

# **INSTRUCTION MANUAL**

**Models 802D**

**Permeabilized Fiber Test Apparatus**

April 16, 2009, Revision 0

Copyright © 2009 Aurora Scientific Inc.

**Aurora Scientific Inc.**

360 Industrial Parkway S., Unit 4  
Aurora, Ontario, Canada L4G 3V7

Tel: 1-905-727-5161

Toll Free: 1-877-878-4784

Fax: 1-905-713-6882

Email: [info@AuroraScientific.com](mailto:info@AuroraScientific.com)

Web Site: [www.AuroraScientific.com](http://www.AuroraScientific.com)

## Table of Contents

Table of Contents .....	1
List of Figures .....	2
List of Tables .....	2
1.0 Introduction .....	3
1.1 Specifications .....	6
2.0 Apparatus Setup .....	7
2.1 Unpacking .....	7
2.2 Instrument Checkout and Positioning at Bath 1 .....	7
2.3 Attaching the 802D to an Inverted Microscope .....	9
2.4 Attaching the XYZ Micrometer Translation Stages to the 802D .....	10
2.4.1 Mounting the Motor Mount XYZ Translation Stage .....	10
2.4.2 Mounting the Force Transducer XYZ Translation Stage .....	12
2.5 Attaching the Motor .....	12
2.6 Attaching the Force Transducer .....	14
2.7 Attaching the Cooling Water Lines .....	14
2.8 Thermoelectric Controller .....	15
2.9 Replacing the Cover Slip .....	16
3.0 802D Bath Controller Software .....	17
3.3 File Menu .....	19
3.4 Sequencer Menu .....	20
3.5 Setup Menu .....	22
Run Mode .....	23
3.6 600A Menu .....	23
3.7 Help Menu .....	24
802D Help .....	24
About .....	24
3.8 Creating and Editing Sequences .....	25
3.9 Error Codes .....	30
4.0 Using the 802D .....	31
4.1 Adjusting the Location of the Force Transducer .....	31
4.2 Adjusting the Location of the Lever Arm .....	31
4.3 Attaching a Muscle Fiber to the 802D .....	31
4.4 Adjusting the Resting Tension or Sarcomere Length .....	31
4.5 Measuring the Fiber Length and Cross-section .....	32
4.6 Point-to-Point Movement .....	32
4.7 Sequencer Controlled Movement .....	32
Appendix A – Reversing the Direction of Models: 312C, 315C, 322C .....	33
Appendix B – Fiber Attachment Methods .....	35
Appendix C – Fabrication of Needles for Tying Attachment Method .....	37

## List of Figures

Figure 1	802D Apparatus with 825A TEC Controller and 826A Water Cooler.....	3
Figure 2	Main Window of 802D Bath Controller Software .....	4
Figure 3	Sequencer Window of 802D Bath Controller Software .....	5
Figure 4	802D Apparatus on Bench Stand.....	8
Figure 5	Correct Positioning of 802D Apparatus with respect to Microscope Cutout .....	10
Figure 6	Close-up of Length Controller and Force Transducer in Bath 1 .....	13
Figure 7	Main Window of 802D Bath Controller Software .....	17
Figure 8	Setup Drop Down Menu.....	19
Figure 9	File Drop Down Menu.....	19
Figure 10	Sequencer Drop Down Menu .....	20
Figure 11	Sequencer Window showing Test Sequence Tab.....	20
Figure 12	Select File Window used by the Open Sequence Command.....	21
Figure 13	Setup Drop Down Menu.....	22
Figure 14	Offset Adjustments Window .....	22
Figure 15	600A Drop Down Menu .....	24
Figure 16	Help Drop Down Menu .....	24
Figure 17	About Window .....	25
Figure 18	Sequencer Window showing Test Sequence Tab.....	25
Figure 19	Sequencer Window showing Comments Tab.....	26
Figure 20	Sequencer Window showing Disabled Steps in Sequence .....	27
Figure 21	Sequencer Window showing Disabled Timing for Steps 3 and 6 .....	28
Figure 22	Sequencer Waiting for User to Click Next Step.....	28
Figure 23	Scroll Bar Positioned to Show End of Sequence.....	29
Figure 24	Sequencer Comments Tab .....	30

## List of Tables

Table 1	Error Codes .....	30
---------	-------------------	----

## 1.0 Introduction

The 802D permeabilized fiber test apparatus was designed to enable physiology researchers to easily test permeabilized fibers with an ASI model 315C or 322C high-speed length controller and an ASI series 400A force transducer.

The 802D consists of an 8-well aluminum bath plate that includes right-angle prisms on either side of bath 1 and a glass bottom on all 8 wells, 2 TEC heater/coolers, water-cooled heat sink plate, 2 stepper motors for up/down movement and bath-to-bath indexing, micro-processor based electronic control system, software control program (for Windows and Linux), high-speed length controller motor mount, 400A series force transducer mount and micrometer drive XYZ translation stages for motor and force transducer positioning. Also included are mounting screws for the force transducer and a set of Allen keys. The 802D comes complete with a model 825A TEC Controller/Thermocouple Thermometer, a T-type thermocouple for measuring the solution temperature in the bath and a model 826A Water Cooler to control the temperature of the heat sink built into the 802D. All parts are manufactured from corrosion resistant materials (anodized aluminum, stainless steel and Delrin).



**Figure 1 802D Apparatus with 825A TEC Controller and 826A Water Cooler.**

The 802D works by moving the bath plate relative to the fiber. The fiber is clamped between the output tube of the force transducer and the lever arm of the high-speed length controller. In operation the researcher selects the desired bath by either clicking on the bath number on the front panel of the control software, by programming the control software to automatically step between baths or by inserting a bath move command into the test protocol

generator of either our 600A control software or our DMC control software. When a new bath is selected the bath plate moves downwards and then indexes to the desired bath. When it arrives at the new position the plate automatically rises to the operating position. The control software includes a digital display that shows the current bath position and the up/down status of the bath plate. The bath-to-bath index time is approximately 1.2 seconds between adjacent baths and approximately 2.5 seconds from one extreme to the other. This time includes the time required to lower the bath, move to the new position and then raise the bath plate to its working position.

The bath plate is manufactured from aluminum and has eight baths machined in its upper surface. This number allows for test protocols involving multiple solutions without the need to empty and re-fill baths. All baths have a glass bottom to allow for microscope observation of the fiber or video or laser-based sarcomere length measurement. Bath 1 is larger than the rest as it was designed to be the bath in which the fiber is attached to the force transducer and length controller. Bath 1 also includes right angle prisms on both sides of the bath to allow the side of the fiber to be viewed using the microscope. One prism is oriented for observation from above and the other for observation from below.

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. The 802D accurately controls the position of the bath plate with respect the length controller and force transducer. Before using the 802D the researcher must adjust the position the lever arm and force transducer relative to the plate. The bath-to-bath positional accuracy is better than  $\pm 0.05$  mm ( $\pm 0.002$ " ) in both the x (horizontal) and z (vertical) directions.



**Figure 2 Main Window of 802D Bath Controller Software**

The 802D apparatus is connected to a control computer with a USB cable. The apparatus is controlled by the 802D Bath Controller software running on the control computer. Both a Linux and a Windows version of the control software are available. Since most users of the 802D also have an ASI 600A Digital Controller we will focus on the Linux 802D Bath Controller software in this manual. Both software products perform the same functions with only minor differences in the layout of the main window. A separate instruction manual is included with the Windows software.

The Bath Controller software features a programmable motion control sequencer. This sequencer allows the researcher to automate the movement of the bath plate. The sequencer program is easy to use and consists of a multi-step program that has two variables for each step. The first variable sets the desired bath and the second sets the amount of time to wait before proceeding to the next step. A repeat command is also provided. Pressing the Start Test button on the main window starts the sequencer. A Stop Test and a Pause button are also provided to stop/pause the sequence program at any time.

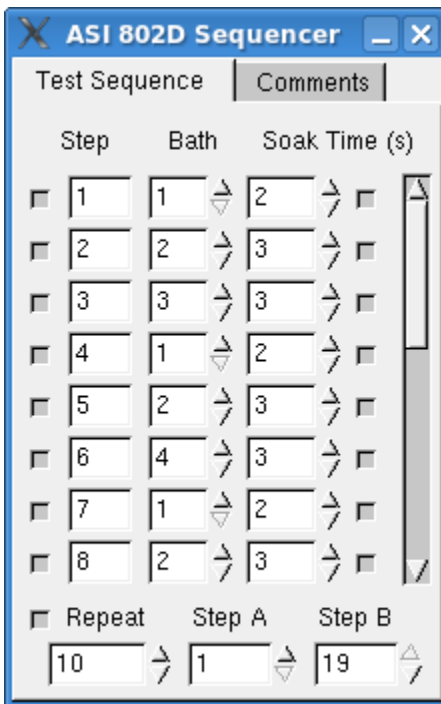


Figure 3 Sequencer Window of 802D Bath Controller Software

The temperature of the 802D bath plate can be controlled from 4 to 38°C by use of the included 825A temperature controller and the built in Peltier temperature control modules. The temperature of the plate is measured by a temperature sensor located on the bottom of the bath plate and liquid temperature is measured by the included T-type thermocouple.

## 1.1 Specifications

### Bath Plate

No. of Baths:	8
Bath Plate Material:	6061-T6 Aluminum
Right-Angle Prisms:	3x3x6mm, aluminized hypotenuse with incolnel overcoat and black paint overcoat.

### Bath Dimensions:

#### Large Volume Bath Plate:

Bath 1:	14mm (0.551") L x 5mm (0.197") W x 3mm (0.118") D
Baths 2 – 8:	14mm (0.551") L x 4mm (0.157") W x 3mm (0.118") D

#### Small Volume Bath Plate:

Bath 1:	14mm (0.551") L x 5mm (0.197") W x 3mm (0.118") D
Baths 2 – 8:	14mm (0.551") L x 3mm (0.118") W x 3mm (0.118") D

### Bath Volume:

#### Large Volume Bath Plate:

Bath 1:	198 $\mu$ l
Baths 2 – 8:	158 $\mu$ l

#### Small Volume Bath Plate:

Bath 1:	198 $\mu$ l
Baths 2 – 8:	120 $\mu$ l

### Centerline Distance Y-axis:

Bath 1 – 2:	8 mm (0.315")
Baths 2 – 6:	6 mm (0.236")

### Angular Movement, Z-axis:

3.0°

### Excursion, Z-axis:

Max. (@ Force Transducer end of Bath):	4.72 mm (0.186")
Min. (@ lever end of Bath):	3.35 mm (0.132")

### Indexing Time:

Bath 1 – Bath 2:	1.2 seconds
Adjacent Baths in range 2-8:	1.1 seconds
Bath 1 – Bath 8:	2.5 seconds

### Thermoelectric Coolers

No. of TECs:	2
Power:	33 W
Voltage:	12 V
Temperature Sensor:	AD590

### Thermocouple Microprobe

Model:	Physitemp IT-18
Temp. Range:	-273 to +150°C
At tip:	-492 to +300°F
Time constant	0.1 seconds
Sensor:	Type T Thermocouple Copper-Constantan
Sheath:	0.025" (0.064mm) diameter. Teflon with sealed tip.

### Stepper Motor

No. of Motors:	2
Voltage:	12 V
Movement:	25 $\mu$ m (0.001")/step
Warm Up Time:	30 seconds to rated accuracy
Power Requirements:	100–240VAC $\pm$ 10%, 50/60Hz, 2 amps max.

## 2.0 Apparatus Setup

### 2.1 Unpacking

Unpack the apparatus from the three shipping boxes. The first box contains the 802D apparatus complete with its stand. The second contains the 825A Temperature Controller and an accessories kit which includes force transducer mounting screws and Allen keys. The third box contains the 826A Water cooler complete with water hoses and the 802D power supply. The 802D is shipped assembled however the researcher must attach the high speed length controller, force transducer, the control cables and the water lines. It is suggested that you keep the shipping foam and box for the 802D just in case you ever need to ship it to another location or back to the factory.

Cut and discard the tie wraps that hold the apparatus onto its stand and those that hold the bath plate to the motor mount. Leave the apparatus on the stand.

Do not move the bath plate by hand as this can damage the mechanism used to position the plate.

The 802D is shipped with a glass cover slip mounted to the bottom of the bath plate. A box of spare cover slips and some high vacuum grease are included in the accessories kit. The cover slip is held onto the bottom of the bath plate using a small amount of high vacuum grease. If the cover slip has been damaged in shipping or is missing you will need to replace it with a fresh slip. Please refer to section 2.9 for instructions on how to replace the cover slip.

### 2.2 Instrument Checkout and Positioning at Bath 1

Prior to mounting the apparatus on a microscope stage or to attaching the motor and force transducer it is recommended that you check the operation of the 802D. During this checkout you will also reposition the bath plate to the Bath 1 position.

Before proceeding you will need to install the control software on the PC. If you have purchased an ASI model 600A with your 802D then the software will be pre-installed on the computer and you are ready to proceed. If the 802D is to be used with a 600A that you already own or with a Windows PC then you will need to install the software as described in chapter 3 of this manual.

Place the 802D and its stand on a lab bench. Connect the 3m (10 ft) long USB cable supplied with the apparatus to the PC and to the 802D. Plug the 802D power supply into an appropriate AC power outlet and then into the mating connector on the 802D. At this point it is not necessary to attach the water or power lines for the thermoelectric controller (TEC) since we are only performing a preliminary checkout of the 802D and won't be temperature controlling the bath plate at this time. Please note however that the TEC controller should never be used with the apparatus if water is not flowing through the heat sink plate. Our aim at this point is to position the bath plate at the bath 1 location prior to attaching the motor and force transducer.

Turn the power on using the power switch located on the end panel of the 802D next to the power connector. The power LED on the 802D should light. Open the 802D control program on the PC. The LED on the 802D will flash when the PC program connects with the apparatus. Note: in some cases the apparatus may move to the nearest bath location when the

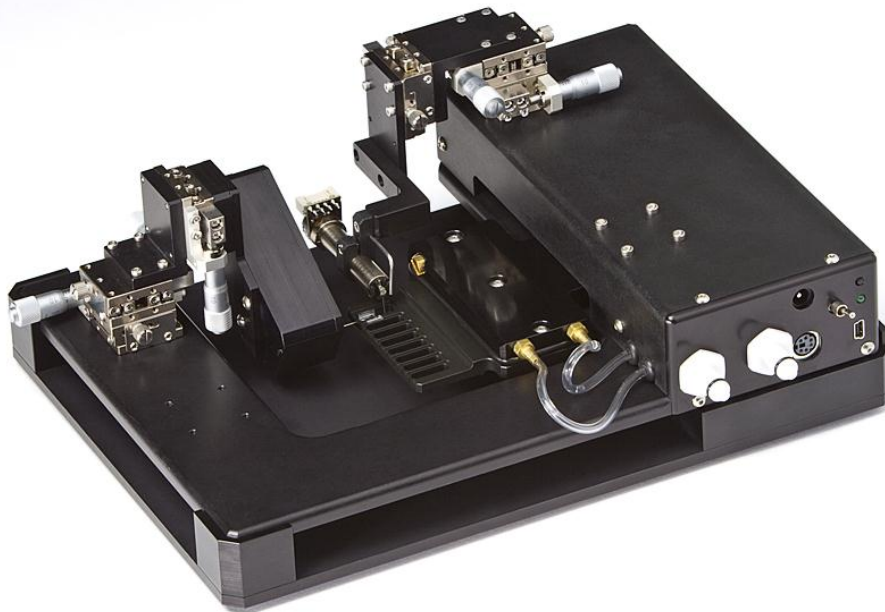


power is switched on, this is normal. The software indicates the up/down status of the bath plate and also the current bath location.

It is important that the apparatus be repositioned prior to alignment of the force transducer and length controller. This repositioning ensures that the apparatus is in the correct location. To reposition the apparatus simply click on the bath 1 on the front panel of the control software. The bath plate should lower, move to bath 1 and then rise. Once this positioning is complete the apparatus is in its home (bath 1) location and you can proceed to attach the motor and force transducer. Note: if the system stops at any other bath then simply click on the bath 1 button again and the system should move to bath 1. Try clicking on other bath buttons and observe the motion of the bath plate.

You will also note that the control software has an Up/Down switch and a Setup/Run switch. The Up/Down button simply moves the plate up or down without repositioning the plate to another bath location. The Setup/Run switch controls the operating mode of the 802D. In Setup mode the bath will not rise at the end of a movement. In Run mode the bath plate will automatically rise at the end of each movement. Try pressing a bath number when the Setup/Run switch is in both positions and observe the difference.

A manual up/down switch is located on the 802D apparatus on the opposite end panel from the power switch. The up/down switch is a spring loaded toggle switch that can be moved either up or down. When pressed up the switch will cause the bath plate to move up slowly. When pressed down the switch will cause the bath plate to move down slowly. The plate will remain at the location it is set to until the up/down switch is pressed again or until a bath movement is made. Further details of the controller software are provided in chapter 3. When you have finished testing the operation return the apparatus to the bath 1 position by clicking on the bath 1 button.



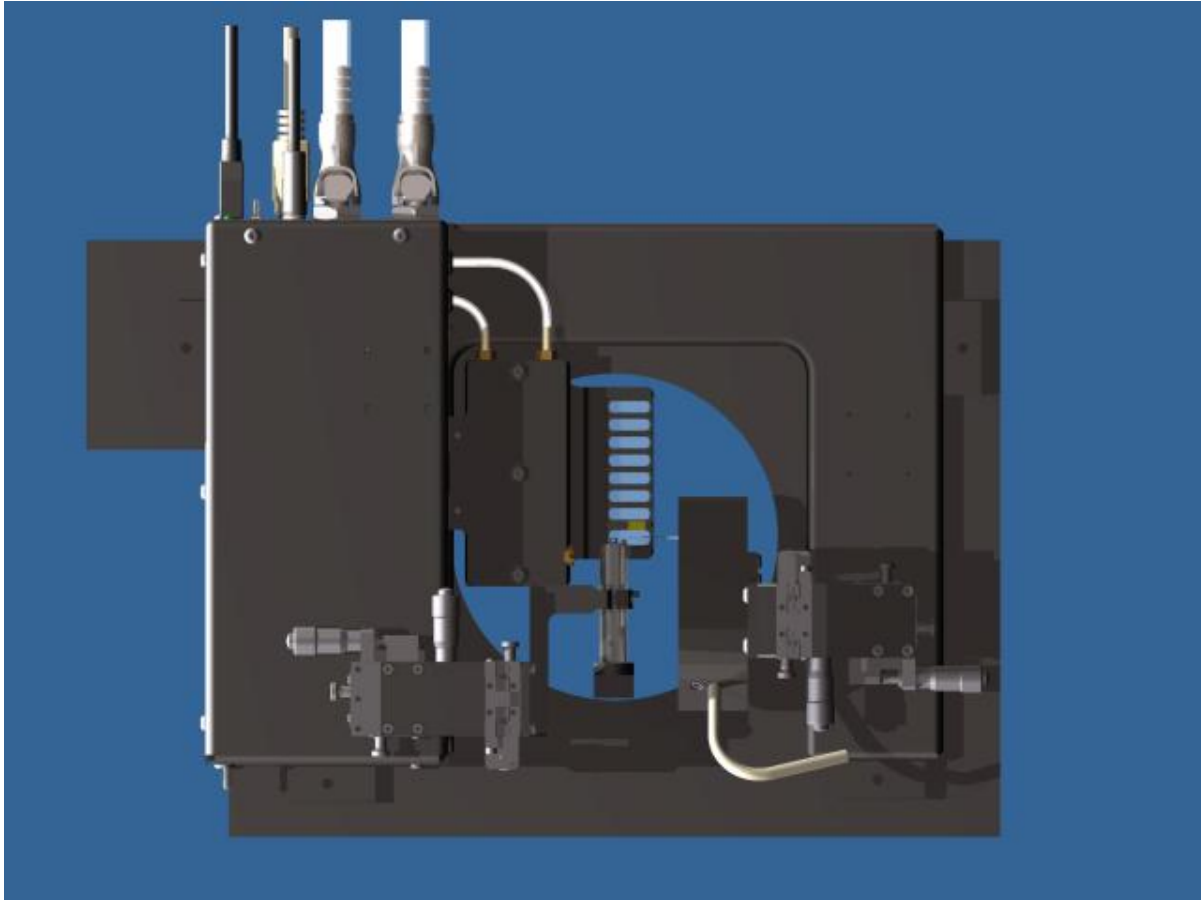
**Figure 4 802D Apparatus on Bench Stand**

### 2.3 Attaching the 802D to an Inverted Microscope

The 802D is supplied with a bench stand. The apparatus should be stored on this stand when not in use and when it is used with a dissection microscope. You will notice that the force transducer protrudes below the lower level of the 802D and when the bath plate lowers it also moves below the lower level of the apparatus. For these reasons it is important that the apparatus be stored on its stand and not be allowed to rest on the force transducer or the bath plate.

When used on an inverted microscope the apparatus must be removed from the stand and placed on the XY stage of the microscope. Please note that the microscope stage must have a minimum cutout that measures 100mm (3.94in) diameter. This cutout is required for clearance of the bottom of the force transducer and for clearance of the bath plate when it lowers and moves.

First remove any cutout inserts from the microscope stage and then carefully place the apparatus on the microscope stage ensuring that there is sufficient clearance for the force transducer to protrude down into the cut out. Please see Figure 5 for correct positioning of the 802D with respect to the stage cutout. Use the PC software to move the bath plate from the bath 1 location to bath 6. Observe the plate while it is moving and ensure that the bath plate doesn't contact the cutout on either end of the plate's range of motion. Adjust the mounting location of the apparatus with respect to the stage cutout if required and then use the software to move the bath plate to bath 7. Once again check the clearance and adjust the mounting location if required. Finally use the software to move the bath plate to bath 8 and once again check the clearance. Also make sure you check the clearance between the force transducer and the edge of the cutout. Use the software to move the plate back to bath 1 and ensure there is no interference. Once the correct position for the apparatus has been determined place the supplied alignment bars on the microscope stage using the double sided tape attached to the back of the bars. These alignment bars will aid in correctly positioning the apparatus whenever it is placed on the microscope. The apparatus has rubber mounting feet on the bottom surface that provide sufficient grip so that it is not usually necessary to clamp the apparatus to the microscope stage.



**Figure 5 Correct Positioning of 802D Apparatus with respect to Microscope Cutout**

## **2.4 Attaching the XYZ Micrometer Translation Stages to the 802D**

The 802D is shipped with the motor and force transducer XYZ stages pre-installed in the default location shown in Figure 4. If this configuration is satisfactory then skip to section 2.5 in the manual for instructions on mounting the motor and force transducer. If for access, fiber mounting or other reasons the location of the motor and/or force transducer needs to be changed then this section will provide details of how the XYZ stages can be moved.

In this manual we will define the X-axis to be the horizontal direction that would shorten or lengthen a fiber; the Y-axis is the horizontal direction in which the bath plate moves and the Z-axis to be vertical direction.

Two XYZ micrometer translation stages are included with the 802D to which the force transducer and the high speed length controller are attached. The force transducer stage includes a Delrin mount plate that electrically and mechanically isolates the force transducer from the rest of the apparatus. A motor mount and a transducer mount are also included and are attached to the XYZ stages when shipped.

### **2.4.1 Mounting the Motor Mount XYZ Translation Stage**

The motor XYZ stage can mount at two locations on the top surface of the apparatus main cover. The best location can be determined by taking into account the required clearance between the apparatus and the microscope, the researcher's preference when

attaching the fiber, the location of other equipment and required access to the baths. The location closest to Bath 1 positions the motor such that the bulk of the motor is off the Bath 1 end of the plate. This position has the advantage of leaving all 8 baths open when the bath plate is positioned at Bath 1. Since Bath 1 is designed as the attachment bath and therefore usually it will contain a relaxing solution it is advantageous to have easy access to all of the other baths while the fiber is resting in the relax solution. This is the default “as shipped” configuration of the 802D. If a high speed length controller was purchased along with the 802D then the length controller will have been factory set to ensure that it moves in the correct direction (a positive control signal to the length controller must always result in a lengthening of the fiber). If an ASI length controller was purchased separately then be aware that you may need to change the direction of operation of the motor in order to use it at the Bath 1 end of the apparatus. Please see Appendix A for instructions on reversing the direction of motion of an ASI High Speed Length Controller.

The alternate mounting location for the motor positions it closest to the Bath 8 end. This location is useful if flash photolysis of caged compounds is being performed as it provides clear access for the flash lamp to the Bath 1 end of the plate. However the disadvantage of this motor location is that when the plate is located at bath 1 the bulk of the motor is located above the other baths thus limiting access to the baths when changing solutions. As with the other mounting location it is important to check the direction of movement of the lever arm and change the direction if necessary. Four M2 tapped holes are provided at each mounting location. When shipped the unused holes have screws in them, simply remove these screws prior to re-locating the motor XYZ stage.

In order to reposition the XYZ stage the Z-axis stage must first be removed in order to gain access to the mounting screws in the X-axis stage. Remove the right-angle bracket, along with the Z stage, motor mount and motor, from the Y-axis stage by removing the four M2 socket head cap screws. Unlock the stage lock screws and turn the X-axis micrometer counter clockwise to uncover the mounting hole in the base of the stage. Remove the two M2 socket head cap (SHC) screws. Push the stage with your finger in the opposite direction to uncover the other screws. Remove these screws and then relocate the X and Y stages to the new mounting location. Reinstall the four screws in the same manner as used to remove them. Ensure that the stage is aligned perpendicular to the front edge of the cover and then firmly tighten the screws. Prior to re-attaching the Z-axis right-angle bracket you must first remove the L bracket from the vertical mounting plate, rotate it 180 degrees and re-attach it to the mounting plate. The motor clamp ring must also be rotated 180 degrees. To mount a 315C motor remove the screw clamping the ring tight around the motor. Rotate the motor so that the arm lines up with the slot in the clamp ring and then slide the motor out of the ring. Now re-insert the motor back through the opposite side of the ring and re-install the ring clamp screw. For a 322C motor remove the arm, then loosen the clamp screw, remove the motor from the clamp. Now re-insert the motor back through the opposite side of the ring and tighten the clamp screw. Re-attach the lever arm. Once the motor direction has been changed the Z-axis bracket can be re-attached to the Y stage. Align the bracket with the Y-axis stage and tighten the screws. Note: it may be necessary to adjust the micrometers on the X and Y stages in order to properly align and mount the stages.

#### 2.4.2 Mounting the Force Transducer XYZ Translation Stage

The force transducer XYZ stage can also be mounted at two locations. One location is at the bath 1 end of the plate and the other is towards the bath 8 end. Four M2 tapped holes are provided in the aluminum plate at each location.

Remove the Z-axis right-angle bracket from the Y-axis stage by removing the four M2 socket head cap (SHC) screws. Unlock the stage lock screws and turn the X-axis micrometer counter clockwise to uncover the mounting screws in the base of the stage. Remove the two M2 screws and then push the stage with your finger in the opposite direction to uncover the other mounting screws and remove them. Re-position the XY stages at the new mounting locations and re-install the mounting screws. Align the stage to be perpendicular to the outer edge of the 802D base plate and then firmly tightened the screws.

It is normally easier to mount the Z stage to the XY stages before attaching the transducer mounting plate. Ensure that you align the bracket with the Y-axis stage and tighten the screws. Note: it may be necessary to adjust the micrometers on the Y or X-axis stages to properly align the stages. It is usually more convenient to attach the transducer to the mount plate before attaching the plate to the Z stage. The transducer attaches to the transducer plate with 4-40 x 1/4" button head screws. The same type of screw is also used to attach the mounting plate to the Delrin mount on the Z stage, see section 2.6 for transducer mounting instructions.

### 2.5 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 (3.5 on the micrometer). The Z stage should be raised (to the 0 mark on the micrometer). Ensure that the 802D controller is powered and that the bath plate is located on bath 1 and that it is in the down position before attaching the motor.

In most cases it is easiest to attach the motor by first removing the motor mount clamp ring from the motor L bracket. The clamp ring is held on with a single 4-40 SHC screw. A second screw can be seen on the clamp ring which is used to tighten the clamp. The procedure for mounting the motor is dependent on the model of the motor you have. For a 312C or 315C motor completely remove the clamp screw and then orient the motor such that the lever arm lines up with the slot in the clamp. Carefully insert the motor into the motor clamp ring and once the arm clears the clamp you can rotate the motor so that the connector is at the top and the arm is pointing down (see figure 7). Reinsert the clamp screw and gently tighten the clamp screw until the motor is held in position. For a 322C motor simply insert the motor into the clamp before attaching the lever arm to the motor. Once inserted and clamped in position you can then attach the lever arm.

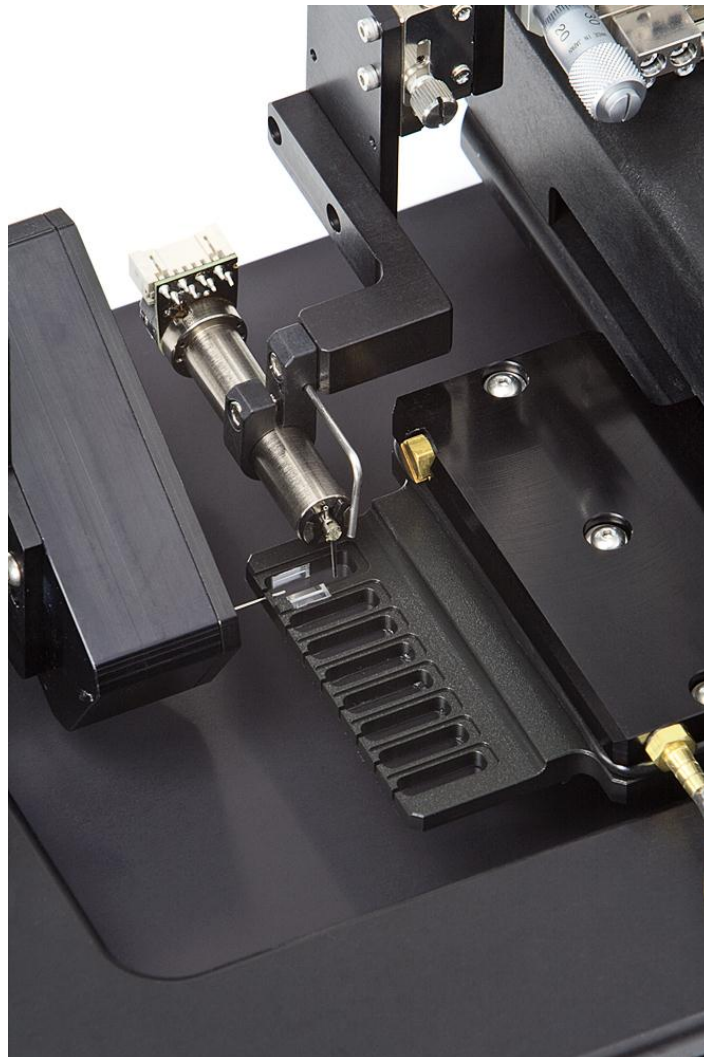
Re-attach the motor clamp ring complete with the motor to the mount. If desired the clamp ring can be rotated to allow the axis of the motor to be tipped. Some researchers prefer a tipped motor orientation as it provides a little more clearance for attaching the fiber and observing the experiment. The 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 bath 1. The X and Y stages may also require adjustment to properly locate the motor. Once the motor is in the desired position tighten both the ring clamp screw and the motor clamp screw. If the motor is not inserted at the correct location in the clamp the Y

stage will not have sufficient range of motion to centre the lever arm in the bath. Adjust as necessary. If the clamp is tipped at too great an angle the back of the motor may interfere with the bath plate. Once again readjust as necessary.

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.

The 802D Bath Controller software should be placed in Setup mode. Push the manual up/down switch on the side of the 802D up and observe the motion of the plate. The plate should rise slowly. Ensure that the plate doesn't touch the motor and that the motor cable doesn't interfere with the plate. Also ensure that the lever arm is not located too close to the side of the bath. The lever arm should be located near the centerline of the bath. If at any time you observe interference between the bath plate and anything else simply release the manual up/down switch and the bath plate will remain in that position. To lower the plate simply press the manual up/down switch down. Also check that when the bath is in the upper position that the lever arm doesn't hit the glass bottom of the bath.

Remember that bath 1 is wider than the rest so it is important to check for clearance in one of the other baths as well.



**Figure 6 Close-up of Length Controller and Force Transducer in Bath 1**

## 2.6 Attaching the Force Transducer

The 802D design calls for the force transducer to be outside of the bath with a fiber mounting hook/wire entering the bath chamber through a narrow vertical slot (700 microns (0.028”) wide) in the end of the bath. 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 exits the force transducer enter the slot. It will not fit and the transducer will be broken. See section 4.3 and Appendix B for information on building a mounting hook/wire and for information on fibre mounting.

As in section 2.5 ensure that the 802D controller is powered and that the bath plate is located on bath 1 and that it is in the down position before attaching the transducer. Refer to Figure 4 for correct orientation of the force transducer in the 802D apparatus. Note that there are two sets of mounting holes drilled in the plate. The upper set is used when the force transducer XYZ stage is located at the bath 1 end of the apparatus (as is seen in figure 4). The lower set of holes is used when the transducer XYZ stage is located at the bath 8 end of the apparatus. Use the four 4-40x1/4" button head screws included in the accessories kit to attach the force transducer to the mounting plate.

Position the X-axis translation stage as far from the bath plate as possible (6.5 on the micrometer dial). Position the Y-axis stage at about the 3.5 mark on the micrometer dial. Raise the Z-axis stage to near the top (0 on the dial). Attach 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 Y-axis stage. Attach the transducer to the disassembled Z-axis stage and then re-attach the bracket complete with the Z stage and the transducer.

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 1. Use a microscope to observe the needle and slot positions relative to each other. The controller should be powered and in Setup mode. Push the manual up/down switch upwards and observe the motion of the plate. The plate should rise slowly. Ensure that the needle is located in the center of the slot and that it doesn't touch the bath plate. 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 anything else simply release the manual up/down switch and the bath plate will stay in that position, then press the switch down to lower the plate.

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 802D base plate. Adhesive backed cable tie downs and cable ties are included in the accessories kit for this purpose. Cable movement can lead to increased noise on the length and force signals.

## 2.7 Attaching the Cooling Water Lines

On the connector end of the 802D 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 lengths of vinyl hose complete with mating

liquid connectors have been supplied along with the 826A Water Cooler. Follow the instructions in the 826A Instruction Manual to setup the water cooler. Connect both hoses to the mating connectors on the 802D apparatus. 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. Operating the thermoelectric coolers (TECs) without water flowing can damage the TECs.

## 2.8 Thermoelectric Controller

Two thermoelectric coolers (TECs) are built into the 802D. These devices are rated at 15 volts and 33 watts. A model 825A TEC Controller is 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 40°C with an accuracy of  $\pm 0.1^\circ\text{C}$ . 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 40°C with an accuracy of 0.1°C.

The 802D includes a temperature cutoff circuit to protect the TECs from overheating in the event of a failure with the 826A water cooler. If the heatsink plate on the 802D exceeds 50°C then the microprocessor in the 802D will open a relay and disconnect the TEC input cable from the TEC modules. When the heatsink plate temperature falls below about 45°C the relay will automatically be activated and the connection between the 825A and the TEC modules will be reconnected.

**Note: the relay is only on when the 802D is powered and the heat sink temperature is below 50°C. Therefore in order to control the plate temperature the 802D must be powered.**

After properly attaching the 825A and 826A to the 802D and powering the 802D you should note that the Process temperature should match the Setpoint temperature within about 3 minutes. Please be aware of the fact that the temperature of the liquid in the bath may take up to 7 minutes to stabilize after a large Setpoint change. It is normal for there to be a temperature offset between the AD590 temperature and the liquid temperature. This offset is greater the farther the Setpoint is from 20°C. The 825A includes a thermocouple meter and the apparatus includes a T-type Teflon coated thermocouple probe that can be used to monitor the temperature of the liquid in the bath. If you note that the temperature in the bath is not the desired temperature but the Process temperature is correct then you will need to readjust the Setpoint to account for the temperature offset. As an example it is common for the Setpoint to be at 39 or even 40°C in order to get a bath temperature of 37°C.

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.



## 2.9 Replacing the Cover Slip

The cover slip is attached to the bottom of the bath plate using high vacuum grease or Dow Corning 111 silicone grease or equivalent. A syringe of high vacuum grease is included in the accessories kit. Apply a small amount of grease to the bottom of the bath plate. Spread it thinly over the plate taking care to get some grease around the entire circumference plus between all of the baths. Take care to keep the grease out of the baths and avoid getting grease on the right angle prism located in bath 1. Place a #1 24x55 mm microscope cover slip (Fisherbrand 12-544-18 or equivalent) onto the bottom of the bath plate. A box of cover slips is included in the accessories kit. Gently press on the cover slip to have it adhere to the grease. If you have applied too much grease and it spreads into the baths then remove the cover slip and discard it. Clean the grease out of the baths and then apply a fresh cover slip to the bottom of the bath.

## 3.0 802D Bath Controller Software

### 3.1 Software Setup

If the 802D is purchased along with an ASI 600A Digital Controller then the bath control software will be pre-installed on the 600A PC. If you will be using the 802D with a Windows PC then please refer to the 802D Bath Controller Software for Windows instruction manual for details on installing and running the software. Both bath control software products are similar and therefore in this manual we will concentrate on the Linux based software.

Attach the 802D to the 600A PC using the supplied USB signal cable. Attach the 802D power supply to the 802D and plug it into an appropriate power outlet.

### 3.2 Starting the 802D Bath Controller Program

Power on the 600A PC and, when prompted, type in the user name and password, note: Linux is case-sensitive so ensure you use lowercase when entering the username and password.

Username: asi

Password: asi

Once the PC finishes the login process you will see an 802D Bath Controller icon on the Desktop. The icon will start the bath control software which is used to control the position of the 802D bath plate. Ensure that the 802D is powered before starting the 802D Bath Controller program you will note a power switch on the end panel of the 802D next to the power plug. It is important that the 802D be powered before starting the program because the program sends commands to the 802D when the program starts; if the controller doesn't respond then the software will automatically close. If this happens check that the power LED on the side of the 802D is lit and also check that the USB cable is attached at the 802D and at the PC. In some cases the program will fail to start if a USB Flash memory stick is attached to the PC. If the control program won't start try removing the USB Flash memory stick and try to start the program again.



Figure 7 Main Window of 802D Bath Controller Software

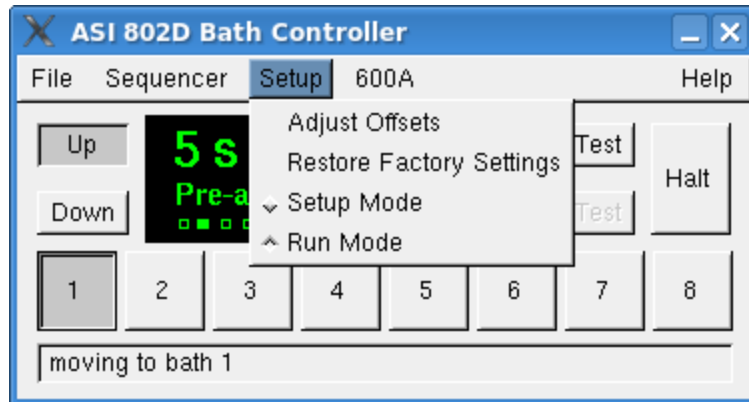
When the program starts the main window (Figure 7) will open. The main window consists of a drop down menu bar at the top of the window, a digital display in the centre, a series of buttons and a status line at the bottom of the window. The digital display shows the current bath position as a large numeral, a graphical indication of the current bath location (squares at the bottom of the display), a timer that indicates the amount of time in the current bath and a label that can be used to indicate the solution in the current bath or any other information that the user wants to associate with that bath location. Note the timer will only start if the bath is in the up position.

Below the display are buttons labeled 1 through 8 which can be used to relocate the bath plate to the desired bath. Bath 1 is used for attaching the fibre and usually contains relax solution. It is also the largest bath and it includes right angle prisms to allow the side of the fibre to be viewed. To the left of the display are Up and Down buttons that indicate the up/down status of the bath plate. These buttons can be used to manually raise and lower the bath plate but only when the plate is centered on a bath. To the right of the display are the Start Test, Stop Test and Halt buttons which are used to start and stop sequences and to halt any movement of the bath plate. At the bottom of the window is a status message area. At the top are the File, Sequencer, Setup, 600A and Help drop down menus. The main window can be repositioned and minimized but its size can't be altered. The program uses the normal Windows-type controls for manipulating the display window.

Motion control can be initiated by using the desired bath button on the software front panel. Simply click on the desired bath button and the bath plate will lower, move to the selected bath and then automatically rise back to the test position.

In addition to the normal point-to-point motion control the program also features a sequencer that allows a series of movements to be pre-programmed and then executed by a single click of the Start Test button. Each step in a sequence is defined by a desired bath location and a delay time at that bath. The sequencer also includes a repeat function. Details of programming the sequencer are given later in this chapter. A sequence can be initiated from any location and if the bath plate is not located at the first bath listed in the sequence then the bath plate will move to the first location and start the delay time associated with that bath. A sequence can be aborted by clicking on the Stop Test button. If for some reason you need to pause the sequence then the Pause button can be used. The sequence will remain paused until you start it again by pressing the Continue button.

The behaviour of the 802D depends on the Setup Mode (see Figure 8) that is accessed through the Setup drop down menu. When the software is in Setup Mode the bath plate will not rise at the end of a movement. In Run Mode the 802D will make complete movements consisting of lowering the plate (if it is up) indexing to the new location and then raising the plate to the operating position.



**Figure 8 Setup Drop Down Menu**

The accuracy of all movements is ensured by the use of a series of very accurate position sensors, one for each bath location plus one each for up and down control. By using position sensors at each location there are no cumulative positioning errors that can build up with successive movements. The position sensors change state from a logic HI to LO within 150 microns (0.006”) of travel. This very short actuation distance coupled with the method that the microprocessor uses to determine position results in the 802D being able to relocate itself on any bath within  $\pm 25$  microns ( $\pm 0.001$ ”) of the correct position. To further aid the positioning accuracy an anti-backlash control is built into the micro code. When moving from a higher bath number to a lower one the plate is indexed past the correct location and then moved back to the correct location. This removes any backlash present in the mechanical drive and increases accuracy.

The following sections provide details of the operation of all parts of the program.

### 3.3 File Menu

The File menu (Figure 9) only contains one item which is Quit. This is used to close the ASI 802D Bath Controller program.



**Figure 9 File Drop Down Menu**

### 3.4 Sequencer Menu

The Sequencer menu (figure 10) contains four sub menu items, Open, Close, Load Sequence and Save Sequence.

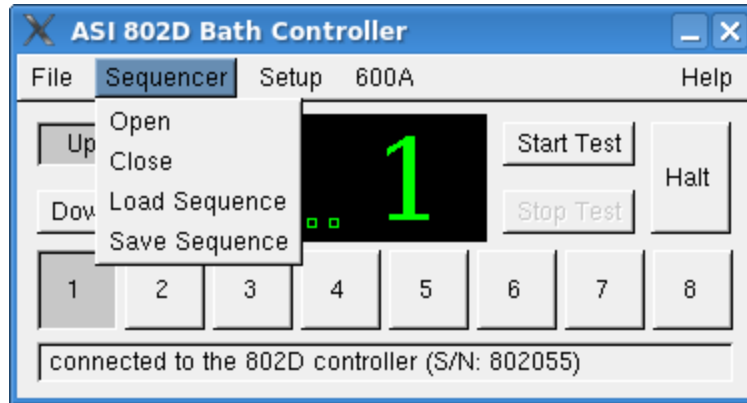


Figure 10 Sequencer Drop Down Menu

#### Open

The Open sub menu item is used to open the currently loaded sequence for editing. Clicking on the Open item will bring up the Sequencer window as seen in Figure 11. Please refer to section 3.8 for details on creating and editing sequences.

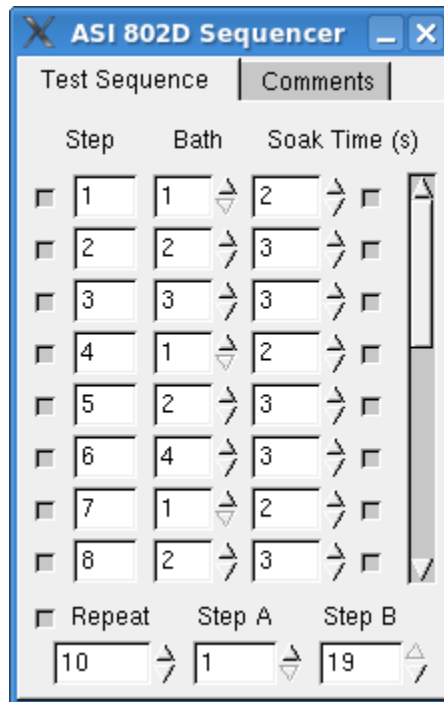


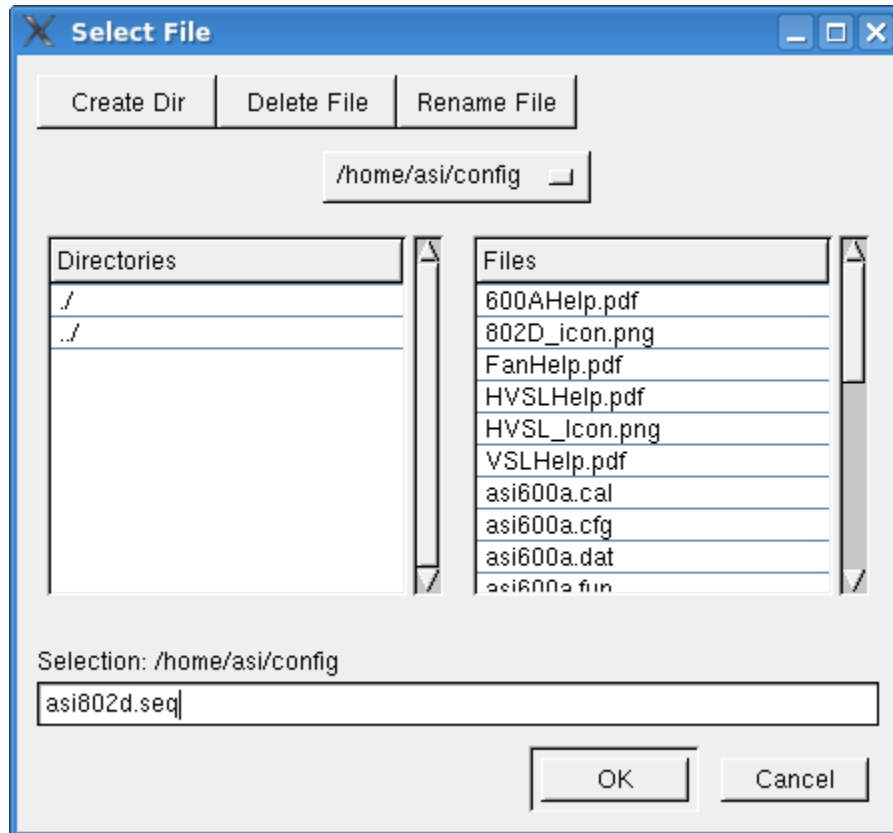
Figure 11 Sequencer Window showing Test Sequence Tab

#### Close

The Close sub menu item is used to close the sequence edit window. The Sequencer window can also be closed by clicking on the X in the upper right corner of the window.

#### Load Sequence

The Load Sequence sub menu item is used to load a previously stored sequence file from disk. Clicking on the Load Sequence item will bring up the Select File window as seen in Figure 12.



**Figure 12 Select File Window used by the Open Sequence Command**

The Select File window allows the user to create directories, delete files, rename files, select the sub directory of interest and to select the sequence file to be opened. In this example the directory is set to /home/asi/config and the file to be opened can be seen to be asi802d.seq. We recommend that you use a unique filename extension to designate 802D sequence files from ASI 600A sequence files. Note: Linux is case sensitive and therefore ASI802D.seq is a different file from asi802d.seq. Once the file of interest has been selected, press the OK button to close the Select File window and to load the selected sequence file. The selected sequence will be automatically opened for edit.

#### Save Sequence

The Save Sequence sub menu item is used to save an edited sequence file to disk. Clicking on the Save Sequence sub menu item will bring up the Select File window and allow the user to either select an existing file to be overwritten or type in a new file name in the Selection text entry box at the bottom of the window.

### 3.5 Setup Menu

The Setup menu (figure 13) contains four sub menu items, Adjust Offsets, Restore Factory Settings, Setup Mode and Run Mode.

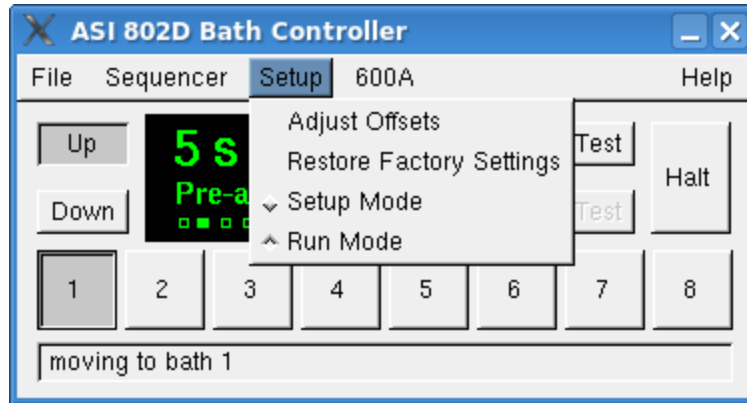


Figure 13 Setup Drop Down Menu

#### Adjust Offsets

The Adjust Offsets sub menu item is used to alter the offset associated with each bath location. Offsets are used to precisely position the bath plate so that the force transducer mounting needle is exactly in the center of the slot at the end of each bath. The Offsets are set at the factory and in most cases they will not need to be altered. However with time and wear it may be necessary to slightly alter the offset values to bring the force transducer needle back to the center of a particular bath. Clicking on the Adjust Offsets sub menu item will open the Offset Adjustments window as seen in Figure 14.

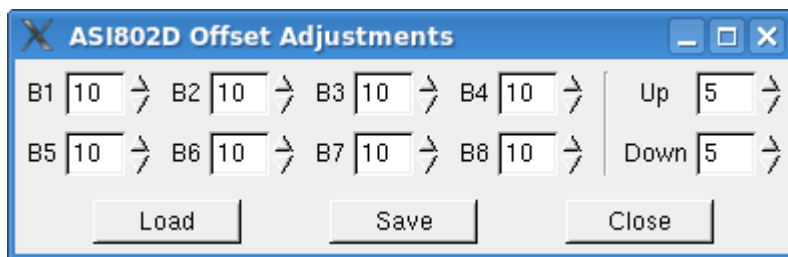


Figure 14 Offset Adjustments Window

After the window opens it is good practice to press the Load button in order for the Bath Controller program to query the 802D to request the current offset values. These values will then be updated in the text entry boxes associated with each bath location. Note that in addition to the eight baths there are also offsets associated with the up and down sensors.

It is highly recommended that a microscope be used when setting offset values. Before adjusting offset values please ensure that you have moved the bath plate from bath 1 to some other bath and then back to bath 1. Once back at bath 1 use the XYZ translation stage to centre the force transducer needle on bath 1. This move off and back is required to ensure that the 802D has positioned itself correctly at bath 1 and that the plate hasn't been moved by accidentally pushing on it with your finger. Let's assume that the force transducer

needle has been centered on bath 1 and that the needle is centered on all of the other baths except bath 3. Press the bath 3 button on the main window to move the bath plate to bath 3. Observe the location of the needle with respect to the slot. If the needle appears to be off centre then an adjustment to the offset may be required. To adjust the offset value simply type in the new value in the text entry box or use the spin buttons. It is recommended that you only change the offset value by one number at a time. A change of one number corresponds to a position change of 0.001" (0.0254mm). Note that when the needle is centered on the slot there is only about 0.007" clearance on either side of it. Press the Save button and then go back to the main window and cause the bath plate to move from the current bath to a different one and then back to the one you are trying to change the offset for (bath 3 in our example). Observe the location of the needle with respect to the slot and change the offset if required. Press the Save button again then move the bath plate off and back and check the position. When complete press the Close button. Note the normal range for offsets is between 5 and 15. If you feel that the offset needs to be set well outside of this range then this may indicate a problem with your 802D and you should contact ASI for technical assistance.

#### Restore Factory Settings

The Restore Factory Settings returns the offset values for all baths and the up and down sensors to the factory settings. Each 802D is adjusted prior to leaving the factory and these values are stored as the factory settings.

#### Setup Mode

The Setup Mode and Run Mode sub menu items are actually radio buttons (this means that only one of the two can be selected at a time).

In Setup Mode the plate does not move up after an index. This is useful when you are checking the offset values as there is no danger of the plate automatically rising and potentially breaking the force transducer.

#### Run Mode

In Run Mode the plate completes a full index cycle including raising the plate at the end of the movement. This is the normal setting when using the 802D.

### 3.6 600A Menu

The 600A menu (figure 15) contains two sub menu items, enable and disable. These menu items are radio buttons so only one can be active at a time. When the enable switch is active the 802D can receive bath movement commands from a 600A Digital Controller. There is only one 802D command incorporated in the 600A protocol command structure and it is the Bath command.

The 600A Bath command operates in a different manner depending on whether a sequence is running on the 802D or not. If a sequence is not running then the 600A Bath command will move the 802D bath plate to the bath specified in the Bath command. If a sequence is running and a 600A Bath command is issued then the 802D will ignore the command unless the sequence is at a step that has manual timing and the 802D is waiting for



a Next Step command. In this case a 600A Bath command is interpreted to be the same as the user pushing the Next Step button.

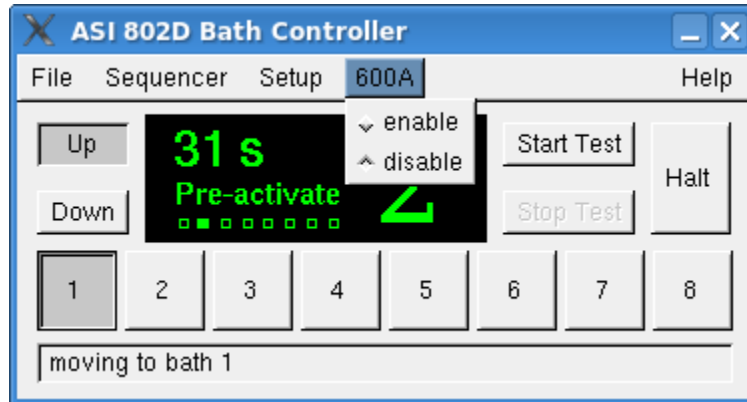


Figure 15 600A Drop Down Menu

### 3.7 Help Menu

The Help menu (figure 16) contains two sub menu items, 802D Help and About.

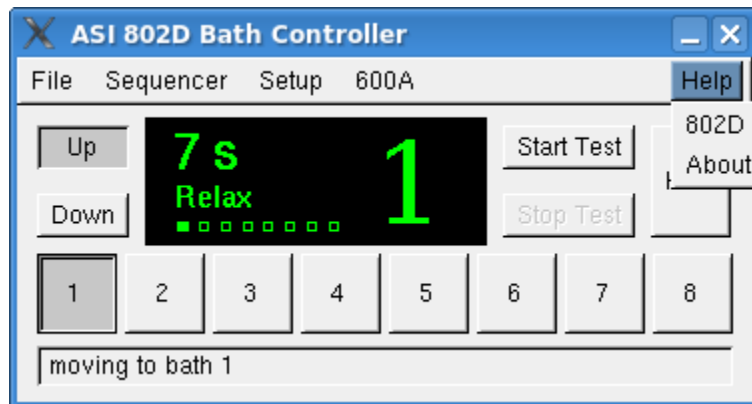


Figure 16 Help Drop Down Menu

#### 802D Help

Pressing the 802D Help sub menu item will cause this manual to be opened in a pdf viewer.

#### About

Pressing the About sub menu item will open the About window which provides contact information, the program version number and the program creation date.

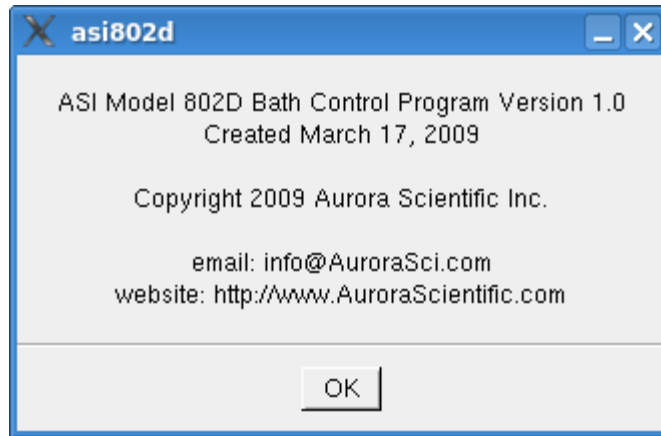


Figure 17 About Window

### 3.8 Creating and Editing Sequences

The Sequencer window has two tabs labeled Test Sequence and Comments, see Figure 18 and 19.

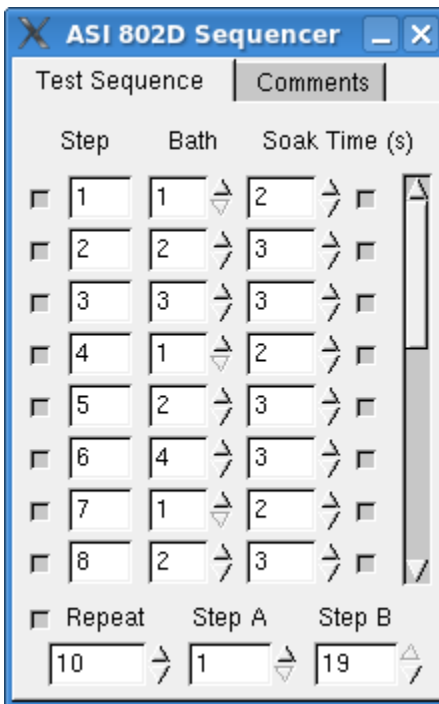
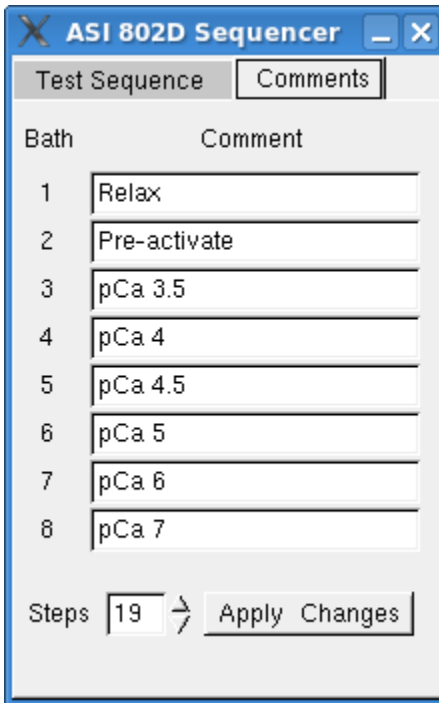


Figure 18 Sequencer Window showing Test Sequence Tab



**Figure 19 Sequencer Window showing Comments Tab**

### Test Sequence Tab

The Test Sequence Tab displays the sequence Step number, Bath number, Soak Time (s) and enable/disable buttons located on each end of each line. Also found on the Test Sequence tab are controls for repeating the sequence.

The Step number is self explanatory however the total number of steps is set on the Comments tab.

The Bath Number can be set to any bath number from 1 to 8. The bath number can either be entered directly in the text entry box or the spin button located to the right of the box can be used to select the bath number.

The Soak Time (s) is the amount of time that the plate will remain up at the selected bath before proceeding to the next step in the sequence. The time is set in seconds and the values can either be typed into the box or the up/down spin buttons can be used.

The enable/disable button on the left side of the line enables the entire step. In Figure 11 all steps are enabled which is shown as the enable/disable button being pressed and the line not being “greyed out”. In Figure 12 the appearance of disabled steps can be seen. In this case steps 4, 5 and 6 are disabled. When the sequence is run the sequence will jump from the end of step 3 to step 7 without executing steps 4, 5 and 6. This feature is useful as it allows you to easily modify an existing sequence without the need to create a new one.

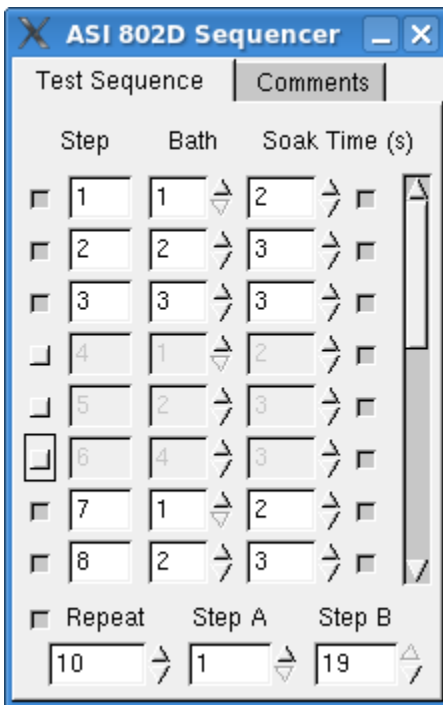


Figure 20 Sequencer Window showing Disabled Steps in Sequence

The enable/disable button on the right side of each line enables automatic or manual timing of that step. In Figure 13 you can see that the automatic timer for steps 3 and 6 is turned off. When the automatic timer is turned off the Soak Time (s) is “greyed out” for that step. In this case the sequencer will remain at step 3 until the user presses the Continue button on the main window of the program. See Figure 14 for a diagram of the main window paused for the user to continue the sequence by clicking on the Next Step button. Note that the status line at the bottom of the window clearly indicates the step that the sequence is at, the time at that step and the message ...waiting for Next Step. Manual timing is often useful when the researcher doesn't know the precise amount of time that will be required to complete the tests in a particular bath. Please note that if the 802D is used with a 600A Digital Controller then a Bath command can be entered in the 600A test protocol to tell the sequencer to continue. If the 802D is paused during a sequence waiting for the Next Step command then any bath command received from 600A will be treated as a Next Step command. This is convenient because it means that the user can use a single test protocol for testing and then advance the sequencer to the next step without having to program the particular step into the 600A test protocol.

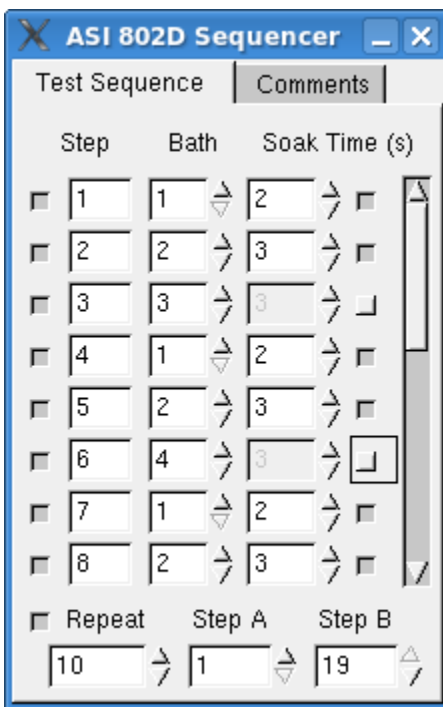


Figure 21 Sequencer Window showing Disabled Timing for Steps 3 and 6

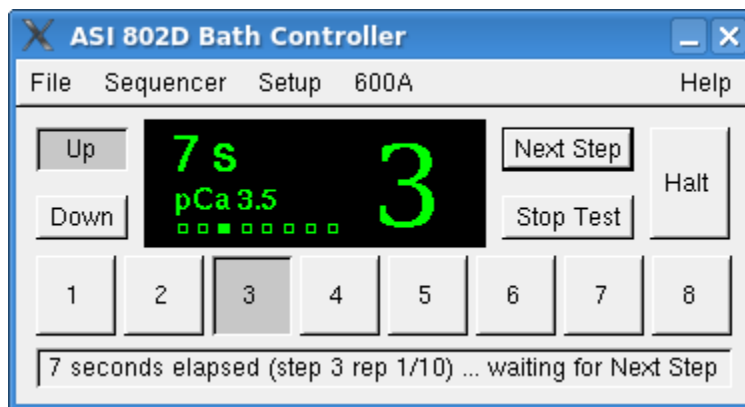


Figure 22 Sequencer Waiting for User to Click Next Step

The Repeat function is enabled/disabled by the button at the bottom of the Test Sequence window just left of the word Repeat. In Figure 13 the button is pressed which enables the Repeat function. The three text entry boxes associated with the Repeat function are the number of repeats, in this case 10, the starting step, Step A, for the repeat which is set to 1 in this case and the ending step, Step B, for the repeat which is set to 19 in this case. This example will repeat the entire sequence from steps 1 through 19 ten times. The Step A and Step B numbers can be any part of the currently defined sequence but Step B must always be greater than Step A. As an example say the researcher wanted to do a pre-conditioning protocol that required the first 6 steps in the sequence then a test routine was to be performed 5 times on the next 10 steps and then a post-conditioning protocol was required for the last 4 steps. In this case the sequence is 20 steps long with just steps 7 through 15

being repeated. Step A would be set to 7, Step B would be set to 15 and Repeat would be set to 5.

A scroll bar is provided on the right side of the Test Sequence window to allow scrolling through the entire sequence. Figure 15 shows the scroll bar positioned to show the end of the sequence.

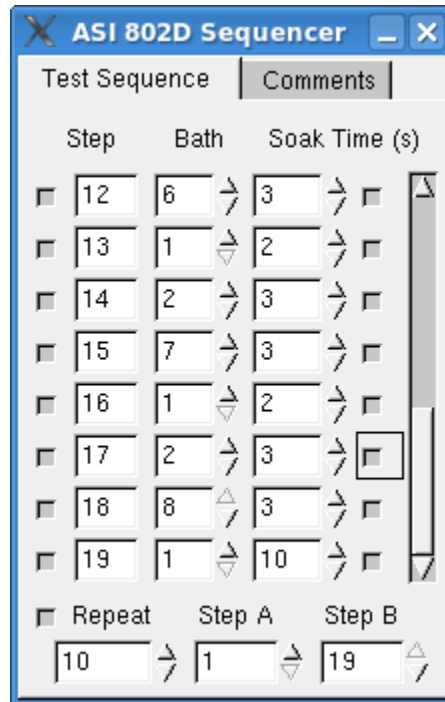


Figure 23 Scroll Bar Positioned to Show End of Sequence

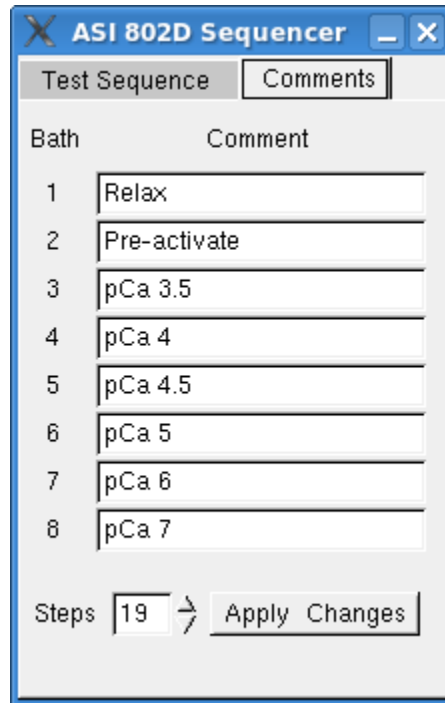
### Comments Tab

The Comments Tab displays a comment that can be associated with each bath and a text entry box to set the number of steps in a sequence.

Comments can be entered to identify the solution in each bath or provide some other comment that is useful to the researcher. The comment is shown on the digital display area of the main window whenever the bath plate is in the raised position at a bath.

The text entry box next to the Steps label is used for setting the total number of steps in a sequence. Either type in the desired number or use the spin buttons provided.

After making changes to the comments or number of steps click on the Apply Changes button to apply your changes. The comments and number of steps are stored in the sequence data file along with the sequence numbers and timing.



**Figure 24 Sequencer Comments Tab**

### 3.9 Error Codes

The 802D Bath Controller monitors eight possible errors. These include: X-direction movement, Z-direction movement, X-direction shorted sensor, Z-direction shorted sensor, X-direction missing sensor and TEC over temperature. All errors will sound a beeper and will flash the power LED indicator located next to the power switch on the 802D end panel.

**Table 1 Error Codes**

Error #	Code Returned in Status Query	Error Description
0	00	No Error
1	01	X Direction Period Timeout
2	02	Z Direction Period Timeout
3	04	X Direction Shorted Sensor
4	08	Z Direction Shorted Sensor
5	10	X Direction Missing Sensor
6	20	Reserved for Future Error Codes
7	40	Reserved for Future Error Codes
8	80	TEC Over Temperature

## **4.0 Using the 802D**

### **4.1 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 prior to setting the controller in Run mode and raising the bath plate. Raising the bath plate without first adjusting the force transducer position can result in breakage of the force transducer or damage to the bath plate.

### **4.2 Adjusting the Location of the Lever Arm**

The lever system motor and lever arm location must be adjusted prior to moving the bath plate. 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. Raising the bath plate or indexing it without first adjusting the lever arm position can result in damage to the lever arm, motor or bath plate.

### **4.3 Attaching a Muscle Fiber to the 802D**

The 802D was designed with the intent that the fiber would be attached in bath 1. For this reason bath 1 is wider than the rest of the baths and also contains right angle prisms that allow the side of the fiber to be viewed.

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. See Appendix B for further information about fiber attachment methods.

The 802D was designed for the lever arm to be in the bath along with the fiber. The lever arm is made from titanium to minimize corrosion and the possibility of contaminating the bath. The lever arm may be coated with a thin layer of epoxy if it is felt that the arm should be sealed from the solutions in the baths.

The 802D 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 exits the force transducer enter the slot. It will not fit and the transducer will be broken.

### **4.4 Adjusting the Resting Tension or Sarcomere Length**

Once the fiber is attached then 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. Use the front panel Length Offset control to change the position of the lever arm. The digital panel meter on the front of the length



controller can be used to monitor the amount of movement. For the 312C/315C a 1 micron movement corresponds to a voltage change of 0.007 volts. For the 322C a micron movement corresponds to a voltage change of 0.003 volts.

#### **4.5 Measuring the Fiber Length and Cross-section**

Fiber length and cross-section can be measured using an optical microscope equipped with a measuring reticule. The right angle prisms located in bath 1 provide a convenient method of obtaining a cross-section measurement in the vertical plane.

#### **4.6 Point-to-Point Movement**

In normal operation the 802D control software should be set to Run mode which results in the controller automatically raising the bath at the end of the movement. Clicking on the bath button of the desired bath on the software control panel will cause point-to-point movement. The bath plate will automatically lower, move to the commanded bath location and then rise.

#### **4.7 Sequencer Controlled Movement**

When the experiment calls for sequences of movement with varying delay times at each bath location the sequencer should be used. Details of sequencer programming can be found in chapter 3.

To initiate a sequence simple click on the Start Test button on the software control panel. The status line on the software will provide information about which step the sequence is on, how many steps are in the sequence and provide a count down timer until the next movement will occur.

To stop a sequence, press the Stop Test button on the software control panel.

## **Appendix A – Reversing the Direction of Models: 312C, 315C, 322C**

Please look at the two photos attached. The Normal photo sets the controller so that a positive control voltage causes the arm to move in a clockwise direction. The Reversed photo sets the controller so that a positive control voltage causes the arm to move in a counterclockwise direction. The motor must be set so that a positive control voltage cause a lengthening of the muscle tissue. If the orientation of your motor is such that a positive voltage causes a shortening of the muscle then you must reverse the movement of your high speed length controller. The following procedure provides instructions for reversing the direction of rotation of the length controller.

- 1) Turn off the power to the length controller electronics and unplug it from the AC source.
- 2) Remove top cover by removing the screw on the top back of the box and then sliding the top cover back and off of the box.
- 3) Look in the front left corner of the box near where the motor connector and offset control are. Identify jumper block JMP1 and JMP12.
- 4) Remove both jumpers from JMP1, rotate the jumpers 90 degrees and replace them on the posts. The gold bars visible on the top of the jumpers should now be parallel to the front panel. Make sure both jumpers are pushed all the way onto the posts.
- 5) Repeat step (4) for JMP12.
- 6) Double check what you did, all the jumpers (2 at JMP1 and 2 at JMP12) should now be in the same orientation (parallel to the front panel). Compare your jumpers to Photo 2 and make sure they are the same. If they are then put the top cover back on and turn on the system. If the arm goes hard to one side immediately turn off the power and check your changes again. It is very important that all 4 jumpers get changed together.



**Photo 1 Normal Jumper Orientation**



**Photo 2 Reversed Jumper Orientation**

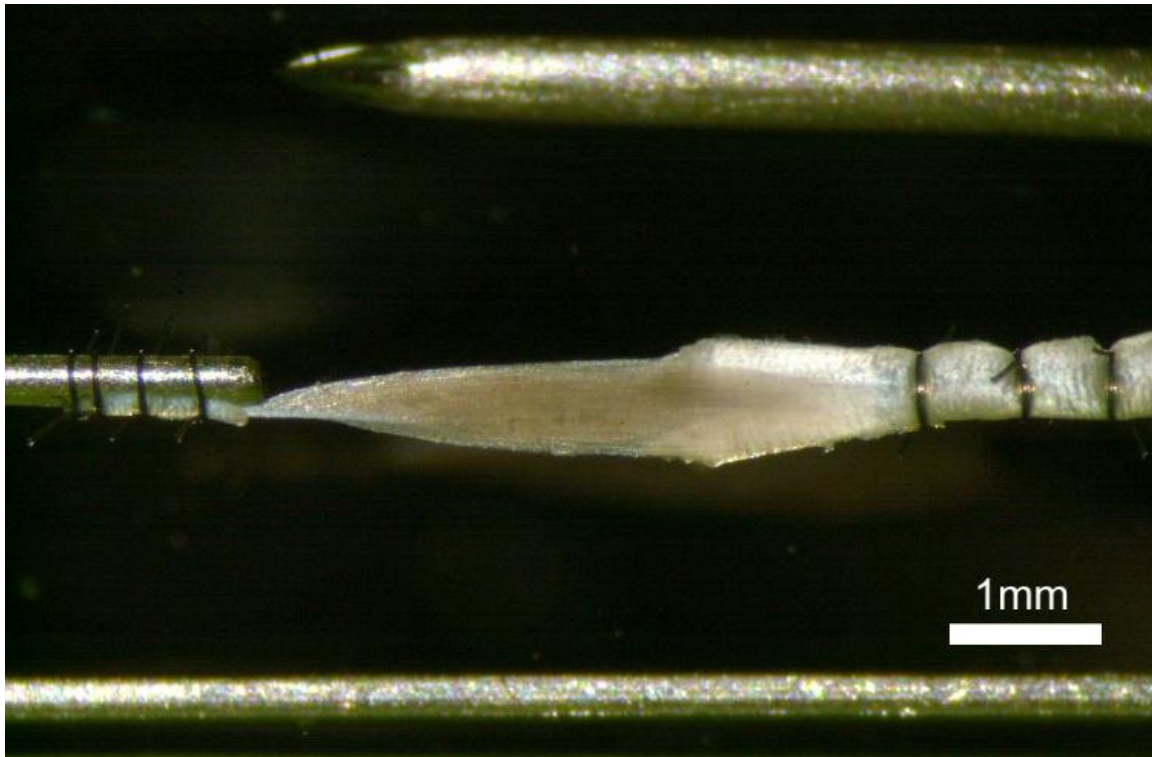
## Appendix B – Fiber Attachment Methods

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 is visible in this photo but the researcher was using a model 400A force transducer and a model 318B 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 3 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 normally the tube will project out of the force transducer tube about 8-10mm, which gives an overall needle length of 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 needle 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 is not as strong and weakens when placed 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 the required amount from 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 prominent research groups using the tying method include Dr. Denis Claflin in 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 such as 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 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 the tissue is attached. Two main types of glue seem to be used, the first is an expanding foam called Great Stuff that is available in the USA. The other is silicone glue.

## Appendix C – Fabrication of Needles for Tying Attachment Method

For the motor (312C, 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 a flat surface. Continue to raise the vise until it is normal to the flat surface (at which time the tubing ought to have a 90 degree 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). You will be happiest if you 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 312C motor is very sensitive to added mass. You may need to make the "L" about 3mm x 3mm when used with a 312C. 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 needle extension.

Use 29 gauge stainless steel tubing (0.013 inch outside diameter) that is readily available from McMaster-Carr or make it 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