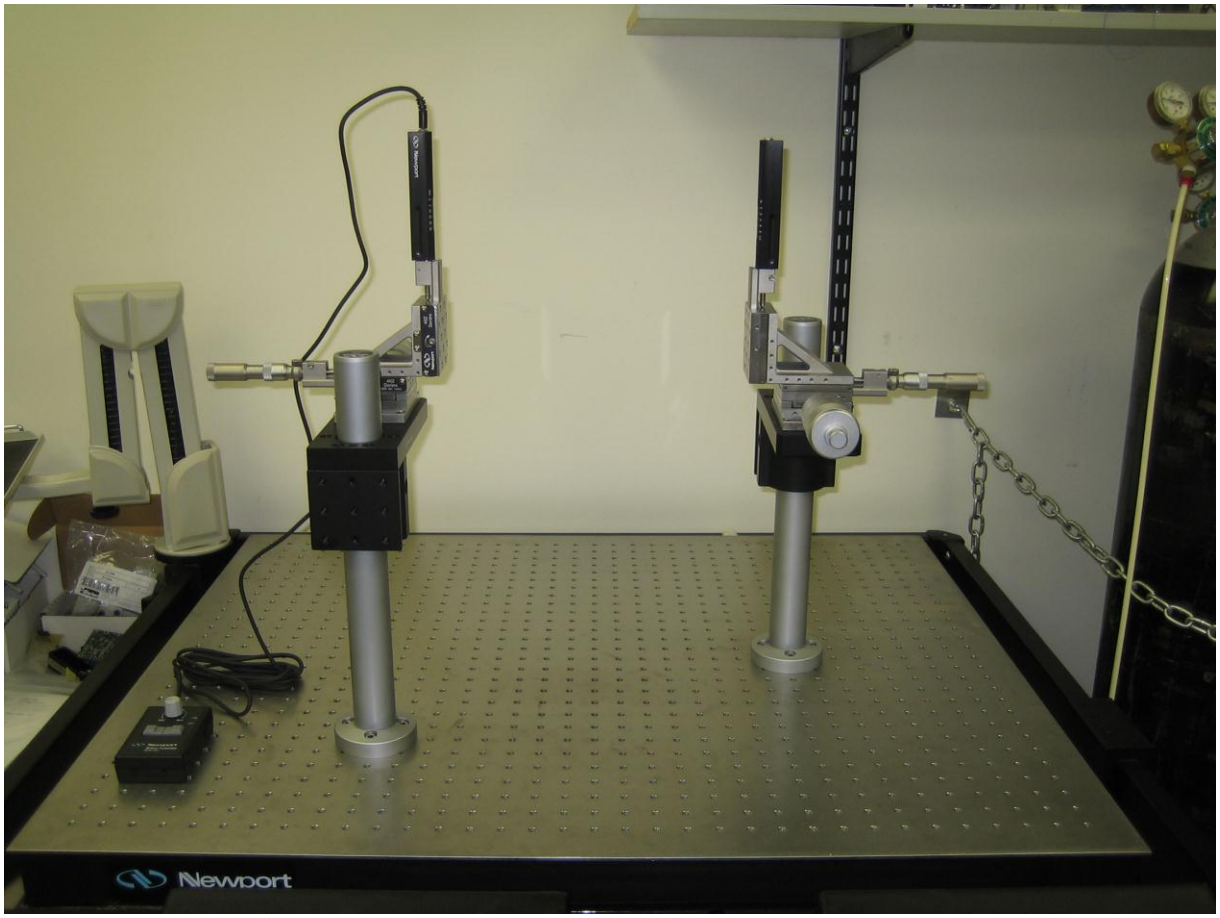

Photo 4 XYZ Translation Stages Mounted to Vibration Table

Photo 3 shows the XYZ stages mounted to a vibration isolation table. The inverted microscope fits between the two stages. The Force Transducer boom mount should be fastened to the left XYZ and the Length Controller boom mount to the right XYZ. Screws and an Allen key are provided to attach the boom mounts to the stages.

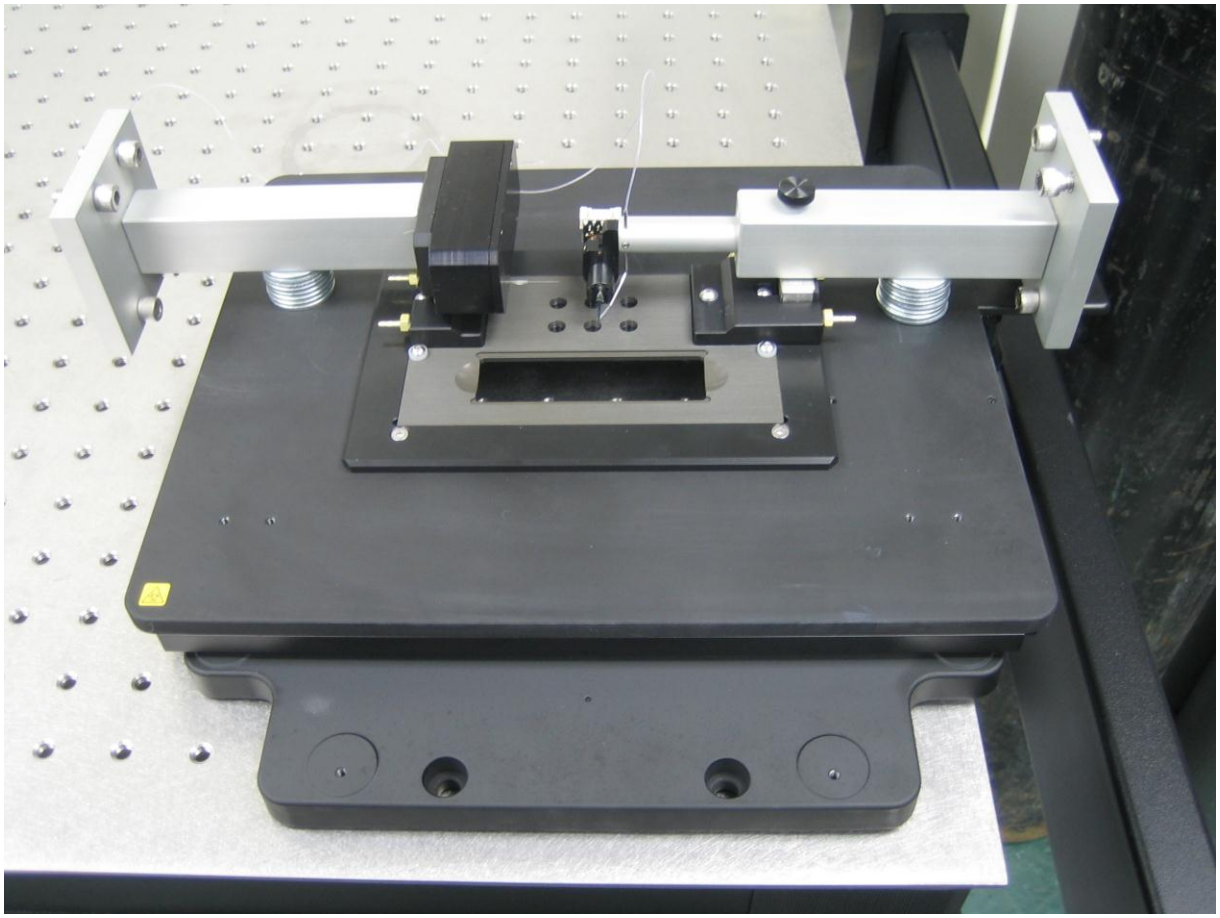

Photo 5 Location of Boom Mounts Relative to 803B

Photo 4 shows the boom mounts in their relative position with respect to the microscope stage and the 803B apparatus. It may be necessary to adjust the position of the XYZ platforms prior to attaching the boom arms. Likewise it will be necessary to make fine adjustments to the XYZ stages once the force transducer and length controller are mounted to the booms.

2.6 Attaching the Force Transducer and Length Controller

The Force Transducer is attached to the left boom mount with the 4-40x¼" socket head cap screws provided. The output tube of the transducer should be located near the bottom and towards the front.

The 312C Length Controller is attached to motor mount provided on the end of the right boom mount. To install the motor remove the lower clamping screw from the motor mount. Rotate the motor so that the arm lines up with the slot in the clamp ring and then slide the motor into the ring. Now re-install the ring clamp screw and tighten the screw to clamp the motor in place. To adjust the angle of the motor simply loosen the clamp ring screw, rotate the motor to the desired position and re-tighten the screw. Once the motor and force transducer are mounted you can reposition the XYZ stages as required and plug the T-type thermocouple into the connector on the front of the 825A.

Once the motor and force transducer are attached it may be necessary to change the distance between the force transducer and the motor. Large changes are best made by loosening the thumb screw on the motor boom and sliding the motor mount rod in or out of the boom. Once the correct position is determined re-tighten the thumb screw. After coarse adjustments have been made the XYZ stages are then used for fine positioning of the motor and force transducer.

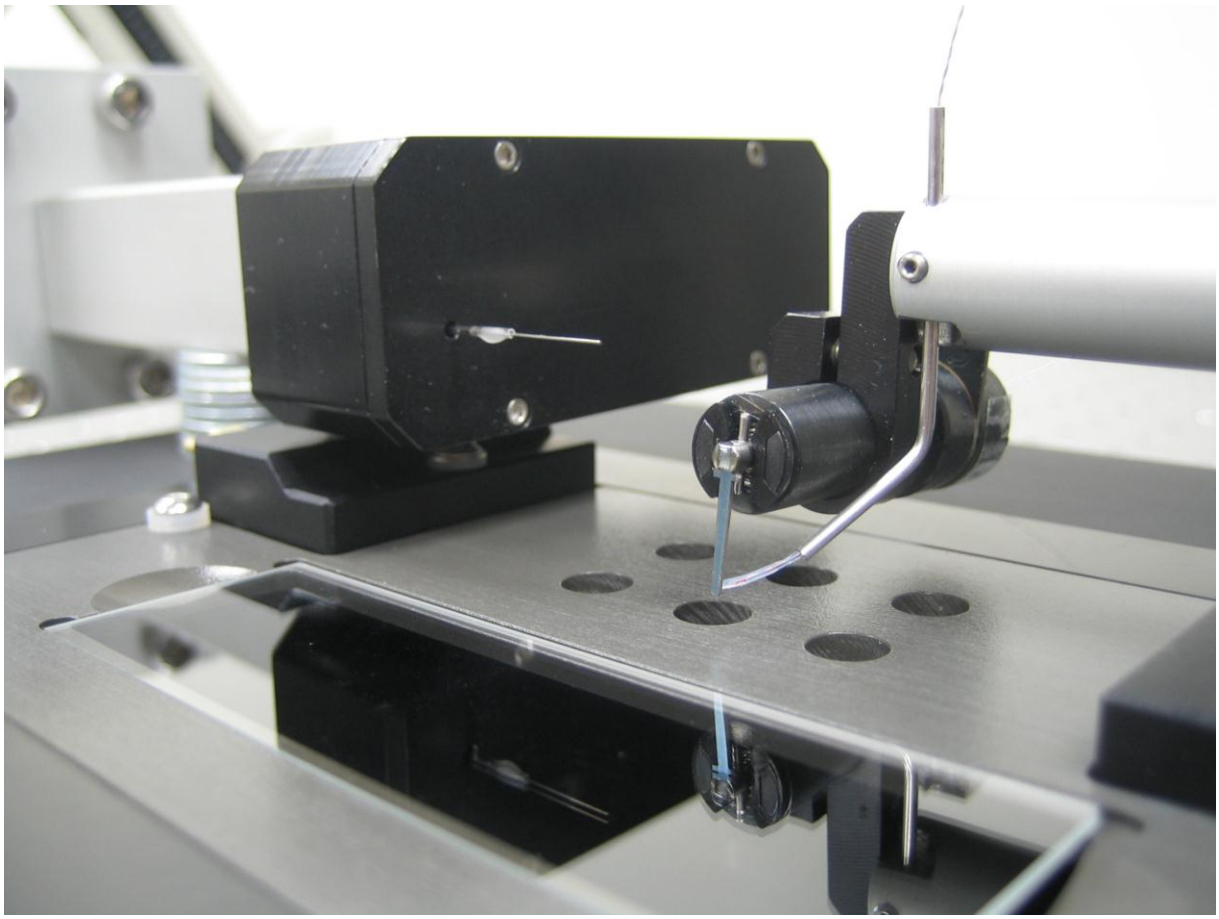

Photo 6 Close up of 312C, Force Transducer and Thermocouple

3.0 Using the 803B

3.1 Attaching a Probe to the Force Transducer

A probe must be built and attached to the force transducer output tube prior to use. Normally most researchers fashion the probe using a pipette puller. For the 803B the probe must exit the force transducer horizontally and then angle downwards to the location of the myocyte. The output tube of the force transducer is a glass tube with an inside diameter of about 0.47 mm (0.018"). If the pipette is this diameter or less than it can be slipped into the output tube and held in place with paraffin wax, shellac or a similar material. We recommend using wax since it is easy to simply warm the wax to the melting point and then the probe can easily be removed. Using permanent glues such as cyanoacrylate or epoxy can make it very difficult to change the probe without breaking the output tube or the force transducer sensor.

We strongly recommend that the force transducer electronics be on whenever attaching or removing anything from the transducer. This allows the user to monitor the output of the transducer and keep their applied loads during attachment/removal under the full scale load of the transducer.

3.2 Attaching a Probe to the Lever Arm

The 312C lever system will also require a probe attached to it. This probe should be kept as small, short and light as possible. The 312C is very sensitive to added mass on the arm so great care must be taken to create a probe of minimal mass. Stainless steel wire or tubing can be used for the probe. Likewise titanium wire could be used or even a pipette pulled to a small size. If the probe needs to be replaced for each cell then it should be attached to the arm with melted shellac as paraffin wax is not strong enough to withstand the forces resulting when the motor accelerates at its maximum rate. If possible it is recommended that a permanent probe be attached to the arm with the minimum amount of epoxy that will hold the probe in place. The lever arm can be situated above the well in the droplet above the plate and this allows the probe to be positioned perpendicular to the arm and positioned horizontally. A probe 3 mm or shorter can easily be fashioned. It is also recommended that the probe be formed in an "L" shape with the vertical portion of the L lying along the length of the lever arm and the short portion of the L pointing horizontally into the droplet.

3.3 Attaching a Cell

The 802B was designed so that the cell is attached in the mounting area and then transferred to one of the 6 wells located in the rear portion of the myocyte plate.

Researchers have various methods for attaching myocytes to the lever arm and force transducer. The two primary methods involve gluing the cell to the probe using either Great Stuff (an insulating foam sealant made by The Dow Chemical Company and available in the USA and Canada at hardware and building supply stores) or silicone glue.

Most methods involve laying a very thin line of the glue across a portion of the microscope slide in the mounting area. The probe attached to the force transducer is then positioned just above the slide to one side of the glue line. The XYZ stages holding the

probe are moved in such a manner as to cause the probe to pass through the glue line. This places a small amount of glue on the tip of the probe. The probe is then moved around the mounting slide until a cell is found. Translating the probe into the cell will cause the cell to attach to the glue on the end of the probe. Once one probe is attached then the other probe is moved into position on the opposite side of the cell. The second probe is brought into contact with the cell and then the two probes are left in this position until the glue is set.

When the glue is set the cell is transferred to one of the 6 wells by moving the microscope's XY stage.

3.4 Adjusting the Resting Tension or Sarcomere Length

Once the cell is attached the resting tension, initial cell length or initial sarcomere length can be adjusted by either moving the Y-axis translation stage for either the force transducer or the motor mount. Another method is to use the length controller to adjust the fiber length/force. Use the front panel Length Offset control to change the position of the lever arm and thus the initial length/force. The digital panel meter on the front of the 312C 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.

4.0 Warranty

The 803B Myocyte 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.