INSTRUCTION MANUAL

Model
600A Digital Controller

Program Version: 3.000

RTAD Version: 3.00

September 10, 2012

Aurora Scientific Inc.

Table of Contents

Table of Figures

Index of Tables

Conventions Used in this Manual

 This warning icon indicates a potential for damage to equipment, personal injury or data loss.

 A note icon indicates an important point to remember.

1.0 Introduction

The 600A Digital Controller was designed to enable physiology researchers to study the dynamic mechanical characteristics of muscle tissue. The system is capable of controlling and taking data from all of our 300C series muscle levers, our 400A series of force transducers, our 700A series of stimulators, our 800A series of apparatus and our 900A series of sarcomere length systems. The 600A can also be used with other manufacturer's instruments.

A unique feature of our 600A is its capability of controlling force or sarcomere length. In order to control force the 600A adjusts the length of the preparation to provide the desired force. This force control mode can generate critically damped step changes in force in as little as 1.0 millisecond. When controlling sarcomere length the 600A must be paired with an ASI HVSL (High speed Video Sarcomere Length) system which provides the sarcomere length signal. Just as with force control, 600A adjusts the length of the preparation to provide the desired sarcomere length.

The 600A Digital Controller is a complete data acquisition and control system that consists of: custom software for data acquisition, instrument control and data analysis, a computer (PC) running a real-time version of Linux, a data acquisition card, a model 604A Signal Interface and all required interconnecting cables.

 The 600A software has an easy-to-use graphical user interface that collects high-resolution length and force data while controlling the length and force of the muscle tissue under test. The 600A software can also log sarcomere length (SL) data and up to two other analog input channels. Collected data can then be plotted and analyzed within the program. The 600A program also includes a real-time scrolling display that can be configured to show length, force, SL or the auxiliary analog input channels. This scrolling display can be frozen at any time and then up to 4 hours of data can be reviewed.

A unique feature of the 600A Digital Controller is that the PC uses a real-time version of the Linux operating system. The real-time operating system maintains control over all time-critical data acquisition and control tasks with a timing accuracy of better than 10 microseconds, regardless of what other programs and processes are being run on the computer. This is not the case with other PC or Apple operating systems such as Windows, DOS or the Apple OS.

The PC supplied with the 600A system features a compact tower case, a 64x6 six-core processor, 4 GB RAM, large hard disk and a built-in Ethernet adapter. Included within the PC is a high-speed data acquisition card. The card features 16-bit accuracy on the eight differential analog input channels (A/D) and on the two analog output channels (D/A). The use of a 16-bit A/D and D/A ensures that the 600A Digital Controller measures and controls length and force to the resolution of our instruments. The A/D card also provides control of a stimulator and the ability to trigger other instruments.

A model 604ASignal Interface is included with the system. This interface allows the researcher to easily connect a length controller, a force transducer and a stimulator to the A/D system. The 604A is housed in a 19" rack-mount case that is 1U (1.75") high. All connections

to the A/D card are available via front panel-mounted BNC connectors. In order to make the system as "turn-key" as possible we have also included BNC to BNC patch cables to make the necessary connections between a length controller, a force transducer, a stimulator and the 600A Digital Controller.

1.1 What's New in Version 3.000

Version 3.000 of the 600A Program comes with many new features and improvements. Some of the key improvements are a completely redesigned and upgraded Force and SL Control system, increased data acquisition and control speed, new automatic protocol checking to help avoid common mistakes, channel colours that are persistent across all graphs, and new light and dark themes for the graphs.

Redesigned Force and SL Control
The Force and SL Control window has been completely redesigned with great improvements to the functionality, stability and ease of use. One of the biggest improvements is the new feedback control algorithm which provides far more stable control of either force or sarcomere length and also provides an easy-to-use tuning interface. Another new feature is that the feedback control parameters can be changed at any time, even in the middle of a protocol. This makes it much easier to setup the force and sarcomere length control. Section 5 provides greater detail of the new Force and SL Control.

Increased Sample Rate
The sample rate of the program has increased from 10,000 samples per second to 20,000 samples per second. This allows the users to sample up to four channels and save the data to file at a rate of 20 kHz. This increased data rate is also available for control outputs thus providing an increase in performance of the control section of the program.

Protocol Auto-Checking
The protocol window now contains auto-checking functionality which looks for common mistakes and warns the user. An example of this is the auto-checking routine will detect when a protocol command is programmed to start before the previous command has finished executing.

Burst Window
Burst is a completely new function in version 3.000. It allows the user to sample data at the maximum speed of the A/D card (250,000 Hz). This increases the normal maximum rate of 20,000 samples per second to 60,000 samples per second on four channels. This is very useful when tuning a high speed length controller or capturing a transient event.

Plot Themes
Two plot themes are available in the new version with the addition of a dark theme along with the standard white background theme. The dark theme replaces the black lines on white background of the original light theme, with green lines on a black background. This setting provides more options when displaying your data and generally makes it easier to see the data.

Display Colours

The Display Settings window has changed to accommodate colours that are persistent to a channel as opposed to a graph. As an example if SL is set to blue then it will show as blue on all plots (Main window, Scope, and Analysis) even after the program has been restarted.

Protocol Menu

Changes in the menu structure in the protocol window have been made in order to make it easier to navigate the growing list of functions. The functions are now broken up into subcategories of Length, Force, SL, Stim, and Control.

2.0 Specifications

Model: 600A Digital Controller

Personal Computer

Processor:	3.3 GHz AMD 64x6
RAM:	4 GB
Hard Disk:	500 GB
DVD ROM:	DVD-R, CD-R
USB Flash Disk:	4 GB
Ethernet Card:	1 Gbit/sec
Keyboard:	USB type, 101 key
Mouse:	USB type, Microsoft compatible.
Power Requirements:	120/240VAC ±10%, 50/60Hz, 5 amps max., switch selectable.
Dimensions:	mini-tower case.
Weight:	4 kg.

A/D Card

Analog Input

Number of Channels:	8 differential
Resolution:	16 bits (1 part in 65535)
Maximum Sampling Rate:	250,000 samples per second

Analog Output

Number of Channels:	2
Resolution:	16 bits (1 part in 65535)
Maximum Output Rate:	833,000 updates per second

Digital Input/ Output

Number of Channels:	24
Type:	TTL

Interface:	68 conductor ribbon cable
Dimensions:	PCI Bus

604A/C A/D Signal Interface

Input Connector Type:	BNC – isolated, male
Number of A/D Inputs:	8: Length In, Force In, Aux 1-6
Number of D/A Outputs:	2: Length Out, Force Out
Digital I/O:	2 Inputs: Trg In 1, Trg In 2
	4 Outputs: Trg Out 1, Trg Out 2, Stimulator, Inhibit

Operating System

Type:	Linux - RTAI real-time
Kernel:	2.6.37.6-rt
Interface:	GUI (Windows-type)

ASI600A Software

Version:	3.000
RTAD Version:	3.00

Programming Language: C
Interface: GUI (Windows-type), drop down menus

Main Window Menu
File: Save Data, Exit
Edit: Display Settings, Show Controls
Calibrate: Models Attached, Length, Force, Force and SL Control
Setup: Open, Close
Protocol: Open, Close
Analysis: Open, Close
Scope: Open, Close
Burst: Open, Close
Help: ASI600A Help, About

Protocol Window

Protocol Functions
Length: Step, Ramp, Square, Sine, Sweep, Sample, Hold, Read Larb, Write
                        Larb, Send Larb, Larb
Force: Step, Ramp, Square, Sine, Sweep, Sample, Hold, Clamp
SL: Step, Ramp, Sample, Hold, Trigger, Track
Stim: Stimulus, Trigger 1, Trigger 2
Control: Data Enable, Data Disable, Data Burst, Bath, Repeat, Stop

Stimulus Tab
Profile 1-10:   Pulse Width, Pulse Frequency, Frequency Duration, Train
                        Frequency, Train Duration.

Sequencer Tab
Sequencer Window: Clear Sequence, Verify Sequence, Load, Save As, Save.
Settings: Sequence File, Protocol File Directory, Data File Directory,
                        Sequence Number, One Data File per Frame or Test.
Sequence: Enable, Protocol File Name, Browse, Delay Time, Data File Name,
                        File Status.

Analysis Window
File: Load Data, Load Current, Export Data, Close
Signals In: Length (L), Force (F), Velocity (V), dF/dt, SL, Aux1, Aux2
Signals Out: Length (L), Force (F), Velocity (V), dF/dt, SL, Stimulus-Triggers
                        (Stim), Digital Readout (readout)
Unit Controls: Length [um, mm, cm, Lo, Lf, volts],
                        Force [mN, N, gm, kg, Fmax, Pa, kPa, volts],
                        Time [ms, s]
Plot Zoom Controls: T Start, T End, un-zoom data (Unlock T) button. User can zoom
                        by using the cursor to select an area on the graph to zoom
                        Filter Controls: Filter Length (1-16)
                        Frame Control: Choose frame to plot
                        Auto Load Test: Toggle button to set analysis to automatically plot
                        latest data

Lock T Interval: Toggle button to allow the selected time interval of the data to be automatically shown for each new test

Burst Window

Channel to plot:	Length In, Length Out, Force In, Force Out, Aux 1, Aux 2
Units:	[V, mV, bits, mm]
File Control:	Save As, File Name, Close

Scope Window

File:	Open log file, Close
Edit:	Show Controls, Cursor Readout, Colour
Time Base:	Length of time for data displayed on the plot
Channel (1 or 2):	Select channel to sample [Lin, Lout, Fin, Fout, Aux1, Aux2, SL]
Units:	Length (L) [um, mm, Lo, Lf, V]
	Force (F) [mN, N. gm, kg, Fmax, V, Pa, kPa, MPa]
	Sarcomere Length (SL) [um, SLo]
V Scale:	Select a scale for the vertical axis [Auto, Fixed]

Help

600A Help:	Opens a .pdf version of this manual on-screen
About:	Shows information about the program including version number of the software and the real time A/D driver (RTAD)

3.0 Quick Start Guide

This quick guide will help you get your system up and running in as little time as possible, including both setup and explanation of basic software features.

3.1 Instrument Setup

The 600A Controller PC has a compact tower case that can be oriented to stand as a tower or to lay on its side depending on the available space. Placement of the computer should take into account the following criteria.

- Adequate ventilation for cooling.
- Protection from water, dust, dirt, vibration, and shock.
- Within a maximum of 2m (6 ft.) from the force transducer and length controller electronics to allow for connection.

A keyboard and mouse are supplied with the PC. Plug each into an available USB port on the back panel of the PC.

We suggest using a wide screen LCD monitor for maximum effectiveness. This may have been supplied by us or you can use any other modern-day monitor designed for use with a PC. The screen resolution is automatically set to the maximum available resolution of the monitor when the PC boots up.

In order for the PC to properly recognize the monitor you must ensure that the monitor is switched on **before** booting the PC.

Connect the monitor to a video port on the back panel of the PC (VGA, DVI, or HDMI). Plug the supplied AC power cord into the back of the PC and into an appropriate AC outlet.

The 600A is shipped completely configured; you do not need to install any software. The 600A PC runs under a real-time version of the Linux operating system and therefore you should not attempt to load Windows or Mac software on the 600A.

The 600A is a high performance data acquisition system that provides capabilities not normally found in PC programs. For this reason it is important that you **do not change the BIOS settings on the PC or make significant changes to the operation of the window manager.** Changes to BIOS settings and window manager settings can cause program malfunctions.

If you want to network the PC an Ethernet port is available on the back of the PC. The network is set for a DHCP server and therefore the PC will look for this server to obtain an IP address. Please contact Aurora Scientific Inc. (ASI) if you have any questions about the setup of the PC including the network setup.

See the 604A A/D Signal Interface Manual for instructions on connecting your length controller and force transducer (or a dual-mode lever system) and stimulator to the A/D Signal Interface. The 600A Digital Controller comes with all cables required to make these connections. The 604A manual also provides instructions on connecting the ribbon cable between the back of the 604A Interface and the data acquisition card located in the PC.

Take reasonable care to route the ribbon cable and the BNC signal cables away from AC power cords in order to minimize unwanted electrical noise from getting into your data.

New 600A Controller PCs come with a 604A A/D Signal Interface but older models came with a 604C. These products are equivalent in operation and the model number is dependent on the data acquisition card installed in the PC. Any time this manual refers to 604A it also applies to the 604C.

3.2 Software Registration and Activation

The ASI600A program **must be licensed** before it will run on your new PC.

The following procedure will license your software and it can be completed in about 5 minutes.

You will need:
- 600A PC with the ASI software to be activated
- PC with an internet connection (does not have to be the 600A)
- Product Key found on the serial number tag for the software.

1. Click on the **Activate Software** icon located on the desktop.
2. When the Activate window opens follow the on-screen directions as shown in Figure 1 (remember Linux is case sensitive). The first step will be to enter the Product Key which is a 5 character key with the format of three numbers followed by a letter and then a final number, e.g., 017A5. Ensure you enter the Product Key and not the serial number.
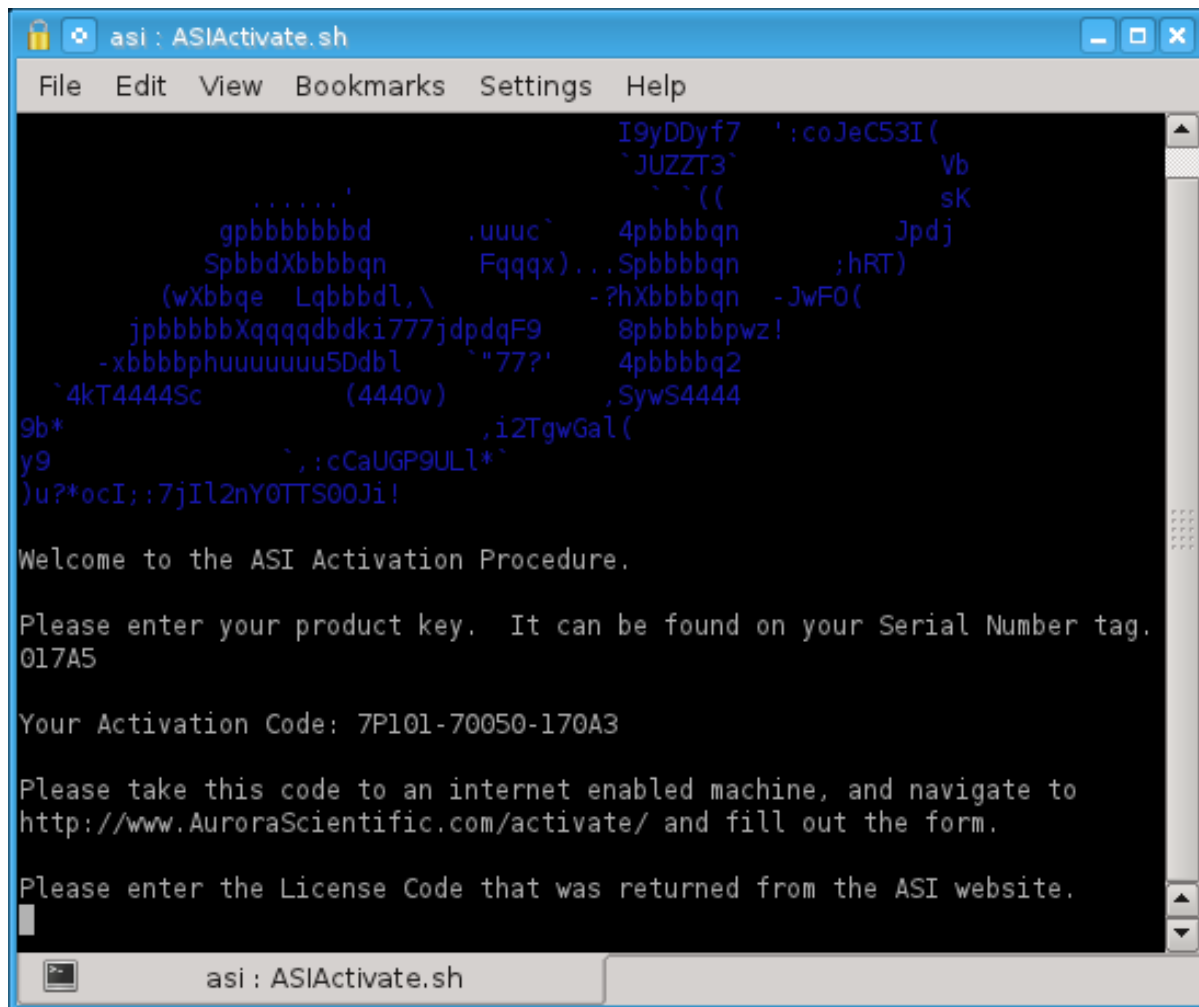
Figure 1 ASI Activate Window

3. After pressing the Enter key the ASI Activate program will provide you with an Activation Code which is an alpha-numeric character string consisting of three groups of 5 characters each separated by dashes, e.g., 7P101-70050-170A3.
4. On the PC with internet access, point your web browser to: http://www.AuroraScientific.com/activate
5. Enter the Activation Code returned by the ASIActivate program, your contact details and then click on the Activate! button. See Figure 2.
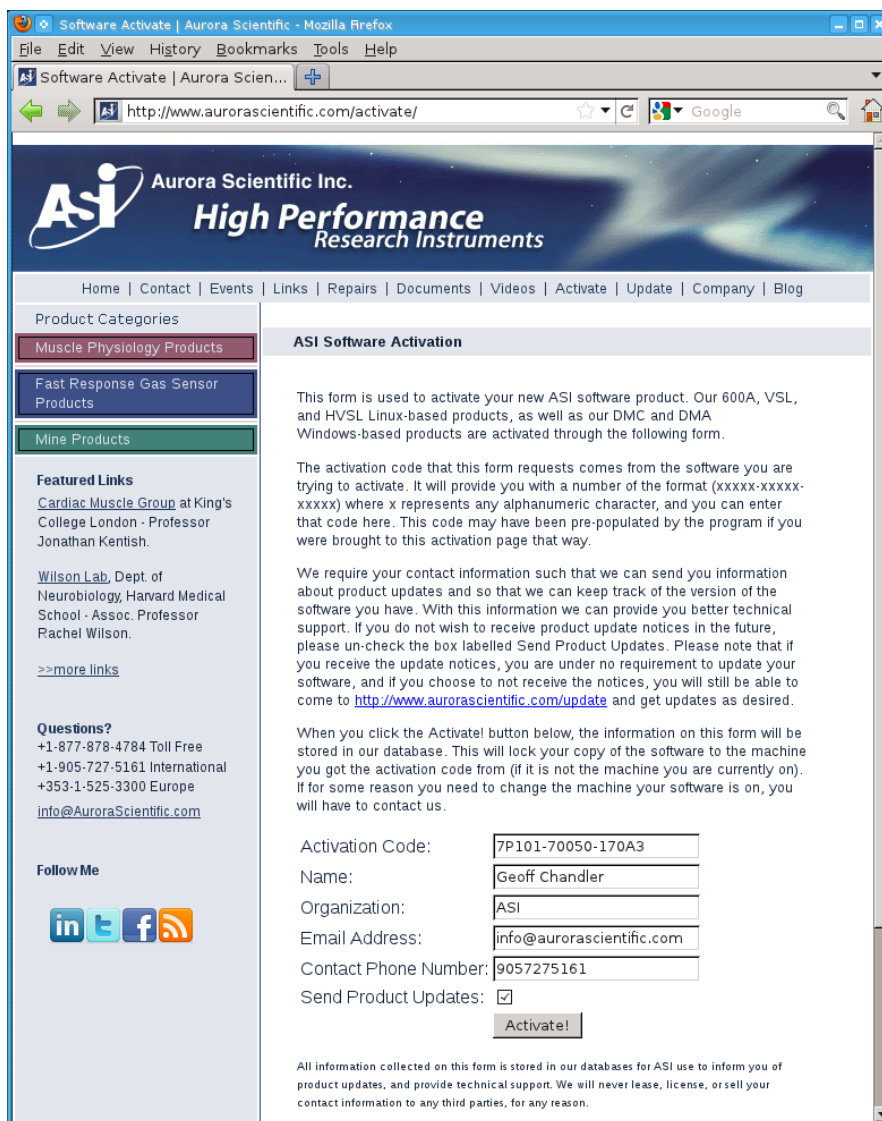
Figure 2 ASI Product Activation Website

6. Copy or write down the license code returned by the webpage.
7. Enter the license code, and then enter the root user's password (By default this password is set to "root". If you have changed it, enter your new one.)
8. Click on the desktop icon to start the 600A program to test that the license was installed correctly.
9. Repeat as required for additional ASI software. For example, if you have a PC with both 600A and HVSL software, you must complete this procedure twice.

Should you encounter any issues with this procedure, please contact ASI by phone or email, and we will be happy to help you with this process.

3.3 Verification of Correct Operation in Force Mode

The ASI600A program requires that the force transducer and length controller signals have the correct polarity and that the two devices are connected correctly. **If the signal polarity is reversed or the devices are not connected correctly then the force feedback will not work properly which could result in damage to the force transducer and length controller.**

The system assumes that tension on the force transducer will result in a positive output voltage. The program also assumes that a positive control signal to the length controller will produce a positive output voltage and that the resulting motion results in the muscle tissue being lengthened. It is strongly suggested that you initially check out the operation of the system using a short length of rubber (elastic) band in place of the muscle tissue. All ASI high speed length controllers have internal jumpers that allow the operation of the length controller to be reversed. If you have purchased your 600A along with a length controller and apparatus then the jumpers will have been set correctly at the factory.

If an Aurora Scientific Inc. series 400A force transducer is used then the transducer Force Out connector can be connected directly to the 604A Force In connector. If using another manufacturer's transducer then the polarity of the transducer output signal must be determined prior to connecting the transducer. The 600A assumes a force signal level ranging from –10 volts to +10 volts. It also assumes that the force transducer output increases with increasing tensile force (this is the standard for series 400A force transducers). If the force transducer signal polarity is reversed (output decreases with increasing tension or negative output voltage for tension) then jumpers located within the 604A interface must to be changed. Open the 604A interface by first removing the two Philips head screws located at the top edge of the box on the back panel. Once the screws are removed simply slide the cover backwards and remove it. You will now see two sets of jumpers located near the Length In and Force In BNC connectors. Remove the jumpers, rotate each of them 90° and then reattach them. This reverses the force input connections. Likewise if the length controller produces a negative output voltage for a positive input voltage you can reverse the Length In signal with the jumpers provided.

Older 604A and 604C Signal interfaces used slide switches instead of jumpers. On these units slide the switch from one position to the opposite position in order to reverse the input signals.

3.4 Linux and Desktop Overview

Once all components of the system have been connected together then turn on the monitor and then the PC. The PC should go through the normal power on self-tests and then the Linux operating system should be loaded. Once loaded, a Windows-like desktop should appear with a task bar at the bottom of the screen and several icons located on the desktop. The desktop is shown in Figure 3. The most frequently used icons will be *ASI Log*, *ASI600A*, *Home,* and *data*. The taskbar at the bottom of the screen shows the current windows that are open and

provides a simple method of switching between the windows. Simply click on the taskbar item that you want to switch to and that window will become the current and top one. The most frequently used icon at the bottom of the screen is the *K* icon at the far left of the screen. The *K* icon is similar to the Start button in Windows. It provides access to programs and also allows you to shut down the computer properly. Just like Windows, Linux must be shut down properly since the file system is updated during the shutdown procedure.

For specific actions, like licensing software, you may need to obtain superuser privileges. This is done by clicking on the xterm icon on the desktop and typing the following:

> *su*

Password: *root*

If you change the root password (superuser password) there is no way for ASI to recover the password if you forget or lose it. Therefore please record the superuser password and store it in a safe place.

Many users will elect to connect the ASI600A to a network. Because the 600A is shipped with no login password and the root password is not secure. **We strongly suggest that you set a secure password before connecting the 600A to a network**. A secure password should not be a recognizable word, it should be a minimum of 6 characters containing letters (both upper and lower case), numbers and at least one special character (&, ^, $, #, @, !). See Appendix E – Network Security for instructions on changing passwords and network setup.

Figure 3 ASI600A Desktop

A brief description of the main icons on the desktop is provided below.

Activate Software

Used to start the software activation program. You must activate ASI software before it can be used. See Section 3.2 Software Registration and Activation of this manual for instructions or refer to the Activation Instructions pdf file located on the Desktop.

ASI Log

Used to start the ASI Log program which creates a running log of the operation of the 600A program as well as allowing the user to enter any comments pertinent to the experiment. All comments are time stamped and stored in a daily log file. Starting with version 3.000 of 600A this program is automatically started when 600A starts.

ASI600A

Used to start the ASI600A program in four channel mode where the A/D channels collected will be Length In and Force In, Aux1 and Aux2.

installer

Used to install a new version of the ASI600A software.

xterm

Used to open an xterm. An xterm can be used to type commands to the Linux system and is similar to the Windows CMD command that opens a command window. An xterm must be opened in order to log on as superuser (su). Later in the manual you will note times when you are required to open an xterm and log on as superuser in order to perform program upgrades, system maintenance, change passwords, etc.

Home

Used to open the ASI600A home directory. This is similar to Windows Explorer looking at the C:\ drive.

data

Used to open the ASI600A data directory. This is similar to Windows Explorer.

ASI Website

This icon opens a web browser at Aurora Scientifics' home page on the Internet. Note: the 600A PC must be connected via the Ethernet port to an Internet connection for this link to work correctly.

Trash

Used to delete files. This is similar to Windows Recycle Bin. To use simply drag files on top of the Trash can or select a file, right click and delete.

Like other modern operating systems Linux must be properly shut down when you are finished working with it. **Failure to shut down the 600A PC correctly can result in program or data corruption and loss.** To shut down the PC click the "K" Icon located in the bottom left corner of the Desktop. This opens the Windows equivalent of the "Start" menu. From here you can click on the Leave icon and then click Shutdown. The PC will then shut down safely and power itself off when done. The shutdown icon is shown in Figure 4.
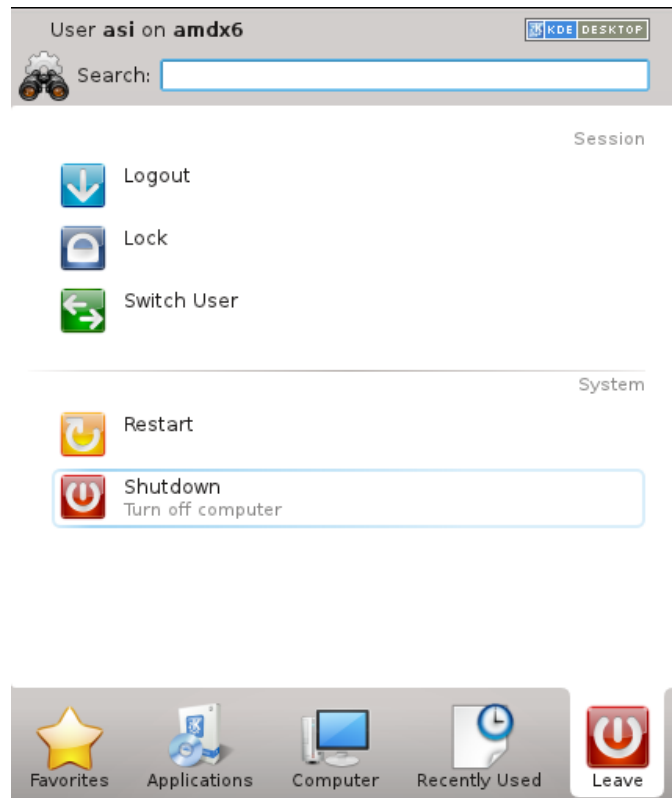
Figure 4 K Menu Showing Shutdown Controls

3.5 Starting the ASI600A Program

To start the ASI600A program click on the icon labeled ASI600A. When the program starts a window will open and you will be on the main page of the program. The main window provides access to all of the menu items and also contains a large graphing area for the display of data taken during a test. The main window is displayed in Figure 5.
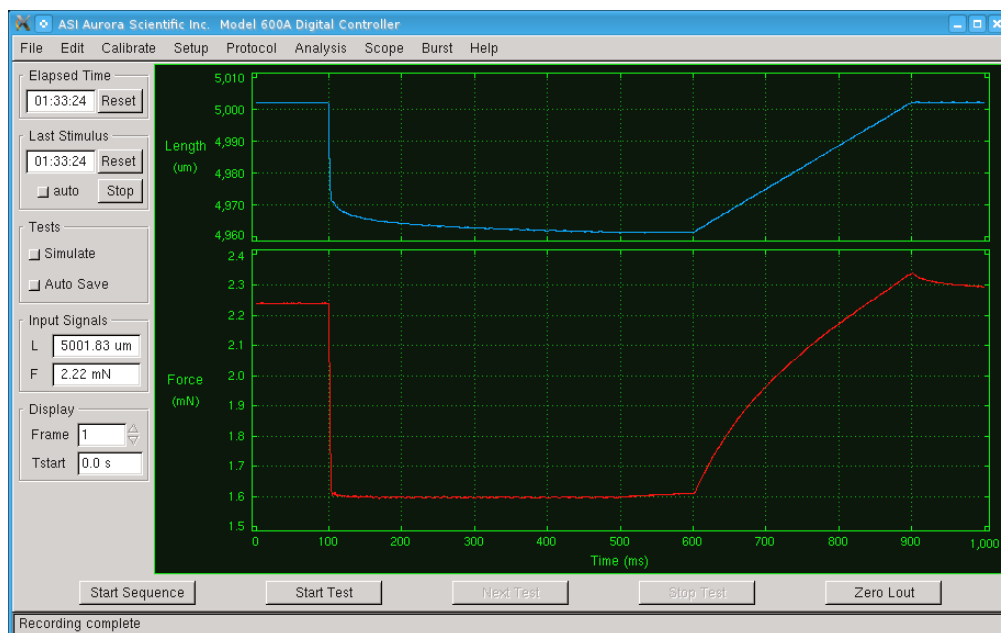
Figure 5 ASI600A Main Window

A menu bar is located along the top of the screen which opens a drop-down menu for each heading. A detailed description of each command is provided in the next chapter. At the bottom of the Main window is a box that displays program status messages.

Located near the bottom of the main window are Start Sequence, Start Test, Next Test, Stop Test and Zero Lout buttons. These buttons are used to start a sequence of test protocols, start a single protocol, advance to the next test in a sequence, stop a test protocol, and zero the length command output to the length controller.

The left side contains several text boxes that display the current values of an Elapsed Time timer, a Last Stimulus timer, digital readouts of the current Length In and Force In signals as well as the current Frame number and the start time of the current Frame. The units for Lin and Fin are set using the Edit→ Display Settings menu.

Also located on the left side of the window are Simulate and Auto Save buttons. When the Simulate button is pressed the currently defined protocol will be plotted in the graph area of the main window when the Start Test button is pressed.

When the Simulate button is pressed test protocols will not execute, instead the current test protocol will be shown on the main graph area.

When the Auto Save button is pressed data will automatically be saved after a test completes.

Data is automatically saved when running sequences and therefore the state of the Auto Save button doesn't matter when running sequences.

3.6 Using the ASI600A Program – Quick Start Guide

Now that that program is up and running we will go through the main steps required to set up an experiment, control a lever system (length controller) and take data. For this example we will assume that the user has a 315C high-speed length controller and a 403A force transducer. If your instruments are different then substitute your actual model number in the instructions.

If you are connecting the 600A Digital Controller to a dual-mode lever system (a lever system that controls both length and force) then you will not have an external force transducer and you must select the *None* option under the force transducer model in Calibrate→ Models Attached. When connected to a dual-mode lever system the program controls force using the Force Out connector on the 604A.

When connected to a high-speed length controller and an external force transducer the program controls force by measuring the current force and then changing the length to obtain the desired force (force feedback). The force control mode used by the 600A program is determined during program setup (see steps 1 through 4 below). Ensure that you select the correct models. Also ensure that your lever system and force transducer are connected correctly to the 604A Signal Interface (see the Instruction Manual for the 604A for connection instructions). If you are electrically stimulating the muscle tissue then ensure that the stimulator is also connected correctly.

It is recommended that while you are becoming familiar with the program you use an elastic (rubber) band connected between the lever arm and the force transducer instead of using muscle tissue.

Use the following procedure to get the program up and running and to perform your first tests. Once you have completed this Quick Start Guide you will be familiar with the operation of the program. More details about using the program are provided in chapters 4 through 7 of this manual.

1. Turn on the PC, log in, and start the ASI600A program (Section 3.4 Linux and Desktop Overview). Set the size of the main window.
2. Turn on the length controller and force transducer electronics. Attach either muscle tissue or an elastic (rubber) band between the lever arm and the force transducer.
3. Click on the Calibrate Menu heading.
4. Select Models Attached and the display should be as shown in Figure 6. Click the button next to 315C and the button next to 403A (substitute your model numbers if different). This sets the default calibration values for length and force to the settings that correspond to the selected instruments. If you are using instruments that are not listed on the Models Attached screen then make sure you perform the Length and Force calibrations before proceeding to step 5. If you are using a dual-mode lever system (measures and controls both length and force) select the model number under Lever Systems and then select "None" under Force Transducer. Exit the Models Attached screen by clicking on OK.

It is a best practice that you perform a proper calibration of your length controller and force transducer. For the purposes of this quick start guide you can use the default calibration values for now.
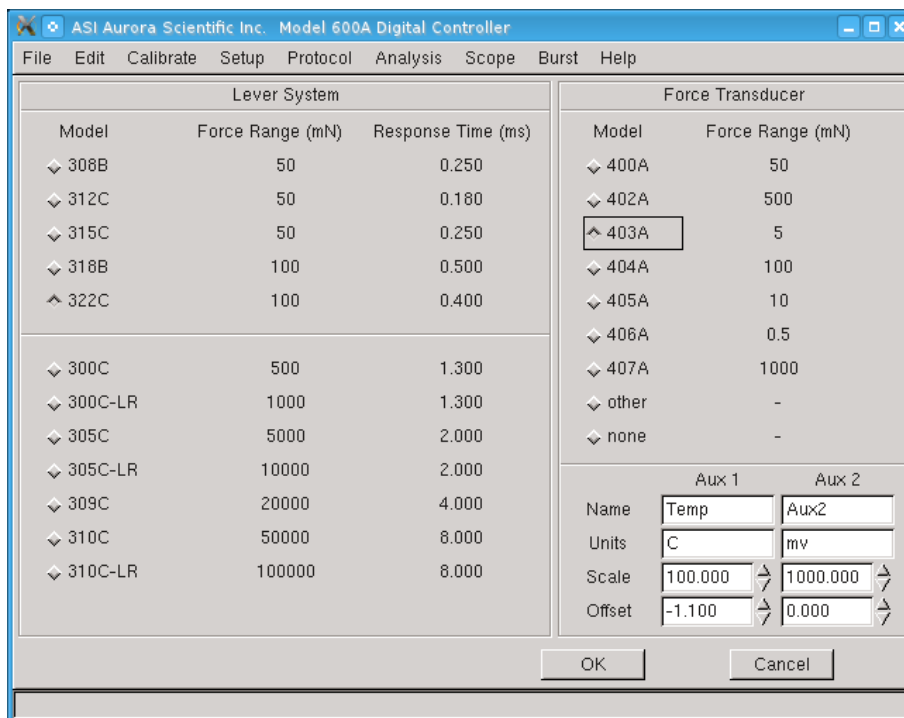
Figure 6 ASI600A Models Attached Window

5. Click on the Setup Menu heading and then click on Open. The Setup window will open and should appear as in Figure 8. You will note the Setup window is divided into five sections, Set Reference Length, Fiber Cross Section, Other Parameters, Set Zero Load and Notes.

6. In the Set Reference Length section press the Zero Lout button and then enter the current muscle tissue length in the Reference Length text box. Now press the Record Lin button. After pressing the Record Lin button the program will sample the current Length In value and display this value, in mV, in the Lin – Length In text box. At the same time the program will display the reference length in the Lref – Ref. Length text box.

We refer to the muscle tissue length when Lout is set to zero as the reference length (Lref). Lref must be set correctly or the control system will not operate as desired. There are two distinct steps required to set Lref correctly, i) measure the actual length of the preparation and record this length in the box to the right of Reference Length, ii) press the Record Lin button to tell the program to associate the current Lin signal with the Reference Length you set. What you are doing in this step is giving the computer a datum from which all length movements and measurements will be based.

After setting the reference length do not adjust the Zero Offset potentiometer control on the front of the length controller or the X stage controls that affect the overall tissue length. If you do you will create a length offset between the actual position and the position that the computer reports. This offset can result in incorrect data being recorded and the system not controlling to the desired length, force or sarcomere length. **If you change the Length Offset potentiometer on the Length Controller or the X stage position then you must perform step 6 again.**
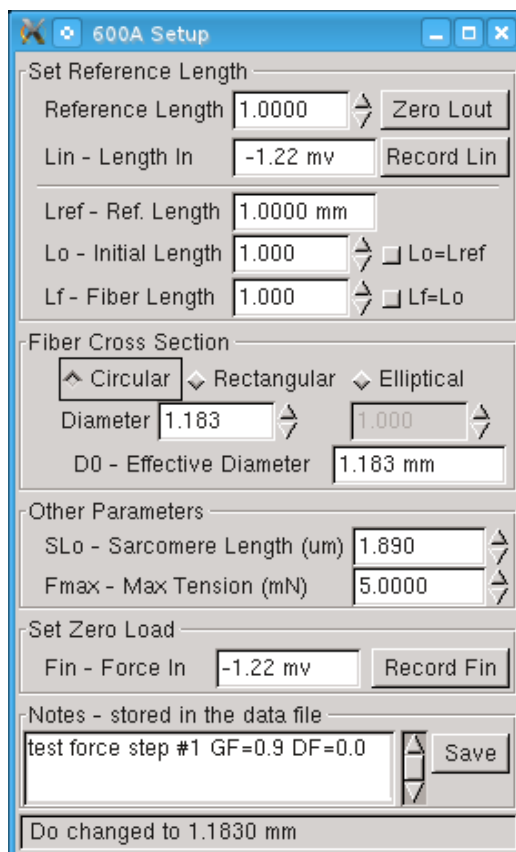
Figure 7 ASI600A Setup Window

7. The lower half of the Set Reference Length section allows you to enter the initial length (Lo) and the fiber length (Lf). You have the option of manually entering Lo and Lf or having the program set them automatically. Click on the Lo=Lref button to set Lo equal to Lref. Lo is used for plotting length in terms of L/Lo but it is also used in test protocols for controlling length. If you want to work in units of fiber lengths then either enter the fiber length (Lf) or click on the Lf=Lo button to set them equal.

In most cases Lo is equal to Lref.

Do not set Lo to zero. Unexpected operation of the length controller can result from improperly setting the Lo value. Lo must always be set to a positive, non-zero value (the initial length of your tissue). This value is used in length control

functions and therefore if it is set incorrectly unexpected movement can occur.

8. In the Fiber Cross Section area of the Setup window the fiber cross section is set by first choosing the type of cross section: circular, rectangular or elliptical and then entering the dimensions corresponding to the type of cross section you selected. The program will automatically calculate the Effective Diameter (Do). Do is the diameter of a circular cross section that has the same cross sectional area as the tissue.
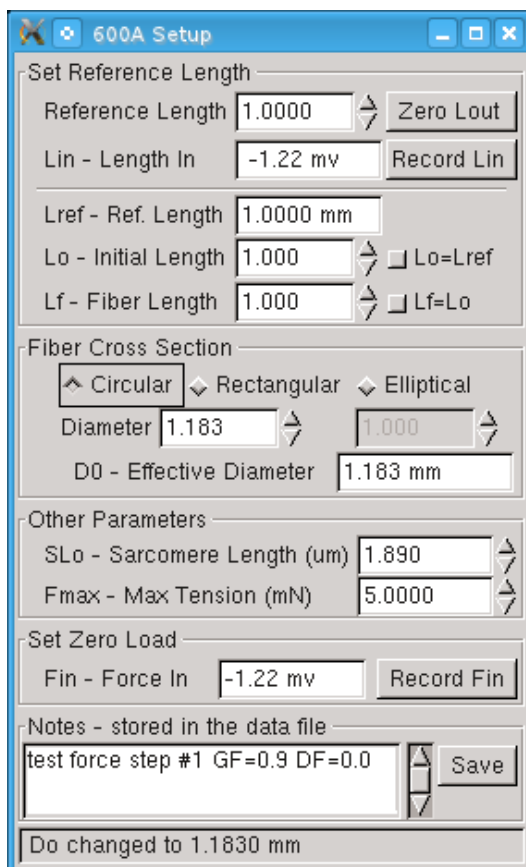
Figure 8 ASI600A Setup Window

9. The Other Parameters section can be skipped for now or you can set the initial sarcomere length (SLo) if desired and the maximum tension the muscle can develop (Fmax).

SLo and Fmax are used for plotting normalized sarcomere length and force (SL/SLo and F/Fmax). Both SLo and Fmax can also be used in protocol control functions. For this reason it is important that these parameters be positive and non-zero. **If SLo and Fmax are not known then enter 1.0 for each of these parameters, do not set either of them to zero.**

10. Near the bottom of the Setup window is the Set Zero Load section. Set the muscle to the desired baseline force and then press the Record Fin button. This will record the current force and display this value, in mV, in the Fin – Force in text box. As the user you can choose what to use as the zero force, options include but are not limited to:

a. Force before attaching the muscle tissue to the force transducer with the force transducer hook in air.

b. Force before attaching the muscle tissue to the force transducer with the force transducer hook in liquid.

c. Force with the tissue attached to the hook but with the muscle slack.

d. Force with the muscle set to Lo.

e. Force with SL set to a particular value.

f. Regardless of which option you choose after pressing the Record Fin button you will note that the force is set to 0 mN. The Set Zero Load function is similar to Set Reference Length in that it provides a datum to the computer for force control and reporting.

11. The last section is the Notes section at the bottom of the window. If desired enter comments in the Notes section and these comments will be stored in all data files.

12. Either leave the Setup window open or close it using the X control in the top right corner.

13. The next step is to create a test protocol. Select Protocol→ Open to open the Protocol editor.

14. The protocol editor allows the user to create a series of control events based on time. The protocol window is a standalone window that can remain open during testing. This allows for easier viewing of the currently defined protocol and allows the user to quickly and easily modify the protocol and then run it. The Protocol Window features three sub menus called Protocol, Stimulus and Sequencer. Click on the headings at the top of the window to switch between these windows.

There are four main headings associated with creating a protocol (see Figure 9) these are:

Step   Time   Function   Parameters

A test protocol consists of at least three steps but it can have many more. Each step has a start time (Time), a function and parameters (if required by the particular function chosen).

At the top of the protocol window are text boxes showing the current protocol file name, the current Lo value and the current Fmax value. None of these text boxes can be modified as the contents of the boxes come from other parts of the program. Below the text boxes is an area showing the contents of the protocol file and below this are control buttons and text entry boxes located near the centre of the screen. Lmin and Lmax are also set on the protocol window and will be discussed in steps 18 and 19 below. At the bottom of the screen are: Sampling Rate, Comment text box and control buttons that start a test (Start Test), load a protocol (Load), save a protocol to a new file name (Save As), and save the current protocol to the currently defined name (Save).
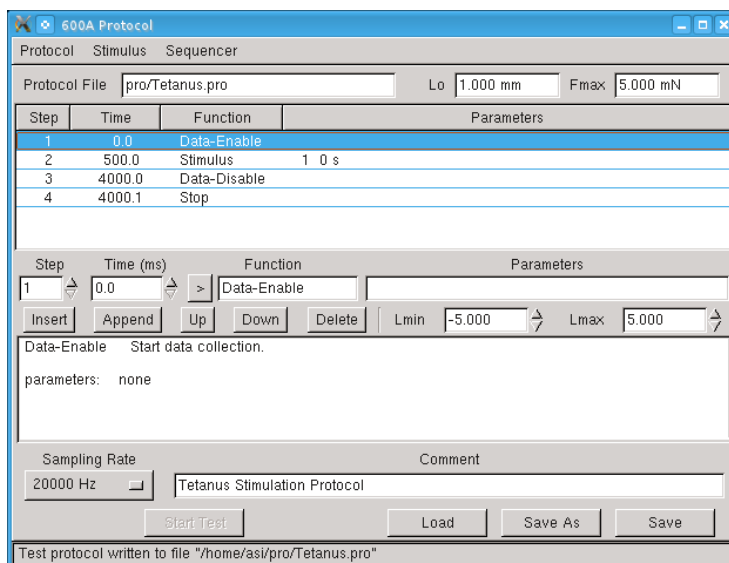
Figure 9 Protocol Editor Screen

15. For this example we will create a simple tetanus stimulation protocol that controls the stimulator and collects data. The protocol will start data collection, issue a stimulation timing pattern and then disable data collection. Enter the protocol shown in Table 1 below or Press Load and load the protocol file Tetanus.pro.

Table 1 Example Test Protocol – Tetanus.pro

Step	Time (ms)	Function	Parameters
1	0.0	Data-Enable	
2	500.0	Stimulus	1 0 ms
3	4000.0	Data-Disable	
4	4000.1	Stop	

16. As you enter the protocol you will note that the Step number is automatically completed for you. Also note the help text that is displayed in the box near the bottom of the screen. This test box provides a brief description of each function including the expected number of parameters, the valid units and an example of the function's use. A very important note about protocols is that you must ensure that Time increases for each successive step. The program will parse the protocol before saving it and if the times don't increase an error will be generated. If the number of parameters entered for a function or the units are incorrect then the step will not update when the Enter key is pressed. Error and status messages are printed in the text box at the bottom of the screen. There are several possible units that can be used for functions that include length, force or time. The units of length are: um, mm, Lo, Lf and volts. The units of force are: mN, N, gm, kg, Pa, kPa, Fmax and volts. The units of time are: ms and s. Parameters are also case sensitive so take care to enter units correctly. Test protocols can be of any length and can contain multiple occurrences of the same function if desired.

Functions can't start exactly at the end time of a previous function there must be at least 0.1 ms between the end of one function and the start of the next. For example: if a Length-Ramp starts at 100.0 ms and has ramp duration of 50.0 ms

the next function cannot start until 150.1 ms. (100.0 + 50.0 + 0.1). Failure to respect the timing in a protocol can cause the program to perform unexpected operations and in some cases the PC can "lock-up".

All protocols must include at least one Data-Enable and one Data-Disable command.

17. Stimulus is a special function in that the parameters are a stimulus profile number and a delay time (the delay controls the actual time that the first pulse of the stimulation is produced, the actual time of stimulation is the Time associated with the Stimulus command plus the delay time). The stimulus profiles are defined by clicking on the Stimulus tab at the top of the Protocol window. Up to 10 stimulus profiles can be defined.

Stimulation can continue after a test protocol is finished! If the Train Duration specified in the stimulation profile is greater than the protocol's test duration then stimulation will continue to be active after the protocol completes. Please see Section 4.6.2 Stimulus for further details on stimulation control.

18. Enter Lmin; this controls the minimum length that the program can command the lever system to move to. The Lmin value is set as a percentage of Lo, a typical value might be 0.10 which corresponds to a minimum length of 10% of Lo. This control is used to limit the movement of the lever to ensure that the muscle is not shortened beyond a safe limit.

Make sure that Lmin is set to a realistic number. If it is set incorrectly the program may not function as expected.

Lmax and Lmin take priority over all control commands. If you tell the system to go to a length outside of the Lmax/Lmin range the program will clamp the length at the limits specified by Lmax and Lmin. The available range of Lmin is -10Lo to +10Lo. **In all cases Lmin must be less than Lmax.**

Enter Lmax; this controls the maximum length that the program can command the lever system to move to. The Lmax value is set as a percentage of Lo, a typical value might be 1.25 which corresponds to a maximum length of 125% of Lo. This control is used to limit the movement of the lever to ensure that the muscle is not stretched beyond a safe length.

Make sure that Lmax is set to a realistic number. If it is set incorrectly the program may not function as expected.

Lmax and Lmin take priority over all control commands. If you tell the system to go to a length outside of the Lmax/Lmin range the program will clamp the length at the limits specified by Lmax and Lmin. The available range of Lmin is -10Lo to +10Lo. **In all cases Lmax must be greater than Lmin.**

19. When you have finished entering the protocol you must save it before you can start a test. Either press Save As to save the protocol file to a new file name or simply press Save to overwrite the existing protocol file. The program will not run a protocol that hasn't been saved.
20. The Stimulation profiles should be entered before executing a protocol which includes stimulus or trigger commands. Click on the Stimulation tab at the top of the Protocol window to switch the display to the Stimulation setup window, see Figure 10.
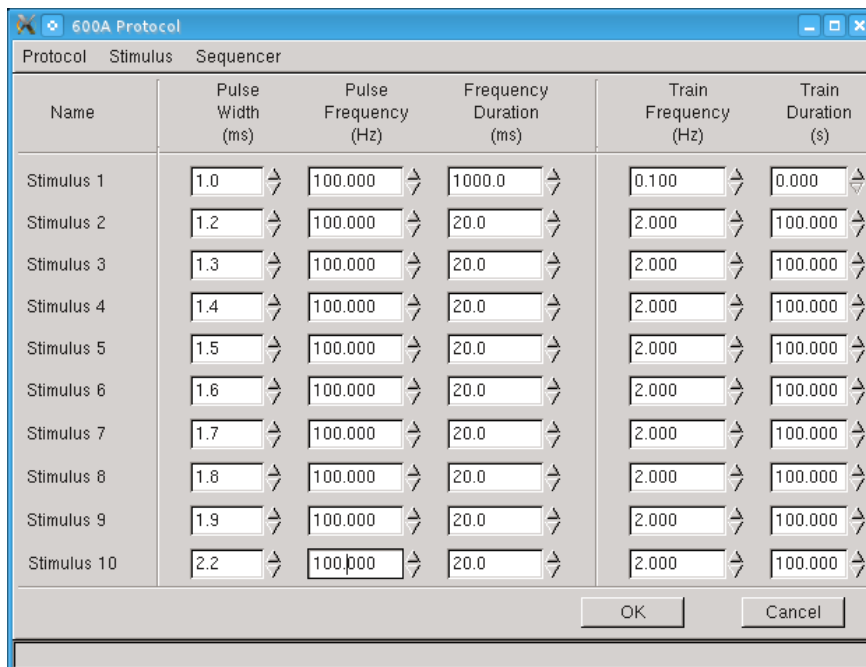
Figure 10 Stimulus Window

Continuing with the tetanus example, a typical stimulus profile would be as shown in Table 2 Stimulus Profile 1. In this stimulus profile the Pulse Width is 1 millisecond (ms), the Pulse Frequency is 100 Hz and the Frequency Duration is 1000 ms. Since this stimulation pattern is intended to occur only once the Train Frequency has been set to 0.1 Hz and the Train Duration to 0 sec.

Stimulus profiles are stored along with the protocol in the protocol file. Make sure you resave the protocol after changing stimulus profiles. **Failure to resave the protocol file could result in unexpected operation.**

Table 2 Stimulus Profile 1

Pulse Width (ms)	Pulse Frequency (Hz)	Frequency Duration (ms)	Train Frequency (Hz)	Train Duration (s)
1.0	100.0	1000.0	0.100	0.000

21. On the main window press the Simulate button and then press Start Test to review the protocol that you just entered and saved. If you are satisfied with the protocol and there are no errors then you can turn off the Simulate button on the main screen in preparation for running the test.

22. Before running the protocol you may want to start Scope. Scope provides a real-time scrolling output on a separate window that resembles an oscilloscope or a strip-chart recorder. Scope is very useful at providing a long-term record of what length, force, sarcomere length and auxiliary data has done over a time period of up to 4 hours. The main screen provides a numerical output of the current Lin and Fin. This readout is useful to see what these values are at any given time but for slow trends (like monitoring the contraction of a skinned muscle fiber) it is very useful to have the Scope function. Click on Open on the Scope menu to open the Scope window. Scope runs independently of the main control and graphics section of the program and because we are using a real-time version of Linux there is no concern that Scope will interfere with the main control program. Scope runs at a constant 100 Hz. In addition to buffering up to 4 hours of data Scope can also be used to record data at low sample rates (1 to 100 samples per second). See Section 7.0 Scope for a more detailed description of Scope.

23. Press the Start Test button on either the bottom of the Protocol window or the bottom of the Main window to execute the protocol. The protocol will start as soon as you press the button and the results will start to appear on the Main window as soon as the protocol starts. If the protocol includes a Repeat function then the results of each execution of the protocol will be plotted as soon they occur. The graph for each repetition is defined as a Frame and there is a button near the bottom of the main screen that allows the user to scroll through the Frames. If the protocol has more than one pair of Data-Enable and Data-Disable functions then each Data-Enable function will also generate a new Frame.

24. Once the protocol is complete the data can be saved by clicking on File→ Save Data. Alternatively you can automatically save the data at the end of each test by pressing the Auto Save button on the Main window.

25. Analysis can be used to review the stored data, zoom in on a selected area and perform calculations. To open the Analysis window click on Analysis→ Open. See Section 6.0 Analysis for more details on using Analysis.

26. The ASI600A program can be closed either by clicking on Exit under the File menu or by clicking on the X in the upper right corner of the Main screen window.

4.0 Main Program

The ASI600A Digital Controller program uses a familiar Windows-type interface that consists of a Main window containing a graphics window, control buttons, several text readouts and a series of drop-down menus. The Main window can be moved, re-sized, minimized, maximized and closed by clicking on the various window controls provided. In the upper right corner of the window are minimize, maximize and close buttons. Clicking anywhere on the window will bring the focus to the window. The window can be dynamically re-sized by clicking on a border or a corner of the window. Most of these controls are similar to those found on Windows or Apple operating systems. Details of the Main program window and program operation will be presented in the following sections.

4.1 Main Screen

The main screen consists of a menu bar at the top, a large graphics window located in the centre, text readouts and control buttons located on the left border, control buttons below the graphics window and a status line at the bottom of the window (see Figure 11).

4.1.1 Graphics Area of Main Window

The graphics area of the Main window can be configured by the user to show the data channels of interest. Configuration of the graphics area is done using the Edit→ Display Settings window. Using the Display Settings window the user can select to plot up to four different data channels, choose the location of each channel in the graphics area, set the size of the plot relative to the others, and also set the units and colour for the data on each channel. The available data channels include: Length In, Force In, SL, Aux 1, Aux 2 and Stimulus. Each graph consists of time along the x-axis versus the chosen data channel on the y-axis. The relative size of each channel's graph can also be set through the Display Settings window. The Main window's graphics area is updated in real time as the data is being collected. The graphics area is also updated whenever the user makes changes through the Display Settings window. Please see Section 4.3.1 Display Settings for further details on the window.

4.1.2 Control Buttons

The main screen includes five control buttons located beneath the graphics window. These buttons are: Start Sequence, Start Test, Next Test, Stop Test and Zero Lout (see Figure 11 ASI600A Main Screen).

Start Sequence
The Start Sequence button is used to start a sequence of test protocols. The Reference Length must be set using Setup→ Open before the Start Sequence button will operate. Upon clicking the Start Sequence button the program will execute the first enabled protocol in the sequence and then either wait for the Next Test button to be pushed to advance to the next protocol or automatically advance to the next protocol in the sequence depending on how the sequence timing is set. Results of each protocol are plotted on the Main window as the protocol is executing. The results will automatically be plotted on the Analysis window if the Auto Load Test button on the Analysis window is pressed.
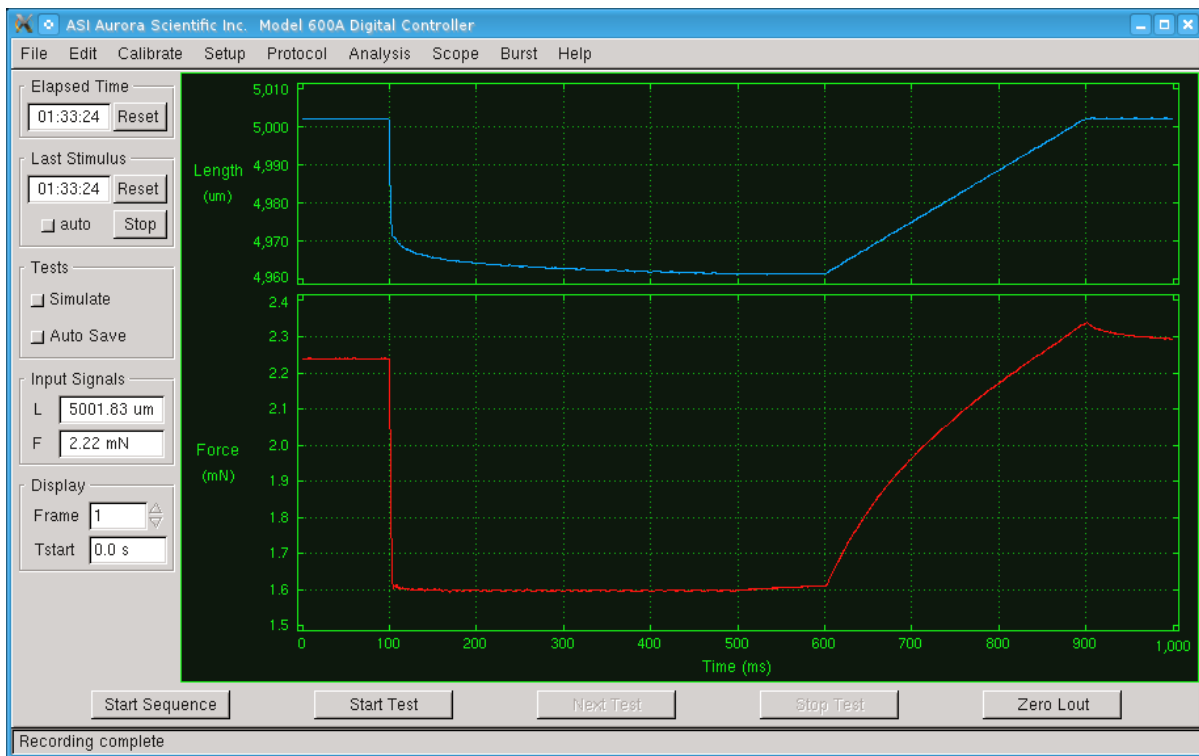
Figure 11 ASI600A Main Screen

Start Test

The Start Test button is used to start a test protocol. The Reference Length must be set using Setup→ Open before the Start Test button will operate. Upon clicking the Start button the program will execute the function listed for each step in the test protocol at the times specified in the protocol. The data is plotted on the Main window as the protocol executes. The results will automatically be plotted on the Analysis window if the Auto Load Test button on the Analysis window is pressed.

Next Test

The Next Test button is used to start the next protocol in a sequence. The Next Test button will only become active after a sequence of two or more protocols has completed the first protocol in the sequence and the timing in the sequence is set to wait for user input. The status line will show the current protocol in the sequence and the total number of protocols to be executed. If the timing in the sequence is set to automatically advance to the next protocol then there is no need to press the Next Test button.

If automatic timing is selected and the timer is counting down to the start of the next protocol in a sequence, then the user can bypass the delay time by pressing the Next Test button. When Next Test is pressed the countdown timer will be set to zero and the next protocol in the sequence will start immediately.

Stop Test

The Stop Test button is used to stop a test protocol or a sequence of test protocols. The button freezes all control action of the program as soon as the button in pressed. If the length out is set to 150 microns when the Stop Test button is pressed then it will stay at this level. The

graph presented on the Main screen will show the data collected from all channels up to the point at which the Stop Test button was pressed.

Zero Lout

The Zero Lout button is used to zero the control output to the length controller. The control output is labeled Length Out on the front of the 604A Signal Interface box. There are several reasons for wanting to zero the Length Out signal. Normally Length Out would be zeroed prior to attaching a muscle to the lever system. This provides a known starting point for all tests. Depending on the structure of any particular test protocol the protocol may end with Length Out at some non-zero value. The user may want to re-zero the Length Out signal prior to starting another protocol.

It is best practice to press the Zero Lout button after a protocol or sequence has been stopped using the Stop Test button. This will ensure the length controller is set to a known location before continuing testing.

4.1.3 Text Readouts

Six text readouts are located on the left side of the Main screen. The first two readouts are timers that can be used to time experiments and to measure the time since the last stimulation. Below the timers are two text readouts that show the current values of Length In (L) and Force In (F) in the units selected by Edit→ Display Settings. These readouts provide a convenient way of monitoring the current status of the lever system, the force transducer and the 600A controller. Please note that Scope provides a graphical readout of these same values. Below L and F are two readouts showing the Frame number and Frame starting time in seconds. The Frame and Time readouts are used primarily if the test protocol includes a Repeat function. Each repetition of the protocol is defined as a frame and the Frame readout in conjunction with the up/down arrows allows the user to view the data from each repetition of the protocol. The Time readout indicates the start time of the Frame currently being displayed. Note: a frame is also created each time a Data-Enable command is processed in a protocol.

Also located on the left side panel are two control buttons listed under the Tests heading near the middle of the section. The first button labeled Simulate is used to check the timing of a protocol. If the Simulate button is pressed then when either the Start Test or the Start Sequence buttons are pressed the output control signals and the Stimulus and Triggers will be displayed in the Main window graphics area. No control signals will be issued to the length controller. The second button under the Tests heading is Auto Save and this is used to cause the program to automatically save the data after each Start Test button push. Data is saved to sequentially named data files. If the Auto Save button is not depressed then the user must manually save the data after the test finishes by using the File→ Save Data function.

Data is automatically saved when sequences are run. The format of the data file names are set on the sequence window.

4.1.4 Status Line

A status line is located at the bottom of the screen that provides information about program operation.

4.2 File Menu

The File menu contains commands to save data files and exit the program, see Figure 12.

Save Data

Opens a Save As dialog box that allows the current data to be saved to disk. Note: the data file is stored in ASCII format and the structure of the file is provided in Appendix A - ASI600A File Structure.

Exit

Closes the ASI600A program.
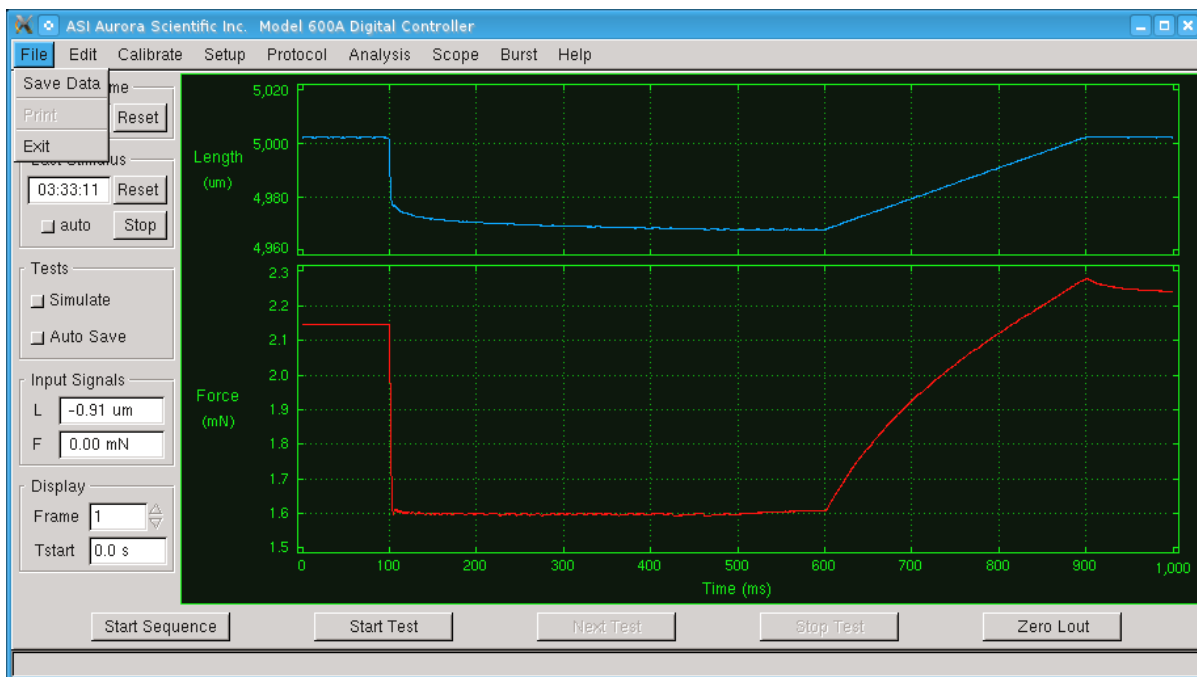
Figure 12 File Drop Down Menu

4.3 Edit Menu

The Edit menu contains two commands, Display Settings and Show Controls. Both of these are used to change the appearance of the program.

4.3.1 Display Settings

The Display Settings window is split into two main regions, the top part of the window is labeled Signals & Sizes and it is used to select the signal to be plotted on each of the four graph locations and the relative vertical size of each graph, the bottom part is labeled Units & Colors and it is used to set the units and colours of the different signal types.

Units cannot be selected for Stim and Aux channels. Stimulus units are always defaulted to 0 or 1 (off or on). Aux channel units can be set in Section 4.4.1 Models Attached.

The choices for each plot location are: Length, Force, SL, Aux1, Aux2, Stimulus and Off.

Plot location 1 can't be turned off as the user must always plot at least one data channel. Therefore Off doesn't appear in the plot location 1 drop down menu for signal selection.

The vertical sizes available are: 1, 2, 3, 4, 5 and 10. The relative size of each plot is taken as a ratio of the size number for that particular plot location divided by the total size of all plot locations that are not set to off.
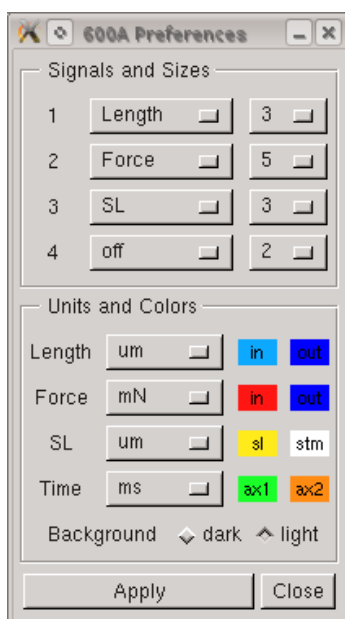
Figure 13 Display Settings Window

The display colour for each channel can be set by clicking the respective colour block found to the right of the signal units. Beside the Length and Force signals are blocks that say in and out. These represent Length In, Length Out, Force In, and Force Out respectively. Below these are blocks with labels sl (sarcomere length), stm (stimulus), ax1 (Aux1), ax2 (Aux2). Clicking any of these blocks opens the colour selection window seen in Figure 14. To set a colour, pick the desired colour or enter its numerical value and then click okay.

At the bottom of the display settings window is a background colour option. This allows the user to pick a light background (black lines on a white background) or a dark background (green lines on a black background). These two backgrounds provide for a greater degree of flexibility in plotting data. It is often much easier to find colours that will display well on a dark background as oppose to a light background.

The program automatically scales the display axes to the range of the collected data. Plot manipulation, zooming, etc. is provided under the Analysis section of the program that is discussed in Section 6.0 Analysis.

.
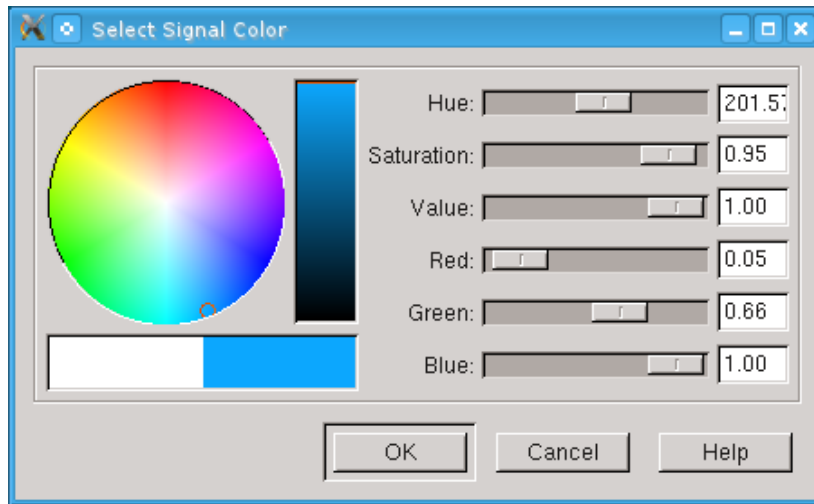
Figure 14 Select Signal Color Window

4.3.2 Show Controls

Click on Edit→ Show Controls to hide or show the controls on the left side of the main window. Hiding the controls maximizes the area on the window for displaying test data, see Figure 15.
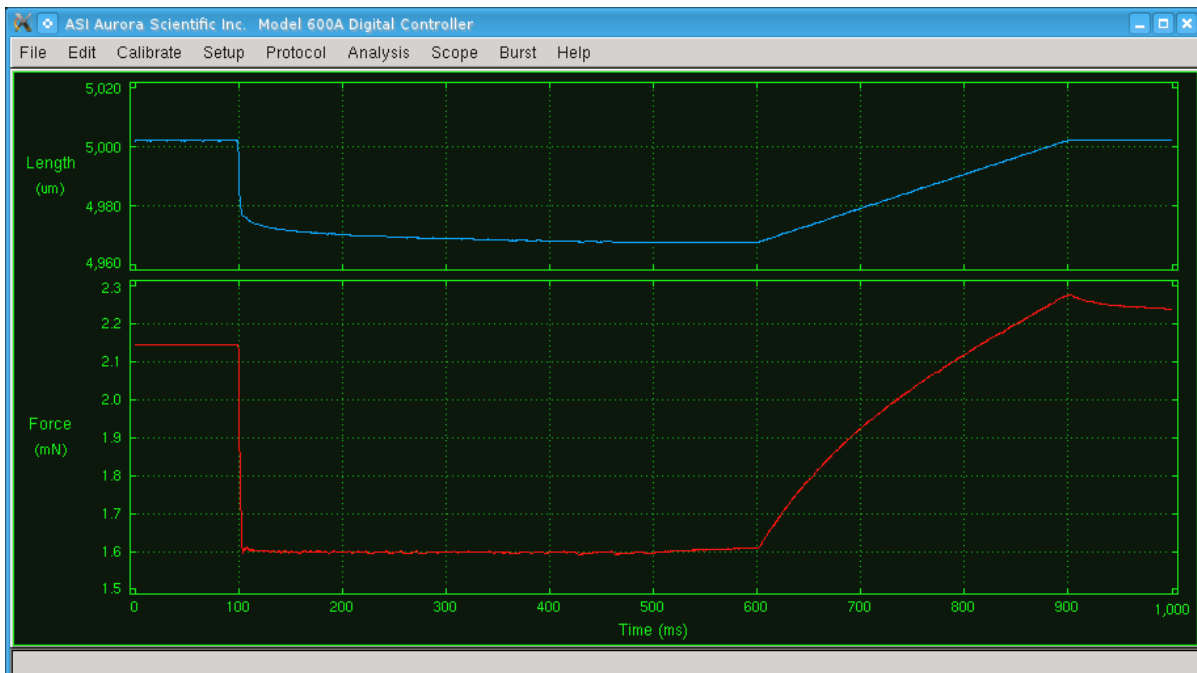
Figure 15 Main Window with Controls Hidden

4.4 Calibrate Menu

The Calibrate menu contains commands to set the models of lever system and force transducer that will be used with the 600A controller, to calibrate both length and force and to provide force and sarcomere length feedback control, see Figure 16.

4.4.1 Models Attached

The Models Attached menu item allows the user to set the model of lever system and force transducer that is connected to the 600A (see Figure 17). If you are using an ASI instrument then check the appropriate model number and the length and force calibrations will be set to the factory specifications. When selecting a dual-mode lever system (models: 300C, 300C-LR, 305C, 305C-LR, 310C and 310C-LR) please ensure that you select *none* under the Force Transducer heading. This will tell the program that you are using the force transducer built into the lever system and not an external transducer. If you are using a length controller or force transducer built by a different manufacturer select the ASI model closest to your device and then perform a manual length and force calibration.
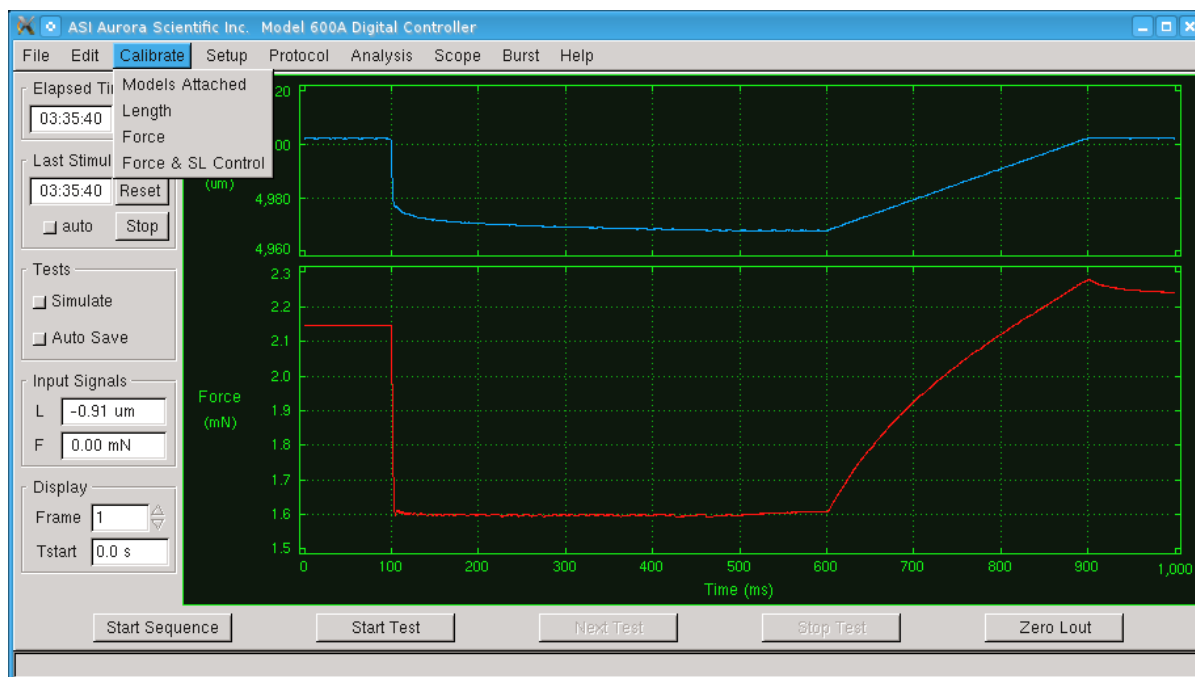
Figure 16 Calibrate Drop down Menu

The Models Attached screen also has an area where you can set the Name, Units, Scale factor and Offset for the two auxiliary input channels that are available. See Figure 17 for an example of auxiliary channels measuring temperature and Aux 2. The Scale factor and Offset are selected to convert volts to the units you specified in Units. Let's assume that a temperature input is attached to Aux 1 with a signal of 10mV for every °C. To calculate the Scale for this signal you would simply convert the 10 mV to read in volts (0.01V) and then invert the 0.01V/°C to obtain 100°C/volt, you then enter 100 in the Scale factor text entry box. Often there will be a small offset present on the signal and this can be compensated for by entering, in the Offset text entry box, the difference between what the output reads in the 600A program and what you know

it to be. In the example shown in Figure 17 you will note the offset is -1.10°C which compensates for the offset seen on this particular temperature sensor.
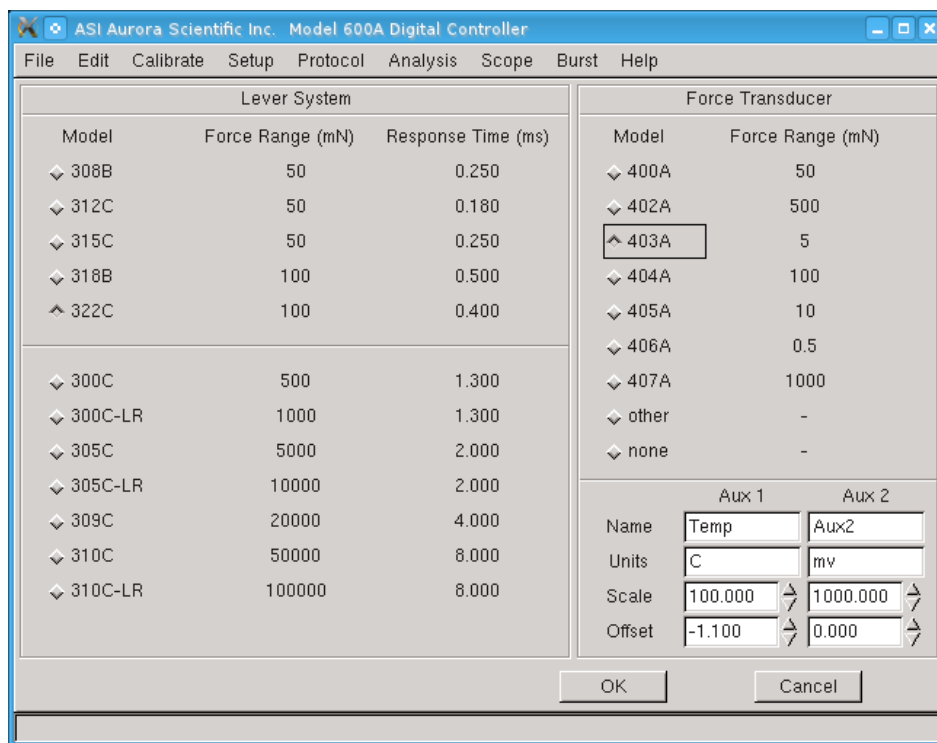
Figure 17 Models Attached Window

4.4.2 Length Calibration

The Calibrate→ Length menu item opens the Length Calibration window (see Figure 18). This window provides a simple means of calibrating the length channel input and output. The calibration screen can be used to perform multipoint calibrations or you can simply enter a known calibration constant. When doing multi-point calibrations the data and a linear curve fit are plotted when the calculate button is pressed at the end of the calibration. The program calculates the calibration constants from the sampled data and displays the calculated values on the right side of the screen under Calibration Factors.

To calibrate a lever system you will require an independent means of measuring the length (or position change) of the lever arm. At the start of calibration you will note that there is a Length In value next to the 0.00 value under the Length Out (v) column while all other boxes in the Length In (v) column are blank. Upon entering the Length Calibration screen the program sets Length Out to 0.000 V and records Length In. This value is then displayed in the Length In column. Now set the Length Out voltages to your desired values or use the default values shown. Use the up/down arrows to the right of the Length Out (v) column to set the length out voltage or simply type in the desired voltage in the text box. Press the radio button to the left of the desired Length Out (v) setting which causes the system to measure the current length input. Manually measure the actual length and enter the actual length in the column labeled Length

(mm). Repeat this procedure for up to five Length Out voltages. Once complete press the Calculate button at the bottom of the screen. The program will plot the calibration data, fit a curve to the data and then automatically calculate Length Out and Length In Scale factors and Offsets (see Figure 19).

Alternatively, if you know the input and output scale factors and offsets you can enter them directly in the text boxes on the right side of the screen.

Figure 18 Length Calibration Window

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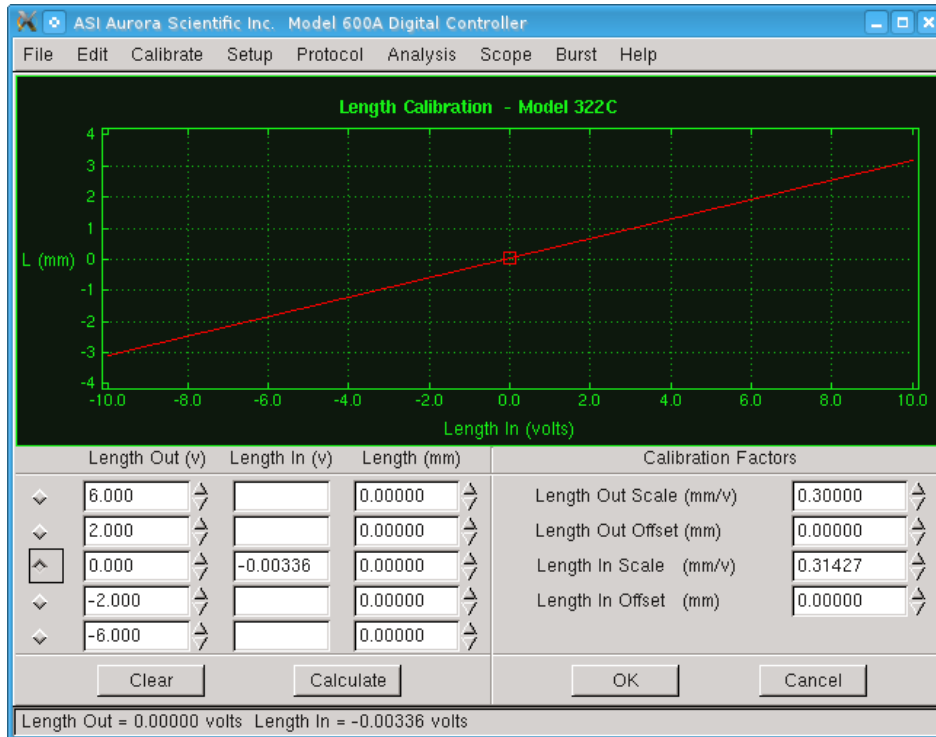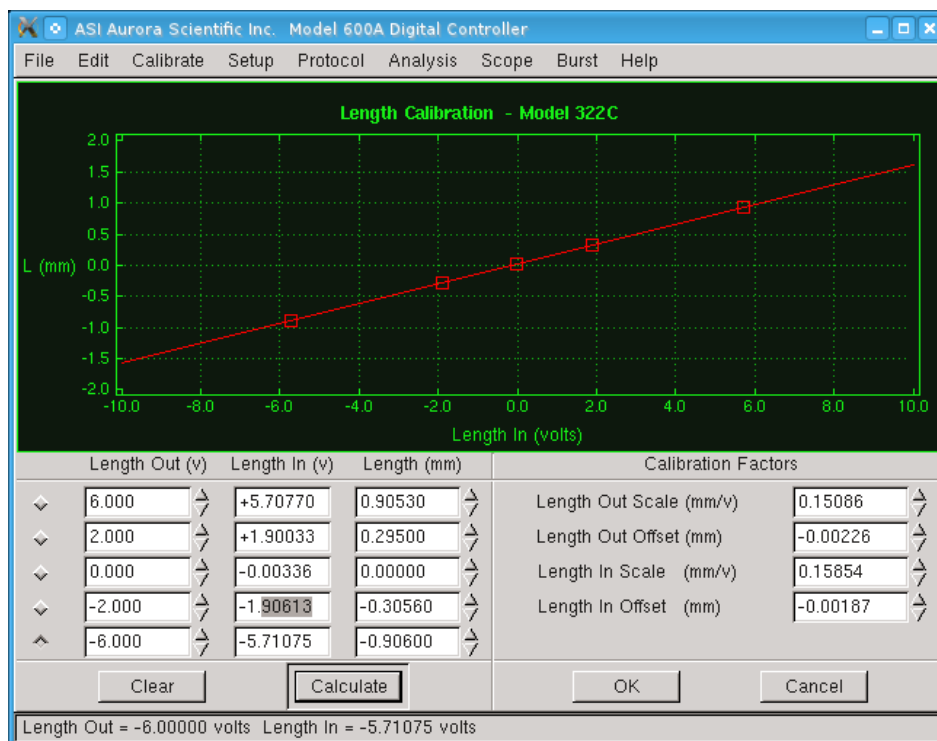**Figure 19 Length Calibration Window Showing Completed Calibration**

4.4.3 Force Calibration

The Calibrate → Force menu item opens the Force Calibration window (Figure 20). This window provides a simple means of calibrating the force. The calibration screen can be used to perform multipoint calibrations or you can simply enter a known calibration constant. When doing multi-point calibrations the data and a linear curve fit are plotted when the calculate button is pressed at the end of the calibration. The program calculates the calibration constants from the sampled data and displays the calculated values on the right side of the screen under Calibration Factors.

To calibrate the force transducer you will require a series of known masses that can be attached to the force transducer. Start with no mass attached to the transducer and press the square radio button in the Rec column next to the 0.0 gm line. Pressing the radio button causes the program to input the current Fin signal. Now apply a series of known weights to the force transducer, enter the actual weight and press the corresponding radio button. Repeat this procedure for up to five weights. Once complete press the Calculate button at the bottom of the screen. The program will automatically calculate the Force In Scale factor and the Offset. If you are calibrating a dual-mode lever system then the Force Controller information will be un-grayed and a calibration of the control signals can also be performed.

Alternatively, if you know the input scale factor and offset for your transducer you can enter them directly in the text boxes on the right side of the screen.
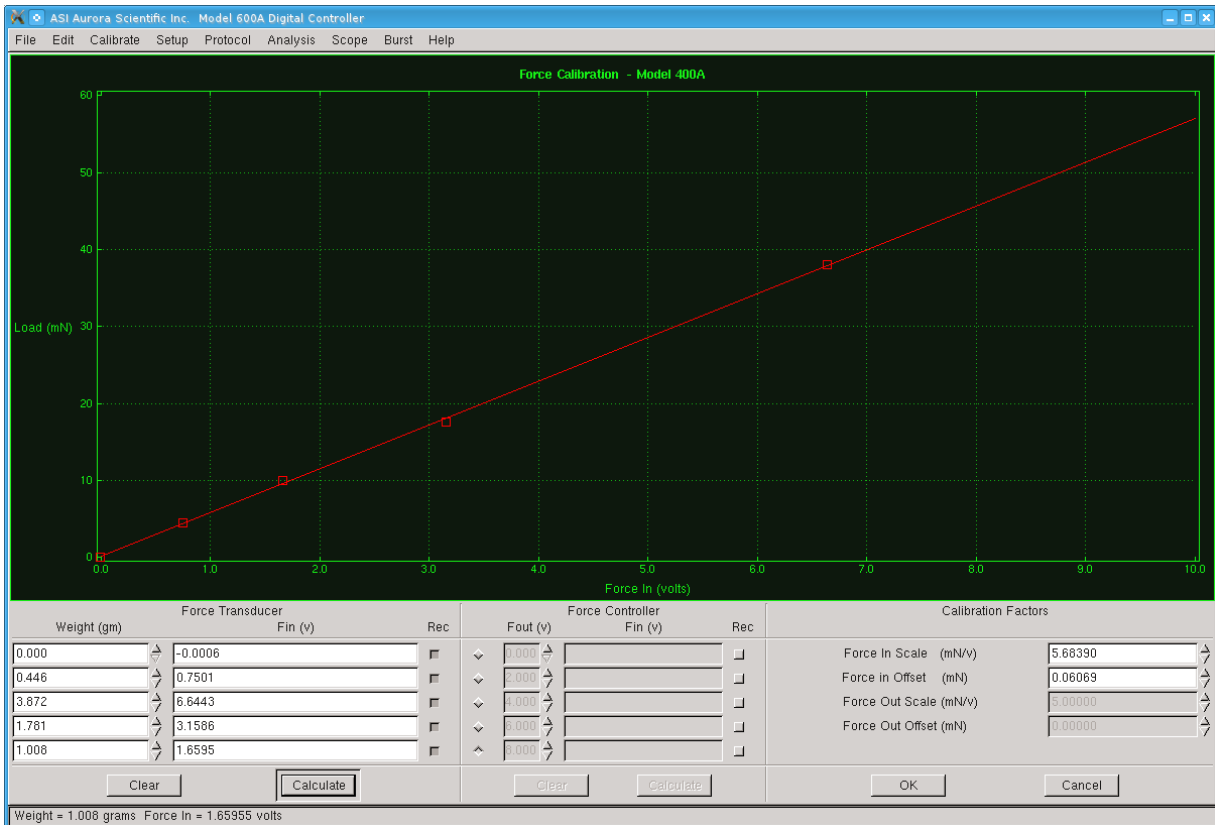
Figure 20 Force Calibration Window Showing Completed Calibration

4.4.4 Force & SL Control

The Calibrate→ Force & SL Control menu item opens the Force & SL Control Window. Please refer to Section 5.0 Force and SL Control for information on this subject. This window is used to calibrate and tune the force control and sarcomere length feedback control functions of the 600A program (see Figure 21).
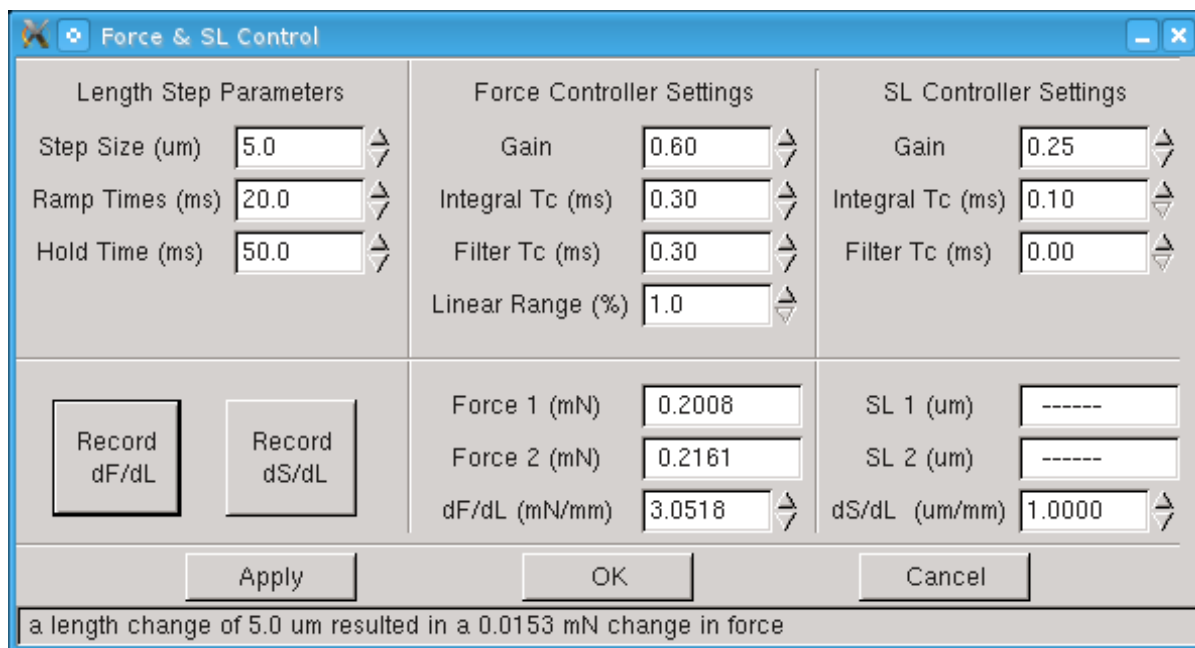
Figure 21 Force & SL Control Window

4.5 Setup Menu

Click on the Setup Menu heading and then click on Open and the Setup window will open as shown in the left window of Figure 22. The Setup window is divided into five sections, Set Reference Length, Fiber Cross Section, Other Parameters, Set Zero Load and Notes.

Set Reference Length

The Reference Length is defined as the muscle tissue length when Lout is set to zero. In most cases this will also be the initial length (Lo) of the tissue. Setting the Reference Length provides the datum used by all subsequent length measurements and length movements. Reference Length must be set before any test protocols are run. In order to correctly set the Reference Length the researcher must have an independent means for measuring the actual length (Reference Length) of the muscle.

The Reference Length must be entered (in mm) **before** pressing the Record Lin button. This step must be performed correctly or the control system will not operate as desired.

In order to Set Reference Length start by pressing the Zero Lout button. Next measure the length of the tissue and enter this value in the Reference Length text box. Now press the Record Lin button. After pressing the Record Lin button the program will sample the current Length In value and display this value, in mV, in the Lin – Length In text box. At the same time the program will display the reference length in the Lref – Ref. Length text box.

After setting the reference length do not adjust the Zero Offset potentiometer control on the front of the length controller or the X stage controls that change the overall tissue length. If you do you will create a length offset between the actual

position and the position that the computer reports. This offset can result in incorrect data being recorded and the system not controlling to the desired length, force or sarcomere length. **If you change the Length Offset potentiometer on the Length Controller or the X stage position then you must set the Reference Length again.**

The lower half of the Set Reference Length section allows you to enter the initial length (Lo) and the fiber length (Lf). You have the option of manually entering Lo and Lf or have the program set them automatically. Click on the Lo=Lref button to set Lo equal to Lref. Lo is used for plotting length in terms of L/Lo but it is also used in test protocols for controlling length. Do not set Lo to zero. If you want to work in units of fiber lengths then either enter the fiber length (Lf) or click on the Lf=Lo button to set them equal.
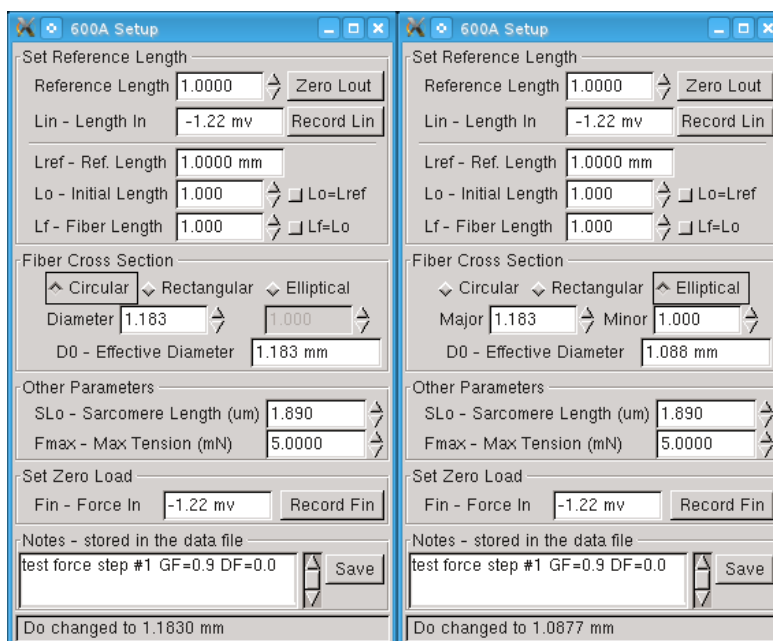
Figure 22 ASI600A Setup Window Showing Different Cross Section Calculation

Lo - Initial Length

The Initial Length (Lo) is normally defined as the tissue length under specified physiological conditions such as the overall length of the tissue when the sarcomere length is set to the resting sarcomere distance, the overall length when the tissue is at some low resting tension or the overall length which produces maximum tetanic force when stimulated. Lo is set in units of mm. The program uses Lo in two key ways: (1) it is used to plot normalized length (strain) as a percentage of Initial Length, i.e., L/Lo; (2) it is used in length control functions in test protocols to allow length changes in terms of a percentage length change (0.9 Lo being a length change to 90% of the initial length). The Initial Length can be entered in the text entry box or the button labeled Lo=Lref located to the right of the text entry box can be pressed which will automatically set Lo equal to the reference length (see Figure 22).

Lf - Fiber Length

The Fiber Length (Lf) is normally determined by the researcher and can be entered in the text entry box. For the case of whole muscle and fiber bundles Lf is less than Lo whereas for single fiber experiments Lf may be equal to Lo. In the case where Lf is equal to Lo you can click the button labeled Lf=Lo located to the right of the text entry box which automatically sets Lf equal to Lo. Note it is possible to have all three lengths equal where Lref = Lo = Lf. Fiber Length is set in units of mm. The program uses Lf for plotting normalized length (strain) as a percentage of the fiber length, i.e., L/Lf.

Fiber Cross Section

In the Fiber Cross Section area of the Setup window the fiber cross section is set by first choosing the type of cross section: circular, rectangular or elliptical and then entering the dimensions corresponding to the type of cross section you selected. The program will automatically calculate the Effective Diameter (Do). Do is the diameter of a circular cross section that has the same cross sectional area as the tissue. See Figure 22 for examples of Circular and Elliptical cross section setup windows.

Other Parameters

The Other Parameters section is used to set muscle and control parameters for a particular set of experiments. The muscle parameters include: initial sarcomere length (SLo) and maximum tension the muscle can develop (Fmax).

⚠️ Do not set either SLo or Fmax to zero, if these values aren't known then enter 1.0.

SLo - Initial Sarcomere Length

The initial Sarcomere Length (SLo) is normally defined as the sarcomere length under specified physiological conditions such as the sarcomere length when the tissue is slack, the sarcomere length when the tissue is at some low resting tension, or the sarcomere length which produces maximum tetanic force when the tissue is stimulated. SLo is set in units of microns (um). The program uses SLo in two key ways: (1) it is used to plot normalized sarcomere length (strain) as a percentage of Initial Sarcomere Length, i.e., SL/SLo; (2) it is used in sarcomere length control functions in test protocols to allow sarcomere length changes in terms of a percentage length change (0.9 SLo being a sarcomere length change to 90% of the initial sarcomere length). The Initial Sarcomere Length is entered in the text entry box next to the SLo – Sarcomere Length (um) text.

Fmax – Max Tension

The maximum tension (Fmax) is normally defined as the maximum tension that is measured during a tetanic contraction. Fmax is set in units of milliNewtons (mN). The program uses Fmax in two key ways: (1) it is used to plot normalized force as a percentage of maximum force, i.e., F/Fmax; (2) it is used in force control functions in test protocols to allow force changes in terms of a percentage force change (0.9 Fmax being a force change to 90% of the maximum force). The maximum tension is entered in the text entry box next to the Fmax– Max Tension (mN) text.

Set Zero Load

Near the bottom of the Setup window is the Set Zero Load section. Ensure that the muscle is slack and then press the Record Fin button. This will record the current force and display this value, in mV, in the Fin – Force in text box. As the user you can choose what to use as the zero force, options include: force before attaching the muscle tissue to the force transducer with the force transducer hook in air, force before attaching the muscle tissue to the force transducer with the force transducer hook in liquid or, force with the tissue attached to the hook but with the muscle slack. Regardless of which option you choose after pressing the Record Fin button you will note that the force is set to 0 mN. The Set Zero Load function is similar to Set Reference Length in that it provides a datum to the computer for force control and reporting.

Please note that if the Set Zero Load function is not used properly then undesirable control actions may result when performing force clamps. An example of this would be the following. Assume that the user sets the Zero Load when the muscle is at a resting tension of 2 mN. A protocol is then created that includes a force step to 1 mN. Since the program has been told that 2 mN is actually zero force the force command will actually result in the muscle being stretched (force increased from 0 to 1 mN according to the program) whereas in reality the force has increased from 2 to 3 mN. The user wanted the force to decrease to 1 mN but due to the faulty use of Set Zero Load an actual force of 3 mN resulted even though the program reports the force as 1 mN.

Ensure that the Force Offset control potentiometer on the front panel of the force transducer is not adjusted after setting the zero load. If the control is changed then Set Zero Load should be executed again.

Notes

The last section is the Notes section at the bottom of the window. If desired, enter comments in the Notes section and these comments will be stored in all data files.

4.6 Protocol Menu

The Protocol menu item provides an Open and a Close command. Opening the Protocol window provides access to the Protocol editor, Stimulus setup and the Sequencer windows. The Protocol window allows the user to create and edit test protocols. The Stimulus window is used to define stimulation and trigger profiles. The Sequencer window is used to create, edit and save protocol sequences.

4.6.1 Protocol Editor

Allows the user to define and edit a test protocol (see Figure 23). Test protocols are the heart of the control system and they provide all the functions necessary to generate any muscle physiology test protocol desired. The Edit Protocol screen can be divided into the following four sections: a) the top portion of the screen displays the test protocol, b) the middle section allows the user to edit selected lines of the protocol as well as set the Lmin and Lmax values, c) a text section is below the editing section and it displays information about the selected function and d) at the bottom the Sampling Rate can be set, comments can be entered and buttons are provided to

start the protocol as well as load and save protocols. A status line is provided at the very bottom of the window.

All test protocols are created in order of steps. Each step includes a start time, a function and function parameters. A step cannot start before a previous step has completed. The program checks the protocol to ensure that each step starts at a later time but the user should still take care to ensure that the function called on the current step completes before the start time of the next step. The minimum time between instructions is set in the program to be 0.1 ms. Therefore when creating an instruction it must have a start time that is a minimum of 0.1 ms after the time when the previous command finished. All functions start at the start time set except for Stimulus and Trigger functions which are special and can be delayed to start during a following command.
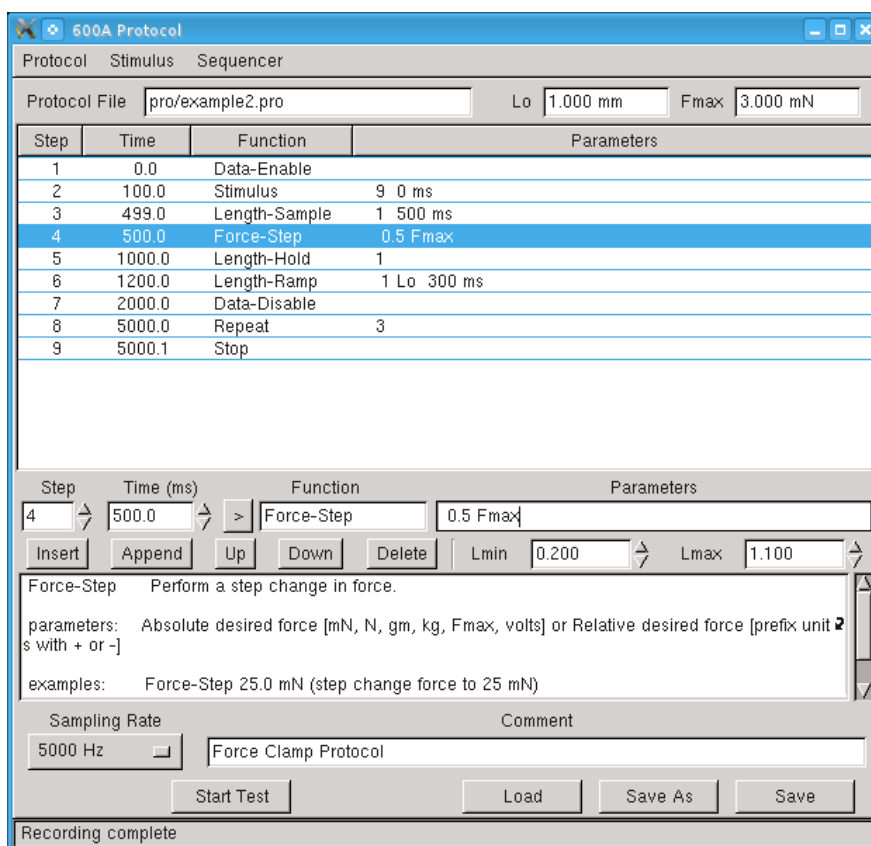
Figure 23 Protocol Window

Absolute vs. Relative Control
The 600A program can operate in either absolute or relative units of length and force. When operating in absolute mode the desired length, force or sarcomere length is set in the protocol function. An example of absolute mode is the current length of the tissue is 9mm and the researcher wants to shorten the tissue to 8mm, an absolute function would simply specify the new length as 8mm. The absolute mode can be thought of as change **to** the new value. When operating in relative mode the researcher must think in terms of changes to the current value of length, force or sarcomere length. An example of relative mode is the current length of the tissue is 9mm and the researcher wants to shorten the tissue by 1mm, a relative function would specify the new length as a change of -1mm. The relative mode can be thought of as a change **by** the

given value. The length, force and sarcomere functions listed below show examples of both absolute and relative modes.

Absolute Control

The 600A program's default setting is to make all control tasks absolute in nature. This means that the program knows the current length of the preparation and when you request a length, force or SL change the program will take this request as an instruction to change to the new absolute unit. The following is a simple example. We will assume that the preparation is 5.0 mm long and that Lref = Lo = 5.0 mm. The test protocol calls for the tissue to be shortened by 1.0 mm to a new length of 4.0 mm. The normal operation of the protocol functions are in absolute units so in order to create this shortening the protocol function would be

Length-Step 4.0 mm

Functions can also be structured in terms of fractions of a reference quantity; in this case the initial length (Lo) is 5.0 mm so a shortening to 80% of Lo (0.8 Lo) will also cause the tissue to shorten to 4 mm.

Length-Step 0.8 Lo

Relative Control

Since the introduction of version 2.0, 600A has provided the capability to structure functions in relative units as well as the standard absolute units. Many researchers have told us they prefer to structure protocols in relative terms such as a shortening **by** 20% as opposed to a shortening **to** 80%. For this reason we enabled the use of relative units in control functions. Even though the program can now be operated in relative mode we feel that it is a best practice to set the datum for the program (Lref and zero force) correctly whenever a new preparation is attached as this provides maximum control options when using the program.

Relative functions change the quantity **by** an amount not **to** an amount. All functions have the same structure whether being used in absolute or relative modes. In order to cause a relative change simply add the prefix + or – to the quantity. Using the example above, in order to shorten the preparation by 1.0 mm to a new length of 4.0 mm, the protocol function in relative mode would be

Length-Step -1.0 mm

Similarly the same shortening can be structured in terms of fractions of a reference quantity in relative mode so a shortening by 20% (-0.2 Lo) will also cause the tissue to shorten by 1mm.

Length-Step -0.2 Lo

Functions

The protocol functions are organized into categories (See Figure 24). The following is a list of all available functions, a brief description of the function and a list of the required parameters and units that must be included with the function. An example of the function's

usage is included. These examples are all shown as a time followed by the function name, parameters and units.

Length	**Force**	**SL**	**Stim**	**Control**

Figure 24 Protocol Dropdown Menus

Length Functions
Length-Step
>   Description: Perform a step change in length.
>   Parameters: 1, Absolute desired length [um, mm, Lo, Lf, volts] or Relative desired length [prefix quantity with + or -]
>   e.g.: 1) Absolute: 20.0 Length-Step 105.0 um   (step change the length to 105 um)
>   2) Relative: 20.0 Length-Step +5.0 um   (stretch by 5 um)

Length-Ramp
>   Description: Perform a ramp change in length.
>   Parameters: 2, Absolute desired length [um, mm, Lo, Lf, volts] or Relative desired length [prefix quantity with + or -], ramp time [ms, s]
>   e.g.: 1) Absolute: 30.0 Length-Ramp 105.0 um 22.0 ms (ramp change length to 105 um over 22 ms time)
>   2) Relative: 30.0 Length-Ramp -5.0 um 22.0 ms (ramp shorten by 5 um over 22 ms time)

Length-Square
>   Description: Change length using a square wave pattern.
>   Parameters: 3, frequency [Hz], amplitude [um, mm, Lo, Lf, volts], duration [ms, s]
>   e.g.: 20.0 Length-Square 55 Hz 0.2 mm 0.12 s

Length-Sine
Description: Change length using a sine wave pattern.
Parameters: 3, frequency [Hz], amplitude [um, mm, Lo, Lf, volts], duration [ms, s]
e.g.: 20.0 Length-Sine 55 Hz 0.2 mm 0.12 s

Length-Sweep
Description: Change length using a sweeping sine wave pattern.
Parameters: 4, start frequency [Hz], end frequency [Hz], amplitude [um, mm, Lo, Lf, volts], duration [ms, s]
e.g.: 20.0 Length-Sweep 10 Hz 100 Hz 0.2 mm 10 s

Length-Sample
Description Sample and store the length after a specified delay.
Parameters: 2, sample number [1-9], delay time [ms, s]
e.g.: 20.0 Length-Sample 3 50 ms   (measure length 50 ms after function start and store value in sample number 3)

Length-Hold
Description: Perform a step length change to the length value stored in the specified sample number.
Parameters: 1, sample number [0-9]   (note: 0 corresponds to current length)
e.g.: 20.0 Length-Hold 1   (hold length at value stored in sample number 1)

Read-Larb
Description: Read a file containing an arbitrary length waveform.
Parameters: 3, filename, length-units [um, mm, Lo, Lf, volts], sampling interval [ms, s]
e.g.: 20.0 Read-Larb pro/wave1.dat Lo 0.2 ms

Write-Larb
Description: Write an arbitrary length waveform to a file.
Parameters: 3, filename, length-units [um, mm, Lo, Lf, volts], sampling interval [ms, s]
e.g.: 20.0 Write-Larb pro/wave1.dat Lo 0.2 ms

Send-Larb
Description: Send a waveform (created by Read-Larb) to the real-time control module.
Parameters: none
e.g.: 20.0 Send-Larb

Length-Arb (shown as Larb in the dropdown menu)
Description: Output a length waveform (see Send-Larb) to the digital to analog converter (DAC).
Parameters: none
e.g.: 20.0 Length-Arb

Force Functions
Force-Step
Description: Perform a step change in force.
Parameters: 1, Absolute desired force [mN, N, gm, kg, Fmax, Pa, kPa, volts] or Relative desired force [prefix quantity with + or -]
e.g.: 1) Absolute: 20.0 Force-Step 25.0 mN (step change force <u>to</u> 25 mN)
2) Relative: 20.0 Force-Step +25.0 mN (increase force <u>by</u> 25 mN)

Force-Ramp
Description: Perform a ramp change in force.
Parameters: 2, desired force [mN, N, gm, kg, Fmax, Pa, kPa, volts], ramp time [ms, s]
e.g.: 1) Absolute: 30.0 Force-Ramp 25.0 mN 22.0 ms (ramp change force to 25 mN over 22 ms time)
2) Relative: 30.0 Force-Ramp -5.0 mN 22.0 ms (ramp decrease force by 5 mN over 22 ms time)

Force-Square
Description: Change force using a square wave pattern.
Parameters: 3, frequency [Hz], amplitude [mN, N, gm, kg, Fmax, Pa, kPa, volts], duration [ms, s]
e.g.: 20.0 Force-Square 55 Hz 0.2 mN 0.12 s

Force-Sine
Description: Change force using a sine wave pattern.
Parameters: 3, frequency [Hz], amplitude [mN, N, gm, kg, Fmax, Pa, kPa, volts], duration [ms, s]
e.g.: 20.0 Force-Sine 55 Hz 0.2 kg 0.12 s

Force-Sweep
Description: Change force using a sweeping sine wave pattern.
Parameters: 4, start frequency [Hz], end frequency [Hz], amplitude [mN, N, gm, kg, Fmax, Pa, kPa, volts], duration [ms, s]
e.g.: 20.0 Force-Sweep 10 Hz 100 Hz 0.2 Fmax 10 s

Force-Sample
Description: Sample and store the current force after a specified delay.
Parameters: 2, sample number [1-9], delay time [ms, s]
e.g.: 20.0 Force-Sample 4 50 ms (measure force 50 ms after function start and store value in sample number 4)

Force-Hold
Description: Perform a step change to the force recorded by Force-Sample.
Parameters: 1, sample number [0-9] (note: 0 corresponds to current force)
e.g.: 20.0 Force-Hold 4 (hold force at value stored in sample 4)

Force-Clamp
>Description: Monitor force and when it reaches a specified value then clamp the force for a specified time period.
>Parameters: 3, clamp force [mN, N, gm, kg, Fmax, Pa, kPa, volts], delay time [ms, s], duration [ms, s]
>e.g.: 20.0 Force-Clamp 28.0 mN 50 ms 1.0 s (wait 50 ms then monitor force and when it reaches 28 mN hold the force for 1 s)

SL Functions
SL-Step
>Description: Perform a step change in sarcomere length.
>Parameters: 1, desired length in um
>e.g.: 20.0 SL-Step 2.3

SL-Ramp
>Description: Perform a ramp change in sarcomere length.
>Parameters: 2, desired length in um, ramp time [ms, s]
>e.g.: 30.0 SL-Ramp 2.4 22.0 ms

SL-Sample
>Description: Sample and store the current value of sarcomere length after a specified delay.
>Parameters: 2, sample number [1-9], delay time [ms, s]
>e.g.: 20.0 SL-Sample 6 50 ms (wait 50 ms then sample SL and store the value in sample number 6)

SL-Hold
>Description: Perform a step change to the sarcomere length recorded by SL-Sample.
>Parameters: 1, sample number [1-9]
>e.g.: 20.0 SL-Hold 6   (change SL to value stored in sample number 6)

SL-Trigger
>Description: Send a trigger to VSL/HVSL to start recording.
>Parameters: 1, delay time [ms, s]
>e.g.: 20.0 SL-Trigger 5 ms   (wait 5 ms then trigger VSL/HVSL)

SL-Track
>Description: Turn ROI tracking in VSL on/off
>Parameters: 1, on-off switch [0 –off, 1 –on]
>e.g.: 100.0 SL-Track 1 (turn tracking on)

Stim Functions
Stimulus
>Description: Start issuing stimulation pulses.
>Parameters: 2, stimulus pattern number [valid range: 1-10], delay time [ms, s]
>e.g.: 20.0 Stimulus 1 5 ms   (Note: stimulation profile is defined in Protocol→ Stimulus)

Trigger1
 Description: Start issuing trigger pulses from port labeled Trigger Out 1.
 Parameters: 2, trigger pattern number [valid range: 1-10], delay time [ms, s]
 e.g.: 20.0 Trigger1 8 5 ms (Note: trigger profile is defined in Protocol→ Stimulus)

Trigger2
 Description: Start issuing trigger pulses from port labeled Trigger Out 2.
 Parameters: 2, trigger pattern number [valid range: 1-10], delay time [ms, s]
 e.g.: 50.0 Trigger2 9 15 ms (Note: trigger profile is defined in Protocol→ Stimulus)

Control Functions
Data-Enable
 Description: Start data collection.
 Parameters: none
 e.g.: 0.0 Data-Enable

Data-Disable
 Description: Stop data collection.
 Parameters: none
 e.g.: 100.0 Data-Disable

Data-Burst
 Description: Data collection at highest possible rate.
 Parameters: 2, delay [ms, s], duration [ms, s]
 e.g.: 100.0 Data-Burst 500 ms 200 ms (delay 500 ms, then turn burst on for 200 ms)

Bath
 Description: Move ASI802D apparatus to desired bath.
 Parameters: 2, bath number [1-8], delay time [ms, s]
 e.g.: 20.0 Bath 3 2.0 s (wait 2 seconds then change 802D to bath 3)

Repeat
 Description: Repeat the entire protocol the desired number of times.
 Parameters: 1, number of repetitions [no units]
 e.g.: 1000.0 Repeat 5

Stop
 Description: Ends the test protocol
 Parameters: none
 e.g.: 350.0 Stop

Control Buttons on the Protocol Screen
Insert
 Inserts a line in the protocol at the highlighted location. The inserted line contains the
 same functions and parameters as in the highlighted line.

Append

>Appends a line to the end of the protocol.

Delete

>Deletes the highlighted line in the protocol.

Start Test

>Starts the currently loaded protocol executing. This is the same button as the Start Test button on the main screen.

Load

>Loads a protocol file saved on disk. Once a protocol has been loaded it can be executed. The file is stored in ASCII format and the file structure is provided in Appendix A.

Save As

>Save As saves a protocol to a new file name. Any edited protocol file must be saved using either Save As or Save before the protocol can be executed. The protocol file is stored in ASCII format and the file structure is provided in Appendix A.

Save

>Saves a protocol file to the currently defined protocol file name. The file is stored in ASCII format and the file structure is provided in Appendix A.

Up

>Moves the selected protocol function up one position.

Down

>Moves the selected protocol function up one position.

Lmin and Lmax

>Lmin and Lmax are used to set limits on the movement of the lever system. These limits are a hardware limit; 600A will not exceed these limiting values even if told to do so in a protocol. Both Lmin and Lmax are set in terms of fractions of the initial length (Lo) and are absolute limits not relative ones. Thus Lo must be set properly in order for Lmin and Lmax to perform correctly.

>Lmin is the minimum length that the controller can move to and has the range of -10Lo to +10Lo but a typical value would be in the range 0.0 to 0.5. A setting of 0.2 Lo means that the length controller can move towards the force transducer such that the minimum length of the tissue will be 20% of the Lo value (a shortening by 80% of Lo). Be aware that an Lmin value of 0.0 corresponds to the lever arm touching the force transducer.

>Lmax is the maximum length that the controller can move to and has the range of -10Lo to +10Lo but a typical value would be in the range 1.01 to 1.5 (1% longer than the initial length to 50% longer than the initial length). Avoid setting Lmax equal to Lo (i.e., 1.0 Lo) as the controller may have trouble controlling to the initial length.

Be careful when setting the values of Lref, Lo, Lmin and Lmax. Improper settings could result in damage to the force transducer or length controller. For example, assume that the user incorrectly sets Lref and Lo to 2.00 mm but the preparation is only 1.00 mm long. If Lmin is then set to 0.2 Lo the lever arm will be allowed to move to 0.2 x 2.0 mm = 0.4mm which is a shortening of 1.6mm. However the preparation is only 1mm long so this actually means the lever arm can move to -0.6mm past the point where the preparation is connected to the force transducer. This action will almost certainly result in damage to the force transducer and possibly also the length controller.

In all cases Lmin must be less than Lmax. Both Lmin and Lmax are stored in the protocol file. Whenever a protocol is loaded Lmin and Lmax are set to the values found in the protocol. Therefore it is important to set the correct Lmin and Lmax values before saving the protocol.

Setting Lmin and Lmax values tightly around the desired range of lengths for the tissue is especially important if the protocol contains either force or sarcomere length commands. In both of these cases the length is driven by the feedback algorithm that is trying to match the current force/SL with the commanded value. This means that without proper Lmin and Lmax values the length controller is free to use its entire range of motion to achieve the desired force or sarcomere length.

Improper setting of Lmin, Lmax, Lo and Lref can result in the program not being able to run a protocol. If the settings of these parameters results in an Lref value that is outside of the currently defined Lmin and Lmax range the program will show a warning message and not execute the protocol.
Under all circumstances Lmin x Lo < Lref < Lmax x Lo.

Sampling Rate
The protocol editor also includes a control to set the sampling rate for data collection. This sampling rate is used for writing data to disk and for graphing the results of a test. The A/D hardware always samples at a higher rate and then block averages the data down to the selected sampling rate. The maximum sampling rate is 20 kHz with the minimum of 10 Hz.

Sample rates higher than 20 kHz can be achieved using the burst functionality. Please refer to Section 4.9 Burst Menu for more information.

Example Protocol
As an example we will create a test protocol that will demonstrate stimulus, length and, force control. The protocol enables data collection, stimulates the muscle, performs a force step, samples the length and holds it and then ramps the length back to the original length before disabling data collection. The protocol then waits for 3.0 seconds and repeats the test 3 times. This example protocol is shown in Figure 25.

Enter the protocol as shown in Figure 25 or load protocol file *example2.pro* which is located in the *home/asi/pro directory*. As you enter the protocol you will note that the Step

number is automatically assigned for each line. Also note the help text that is displayed in the box near the bottom of the screen. This test box provides a brief description of each function including the expected number of parameters, the valid units and an example of its use.

If the number of parameters entered for a function or the units entered are incorrect the program will not accept that protocol line when you press the Enter key. In this case the selected line will not be updated.

The functions in the protocol editor must occur one after then other. The start time of each step must be greater than the end time of the previous step. Since this is a common error, if you attempt to save or run a file that has this issue 600A will give a warning message.

The minimum time between functions must be 0.1 ms. The program will check the protocol before saving it and if there is a timing error a message will appear. For example: if a Length-Ramp starts at 100.0 ms and has a ramp duration of 50.0 ms the next function cannot start until 150.1 ms (100.0 + 50.0 + 0.1).

Failure to respect the timing in a protocol can cause the program to perform unexpected operations and in some cases the PC can "lock-up".
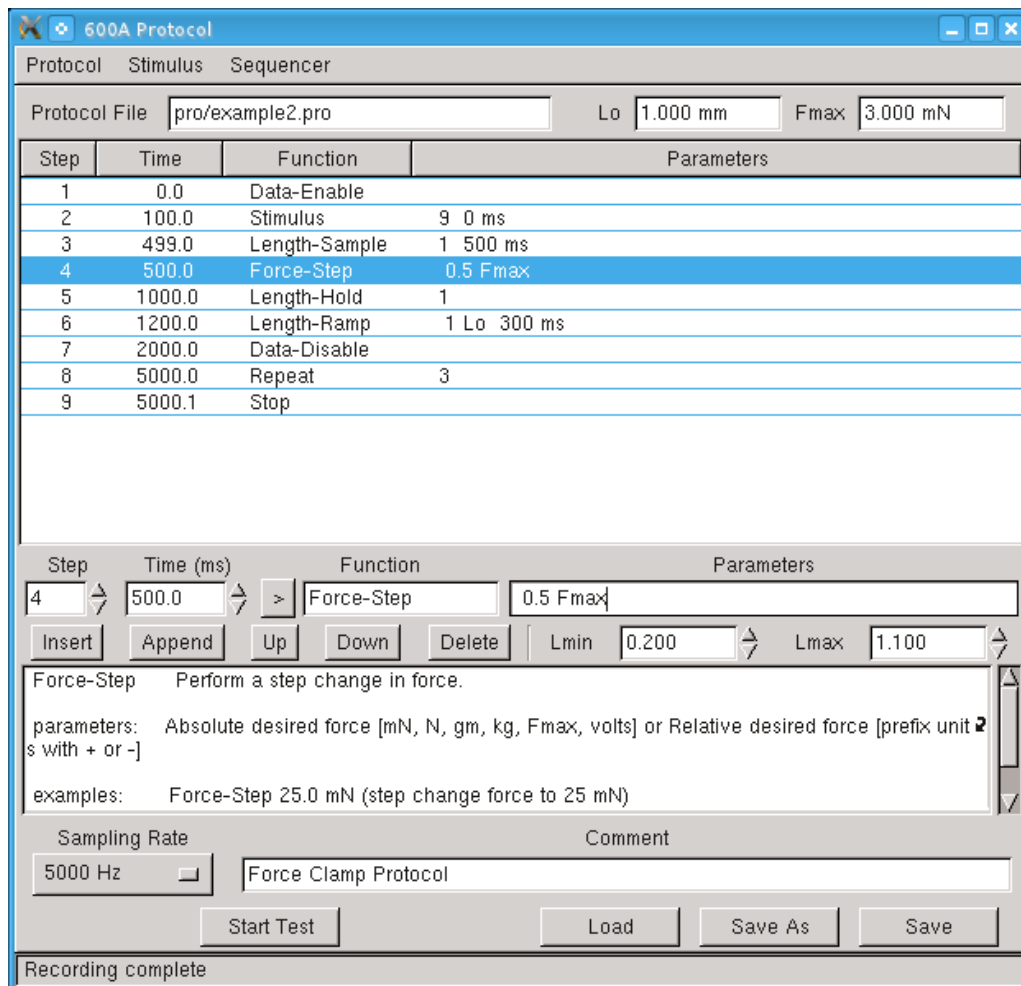
Figure 25 Example Test Protocol

Test protocols can be of any length and can contain multiple occurrences of the same function if desired. At the bottom of the screen there is a status line which displays error and status messages.

Units

Please note that for any function that includes length, force or time there are several possible units that can be used. The units of length are: um, mm, Lo, Lf and volts. The units of force are: mN, N, gm, kg, Fmax, Pa, kPa and volts. The units of time are: ms and s. As mentioned earlier in this section units can also have a + or – prefix which makes the unit relative instead of absolute.

Delay Time in a Function

Stimulus, Trigger and the Sample functions for length, force and SL are special functions in that the parameters include a delay time. The delay time is included to allow a stimulus/trigger/sample to be processed before the start of a length or force command but the action resulting from the stimulus/trigger/sample can be delayed to occur during the length, force or SL command. Stimulation, triggers and sampling usually occurs during some other function such as a length or force function. Since our system doesn't allow a function to start before the

previous function has completed we needed a method of delaying the start of a stimulus, trigger and sample command. Thus we included the delay time to get around this limitation. Normally the user would specify the stimulus/trigger/sample function and set the delay time so that the stimulus/trigger/sample occurs during the following function.

Stimulation and Trigger functions include two parameters; a Stimulus number and a delay time. There are 10 possible Stimulus numbers defined on the Stimulus window and these are specified using the first parameter in the command as 1-10.

Stimulus, Trigger1 and Trigger2 all use the same stimulus profile table. In most cases the researcher will dedicate most of the Stimulus profiles to the Stimulus command and one or two of the profiles to trigger commands.

Sample functions include two parameters; a sample number and a delay time. There are 10 possible sample numbers (memory locations) for each type of sample command and these are specified using the first parameter in the command as 0-9. The zero (0) memory location is a special location in that it corresponds to the current value of length, force or SL. In most cases when you want to sample the current length, force or SL you should choose a memory location between 1 and 9.

Editing

When editing or creating a protocol note the step is not updated until the user clicks on the parameter box and then presses the Enter key on the keyboard (make sure you use the main Enter key and not the one next to the numeric keypad).

If you are satisfied with the protocol and there are no errors then either press the Save As button or the Save button to save the protocol. Protocols must be saved before they can be run. Likewise the protocol must be saved prior to using the simulate function that allows you to review the protocol.

The protocol can be reviewed by clicking the Simulate button on the main window and then clicking on the Start Test button. This action will plot the protocol on the main screen in the data graph area. When the Simulate button is pressed no control functions will be generated.

4.6.2 Stimulus

The Stimulus menu item found at the top of the protocol window allows the user to open the Stimulus definition window as shown in Figure 26. The Stimulus window is used to define both stimulation and trigger profiles. Up to 10 separate stimulus profiles can be defined and these are stored along with the protocol information in the protocol file. Thus an unlimited number of profiles can be defined but only 10 can be associated with any single protocol. Stimulus and trigger commands can be called at any time in a test protocol and can even be called repeatedly if desired.

The ASI600A program provides all the signals necessary to completely control the electrical stimulation of muscle when used with an Aurora Scientific Inc. 700A series Stimulator.

When using other manufacturer's stimulators the program can provide a TTL trigger pulse to start the stimulator.

Referring back to the protocol defined earlier in this chapter (Figure 25) it can be seen that we have selected Stimulus Profile 9 and the stimulus function starts at 100 ms and is not delayed (delay set to 0 ms). This means that the stimulus will start at the 100 ms mark of the test and will use the timing defined by stimulus profile 9.

Stimulus profiles are defined by clicking on the Stimulus sub-heading under the Protocol main menu heading. Up to 10 stimulus profiles can be defined. Stimulus and Trigger are the same type of function and either function can call any table entry in the Stimulus table.

The stimulus profiles are stored in the protocol file. This means you don't need to reenter the stimulus profile when you read in a protocol. The table will automatically update with the stimulus profiles that were defined when the protocol was saved.

Figure 26 Stimulus Profiles Screen

The Stimulus Profile screen includes the following parameters: Pulse Width, Pulse Frequency, Frequency Duration, Train Frequency and Train Duration. Table 3 shows an example Stimulus Profile and Figure 27 is this profile plotted. Together the table and figure may help in understanding the various stimulation control parameters.

Pulse Width

The Pulse Width defines the width of each individual stimulation pulse. In our example each pulse is 1.0 millisecond (ms) wide.

Table 3 Example Stimulus Profile 2

Name	Pulse Width (ms)	Pulse Frequency (Hz)	Frequency Duration (ms)	Train Frequency (Hz)	Train Duration (s)
Stimulus 2	1.0	100.000	50.0	10.00	0.200

Pulse Frequency

The Pulse Frequency is the rate at which the individual pulses occur. This parameter sets the start of each individual pulse (in the example of Table 3 the Pulse Frequency is set to 100 Hz or one pulse every 10 ms). Examination of Figure 27 shows the 1.0 ms wide Pulse Width with a pulse starting every 10.0 ms at times 10, 20, 30, 40 and 50 ms.
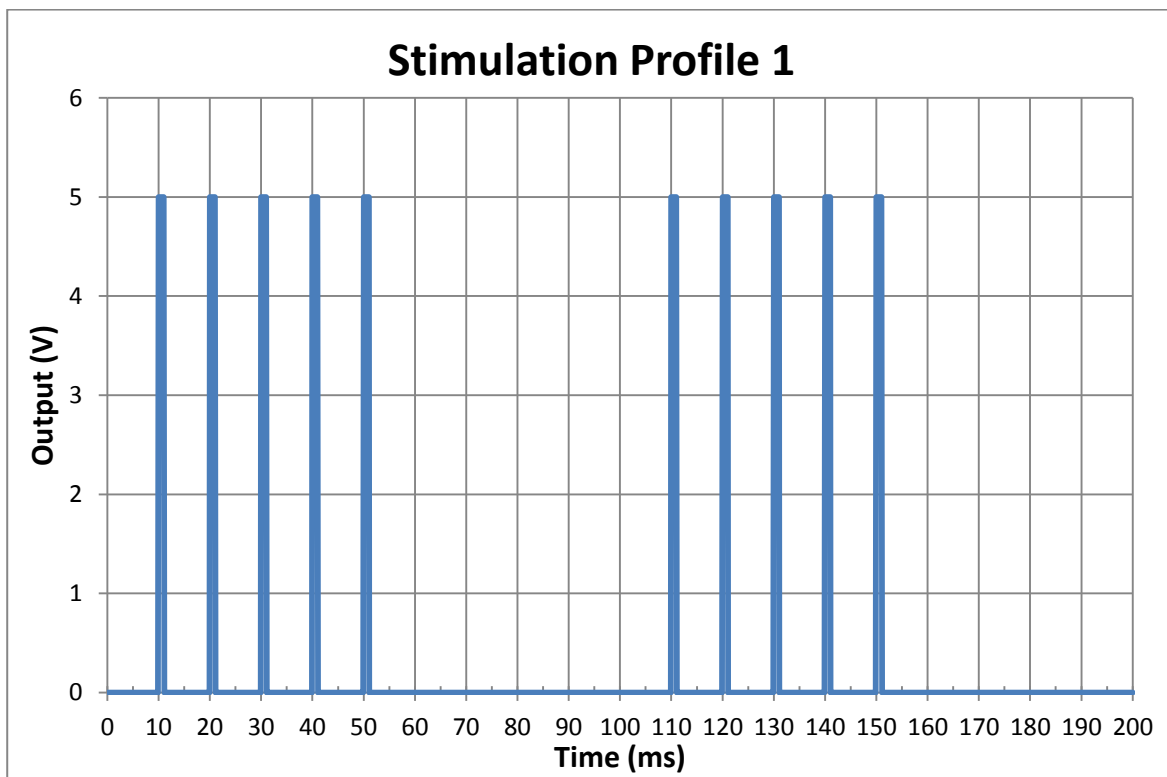
Figure 27 Stimulus Profile Corresponding to Table 3

Frequency Duration

Frequency Duration is the time that the pulses set by Pulse Width and Pulse Frequency are active. In our example Frequency Duration is set to 50 ms and therefore the 100 Hz stimulation pattern will end at the 60.0 ms mark of the test (10.0 ms start time + 50.0 ms Frequency Duration). Since the Frequency Duration ends exactly at the time when the next pulse

should be produced this pulse is not produced. If the user wanted a pulse to occur at the 60 ms mark then the Frequency Duration would need to be set to a minimum of 51.0 ms which would provide sufficient time for the program to produce this next pulse.

Train Frequency

Train Frequency is the rate at which the pulse trains get repeated. In the example the Train Frequency is set to 10.0 Hz or a train starting every 100 ms. Examination of Figure 27 shows a train starting at 10 ms and another 100 ms later at 110 ms into the test.

Train Duration

Train Duration is the length of time that the trains will be output. The example shows a Train Duration of 0.200 sec and this is shown on Figure 27.

Caution – Do not assume that the program is not generating stimulus pulse simply because a protocol has finished.

If the Train Duration is set to a time longer than the test protocol duration then stimulation will continue after the protocol finishes. This feature can be useful if the researcher requires stimulation to continue after a protocol finishes. The pacing of heart tissue is a good example of this.

Example of Heart Stimulation

Assume that the heart tissue must be stimulated continuously for 30 minutes. During this time the researcher will run a series of test protocols on the tissue. A simple method of achieving this continuous stimulation would be to create a protocol that consists of a Data-Enable function a Stimulus function, a Data-Disable function and a Stop function as shown below.

0.0	Data-Enable
0.0	Stimulus 2 0 ms
100.0	Data-Disable
101.1	Stop

In this example the Stimulus function calls Stimulus profile 2 which is as follows.

Table 4 Heart Pacing Stimulus Profile

Name	Pulse Width (ms)	Pulse Frequency (Hz)	Frequency Duration (ms)	Train Frequency (Hz)	Train Duration (s)
Stimulus 2	0.3	100.000	50.0	2.00	900

This profile defines a Train Frequency of 2.0 Hz (1 heart beat every ½ second) and a Train Duration of 900 seconds (15 minutes). When this protocol is run it will complete 100 ms after it started but the stimulation will continue for 15 minutes longer. The first set of pulses will start at the 0.0 ms mark which is exactly when the protocol starts and then will repeat every ½ second for 15 minutes even though the protocol ends after only 100 ms.

The researcher would typically want to run different tests on the tissue during the 15 minute test period. In order to do this the researcher has three options, 1) load and run a test protocol of interest whenever desired or, 2) use a sequence of protocols to automatically load and run different protocols or, 3) create a 15 minute test protocol that coordinates all of the tests with the stimulation. Although all three options will work the best one is to create the protocols required for all of the desired tests plus the long term stimulation and then combine these in a single sequence. Sequences are discussed in the next section. Regardless of the option chosen the test protocols will require synchronization of the stimulation with other functions such as length, force or SL controls. The 600A program can only synchronize control functions and stimulus commands within a single protocol. Therefore, for our heart pacing example, each test protocol will need to restart the stimulation in order to be able to synchronize the control functions and the stimulus. An important feature of the Stimulus function is that the execution of a Stimulus command will stop any stimulus command currently in effect. Therefore all other protocols used for testing the heart tissue would also need to include a stimulus command in the test protocol. Since we can't guarantee that the Stimulus function in the current test protocol will exactly synchronize with the Stimulus function that is currently in effect we can handle this by pacing the heart for a sufficient number of cycles in each subsequent test protocol before we start collecting data. In our heart pacing example we therefore start the Stimulus function and ensure several stimulations occur before the data collection. Below is a simple protocol that starts stimulation and provides 6 stimulation events prior to starting data collection and then a further 2 stimulation events prior to performing a quick release followed by a re-stretch. Data collection starts at the 3 second mark and ends at the 5 second mark for a total of 2 seconds of data collected.

0.0	Stimulus 2 0 ms
3000.0	Data-Enable
4025.0	Length-Step -0.3 Lo
4200.0	Length-Ramp 1.0 Lo
5000.0	Data-Disable
5001.0	Stop

The Stimulus Profile selected was the same as that used in the simple protocol created to start stimulation because once again we want the pacing to continue even after the protocol finishes. You could also choose to use a different stimulus profile if desired. When the Stimulus function is called in the test protocol the timing of the stimulation pulses will change to those specified by the Stimulus Profile specified. In this example as long as the researcher starts a test protocol within 15 minutes of the previous protocol the stimulation will continue. Of course the Train Duration can be specified to be different than 900 seconds if desired. At the end of the test period when the pacing is to be turned off then another protocol can be run that simply contains a stimulation profile with a Train Duration of 0.00 seconds.

4.6.3 Sequencer

The Sequencer window is opened by clicking on the Sequencer menu tab at the top of the Protocol window.

The Sequencer window allows the user to define a series of test protocols that can be executed in a given order. Each protocol is executed in sequence and a data file is automatically created for each protocol in the sequence as soon as that protocol completes.

The Sequencer screen can be divided into the following four sections:
a) The top portion of the screen includes the sequence file name, protocol and data file directory, starting sequence number and a control to determine if the program saves data to a single file per protocol or to separate files for each frame in each protocol.
b) The middle section allows the user to enable/disable each protocol defined in the sequence, to enter the names of the protocols to be included in the sequence, a button for each line that opens a file selector window, delay times and controls as to whether the timing between each protocol in the sequence is automatic or manual, the data file name prefixes to be used when automatically storing data and a File Status box which indicates the results of the verify function shown at the bottom of the screen.
c) Five control buttons are provided below the editing section to clear a sequence (Clear Sequence), verify protocols and file names (Verify Sequence), load an existing sequence (Load), and to save a sequence either to a new file name (Save As) or to the current name (Save).
d) A Status Line is at the bottom of the screen.

Figure 28 shows a sample sequence with six protocols, two of which are repeated twice.

Directory Setup
Upon opening the sequence screen the user should first ensure that the protocol file directory and data file directory are entered correctly. These directory locations are user selectable but must be existing directories. All protocols used in a sequence must be stored in the same directory (e.g. */home/asi/pro*). If protocol files are selected that aren't in the protocol directory then the sequence will fail verification. The Protocol and Data File directories are stored with the sequence file and therefore will reset to the selected directories upon loading a sequence.

Controlling How Sequencer Data Files are Stored
Data file controls are provided to the right of the Sequence Number control at the top of the window. These controls, *one data file per test* and *one data file per frame*, define how the data is stored during the running of a sequence. If the *one data file per test* control is selected then the program will create a single data file for each protocol in a sequence. If the *one data file per frame* control is selected then the program will create a single data file for each frame of each protocol in the sequence.
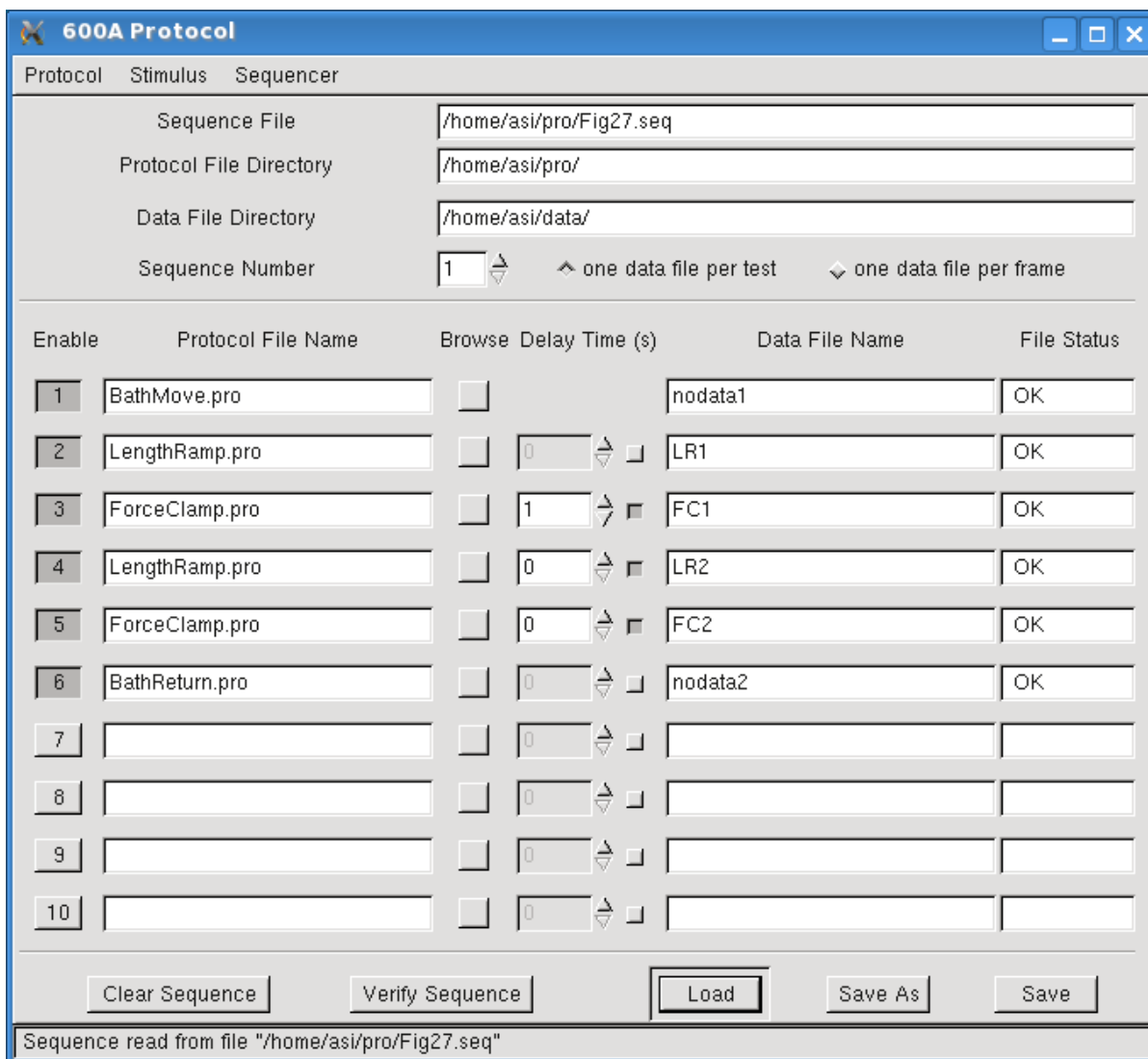
Figure 28 Sequencer Screen

Adding Protocols to the Sequence

To add the protocol files to this sequence, click the square Browse button to the right of the Protocol File Name box which will open a file browser (see Figure 29). Click on the protocol file name to add it to the sequence. Alternatively you can manually enter the name of the file. Be aware that Linux is case sensative.
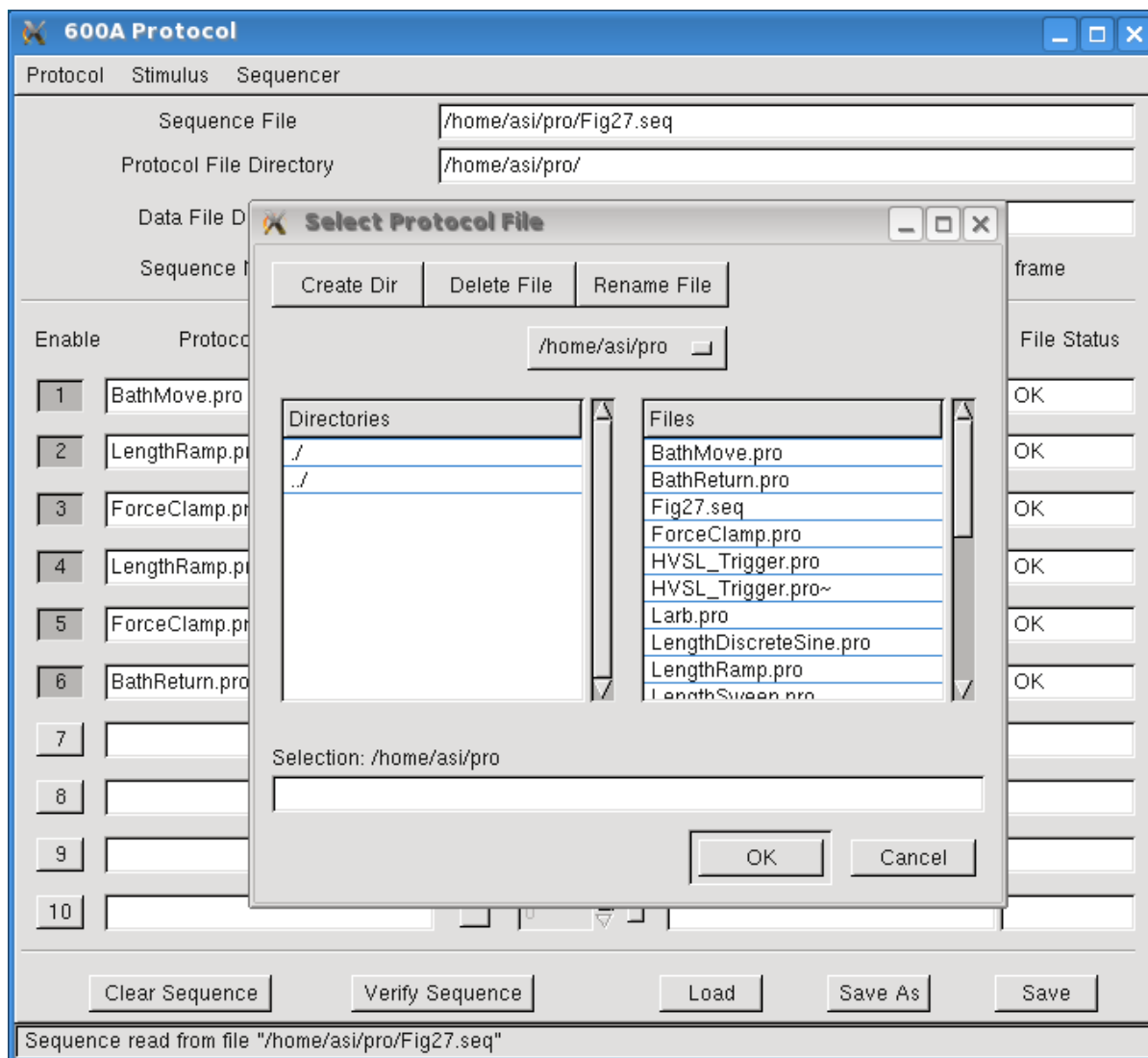
Figure 29 Select Protocol File Window

Controlling Timing in the Sequencer

A Delay Time (s) data entry box and a small square button to its right provide the timing controls for a sequence. At the end of each protocol in a sequence the user has the option to either automatically start the next protocol in the sequence or to allow the user to manually determine when the next protocol starts. When the button to the right of the Delay Time (s) column is pressed then the next protocol will start automatically after the time specified in the number column. A time of 0 seconds causes the next protocol to start as soon as the previous one finishes. Times must be entered in integral multiples of seconds. If finer control of the timing is required then the two protocols should be combined into a single one because the internal timing in a protocol has a resolution of 0.1ms. When the button is not pressed the timing will be manual. When using manual timing the program will stop after each protocol is complete and the Next Test button located at the bottom of the main screen will un-gray. When the user presses the Next Test button the next protocol in the sequence will start immediately.

Setting the Base Filename for Sequencer Data Files

Next enter the prefix for the data file names that will be used when automatically saving the data from each protocol. These data file name prefixes can be whatever the user wants but they must be unique names. In addition the normal limits on file names exist, i.e., no special characters (~, !, @, #, $, %, ^, &, *, (, or)).

A special caution for Linux is you should never include spaces in the file name as this will cause errors. If you want to separate sections of the filename use an underscore such as my_1st_test.
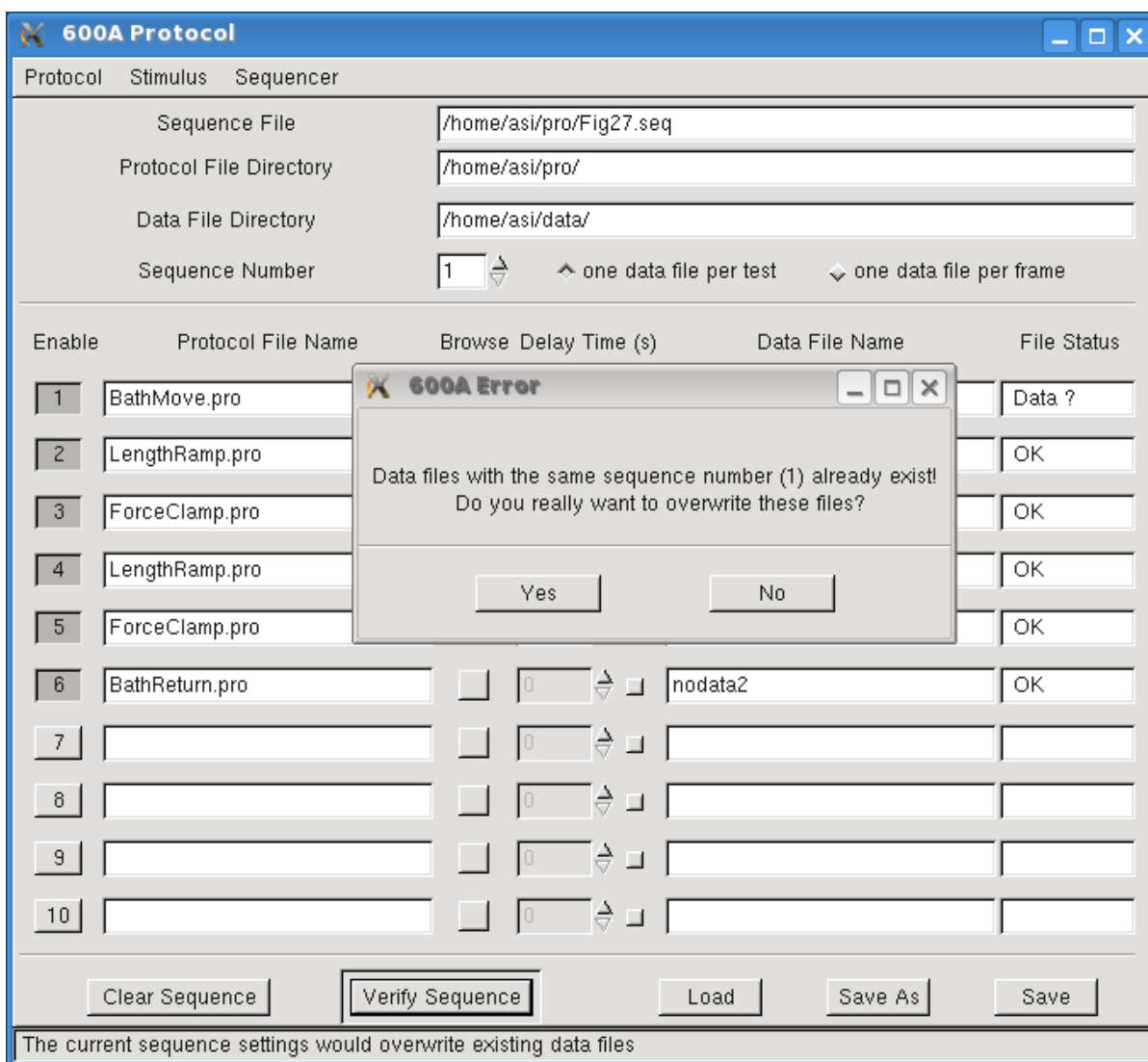
Figure 30 Sequencer Data File Error Screen

After a sequence runs the program will automatically store the data in sequentially number data files that start with the prefix entered in the Data File Name field on the window. An example of file names with appended run numbers is shown in Figure 31. In this figure you will note file names such as FC1_s001.dat, FC1_s002.dat, FC1_s003.dat, etc. Each time the

sequence is repeated the program automatically appends the _s# to the prefix filename that the user has specified for that particular protocol in the sequence. .

The current test number is derived from the largest test number appended to any of the data files defined in the sequence. This means if you turn one or more protocols off for a few tests and then resume them, all of the numbers will still match.

If there are no problems with the Verify Sequence then an OK will appear in the File Status text box to the right of each enabled protocol.

Enabling Protocols in a Sequence

Each of the protocols in a sequence can be enabled or disabled using the Enable button to the left of the protocol file name. Pressing the button enables the protocol. You don't need to enable all of the protocols present on the list. Simply enable the ones you would like to have included in the sequence. When the sequence runs only the enabled protocols will be executed in numerically increasing order. You can have up to 10 protocols defined in a sequence but you don't need to enable all of them. It is often convenient to list all of the main protocols used during an experiment and then save this sequence. Now if a particular experiment calls for protocols 2, 4, and 8 you would only enable these protocols. After enabling these selected protocols you could then save the sequence to a unique name.

Verifying a Sequence

Once you have completed entering the protocol names, timing information, data file names and enabled the protocols of interest press the Verify Sequence button located near the bottom of the screen. The program will check for several conditions and if the protocol files exist, the data file names are unique and the sequence number is correct then an OK will appear in the File Status column on the right side of the window. If there is a problem then the program will pop up an error window as shown in Figure 31. This error window provides two options: Yes or No. Selecting the Yes option will overwrite the existing data files with the same name. Selecting No will close the error window and return the user to the Sequencer editor. At this point the user can either enter new data file names or change the Sequence Number found near the top of the window. After making either of these changes, press the Verify Sequence button again.

Repeating Protocols in a Sequence

You can also have a sequence that repeats a given protocol as many times as required. An example of this would be an experiment that required a pre-conditioning protocol followed by a test protocol and then followed by the same pre-conditioning protocol. In order to repeat a protocol within a sequence you will need to specify the protocol on more than one line of the sequence.

Saving a Sequence

Once you are satisfied with the sequence and it has been verified, press the Save As button to save the sequence to a new filename or press the Save button to save the sequence to

the current sequence name. Sequences that have not been saved cannot be executed. Sequence files are stored in ASCII format and the file structure is provided in Appendix A.
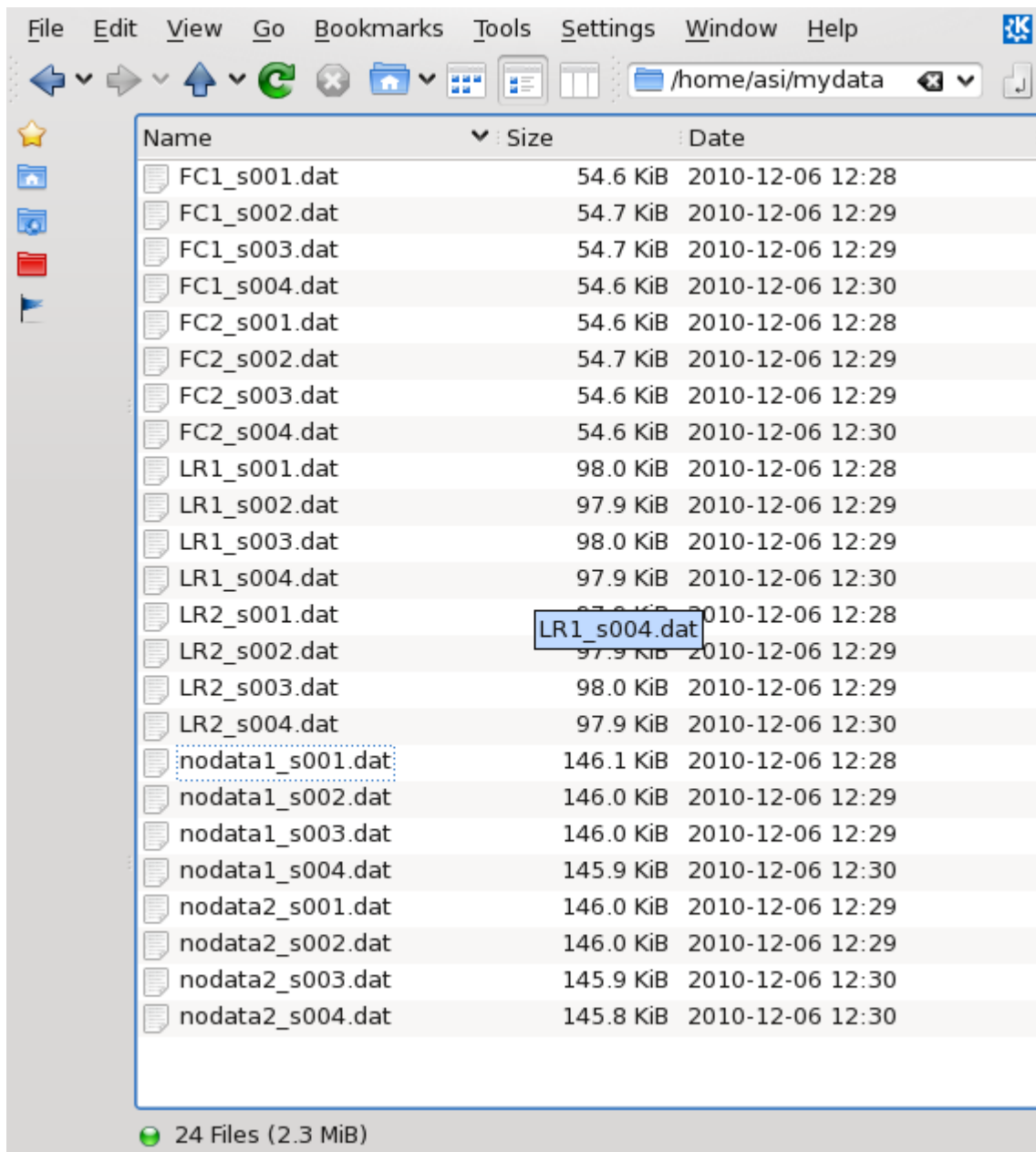
Figure 31 Sequencer Data File List

Running a Sequence

After saving the sequence it can be run by pressing the Start Sequence button at the bottom of the main screen. When a sequence is running the status bar at the bottom of the main window will inform the user of which protocol is executing and will provide a countdown timer to the start of the next protocol in the sequence.

4.7 Analysis Menu

Refer to Chapter 6.0 Analysis.

4.8 Scope Menu

Refer to Chapter 7.0 Scope.

4.9 Burst Menu

The burst menu button on the main window opens the Burst window. The Burst window allows user to view and save burst data. Burst data is sampled at a significantly higher rate; 60,000 samples per second on each of the four channels instead of the normal 20,000 samples per second. Burst data can be collected during a portion of a protocol by use of the Burst protocol function. Figure 32 shows the burst window showing data from a length step.
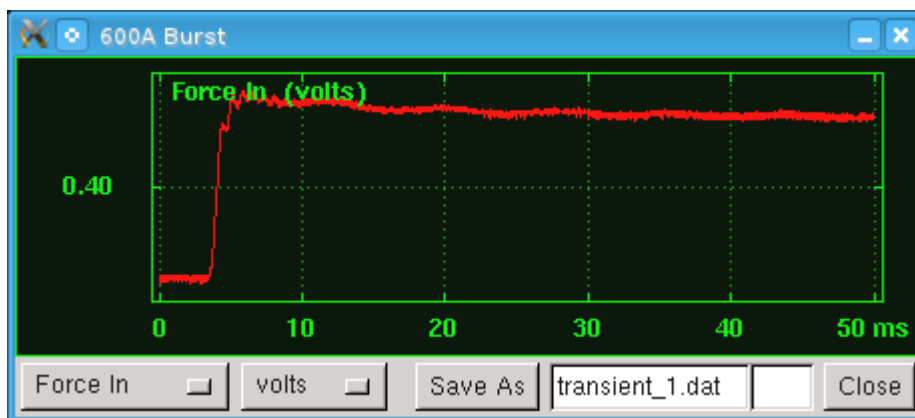
Figure 32 Burst window

See Section 4.6.1 Protocol Editor for information about creating protocols and on the data burst function.

In order to setup a burst recording the user selects the desired data channel, the units of the data and the data file name for the burst data.

4.10 Help Menu

The Help menu contains two commands: ASI600A Help and About.

ASI600A Help
Opens a pdf viewer and displays this manual on the screen. Links are included in the document to make it easier to navigate through the manual.

About

      About provides information about the ASI600A program including the program version number, the real time A/D (RTAD) version number and contact information for Aurora Scientific Inc.

5.0 Force and SL Control

The Force & SL Control window provides an interface for controlling the software feedback control system built into the 600A program. This feedback control system allows the program to control force or sarcomere length using length changes imposed on the tissue by the length controller. Since every tissue has different properties and characteristics; this control panel allows the user to tune the settings to optimize feedback control for each individual experiment.

This section explains how to effectively use the Force & SL Control panel. Starting with the basics of how a feedback control loop works, how to measure the effect of a length change on force or sarcomere length and the function of the control parameters. When properly set up and tuned the force feedback system can produce critically damped force or sarcomere length changes in as little as 1 millisecond.

The Force & SL Control window is not used with Dual Mode Length Controllers (300C, 305C, 309C, 310C) because these instruments have force control built into their electronics.

5.1 Feedback Control Basics

A feedback control loop is necessary when you want to change a parameter that is not directly controlled. In this case, we want to change either the force or the sarcomere length by adjusting the overall length of the tissue.

To understand the use of the Force & SL Control we will use an example of force control, the simplest of which is a force step. A force step simply aims to change the tensile force on a preparation to a certain value, or by a certain value; change force to 3 mN, or decrease force by 2 mN. In this example we want to change the force to 3 mN, we start by reading the current force from the force transducer and then calculate the difference between the current force and the desired force which we will call the force error (F_{error}). The F_{error} value tells us how much we want to change the force by but it doesn't tell us anything about how much we need to move the length in order to obtain this force. So now we need a relationship that ties length change to force change. In the program we call this dF/dL (change in force for a unit change in length) or stiffness. The stiffness is determined by imposing a known length change on the preparation and measuring the resultant change in force. The determination of stiffness is discussed in greater detail in sections 5.2 Determining dF/dL(for force control) and 5.3 Determining dS/dL (for SL control).

Assume we have a muscle tissue that we determine has stiffness (dF/dL) = 25 mN/mm. If this tissue is at a tension of 5mN and we want to do a force step to 3 mN it is obvious that we will need to shorten the tissue length. To determine how far to shorten the tissue the feedback control uses the force error (F_{error}) and the stiffness (dF/dL) and performs a simple calculation to determine the length change (ΔL) that will produce the desired force. The calculation is dividing the force error by the stiffness to obtain the required length change.

Equation 1

$$\Delta L = \frac{F_{error}}{dF/dL} = \frac{2mN}{25mN/mm} = 0.08\ mm = 80\ \mu m$$

It turns out that if you try to eliminate the force error in one step (in our example by moving the full 80 um) the feedback control will overshoot the desired force and may also start a high frequency oscillation (ringing) of the length and the force. In extreme cases this ringing will grow to the point where the feedback system will lose control and the tissue will be damaged. To prevent overshoot and ringing the feedback control includes a gain term in the feedback equation. The gain term is a multiplier of the calculated control response and in the case of Equation 1 it is a multiplier of the 80 um value.

Equation 2

$$\Delta L = G\left(\frac{F_{error}}{dF/dL}\right) = 0.25\left(\frac{2mN}{25mN/mm}\right) = 0.25\ x\ 0.08\ mm = 20\ \mu m$$

Typical values for the gain would be numbers between 0.1 and 0.5 but in all cases less than 1. In most feedback control systems the controller will not use a gain of 1 because this would cause the control system to apply the entire correction in a single step. A high gain term usually results in overshoot and instabilities (ringing or high speed oscillation of the length). A low gain term usually results in a stable but slow response. A large part of tuning the feedback control involves trying different gain factors and observing the results to obtain the best response.

To improve the stability of the feedback control and to eliminate small constant errors we need to add another term to Equation 2 that produces a length change that increases with time. When an integral is added to the control equation the control is improved for the case when large disturbances and noise are present on the signal and when there are time delays in the system. Equation 3 is the result of adding an integral term to Equation 2 and expressing the length correction as a function of time (t).

Equation 3

$$\Delta L(t) = \frac{G}{dF/dL}\left[F_{error}\ (t) + \frac{1}{T_i}\int_0^t F_{error}(t)dt\right]$$

This equation includes an integral time constant (T_i) which controls the magnitude of the corrective action that arises from the integral calculation. Equation 3 is in effect a proportional-integral (PI) controller. A PI controller takes control action based on past and present control errors. The proportional term (controlled by the gain G) is the control action arising from the present value of the error and the integral term (controlled by the integral time constant (T_i)) is the control action arising from past values of the error. The integral time constant determines how far back in time we look for our calculation of the integral control action. An integral time constant of 0.0 ms removes the integrator from the feedback control.

A major issue with many feedback control systems is instabilities that arise from noisy measurements of the error signal. To minimize the chance of these instabilities arising we add a low pass filter to the calculated length change (ΔL) to produce a control signal that is filtered to remove the effect of noise. The amount of filtering that we do is controlled by a filter time constant where larger values produce a greater filtering effect. A filter time constant of 0.0 ms will produce no filter effect.

5.2 Determining dF/dL

In order to determine the stiffness (dF/dL) of a tissue sample a known length change is imposed on the tissue and the resultant force change is measured. The stiffness is the amount of force change per mm of change in the tissue length. As described in section 5.1 above, this value is required for a feedback control to work. If you want to hold the force constant at a certain value, and it is not at that desired value, then the program must be able to determine how much to move the length controller to achieve the desired force.

Ensure that the force transducer and length controller are calibrated before determining dF/dL. See sections 4.4.2 Length Calibration and 4.4.3 Force Calibration for details on these procedures.

5.2.1 Variable Stiffness

So far our discussion has assumed that the stiffness is constant, this is the simplest case and the easiest to control. However the behaviour of muscle tissue can't be described using a constant stiffness. The stiffness varies with force in a more complex manner. At this point our feedback control system does not allow stiffness to be entered as a function of force and therefore, in most cases, the force controller settings will need to be adjusted as the force level changes. There are a few methods of compensating for the variable stiffness: (a) measure the stiffness at the desired force level, (b) determine the range of stiffness exhibited by the tissue from relax to tetanus (full activation) and then manually adjust the stiffness variable as you perform the experiment, (c) determine some "average" stiffness and then adjust the other controller settings to compensate for higher or lower stiffness depending on the protocol you are running. Regardless of the method chosen, experience that comes from use will be your best guide and be prepared to adjust stiffness and control variables as your experiment progresses. Typically this may involve running a particular force control protocol, observing the results, adjusting the stiffness and controller settings and then re-running the protocol to obtain the best results.

5.2.2 Method for Measuring dF/dL

For this method let's assume that we want to measure the passive stiffness of the muscle tissue. The method is the same for measuring active stiffness the only difference is the muscle needs to be activated (stimulated) before the measurement is made.

Start by opening the Scope menu so that you can observe how the Length In and Force In signals change during the measurement of dF/dL. Next mount a tissue sample and adjust the resting tension to the desired level.

⚠️ If the tissue is slack the determination of dF/dL will underestimate the stiffness and the force control will not work properly.
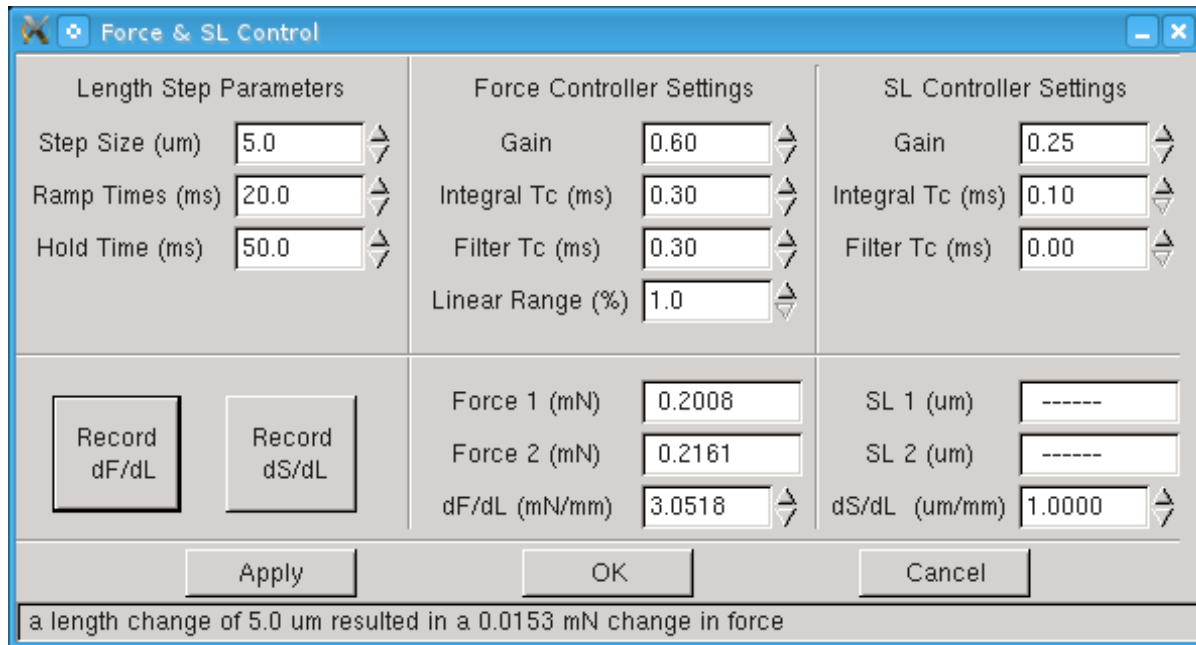
Figure 33 Force & SL Control Window

Now open the Force & SL Control window (Figure 33) by clicking on Calibrate→ Force & SL Control menu item from the main window.   Set the Step Size for the length change that will be imposed during the stiffness measurement. The Step Size can be a positive (stretch) or a negative (shorten) value depending on the conditions under which you are measuring the stiffness. If the tissue is fully activated then you will not likely want to impose a further stretch on the tissue which would risk damaging the tissue. Under this circumstance it would be better to use a shortening movement. Likewise if you are measuring the passive stiffness then the fibre is likely near slack and a stretch movement is more appropriate.

Step Size

Step Size is the change in length to be imposed on the tissue which will produce a force change on the preparation. The step size is always set in microns (um) and can be a positive (stretch) or a negative (shorten) number. Typically the Step Size should be in the range of 0.5-1.0% of the Initial Length (Lo). For a 2mm fibre a step size of 10-20 um would be reasonable.

⚠️ Take care to limit the Step Size as large values can damage the tissue and/or the force transducer especially when the tissue is being stretched.

It is better to start with a small value and observe the change in force resulting from that length change. Then incrementally increase the Step Size value observing the increased force change.   Try to avoid length changes that result in very small force changes (changes that are only a few percent of the full scale range of the force transducer) as there will be more error in the measurement. In general you want to produce force changes that are within the range seen during an experiment.

The length change profile is a ramp from the current length by the Step size followed by a constant length data acquisition period (Hold Time) which is then followed by a ramp length change back to the starting length. The Ramp Time variable determines the slope (time it takes to change the length by the Step Size) for both the initial length change and for returning the tissue back to the starting length. Ramp Time is measured in milliseconds (ms). Based on the type and size of the tissue you may want to vary the Ramp Time. Some tissues exhibit rapid force recovery after a length change and therefore, for these tissues, you may want to make the Ramp Time quite short. Likewise for larger preparations there may be a concern that a rapid stretch would damage the tissue so, in this case, a longer Ramp Time would be appropriate.

The Hold Time is the time that the length is held at the new length. Both length and force are measured before the Step Change and during the Hold Time. These measured changes in length and force are used to calculate stiffness. Some experimentation with Hold Time may be required to obtain the best measure of stiffness. Ensure you use Scope to actually observe the behaviour of the length and force signals during the measurement as this behaviour will provide clues as to changes that may need to be made to Step Size, Ramp Time and Hold Time.

Ramp Time
Ramp time is specified in milliseconds and is the amount of time taken to change the length of the preparation by the specified Step Size. Some experimentation may be required to determine a Ramp Time that yields the best measure of stiffness.

Hold Time
Hold time is measured in milliseconds and is the amount of time taken to hold the length of the preparation after it has been stretched in order for the muscle to stabilize. A Hold Time that is too short may not allow the preparation to stabilize before a force reading is taken.   A Hold Time that is too long may allow the force to change by an unacceptable amount before the stiffness measurement is made.

See Figure 33 for reasonable starting values for Step Size, Ramp Time and Hold Time. Once these parameters are set, click the Record dF/dL button. Three values will update, Force 1, Force 2, and dF/dL. It is recommended that you press the Record dF/dL button a few times to ensure the values remain consistent. A little deviation between measurements is normal and will not adversely affect the force control function.

Force 1 (mN) – Force 1 is the initial force recorded before the length change is made, it is measured in mN. This is a text readout box that shows the measured value, the user can't change this text.

Force 2 (mN) – The force recorded after the length change is made, measured in mN. This is a text readout box that shows the measured value, the user can't change this text.

dF/dL (mN/mm) – Calculated stiffness which is the force change per unit length change, expressed in units of mN/mm. This value can be overwritten by the user. If you have made a series of stiffness measurements at different force levels it is sometimes more convenient to simply enter the stiffness that corresponds to the conditions expected in the next protocol you intend to run.

5.3 Determining dS/dL

The sarcomere feedback control works in a similar manner to the force feedback control. Instead of stiffness we substitute dS/dL or the change in sarcomere spacing due to a change in tissue length. The units of dS/dL are um/mm, the change in sarcomere spacing measured in microns divided by the change in tissue length measured in mm.

⚠️ **Ensure that the 901 HVSL 2 sarcomere length measurement system and the length controller are calibrated before determining dS/dL.** See section 4.4.2 Length Calibration and the ASI 901 Instruction Manual for details on these calibrating these instruments.

To determine dS/dL use the same method as outlined in section 5.2 Determining dF/dL above. As with the stiffness measurement there will be a variability of dS/dL depending on the activation of the muscle tissue. Activated tissue can be expected to have a much higher value of dS/dL than non-activated tissue. As with the determination of stiffness select Step Size, Ramp Time and Hold Time to provide reasonable changes in length and in sarcomere spacing. We recommend using a Step Size in the range of 0.5-1.0% of the Initial Length (Lo).

See Figure 33 for reasonable starting values for Step Size, Ramp Time and Hold Time. Once these parameters are set, click the Record dS/dL button. Three values will update, SL 1, SL 2, and dS/dL. It is recommended that you press the Record dS/dL button a few times to ensure the values remain consistent. A little deviation between measurements is normal and will not adversely affect the sarcomere length control function.

SL 1 (um) – SL 1 is the initial sarcomere length recorded before the length change is made, it is measured in um. On the Force & SL Control window is a text readout box that shows the measured value of SL 1, the user can't change this text.

SL 2 (um) – SL 2 is the sarcomere length recorded after the length change is made, it is measured in um. On the Force & SL Control window is a text readout box that shows the measured value of SL 2, the user can't change this text.

dS/dL (um/mm) – Calculated dS/dL which is the SL change per unit length change, expressed in units of um/mm. This value can be overwritten by the user. If you have made a series of dS/dL measurements at different activation levels it is sometimes more convenient to simply

enter the dS/dL that corresponds to the conditions expected in the next protocol you intend to run.

5.4 Force & SL Control Parameters

There are four parameters used to tune the force feedback control and three to tune the sarcomere length feedback control. The parameters associated with the force controller are listed under the title Force Controller Settings and they are: Gain, Integral Tc (ms), Filter Tc (ms) and Linear Range (%). The parameters associated with the sarcomere length controller are listed under the title SL Controller Settings and they are the same as the force controller parameters with the exception that there is no Linear Range (%) parameter. These control parameters are found in the top centre and top right sections of the Force & SL Control window. There are two distinct sets of parameters and even though they have the same names they are unique to the force and sarcomere length command functions used in protocols. If you place a sarcomere length command in a protocol then the program will use the Gain, Integral TC and Filter TC values found under the SL Controller Settings heading. This is likewise for force control; there are two unique sets of parameters. This allows the researcher to create protocols that have both force control and sarcomere length control in the same test protocol (but not at the same time).

The control parameters for both force and sarcomere length depend on the model of length controller and force transducer being used, the tissue type and size, the tissue stiffness or dS/dL measurement, and the amount of tissue activation. Typically you will find that the control parameters will need to be adjusted for each different protocol you run. In practical terms this means that a protocol to generate a force clamp to 20% of the Fmax value and one to generate a force clamp to 80% of Fmax will need different values for the control parameters.

To further complicate the determination of control parameters you will also find that the individual control parameters affect each other. A change in one of the parameters will often require a change in one or all of the other parameters. Tuning the feedback control is an iterative process where you try a safe set of parameters, observe the results and then adjust the parameters to optimize the results. With time and experience you will find that the tuning becomes faster and easier and you will develop a "feel" for the correct values for the parameters.

The rest of the discussion in this section will refer to the results obtained using the force clamp protocol shown in Figure 34. Initially we will start with the settings shown in the Force & SL Control window of Figure 35.

The protocol we have selected is a force step. Briefly, what this protocol does is:

1. Data-Enable will start the collection of data.
2. Length-Sample will measure the length at 490 ms into the protocol (90 ms start of command + 400 ms delay) and store the measured value in storage location 1.
3. Force-Step will make a step change in the force from the current tensile force to the desired force of 1.6 mN and will hold that force regardless of what the tissue does. This Force-Step command uses force feedback to control the length controller and the parameters that we are discussing in this section only affect this command.

4. Length-Hold will stop the Force-Step command by issuing a length command to keep the length of the preparation at the value measured by the Length-Sample command issued in step 2 of the protocol.

Protocol functions that control length, force and sarcomere length remain active until another function is called or the protocol ends. Therefore to end a force command you need to issue a different force command, a length command or a sarcomere length command. In most protocols force and SL control should be ended by issuing a length command.

5. Length-Ramp will return the length to the initial starting length (Lo) using a ramp function that takes 300 ms to complete.
6. Data-Disable will stop the collection of data.

Located near the middle of the protocol windows are two very important parameters that need to be understood before doing anything with force and SL control. These parameters are Lmin and Lmax and they provide a hardware limit on the range of motion of the length controller. The numbers entered next to the Lmin and Lmax labels are multipliers of the currently defined Lo value which is shown at the top of the Protocol window. For the example shown in Figure 34 Lmin is 0.997 x Lo = 0.997 x 5 mm = 4.985mm. Likewise Lmax is 1.200 x Lo = 1.200 x 5 mm = 6 mm.

It is important to understand that Lmin and Lmax set hardware limits on the length that stay in effect regardless of what either the user tells the length controller to do (via commands in a protocol) or what the feedback control tells the length controller to do (via the feedback calculation of length required to obtain the desired force or SL). Because Lmin and Lmax are hardware limits you can use them to minimize unstable behaviour of the force and SL feedback control. As has been mentioned earlier in this chapter if you specify control settings that are inappropriate for your particular equipment and tissue or if you measure the stiffness (dS/dL when dealing with sarcomere length control) incorrectly, the result can be instability which results in large movements of the length controller. These large movements could damage the tissue and even the equipment. By setting tight limits on Lmin and Lmax you will prevent these large movements from happening.
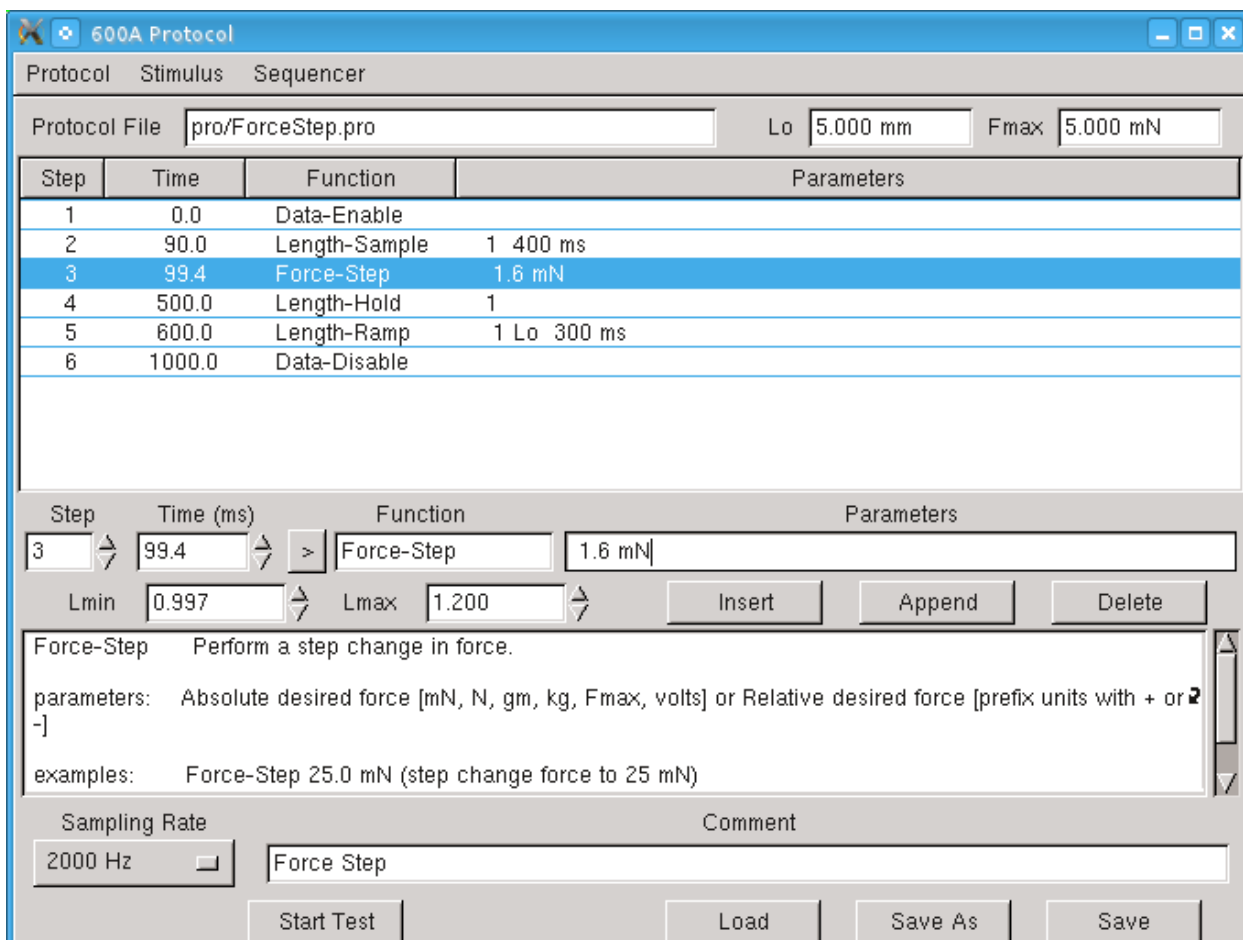
Figure 34 Protocol Window

When first using force or SL feedback control we strongly recommend that you set very tight limits on Lmin and Lmax (we would suggest Lmin = 0.8 and Lmax = 1.1 as reasonable starting values). Limiting the range of movement of the length controller will protect the tissue and the instruments from potential damage should the Force & SL Control become unstable.
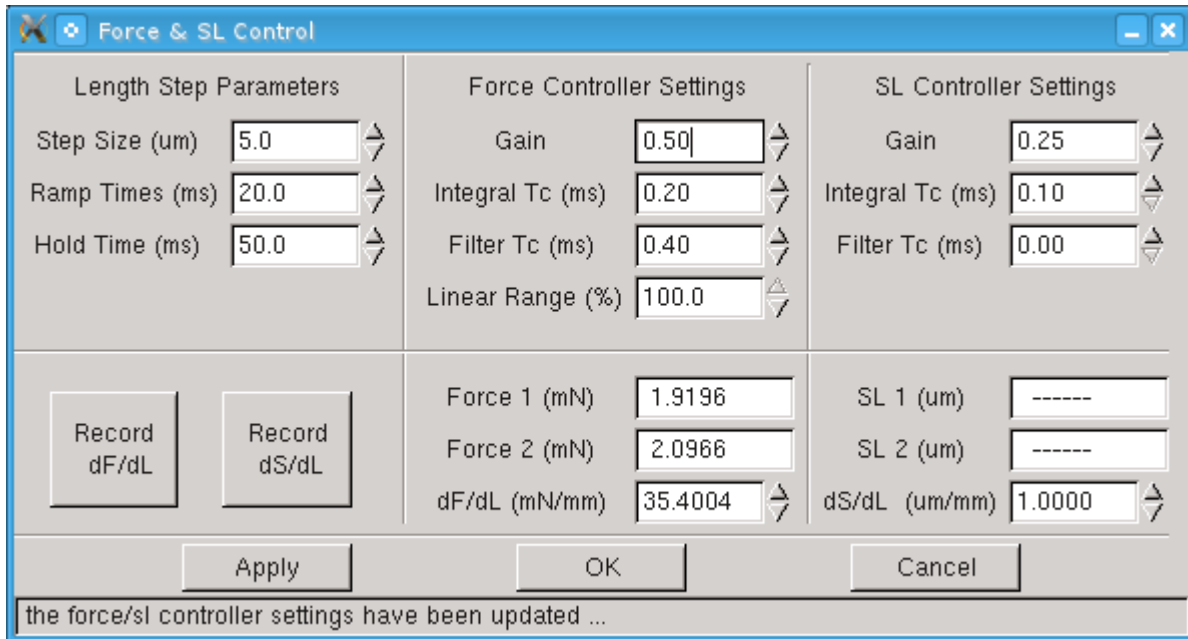
Figure 35 Force and SL Control Window

Several graphs of force control signals are shown on the next few pages. These were all obtained using a 322C high speed length controller and a 400A force transducer mounted on an 801C Small Intact Muscle Test Apparatus. A multi-threaded piece of elasticized string was used in place of the muscle tissue (a rubber (elastic) band provides a good practice fibre). Because the elasticized string could not generate active force we needed to stretch it to create force and then perform force clamps to lower the force to the desired level.

Before attaching muscle tissue or a rubber band to the force transducer, ensure the transducer is turned on. Circuits are included in the force transducer that will produce an alarm sound if you overload the transducer. Listen for this sound and take reasonable caution while mounting the tissue.

5.4.1 Gain

Gain is the first parameter shown in the controller settings sections. The gain parameter, in conjunction with the stiffness (dS/dL for sarcomere control), produces a corrective length control signal that is proportional to the current error between the actual force (SL) and the desired force (SL). The Gain parameter can vary from 0.0 to 1.0 but a safe starting value would be between 0.1 and 0.3.

The Gain parameter is used in conjunction with the measured stiffness (dS/dL for sarcomere control) to generate the length control signal. If you find you need very large (close to 1.0) or very small (close to 0.0) values for Gain then this is a good indication that the stiffness (dS/dL) is not correct for the tissue and experimental conditions.
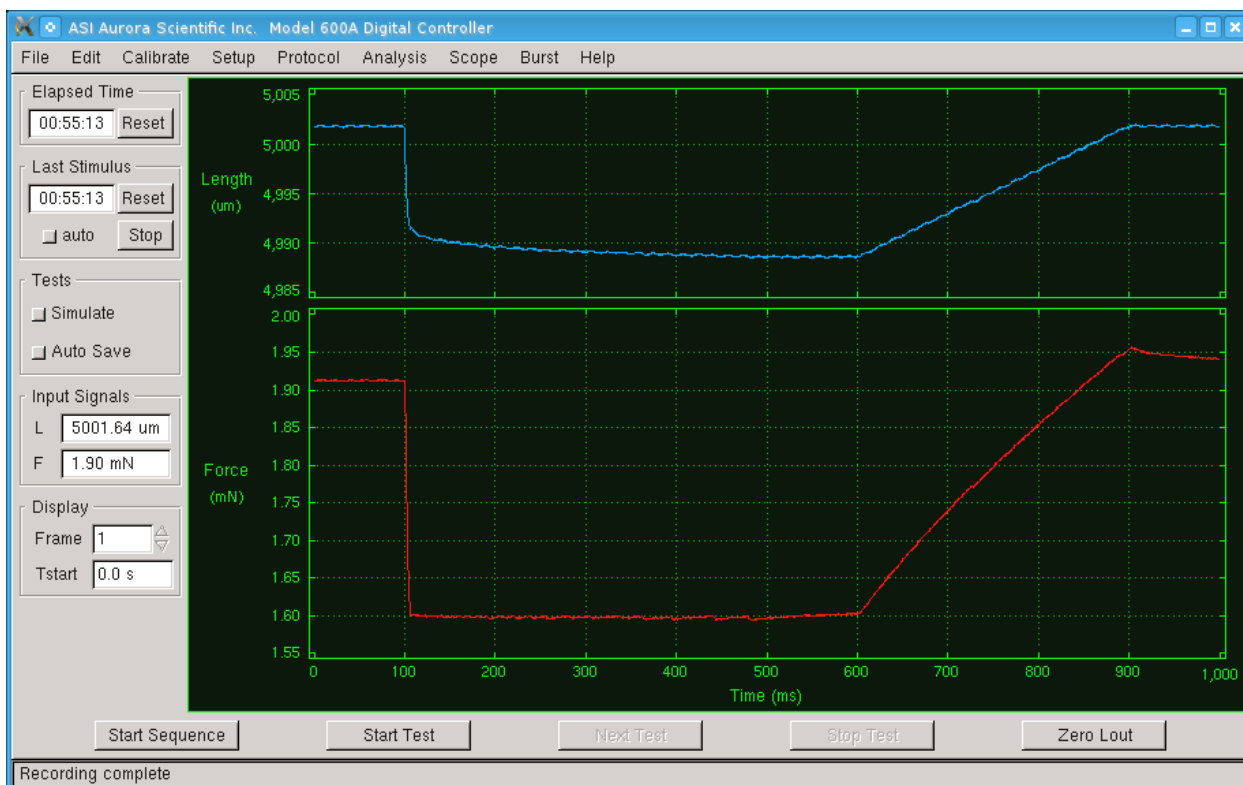
Figure 36 Force Clamp with Gain=0.5, Integral TC=0.2, Filter Tc=0.4

With the protocol shown in Figure 34 and the settings shown in Figure 35 the result of running the protocol is shown in Figure 36. The top graph, shown in blue, is the length of the preparation measured in microns. The bottom graph, shown in red, is the tensile force measured in mN. The figure shows a constant length at the start of the test followed by a rapid clamping of the force which starts at the 100 ms mark. During the force clamp (from 100 ms to 600 ms) the force remains constant. At the 600 ms mark length control is reinstated and the length is ramped back to the starting length.

Examination of the results shows that the force clamp occurs in a short time with no overshoot or oscillation of the force signal during the clamping period. This is a good indication that the Gain and other parameters are set correctly. We suggest you use Analysis to zoom in on the start of the force clamp as this can provide a clearer picture of how good the controller settings are. Figure 37 is the same data shown on the Analysis window with the display zoomed to show the results from 90 to 130 ms of the test. As can be seen there is a stable force clamp that takes about 10 ms to clamp the force to 1.6 mN (clamp starts at 100 ms and is at the 1.6mN level by about the 110ms mark). No overshoot or oscillation of the force signal can be seen.
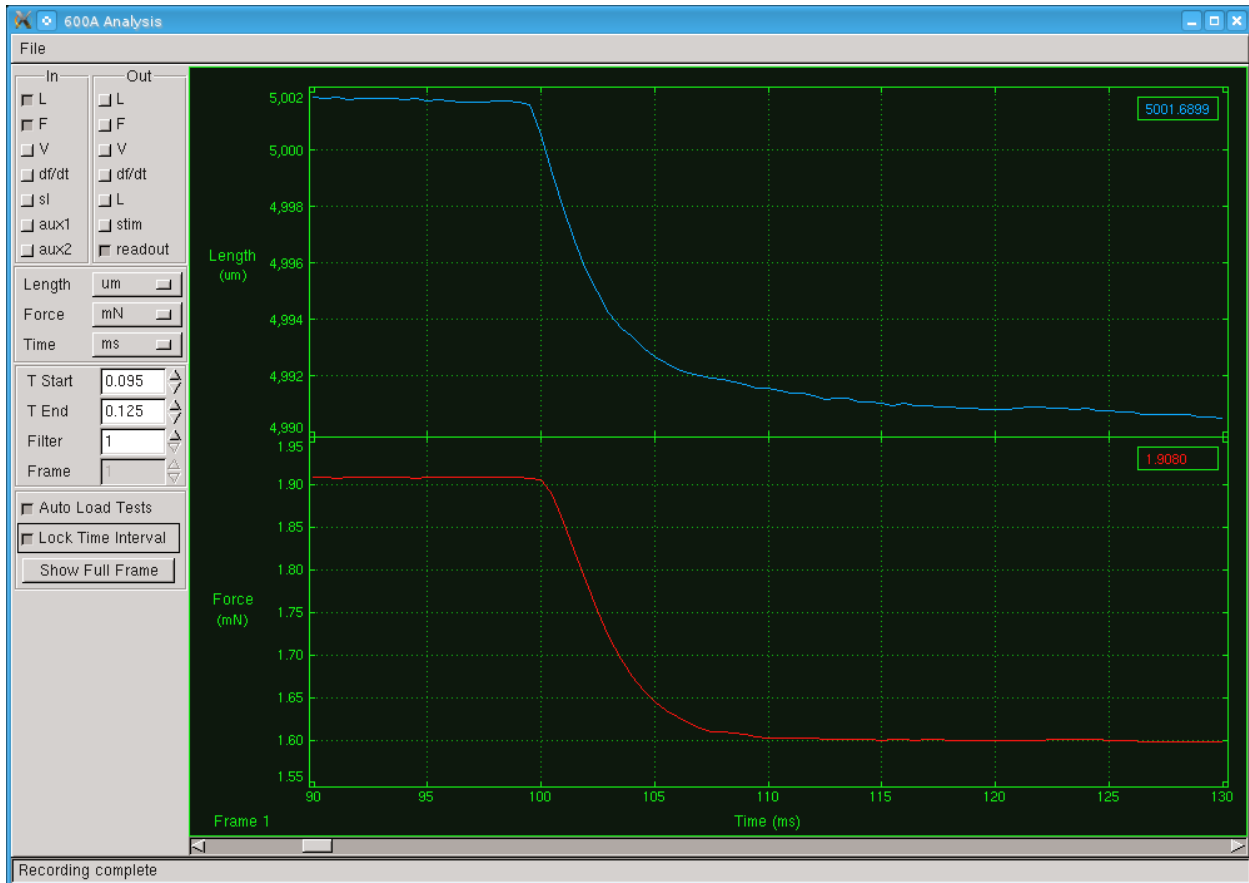
Figure 37 Analysis of Force Clamp with Gain=0.5, Integral TC=0.2, Filter Tc=0.4

Now let's look at the effect of changing the Gain parameter. We may want to improve the performance by reducing the time it takes to clamp the fibre to the desired force. If this is the goal then we will need to increase the Gain. Figure 38 shows the force clamp response when we increase the gain from 0.5 to 0.9. You will note that the desired force is reached in just 4 ms but the force now overshoots the desired force and doesn't settle until about the 108 ms mark of the test, taking a total of 8 ms to stabilize. Thus an increase in the Gain parameter speeds up the control action but leads to overshoot on the force if the Gain is too high.
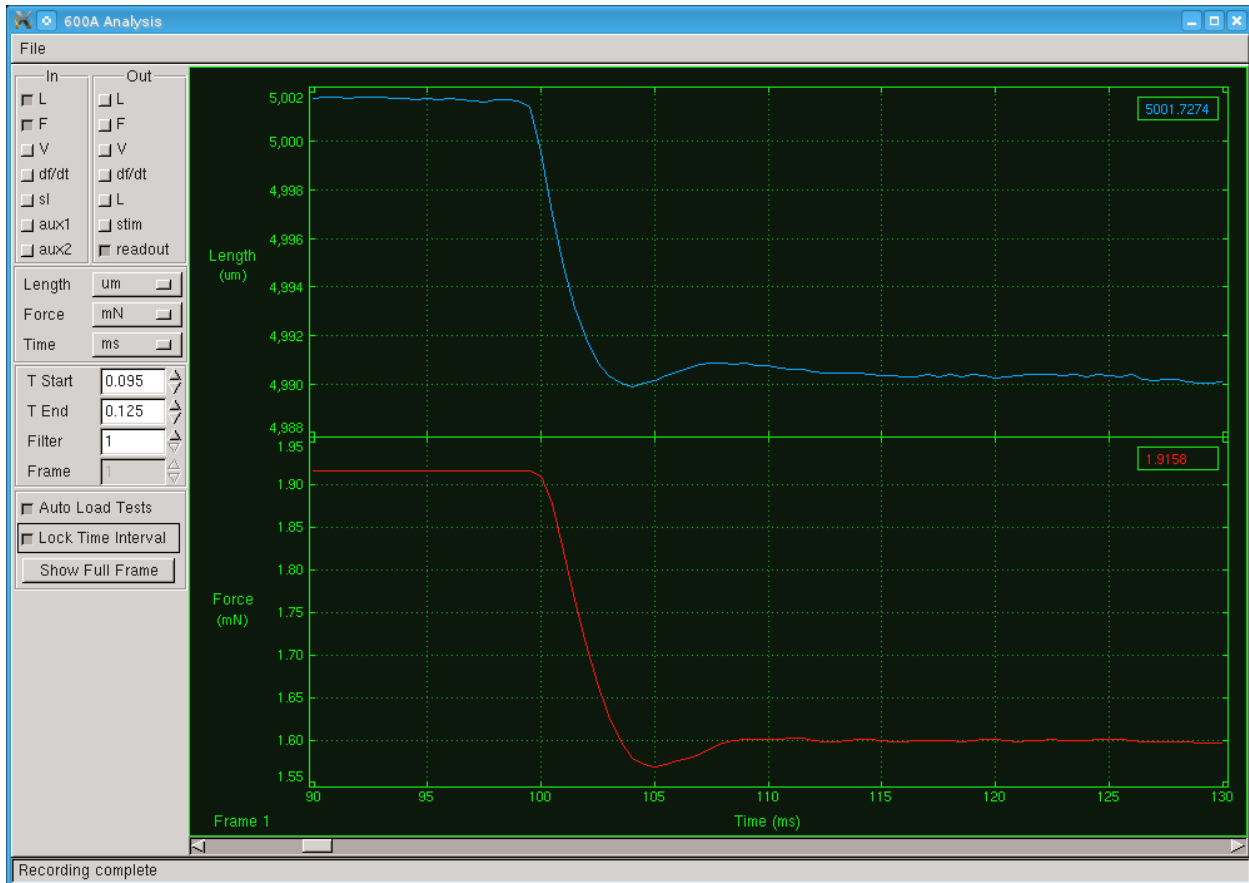
Figure 38 Effect of Increased Gain on the Force Clamp

Gain=0.9, Integral TC=0.2, Filter Tc=0.4

What is the effect of decreasing the Gain parameter? Figure 39 shows the test results when the Gain is set to 0.3. In this case the force clamp is stable (no overshoot, no oscillation) but it takes about 20 ms to reach the desired force.   There is clearly a balance to be had between speed and stability when changing the Gain parameter. A decrease in the Gain parameter slows down the control action and provides more stable results.
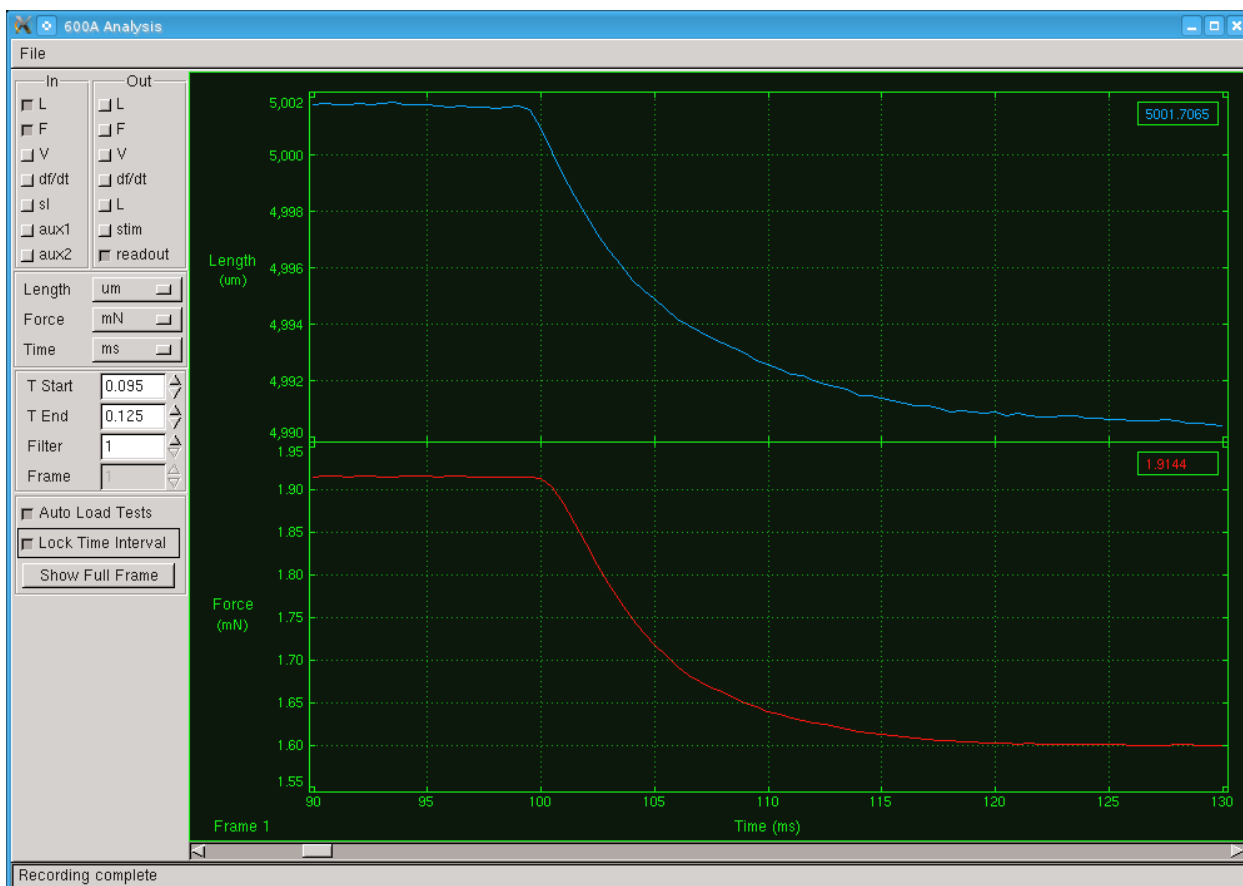
Figure 39 Effect of Decreased Gain on Force Clamp

Gain=0.3, Integral TC=0.2, Filter Tc=0.4

Adjusting the Gain parameter involves finding the highest Gain setting that will provide a stable force without introducing overshoot or oscillation. Figure 40 shows the force clamp when using a Gain setting of 0.6. The length controller is able to change the force to the desired force in about 7 ms. With all other Force Control parameters held constant we have determined the optimal value for the Gain parameter for this particular tissue in just three or four iterations. With experience and time you will learn how to quickly and accurately determine the Gain value based on the response you observe.
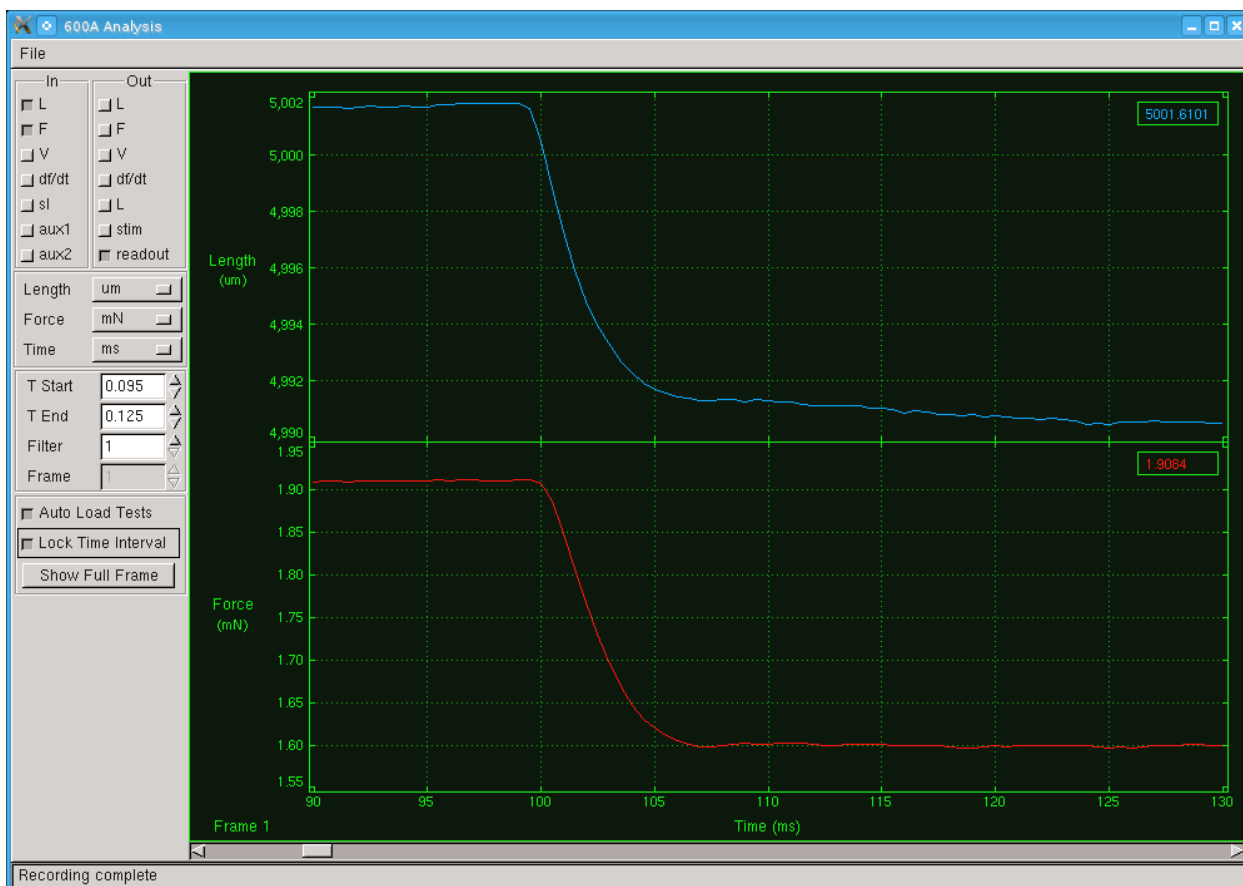
Figure 40 Optimal Gain Setting

Gain=0.6, Integral TC=0.2, Filter Tc=0.4

5.4.2 Integral TC (ms)

Integral TC (ms) is the second parameter in the Force & SL Control window located in both the Force and SL Controller Settings sections. Integral TC stands for the Integral Time Constant and it is used to eliminate small, steady-state errors in the controller. Without the integral term a small, constant difference between the measured force and the desired force would not be eliminated. The addition of an integrator has the disadvantage that it can slow the overall response and also introduce unwanted oscillations in the control signal. Refer back to Figure 37 for the response when the Integral TC is set for 0.2 ms, the Gain set to 0.5 and the Filter TC set to 0.4 ms.

Now let's look at the effect of changing the Integral TC parameter. Some of the reasons for adjusting the Integral TC include: increasing the accuracy of the control and reducing oscillations. Let's first look at what happens when the Integral TC is increased. Figure 41shows the force clamp response when we increase the Integral TC from 0.2 to 0.4 ms. You will note that it takes longer to reach the desired force, 20 ms instead of the initial 10ms. However the response is stable and accurate. Thus an increase in the Integral TC parameter slows down the control action, reduces oscillation and improves accuracy.
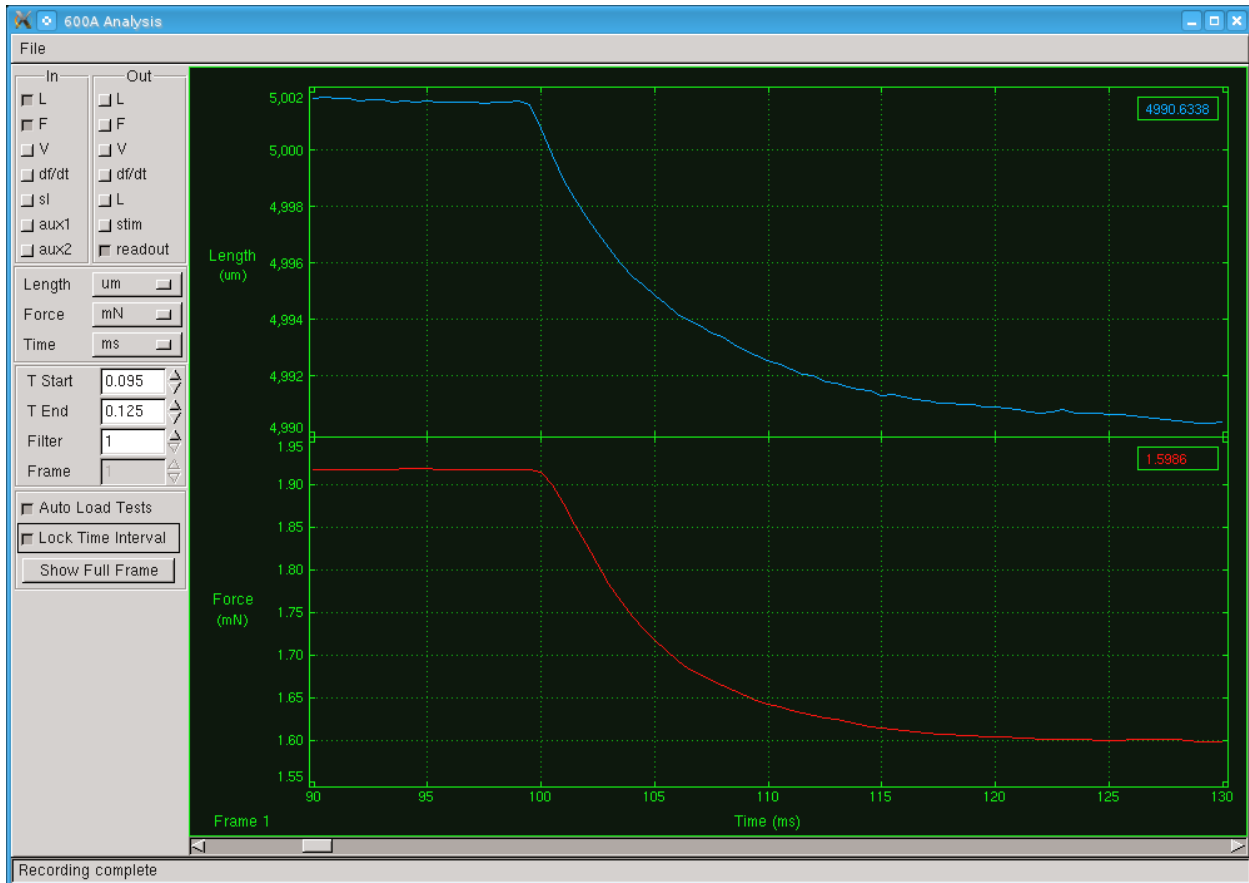
Figure 41 Effect of Increasing Integral TC

Gain=0.5, Integral TC=0.4, Filter Tc=0.4

What is the effect of decreasing the Integral TC parameter? Figure 42 shows the test results when the Integral TC is reduced to 0.1 ms. The most noticeable effect is the force clamp becomes unstable with both an overshoot and an oscillation of the force signal. The less noticeable effect is that the time required to reach the desired force is reduced to 15 ms. As was found when adjusting the Gain parameter, adjusting the Integral TC is also a balance between speed, stability and accuracy. A decrease in the Integral TC parameter speeds up the control action but provides more unstable results.
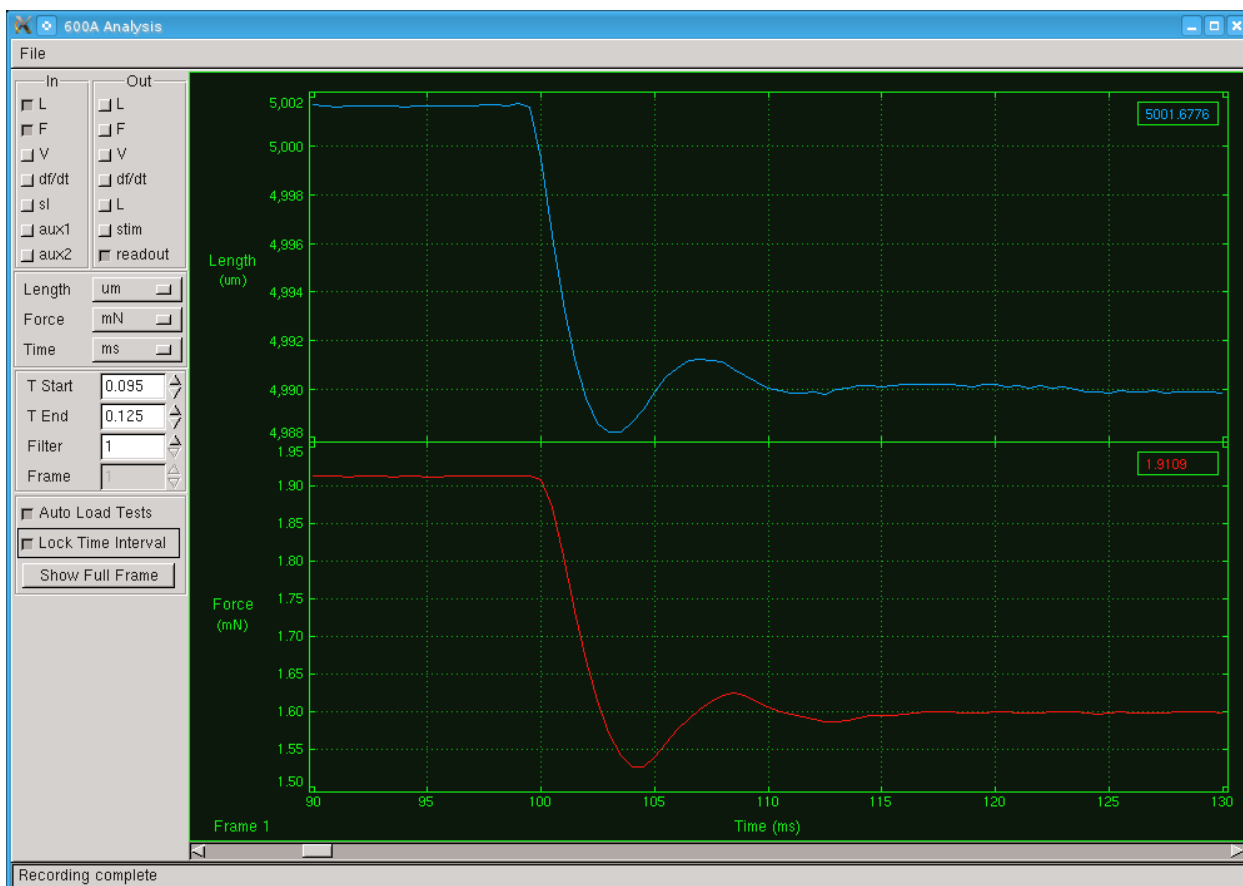
Figure 42 Effect of Decreasing Integral TC

Gain=0.5, Integral TC=0.1, Filter Tc=0.4

With the Integral TC set at 0.15 ms, the overshoot is greatly reduced and the force is clamped in 10 ms. An optimal value would be somewhere between 0.15 and our starting point of 0.2. Figure 43 shows the force clamp when using an Integral TC setting of 0.15. The length controller is able to change the force to the desired force in about 10 ms. With all other Control parameters held constant we have determined the optimal value for the Integral TC parameter for this particular tissue in just two or three iterations. With experience and time you will learn how to quickly and accurately determine the Integral TC value based on the response you observe. Adjusting the Integral TC parameter involves finding the lowest Integral TC setting that will provide a stable force without introducing overshoot or oscillation.
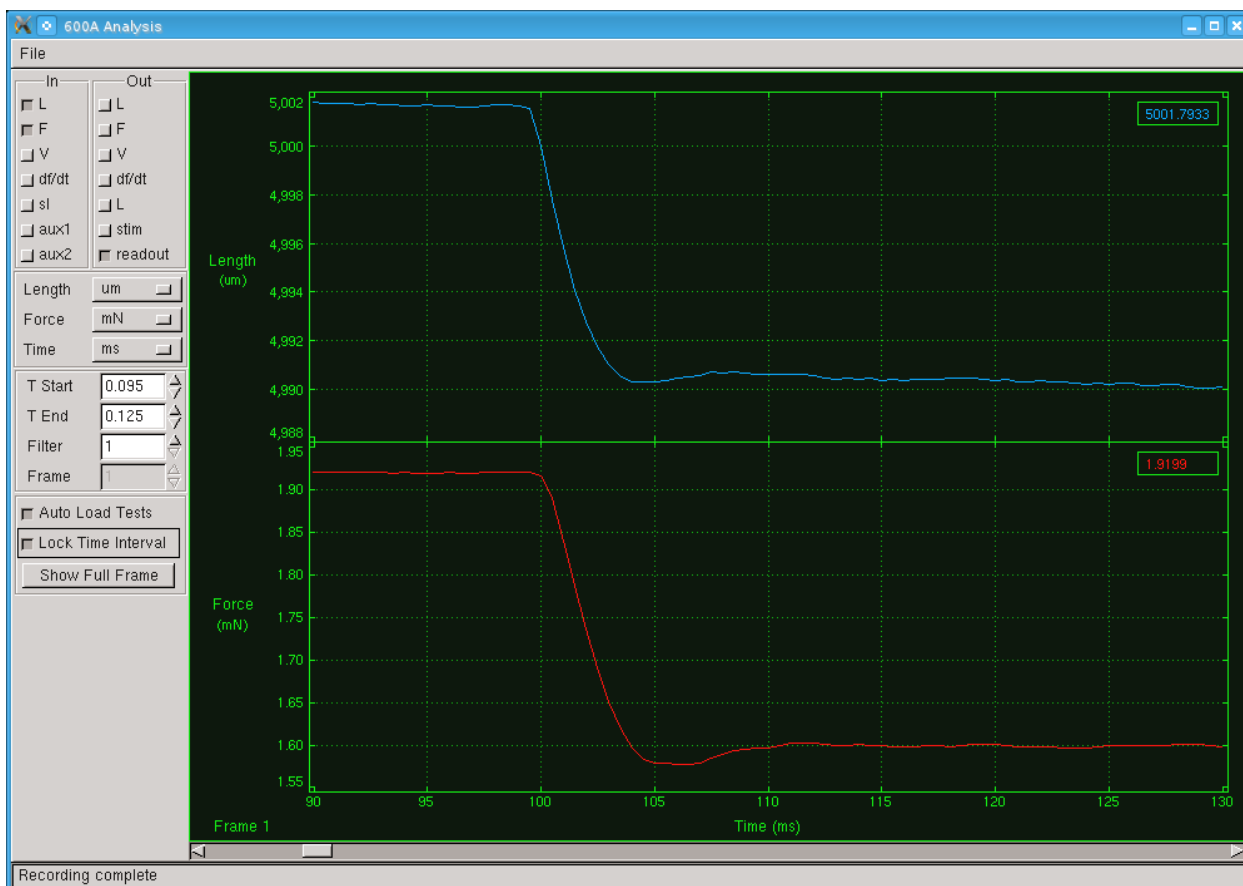
Figure 43 Integral TC Optimal

Gain=0.5, Integral TC=0.15, Filter Tc=0.4

5.4.3 Filter TC (ms)

Filter TC (ms) is the third parameter in the Force & SL Control window located in both the Force and SL Controller Settings sections. Filter TC stands for the Filter Time Constant and it is used to stabilize the control output especially with noisy signals. Without the filter term the control signal will react to noise on the force signal. This reaction can result in major instabilities that make it all but impossible to obtain a force clamp. Since in the real world there is always noise on measurements it is imperative that the Filter TC value is always greater than 0.0. The addition of the filter on the control signal has the disadvantage that it can slow the overall response but this is more than overcome by its ability to stabilize the output. Refer back to Figure 37 for the response when the Gain was set to 0.5, and the Integral TC was set to 0.2 ms and the Filter TC was set to 0.4 ms.

Now let's look at the effect of changing the Filter TC parameter. The main reason for adjusting the Filter TC is to improve the stability of the control which will reduce oscillations. Let's first look at what happens when the Filter TC is decreased. Figure 44shows the force clamp response when we decrease the Filter TC from 0.4to 0.1 ms. You will note the pronounced overshoot and oscillations. Thus a decrease in the Filter TC parameter reduces stability.

Figure 44 Effect of Decreasing Filter TC

Gain=0.5, Integral TC=0.2, Filter Tc=0.1

Figure 45 shows the effect of increasing the Filter TC setting to 0.8 ms. The instabilities are eliminated but the time to reach the desired force is greatly increased to about 25ms.

With all other Control parameters held constant we have determined the optimal value for the Filter TC parameter for this particular tissue in just two or three iterations. With experience and time you will learn how to quickly and accurately determine the Filter TC value based on the response you observe. Adjusting the Filter TC parameter involves finding the lowest Filter TC setting that will provide a stable force without introducing overshoot or oscillation.
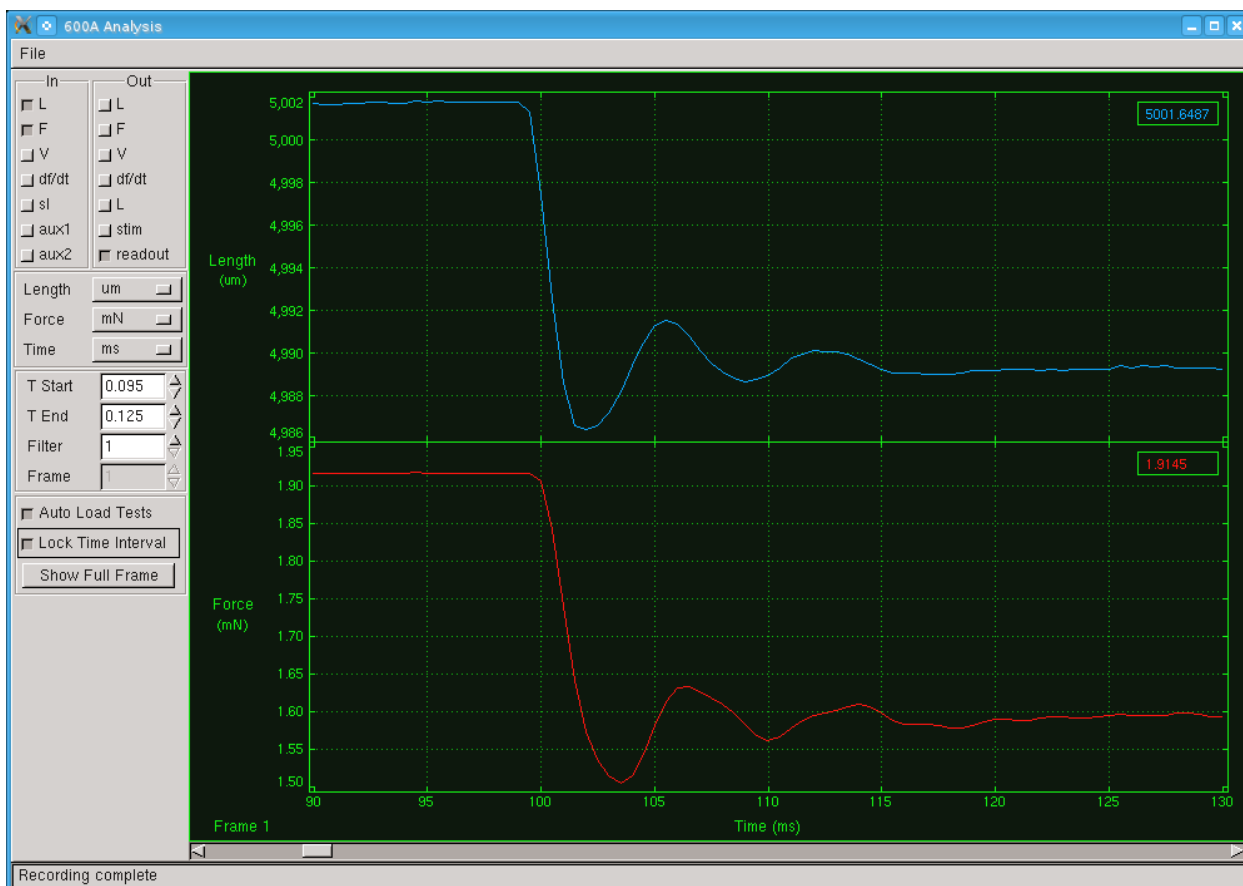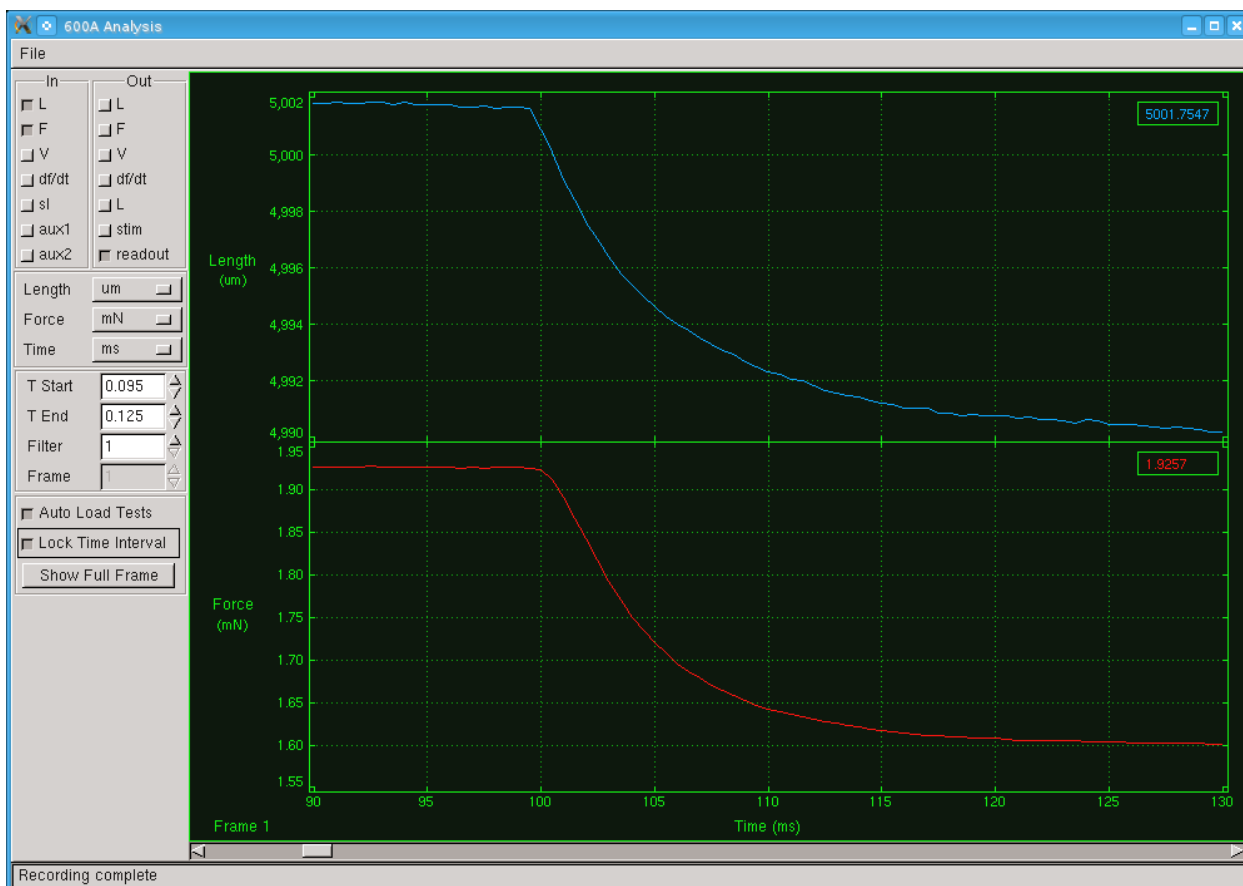
Figure 45 Effect of Increasing Filter TC

Gain=0.5, Integral TC=0.2, Filter Tc=0.8

5.4.4 Linear Range

The Linear Range control parameter is only found in the Force Controller Settings section of the Force & SL Control window, it does not apply for sarcomere length control. As mentioned earlier in this chapter the stiffness of muscle tissue varies with load and with level of activation. We have attempted to compensate for the changing stiffness using a simple method that approximates the load-stiffness relationship as shown in Figure 46. At low forces the stiffness is assumed to be constant and then at some threshold force stiffness starts to increase linearly with force.

The control parameter called Linear Range sets the threshold force. Linear Range is expressed as a percentage in the range from 0-100%. When Linear Range is set to 0% the stiffness varies linearly over the range from zero to Fmax. When Linear Range is set to 100% then the stiffness is constant over the full range of forces from zero to Fmax. The program uses the values Fref and Sref to calculate the slope of the stiffness curve. Fref is the Force 1 value measured when the stiffness was determined and Sref is the measured stiffness dF/dL.
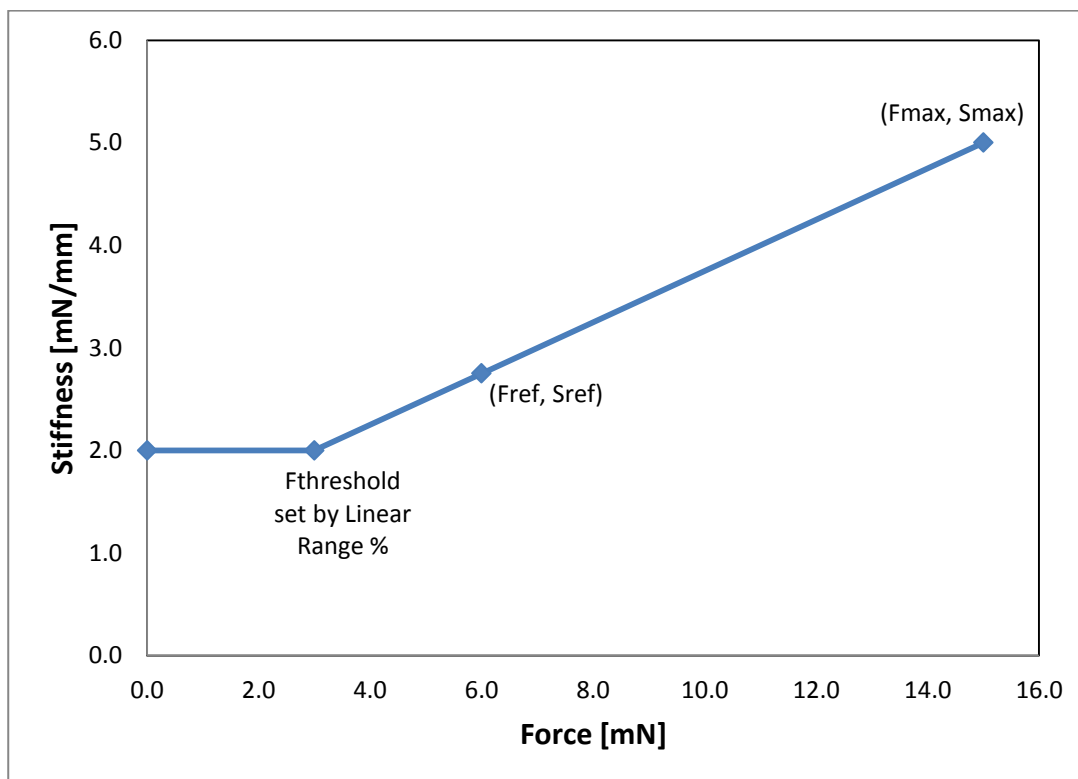
Figure 46 Model of Stiffness as a Function of Force

Initially set Linear Range to 100% and tune the rest of the force control parameters. Then try running protocols with force commands that control forces to different force levels and observe the results. If you find that the force control becomes unstable as the desired force increases then lower the Linear Range percentage. This will have the effect of increasing the stiffness at higher forces which should improve the stability of the force control.

At this point the Linear Range control is experimental and we welcome your feedback to allow us to improve the 600A program. Watch for future releases of the program which will include improvements to the way we handle stiffness as a function of force.

5.4.5 Best Practices for Setting Control Parameters

The following is a quick procedure that you can use when tuning force and sarcomere length control parameters. In the procedure you can substitute sarcomere length whenever you see force and dS/dL whenever you see dF/dL or stiffness.

1) Mount the tissue and make sure you accurately set the reference length, initial length and zero load.
2) Measure dF/dL with the tissue at resting tension or resting SL. This will provide you with the lowest stiffness you can expect with this particular tissue.
3) If you are using a permeabilized tissue then fully activate the tissue in calcium and measure dF/dL again. This will provide you with the highest stiffness you can expect with this particular tissue. At this point you also want to set the Fmax value for your

tissue which you should be able to get from the Scope screen when the fibre is fully activated.

4) Manually set the dF/dL value on the Force & SL Control window to a stiffness value between the two values measured.

5) Set Gain = 0.1, Integral TC (ms) = 0.5, Filter TC (ms) = 0.5 and run a protocol that includes the force control commands you wish to use during your experiment.

6) Observe the Length In and Force In signals using Analysis and zoom into where the force control action takes place. If the response is very slow then increase the Gain parameter and repeat the protocol. Continue to increase the Gain until the response is as fast as possible but still stable.

7) Lower the Integral TC value until you start to see overshoot and other instabilities occur then increase the value until these overshoot and instabilities are gone. Note: at this point you may need to go back and lower the Gain value since changes to Integral TC and Filter TC affect Gain.

8) Lower the Filter TC value until you start to see overshoot and other instabilities occur then increase the value until these overshoot and instabilities are gone. Note: at this point you may need to go back and lower the Gain value and possibly increase the Integral TC value since changes to Filter TC can affect both Integral TC and Gain.

9) Typically you will want the highest Gain and lowest Integral TC and Filter TC that results in fast, accurate and stable force control.

10) If your test will involve force control at different force levels (for example, force clamps to 30%, 20%, 10%, 5%, etc. of Fmax) then you should test the force control protocol at these forces and observe the results. If the force control becomes unstable at higher forces then lower the Linear Range % value and repeat the test. If you can't keep the response stable at varying force levels by using the Linear Range control then you may need to use alternative values for Gain, Integral TC and Filter TC as the force level changes. However you may find that you can't achieve the desired response with these parameters. If so then return these parameters to the values that you had after step (9) and alter the stiffness value shown on the Force & SL Control window. Changing the stiffness value is equivalent to making a major change to the Gain setting; be careful when changing the stiffness. From the range of stiffness you previously measured you may be able to make a reasonable guess of what the stiffness value should be as force varies. Please note that you may need to change Gain, Integral TC and Filter TC when you change the stiffness.

11) Once you have gained some experience with tuning the feedback control you will develop a "feel" for what you need to do to achieve optimal tuning. Often you will only need to make minor changes to the control parameters when you change tissue.

12) Remember that the overall gain value for the control equation is actually the Gain value divided by the stiffness value. Therefore if you have the wrong stiffness you won't be able to properly tune the system regardless of the Gain value you set. Anytime you find you have a gain very close to either 0.0 or 1.0 this is an indication that the stiffness is not set correctly. Gain values close to 0.0 indicate the stiffness is too low whereas Gain values close to 1.0 indicate the stiffness is too high.

13) Integral TC is mostly affected by the model of length controller and force transducer you are using. Once you find an appropriate value for Integral TC it shouldn't change that much (± 0.1 or ± 0.2) for different tissues.

14) Filter TC is mostly affected by the noise on the force transducer signal and therefore the value you need is strongly related to the actual force transducer you are using. Once you find an appropriate value for Filter TC it shouldn't change that much (±0.1 or ±0.2) for different tissues. The most difficult control task is when you are trying to control force to values close to the zero. At this point even small noise on the force signal is large compared to the absolute force and you may need to increase Filter TC to obtain optimum results.

6.0 Analysis

Analysis provides a set of analysis, graphics and storage functions. The Analysis window allows users to load, alter and export test data. The File dropdown menu contains these functions as well as a toggle to show or hide the control panel on the left side. Details of each of these menus are presented in the next section.

Analysis also allows the user to control the variables to be plotted, the units to use, the time base of interest, Zoom controls, Filter Length (for Velocity and dF/dt), a Frame spin button and controls for Auto Loading tests and for Locking and Unlocking the time interval. The colours that are used to plot data are consistent with all of the other plots; they can be set in the display settings menu of the main screen (see 4.3.1 Display Settings for details). The Analysis screen also includes a button to toggle a Digital Readout on/off. All of these controls are discussed in detail in the following sections.
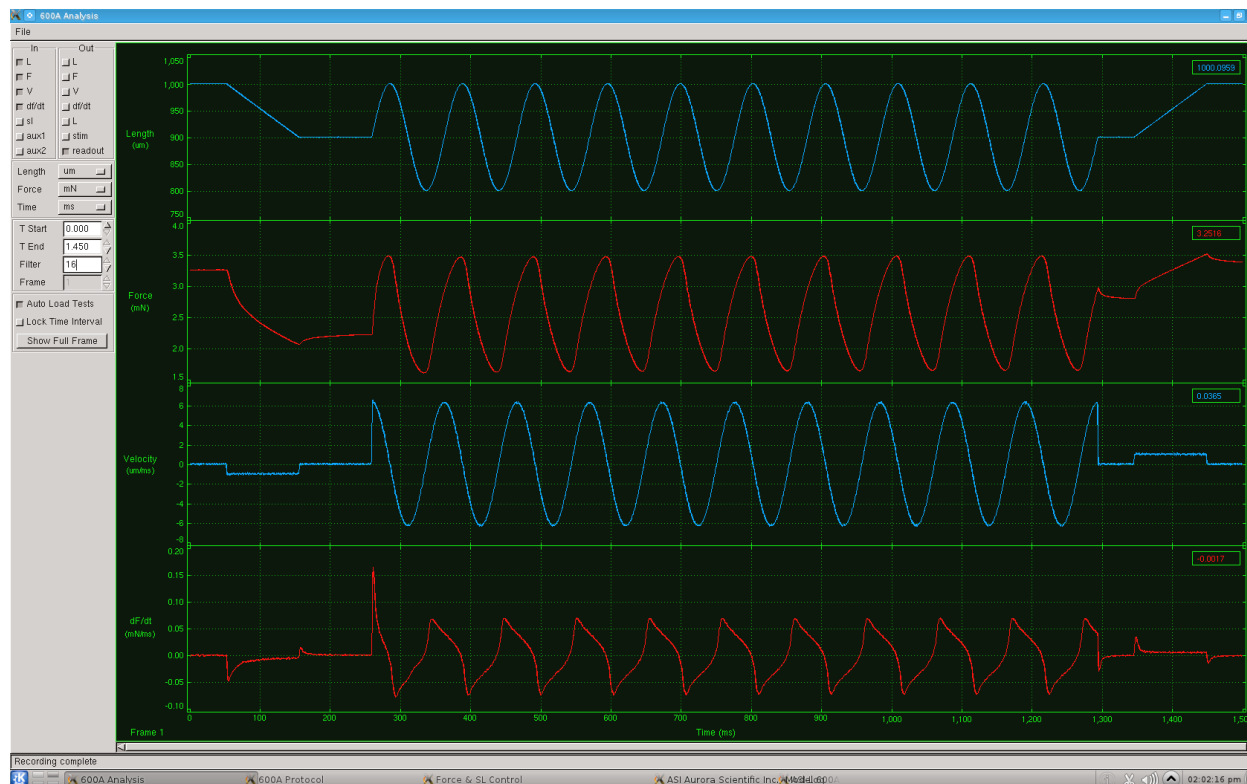

Figure 47 Analysis Screen

6.1 File Menu

The Analysis window File menu drops down to provide access to the following items.

Show Controls

Show Controls is a toggle control that allows the user to hide or show the controls on the left side of the window. Hiding the controls maximizes the data plotting area.

Load Data

Load Data allows the user to open a data file stored on the PC. The data files can be files taken by the main program using the Start Test or Start Sequence button or they can be data files stored using Scope.

Load Current

The Load Current menu item will load the data most recently collected using the main program into Analysis. The data doesn't have to be saved in the main program before importing it into Analysis. This allows you to plot the signal in Analysis and do some preliminary analysis on it before deciding whether you want to save the data or not.

Export Data

The Export Data menu item will output the data currently shown on the Analysis screen to an ASCII text file. The data will be output exactly as shown on the screen with the channels selected, the time base selected, the units selected and the filtering selected. Appendix A shows the file format for these export files.

Close

The Close menu item closes the Analysis window.

6.2 Onscreen Controls

Along the left side of the Analysis window are a series of buttons that control the plotted variables on the Analysis window.

Units Selection

In the upper left part of the window are unit selection controls for Length, Force and Time. Clicking on the button next to the variable allows the user to specify Length, Force and Time units. The current data display is automatically updated whenever the units are changed.

The Velocity and dF/dt are presented in terms of the units specified for length, force and time. Sarcomere length is always plotted in microns (um) and Auxiliary channels are plotted in the units set on the Models Attached window. Stimulus and triggers are plotted as 0/1 (on/off) values.

Plotted Variable Selection

The plotted variables are located to the right of the Units and Color area of the window and are shown as input signals at the top and output signals at the bottom.

Input and output values for a particular variable are overlaid on the same plot.

The graphics window automatically uses the maximum available space to plot the selected variables. In other words, if the user only selects Length as the plotted variable then the length vs time graph will use the entire plotting area. If the user selects Length and Force variables to be plotted then the plotting area is split into two equal size horizontal windows, one for each variable. Thus the number of graphs plotted can range from 1 to 6 depending on the number of variables selected. Length, Force, Velocity, dF/dt and SL are all plotted on individual

graphs. Aux 1 and Aux 2 share a single graph as do Stimulus, Trigger1 and Trigger 2. Clicking on the box next to the variable toggles the plot on and off.

Signals In

L	Plots Length In versus time on the Analysis graphics window
F	Plots Force In versus time on the Analysis graphics window
V	Plots Velocity In (calculated from Length In) versus time on the Analysis graphics window
dF/dt	Plots dF/dt In (calculated from Force In) versus time on the Analysis graphics window
SL	Plots SL In versus time on the Analysis graphics window
Aux 1/Aux 2	Plots the Auxiliary channels 1 and 2 versus time on the Analysis graphics window

Signals Out

L	Plots Length Out (the actual length control values) versus time on the Analysis graphics window
F	Plots Force Out (the actual force control values) versus time on the Analysis graphics window
V	Plots Velocity Out (the derivative of the actual length control values) versus time on the Analysis graphics window
dF/dt	Plots dF/dt Out (the derivative of the actual force control values) versus time on the Analysis graphics window
SL	Plots SL Out versus time on the Analysis graphics window
Stim	Plots Stimulus, Trigger 1 and Trigger 2 versus time on the Analysis graphics window
Readout	Toggles the display of the digital readout for each channel plus the time axis. A dual readout of Y-axis values is presented in the top right corner of each graph. The colour of the readout matches the selected colour for the variable. The time readout is presented on the right end of the time axis. As the cursor is moved back and forth across the plot window the readouts change to show the time and Y-axis value at the cursor location.

Zoom Controls

Below the Color section are graph zoom controls, T Start and T End. There are two methods of zooming: a) enter the desired T Start and T End times and the graph will automatically zoom to the times selected or b) click on the graph and drag a cursor box to define a zoom area (you can use either the left or the right mouse button to define the onscreen zoom area). As soon as the mouse button is released the graph automatically zooms to the times specified by the cursor. To return the graph to the original data press the Unlock T button. You can select the zoom region on any of the displayed graphs and this region then applies to all of the graphs shown.

Filter Control

Below the Zoom controls section is a filter spin button that controls the filter used during the calculation of velocity and dF/dt. Derivatives calculated from actual data can be very noisy.

To reduce the noise a filter is built into the calculation of the derivatives. We use a Savitsky-Golay smoothing filter and the length of the filter can be selected using the Filter Length spin button. A graphic visualization of the effect of the filter length can be seen on Figure 48 and Figure 49. Observe the much smoother velocity and dF/dt data produced when the filter length is increased. Details of the filter are provided in Appendix B – Savitsky-Golay Smoothing Filter.

Frame Control

Below the Filter control is a Frame spin button that controls the Frame displayed on the screen. If the protocol used to collect the data included a repeat function or multiple occurrences of Data Enable and Data Disable then multiple frames will have been created. The Frame spin button allows the user to cycle through these frames displaying the data corresponding to each Data-Enable function.

Auto Load Tests

The Auto Load Tests button is used to control the automatic plotting of data as it is being collected. With the button depressed the Analysis window will automatically plot the most recently collected data.

Lock T Interval

The Lock T Interval button is used to control the zoomed window when automatically plotting data as it is being collected. With the button depressed and the graph zoomed into a particular time region then each subsequent data set will be plotted in the same time window.
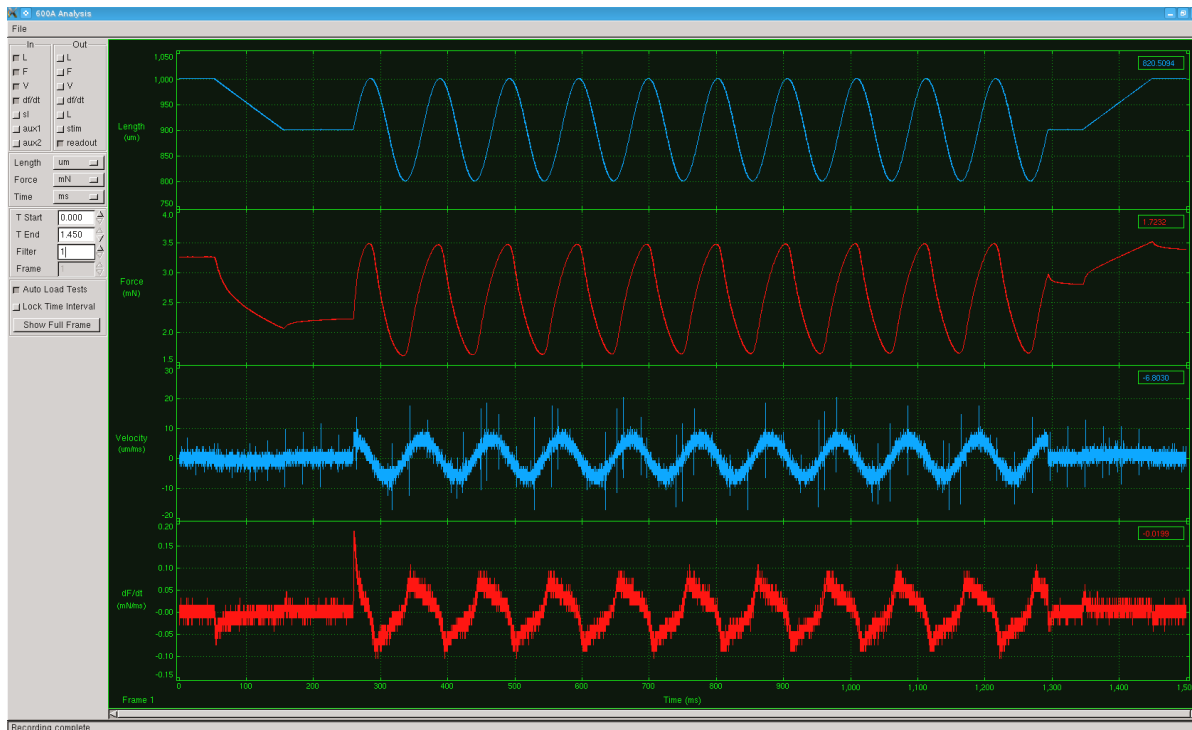
Figure 48 Filter Set to 1
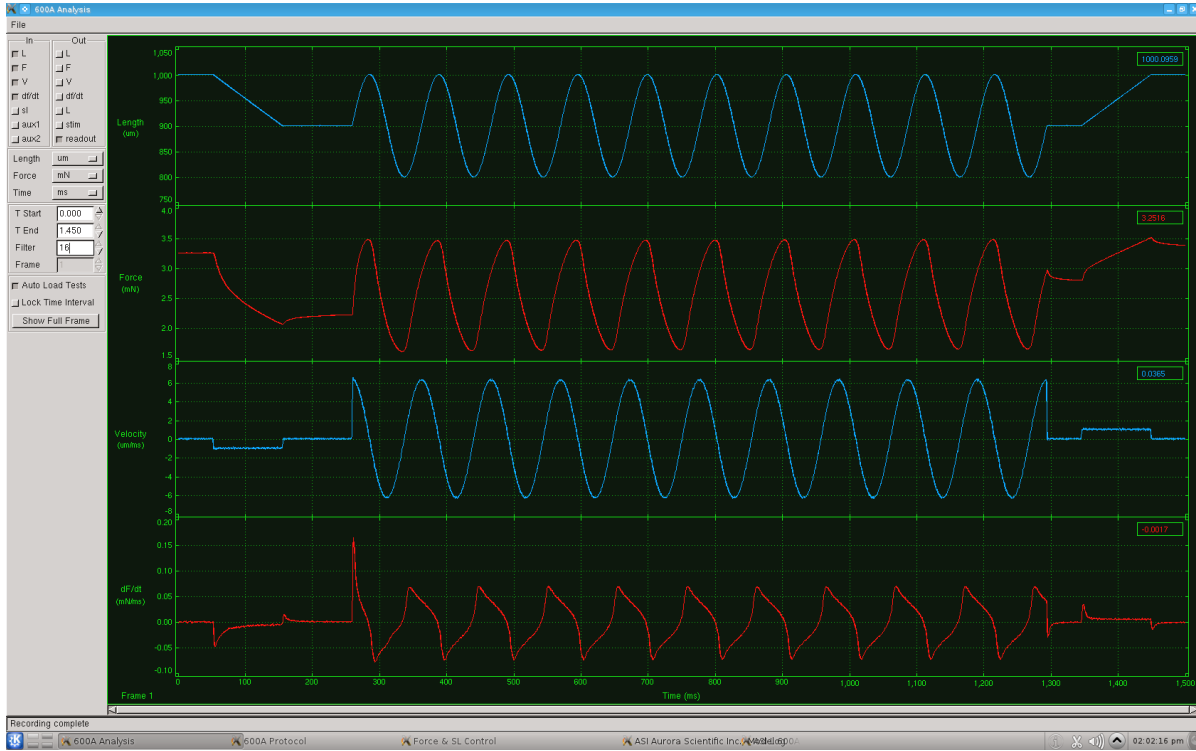
Figure 49 Filter Set to 16

7.0 Scope

Scope provides a real-time output of up to two of the input or output signals in a separate window. Scope starts running in the background as soon as the main program is started, even if Scope is not opened. Scope includes a data buffer that allows the user to review the Scope data taken over the previous 4 hours. Scope also includes controls to specify the variable to be plotted, the time base and the units. A data collection function is also provided that allows data to be streamed to disk for as long as the Record function is active. There is no time limit when recording Scope data to a file (other than the size of the hard drive). Scope also includes controls to freeze the display and then scroll the display back and forth in time. There is a data readout function as well. Whenever the cursor is positioned in the graph area a text readout box appears in the upper right-hand corner of each plot. This box shows the average value of the quantity being displayed at the location in time where the cursor is placed. Scope data is sampled at 100 Hz but the data rate for storage to a Scope data file can be varied from 1 to 100 Hz in set increments.
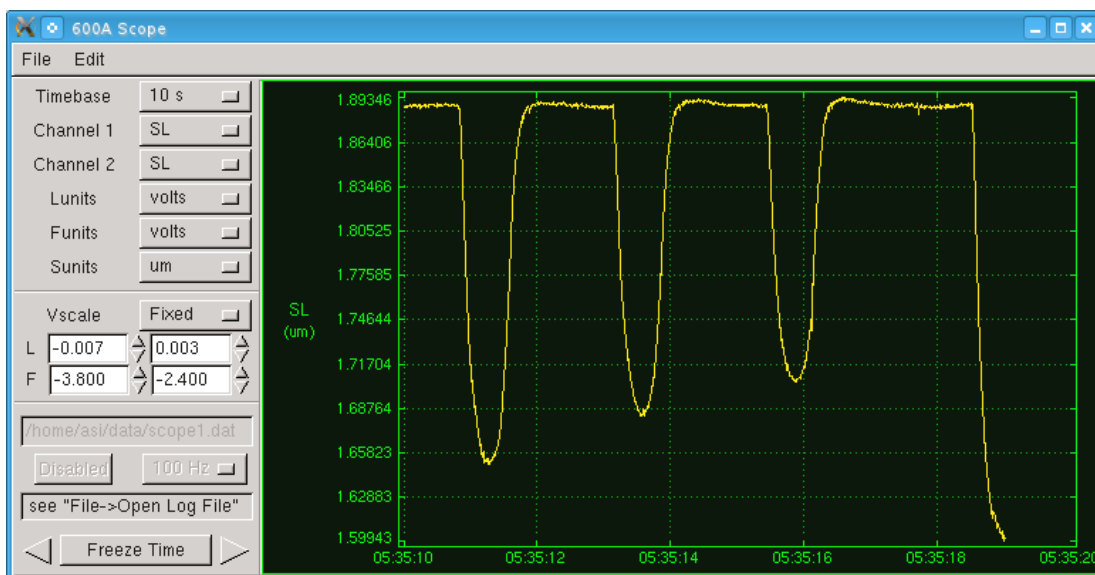
Figure 50 Scope Screen

7.1 File Menu

The Scope File menu contains two menu items, Open Log File and Close.

Open Log File

This menu item opens a file dialog window that allows the user to enter a data file for the storage of Scope data, see Figure 51.

Close

The Close menu item closes the Scope window.
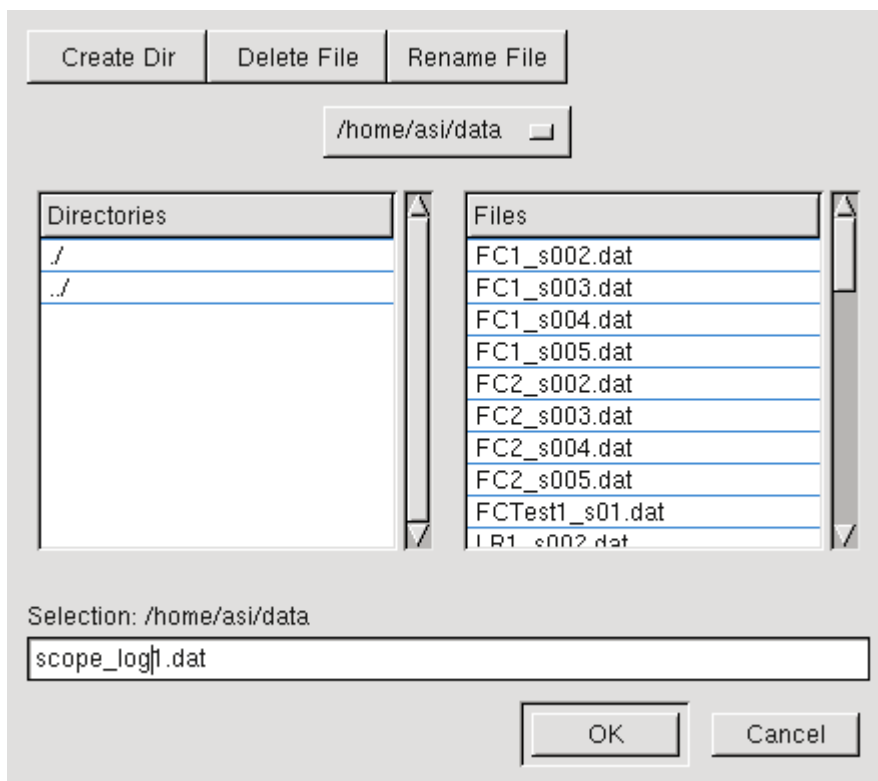
Figure 51 Scope Log File Dialog Window

7.2 Edit Menu

The Scope Edit menu contains three menu items, Show Controls, Cursor Readout and Color.

Show Controls

Show Controls is a toggle control that allows the user to hide or show the controls on the left side of the window. Hiding the controls maximizes the data plotting area.

Cursor Readout

Cursor Readout is a toggle control that allows the user to hide or show the cursor readout located on the right side of the plot area. The readout shows the value of each plotted variable, in the units selected for the current location of the cursor.

7.3 Using Scope

1) Start the ASI 600A program.
2) Select Scope on the main menu and click on Open.
3) The Scope window will open and data will start to be shown on the screen.
4) Select Time Base to choose the amount of data shown on the screen. The time base can vary from 10s to 60 min.

5) Select the Channel 1 and Channel 2 display buttons to choose the variable to be displayed. Options are the same for both Channel 1 and Channel 2 and these include: Lin, Lout, Fin, Fout, Aux1, Aux2, and SL. Note: selecting the same variable to be plotted as both Channel 1 and Channel 2 results in a single graph of that variable that uses the full plotting area.

6) Select Lunits to set the Length display units. Choices include: um, mm, Lo, Lf and volts.

7) Select Funits to set the Force display units. Choices include: mN, N, gm, kg, Fmax, Pa, kPa, and volts.

8) Select Sunits to set the Sarcomere Length display units. Choices include: um and SLo.

9) Select Vscale to set the vertical scale to Auto or Fixed. Auto will automatically scale the data to fit the screen. Fixed allows you to set the vertical limits of the length and force graphs. When Vscale is set to fixed you can enter the minimum L value in the left text box and the maximum L value in the right text box. If Vscale is set to Auto then the text boxes will show the minimum and maximum length scale values that the program is using. You can also enter the minimum F value in the left text box and the maximum F value in the right text box. If Vscale is set to Auto then the text boxes will show the minimum and maximum force scale values that the program is using.

10) Once a log file has been set using File→ Open Log File then this filename will be displayed in the text box beneath the Vscale controls. Also after setting a log file the controls for starting and stopping logging and for choosing the data rate will be un-grayed as is shown in Figure 52.

11) The Sample Rate box allows the user to select the data-sampling rate that is written to disk. Note the Scope displays always runs at 100 Hz. but you can select lower sample rates for writing to a file. The sample rate choices vary from 1 Hz to 100 Hz.

12) Use the Start Button to start recording data to disk.

13) When data is being logged the Start button changes to a Pause Button.

14) Use the Freeze Time and Arrow buttons to first freeze the display and then use the left or right arrow buttons to scroll through the data collected in the past 4 hours (or since the program was started whichever is less). Note: data starts being stored to the internal buffer as soon as the program starts. If for example you opened Scope 15 minutes after starting the program, those first 15 minutes will be in the Scope display buffer. In this case if you froze the scope display as soon as you opened Scope you would be able to scroll back in time through the data that was stored in the buffer since the main program started. Although you can scroll back and see data collected in the past there is no provision for writing this data to disk. You must start the scope logging function in order to collect data with scope.

15) A Status Bar is located above the Freeze Time button that shows status information about Scope including error messages.
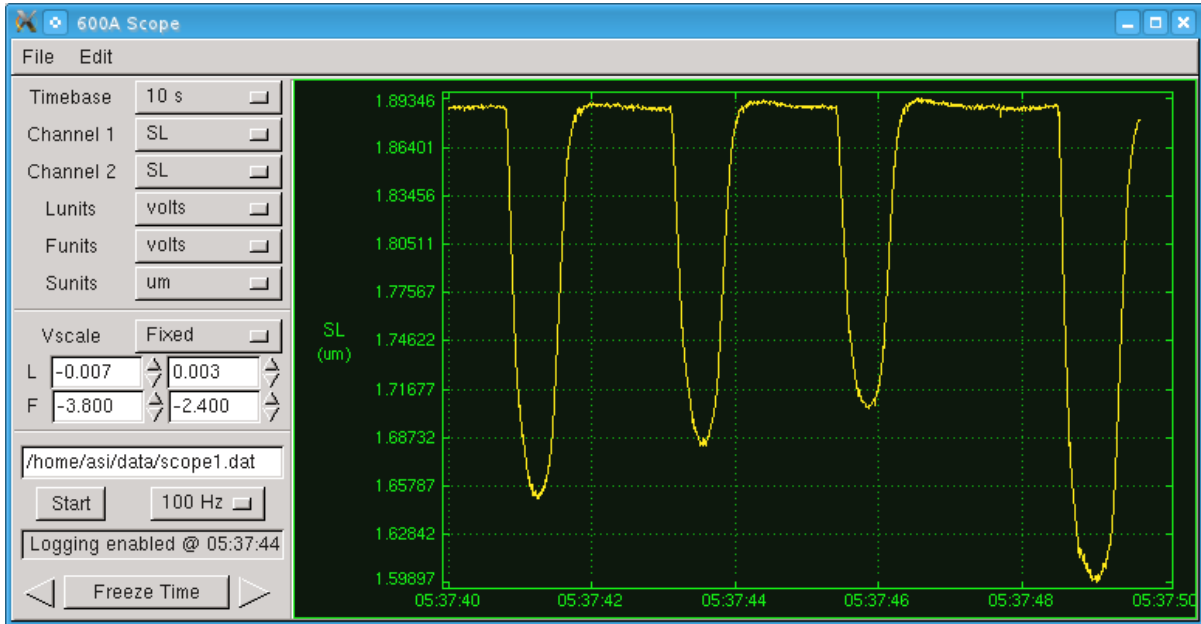
Figure 52 Scope Logging Enabled

8.0 Software Updates

This chapter provides a brief description of the procedure required to update to a newer version of the 600A program.

Aurora Scientific Inc. is striving to make ASI600A the best program for the control and acquisition of muscle mechanics experiments. We are pleased to receive user feedback about the program including suggestions for new features.

New versions of 600A are available from our update website or via email or courier.

<div align="center">

http://www.AuroraScientific.com/update

</div>

If you use our update website you will be asked for user and program information before the update can be downloaded. Once you have received a new version of the program please follow the procedure listed below to upgrade your system.

⚠️ **Do not update the software with the 600A program running**. Close the program prior to proceeding with the update procedure.

1. Navigate to the update website as shown in the link above.
2. Enter the information requested and press the Find Updates! button.
3. All available program updates will be listed, click on the desired update to download it.
4. Once the file is downloaded close all open programs.
5. Copy the supplied update file to the /home/asi directory.
6. Click on the *Installer* icon on the desktop.
7. Browse and select the update file.
8. Press start and the installer will install the file. You may be asked to log in as root during the installation phase. If so then type the current root password and press enter (when the 600A was shipped the root password was set as "root", if you have changed it then enter the new password you created).
9. After the installation is complete you may be asked to reboot the PC. If so please do this before running the new version of the program.

This completes the update procedure. In order to check that the update has been installed start the program and click on Help→ About. Compare the version number listed on the About screen with that shown in the update file name. If they are the same then you have successfully upgraded to the latest program version. If you experience any difficulties while updating your system please contact Aurora Scientific Inc.

9.0 Technical Support, Warranty and Repairs

Aurora Scientific Inc. is dedicated to providing you with products that allow you to meet your research goals. For this reason technical assistance is always free and will be available for the life of your product. If you do have a problem with a product then please know that all ASI products are covered by a three year warranty covering both parts and labour. If you need to return a product to us for repair then consult the final section of this chapter for returns information.

9.1 Technical Support

Please don't hesitate to contact us if you have any technical support issues. Contact us by telephone, email, fax, or regular mail.

Address:	Aurora Scientific Inc.
	Technical Support
	P.O. Box 2724
	Richmond Hill, Ontario, CANADA
	L4E 1A7

Phone:	1 905 727-5161
Toll Free:	1 877 878-4784 (North America)
FAX:	1 905 713-6882
E-mail:	TechSupport@AuroraScientific.com
Web site:	www.AuroraScientific.com

9.2 Warranty

Products manufactured by Aurora Scientific Inc. (ASI) are guaranteed to the original purchaser for a period of three (3) years. Under this warranty, the liability of ASI is limited to servicing, adjusting and replacing any defective parts that are of ASI manufacture. ASI is not liable to the customer for consequential or other damages, labour losses or expenses in connection with or by reason of the use or inability to use the products manufactured by ASI.

Guarantee of parts and components not manufactured by ASI shall be the same as the guarantee extended by the manufacturer of such components or parts. Where possible such parts returned to ASI will be sent to the manufacturer for credit or replacement. Ultimate disposition of these items will depend upon the manufacturer's decision.

All shortages must be reported within ten (10) days after receipt of shipment.

Except where deviations are specified in literature describing particular products, the limited warranty above is applicable to all ASI products, provided the products are returned to ASI and are demonstrated to the satisfaction of ASI to be defective.

Transportation costs of all products returned to ASI must be borne by the customer and products must be returned to ASI within three years after delivery to the original purchaser. ASI cannot assume responsibility for repairs or changes not authorized by ASI or damage resulting from abnormal or misuse or lack of proper maintenance.

Repair or service work not covered under the limited warranty will be billed at current service rates.

NO EXPRESS WARRANTIES AND NO IMPLIED WARRANTIES WHETHER FOR MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR USE, OR OTHERWISE OTHER THAN THOSE EXPRESSLY SET FORTH ABOVE WHICH ARE MADE EXPRESSLY IN LIEU OF ALL OTHER WARRANTIES, SHALL APPLY TO PRODUCTS SOLD BY ASI, AND NO WAIVER, ALTERATION OR MODIFICATION OF THE FOREGOING CONDITIONS SHALL BE VALID UNLESS MADE IN WRITING AND SIGNED BY AN EXECUTIVE OFFICER OF ASI.

9.3 Returning Products to Aurora Scientific Inc. for Repair

There are a few simple steps that must be completed before returning your product to Aurora Scientific Inc.

1. **Obtain a Return Material Authorization number (RMA#).**
Contact our technical support department to obtain a RMA #. We require the serial number of the product along with your contact information, i.e. your name, institution, phone number and email address.

2. **Package your instrument.**
Use the original packaging materials if available. If you do not have original packaging then ensure that the product is wrapped in bubble pack and placed in a sturdy corrugated cardboard box. If you are returning a force transducer please place the transducer head in the plastic protective box and then wrap the plastic box in bubble pack and place it in a small cardboard box which can then be placed in the larger box along with the electronics. For force transducer repairs we require both the transducer head and the control electronics. Please don't send the power cord. When returning a muscle lever system wrap the motor in bubble pack and place it along with the lever arm in a small cardboard box and then place that box in the larger shipping container along with the controller. For muscle lever repairs we require the motor, lever arm, motor cable and control electronics. Please don't send the power cord.

3. **Prepare Customs documents.**
Canadian Clients: no customs documents are required, skip to step 4.
European Clients: no customs documents are required, skip to step 4 and ship to Aurora Scientific Europe.
USA and Rest of the World Clients: You must include a Commercial Invoice (CI) with the shipment. Please click this link to download a blank CI.

You can also prepare the commercial invoice yourself instead of using the downloadable form. Print the document on your company's letterhead and include the following information: Date, Shipper's Name, Address and Phone Number (your company information), Consignee's Name, Address and Phone Number (Aurora Scientific Inc. is the Consignee), Country of Origin of Goods (this will be <u>Canada</u> if you purchased the instrument from Aurora Scientific or <u>USA</u> if your product was purchased from Cambridge Technology), Conditions of Sale (include the following statement: GOODS RETURNING TO FACTORY FOR REPAIR, TEMPORARY IMPORT), Number of Packages (normally 1), Description of Goods (e.g. Model 300B Muscle Lever System, Serial Number 1111), Quantity of Each Item (normally 1) and Value for Customs Purposes (the original purchase price of the instrument).
Place three (3) copies of your CI in an envelope and mark the outside CUSTOMS PAPERS ENCLOSED. Attach the envelope to the outside of the box.

4. Choose a shipper and prepare the waybill.
European Clients: ship your instrument to [Aurora Scientific Europe in Dublin, Ireland](#).
All other Clients: ship your instrument to [Aurora Scientific Inc. in Aurora, ON, Canada](#).
You may ship your instrument back to us via the courier of your choice or via parcel post. If possible we prefer that you ship via FedEx. You are responsible for both the shipping and brokerage charges so please mark the waybill accordingly. Please don't ship freight collect. Shipments sent freight collect will be received but you will be invoiced for the shipping charges when your instrument is returned.

5. Prepare and send a purchase order.
After we receive the instrument we will evaluate it and contact you with the estimated repair cost. We require a purchase order before we can repair and return your instrument. Please fax or email us the purchase order at your earliest convenience.

Return Shipping Address:

Canada, USA, Asia	Europe
Aurora Scientific Inc. 360 Industrial Parkway S., Unit 4 Aurora, Ontario, CANADA L4G 3V7 Attn: RMA Returns Tel: +1-905-727-5161	Aurora Scientific Europe Hilton House 3 Ardee Road Rathmines, Dublin 6, Ireland Attn: RMA Returns Tel: +353-1-525-3300

Appendix A - ASI600A File Structure

Data File Structure

```
ASI 600A Data File
Created: Wed Apr 15 05:49:35 2009
A/D Sampling Rate: 1000 Hz
Comment: Force Clamp
*** Setup Parameters ***
Length Controller: 315C
Force Transducer: 403A
Initial Length: 1 mm
Diameter: 1.4 mm
Maximum Tetanic Tension: 5 mN
ADC0 Offset: 11 mv
ADC1 Offset: 11 mv
DAC0 Offset: 0.6 mv
DAC1 Offset: 1.2 mv
Length Units: mm
Force Units: mN
Time Units: s
Scope Display1: 0
Scope Display2: 6
Scope Timebase: 0
Scope Vscale:   0
Scope Lunit:    3
Scope Lmin:     0.004500
Scope Lmax:     0.007000
Scope Funit:    0
Scope Fmin:     0.012000
Scope Fmax:     0.026000
*** Calibration Parameters ***
```

Model Number	LOoffset mm	LOscale mm/v	LIoffset mm	LIscale mm	FOoffset mm/v	FOscale mN	FIoffset mN/v	FIscale mN/v
308B	0	0.2	0	0.2	0	1	0	1
312C	0	0.15	0	0.15	0	1	0	1
315C	0	0.15	0	0.15	0	1	0	1
318B	0	0.3	0	0.3	0	1	0	1
322C	0	0.3	0	0.3	0	1	0	1
300B	0	0.5	0	0.5	0	50	0	50
300B-LR	0	0.5	0	0.5	0	100	0	100
305B	0	1	0	1	0	500	0	500
305B-LR	0	1	0	1	0	1000	0	1000
309B	0	1.25	0	1.25	0	2000	0	2000
310B	0	2	0	2	0	5000	0	5000
310B-LR	0	2	0	2	0	10000	0	10000
400A	0	1	0	1	0	5	0	5
402A	0	1	0	1	0	50	0	50
403A	0	1	0	1	0	0.5	0	0.5
404A	0	1	0	1	0	10	0	10
405A	0	1	0	1	0	1	0	1
406A	0	1	0	1	0	0.05	0	0.05
407A	0	1	0	1	0	100	0	100
other	0	1	0	1	0	1	0	1

```
*** Test Protocol Parameters ***
A/D Sampling Rate: 1000 Hz
Comment: Force Clamp
Minimum Length: 0.100 (Lo)
Maximum Length: 2.000 (Lo)
PD Deadband:    0.050000 mN
Stimulus 01: 0.3 ms 100.000 Hz 10.0 ms 50.000 Hz 0.100 s
```

```
Stimulus 02: 1.2 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 03: 1.3 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 04: 1.4 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 05: 1.5 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 06: 1.6 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 07: 1.7 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 08: 1.8 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 09: 1.9 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 10: 2.2 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Time (ms)    Control Function    Options
      5.0    Data-Enable
     50.0    Length-Ramp         1.1     Lo    30      ms
     85.0    Length-Sample       114     ms
    100.0    Force-Step          0.2     Fmax
    200.0    Length-Hold
   1000.0    Length-Ramp         1       Lo    60      ms
   1100.0    Data-Disable
      0.0    Stop
*** Force and Length Signals vs Time ***
Time (ms) Lin (mm) Lout (mm) Fin (mN) Fout (mN) SL (um)   Stimulus/Triggers
0.1 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.2 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.3 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.4 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.5 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.6 1.00000 1.00000 0.00015 4.99970 1.66519 0
0.7 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.8 1.00005 1.00000 0.00015 4.99970 1.66519 0
0.9 1.00005 1.00000 0.00046 4.99970 1.66519 0
1.0 1.00000 1.00000 0.00015 4.99970 1.66473 0
1.1 1.00000 1.00000 0.00031 4.99970 1.66473 0
1.2 1.00000 1.00000 0.00015 4.99970 1.66473 0
1.3 1.00000 1.00000 0.00015 4.99970 1.66473 0
1.4 1.00005 1.00000 0.00015 4.99970 1.66473 0
1.5 1.00000 1.00000 0.00015 4.99970 1.66473 0
1.6 1.00000 1.00000 0.00015 4.99970 1.66473 0
1.7 1.00005 1.00000 0.00015 4.99970 1.66473 0
1.8 1.00005 1.00000 0.00000 4.99970 1.66473 0
1.9 1.00005 1.00000 0.00015 4.99970 1.66473 0
2.0 0.99995 1.00000 0.00000 4.99970 1.66473 0
•
•
•
3.0 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.1 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.2 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.3 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.4 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.5 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.6 1.00000 1.00000 0.00015 4.99970 1.66519 0
3.7 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.8 1.00005 1.00000 0.00015 4.99970 1.66519 0
3.9 1.00005 1.00000 0.00046 4.99970 1.66519 0
10.0 1.00000 1.00000 0.00015 4.99970 1.66473 0<-NOTE: Time changes from 3.9 ms to
10.1 1.00000 1.00000 0.00031 4.99970 1.66473 0        10.0 ms because there were
10.2 1.00000 1.00000 0.00015 4.99970 1.66473 0        two Frames in this data
10.3 1.00000 1.00000 0.00015 4.99970 1.66473 0        record.  This occurs when
10.4 1.00005 1.00000 0.00015 4.99970 1.66473 0        there are multiple
10.5 1.00000 1.00000 0.00015 4.99970 1.66473 0        Data-Enable/Data-Disable
10.6 1.00000 1.00000 0.00015 4.99970 1.66473 0        commands in a protocol or
10.7 1.00005 1.00000 0.00015 4.99970 1.66473 0        when there is a Repeat
10.8 1.00005 1.00000 0.00000 4.99970 1.66473 0        function in the protocol.
```

Protocol File Structure

```
ASI 600A Test Protocol File
Created: Thu Apr 26 23:21:59 2012
A/D Sampling Rate: 20000 Hz
Comment: Force Clamp
Minimum Length: 0.900 (Lo)
Maximum Length: 1.100 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 0.3 ms 100.000 Hz 10.0 ms 50.000 Hz 0.100 s
Stimulus 02: 1.2 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 03: 1.3 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 04: 1.4 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 05: 1.5 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 06: 1.6 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 07: 1.7 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 08: 1.8 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 09: 1.9 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Stimulus 10: 2.2 ms 100.000 Hz 20.0 ms 2.000 Hz 100.000 s
Time (ms)      Control Function    Options
     0.0       Data-Enable
    90.0       Length-Sample       1 10010 ms
   100.0       Force-Step           1.5 mN
  2000.0       Force-Sine          20 Hz  0.2 mN  6 s
 10101.0       Length-Hold         1
 10200.0       Length-Ramp          1 Lo   300 ms
 12000.0       Data-Disable
 12001.0       Stop
```

Sequencer File Structure

```
ASI 600A Test Protocol Sequence File
Created: Wed May 23 15:42:12 2012
Protocol File Directory: /home/asi/pro/
Data File Directory: /home/asi/data/
Output File Type (0: one file 1: one file per frame): 0
Protocol 1: 1 0 0 0 LengthSweep.pro LSTest1
Protocol 2: 1 0 0 1 ForceClamp.pro FCTest1
Protocol 3: 1 0 10 1 LengthRamp.pro LRTest1
Protocol 4: 0 0 0 0
Protocol 5: 0 0 0 0
```

Calibration File Structure

```
ASI 600A Calibration File
Created: Wed May 23 10:08:10 2012
```

Model Number	LOoffset mm	LOscale mm/v	LIoffset mm	LIscale mm/v	FOoffset mN	FOscale mN/v	FIoffset mN	FIscale mN/v
308B	0	0.2	0	0.2	0	1	0	1
312C	0	0.15	0	0.15	0	1	0	1
315C	0	0.15	0	0.156935	0	1	0	1
318B	0	0.3	0	0.3	0	1	0	1
322C	0	0.3	0.000636329	0.315927	0	1	0	1
300B	0	0.5	0	0.5	0	50	0	50
300B-LR	0	0.5	0	0.5	0	100	0	100
305B	0	1	0	1	0	500	0	500
305B-LR	0	1	0	1	0	1000	0	1000
309B	0	1.25	0	1.25	0	2000	0	2000
310B	0	2	0	2	0	5000	0	5000
310B-LR	0	2	0	2	0	10000	0	10000
400A	0	1	0	1	0	5	0	5
402A	0	1	0	1	0	50	0	50
403A	0	1	0	1	0	0.5	0	0.5
404A	0	1	0	1	0	10	0	10
405A	0	1	0	1	0	1	0	1

```
406A    0    1    0    1    0    0.05    0    0.05
407A    0    1    0    1    0    100     0    100
other   0    1    0    1    0    1       0    1
```

Setup File Structure

```
ASI 600A Setup File
Created: Wed May 23 15:42:12 2012
Length Controller: 322C
Force Transducer: 400A
Initial Length: 5 mm
Minimum Length: 0.5 Lo
Maximum Length: 1.2 Lo
Fiber Length: 5 mm
Initial Sarcomere Length: 1.89 um
Diameter: 1.183 mm
Maximum Tetanic Tension: 5 mN
PD Deadband: 0.0005 mN
Calibrate FC step size:  10 um
Calibrate FC ramp time:  20 ms
Calibrate FC hold time:  50 ms
Force Gain-Factor:       0.59
Force Integral-Constant: 0.3 ms
Force Filter-Constant:   0.2 ms
Constant Stiffness Range: 100
dF/dL measured at Force:  34275 adu
Fiber Stiffness (adu):   1.15356
Fiber Stiffness (mN/mm): 19.2261
SL Gain-Factor:          0.25
SL Integral-Constant:    0.1 ms
SL Filter-Constant:      0 ms
SL Stiffness (adu):      0.6
SL Stiffness (um/mm):    1
ADC0 Offset: 3.980 mv
ADC1 Offset: 3.980 mv
DAC0 Offset: -1.979 mv
DAC1 Offset: 0.587 mv
Panel 1 size: 2
Panel 2 size: 4
Panel 3 size: 2
Panel 4 size: 1
Panel 1 signal: 0
Panel 2 signal: 1
Panel 3 signal: 6
Panel 4 signal: 6
Background (0/1): 1
Length In color:  895231
Force  In color:  16717330
SL     In color:  16771867
Aux1   In color:  1769257
Length Out color:  255
Force  Out color:  255
Stim   Out color:  16777215
Aux2   In  color:  16747283
Length Units: um
Force Units: mN
Time Units: ms
Sarcomere Length Units: um
Scope Display1: 0
Scope Display2: 2
Scope Timebase: 0
Scope Vscale:   0
Scope Lunit:    0
```

```
Scope Lmin:      4960.000000
Scope Lmax:      5010.000000
Scope Funit:     0
Scope Fmin:      1.400000
Scope Fmax:      2.400000
Scope Sunit:     0
Analysis Lunit: 0
Analysis Funit: 0
Analysis Tunit: 0
Analysis Channels: 10100000000001
Aux1 Name: Aux1
Aux1 Units: volts
Aux1 Scale (units/volt): 1.000000
Aux1 Offset (units): 0.000000
Aux2 Name: Aux2
Aux2 Units: mv
Aux2 Scale (units/volt): 1000
Aux2 Offset (units): 0
```

Analysis Export File Structure

```
ASI600A Export Data File
Created: Thu May 24 11:45:23 2012
    Time        L in        F in
    (ms)        (um)        (mN)
     0.00     5001.770     2.14264
     0.50     5001.860     2.14279
     1.00     5001.840     2.14310
     1.50     5001.800     2.14264
     2.00     5001.880     2.14294
     2.50     5001.840     2.14325
     3.00     5001.920     2.14340
     3.50     5001.900     2.14310
     4.00     5001.910     2.14371
     4.50     5001.910     2.14310
     5.00     5002.000     2.14325
     5.50     5001.960     2.14325
     6.00     5001.950     2.14264
     6.50     5001.910     2.14294
     7.00     5001.950     2.14294
     7.50     5001.960     2.14310
     8.00     5001.920     2.14310
     8.50     5001.880     2.14233
     9.00     5001.930     2.14233
     9.50     5001.840     2.14279
    10.00     5001.890     2.14310
    10.50     5001.850     2.14310
    11.00     5001.860     2.14325
```

Appendix B – Savitsky-Golay Smoothing Filter

The Analysis portion of the ASI600A program includes a smoothing filter for the calculation of velocity and dF/dt. The filter is a Savitsky-Golay filter and it is described below.

savgol: Savitsky-Golay smoothing filter coefficients.

Usage: c = savgol fp d

Remarks: Given a 3-element integer vector fp containing the parameters (nl, nr, M) and an integer scalar d, savgol returns a real vector c of length (1+nl+nr) containing the Savitsky-Golay filter of polynomial order M and derivative order d. The coefficients are returned in reverse order, and may be applied using conv2.

The S-G filter is equivalent to fitting an M-th order polynomial to the points x[j] (j=i-nl:i+nr), and taking the d'th derivative of the polynomial at j=i for i=0:N-1.

Appendix C – Terminology

Lin	Length Input, analog voltage input from a length controller.
Fin	Force Input, analog voltage input from a force transducer.
AUX 1	Auxiliary Input, analog voltage input from auxiliary channel number 1.
AUX 2	Auxiliary Input, analog voltage input from auxiliary channel number 2.
Lout	Length Output, analog control voltage sent to a length controller.
Fout	Force Output, analog control voltage sent to a force controller (only works with dual-mode length controllers).
Stim Out	Stimulator Output, digital control signal sent to a stimulator that controls the timing of the stimulation pulses.
Lref	Reference Length, muscle tissue length when Lout is set to zero. Units are mm.
Lo	Initial Length, tissue length under specified physiological conditions. Units are mm.
Lf	Fiber Length, determined by the researcher. Units are mm.
Do	Effective Diameter, diameter of a circle that produces the same cross sectional area as the fiber.
SLo	Initial Sarcomere Length, sarcomere length under specified physiological conditions. Units are microns (um).
Fmax	Maximum Tension, maximum force measured during a tetanic contraction. Units are mN.
dF/dL	Derivative of Force with respect to Length, change in force for a given change in length. Units are mN/mm.
Protocol	Test protocol consisting of commands that control data acquisition, stimulation, length and force of muscle tissue.
Sequence	Series of Test Protocols run in succession.
xterm	X terminal, similar to a Windows command window. An xterm allows the user to enter commands in the Linux operating system.
RTAD	Real Time A/D device driver, the real-time code segment of the ASI600A program that handles all data acquisition and control signals.

Appendix D – Legacy Force Controls

Legacy is the previous method of using force and SL control protocols. This system works with a less refined control algorithm and is thus less desirable compared to the new one. The purpose of this section is to explain how to update no longer functional protocols.

Old protocols will not behave the same on the new control system because parameters like gain factor, control factor, and damping factor which were previously set in the protocol are now in the Force and SL Control window, making them easier to modify and tune.

These should be removed from the protocol as they are no longer functioning. The functionality of these parameters is moved to the Force and SL Control window, see section 5.0 Force and SL Control for details.

Appendix E – Network Security

⚠️ Many users will elect to connect the ASI600A to a network. Because the 600A is shipped with no login password and the root password is not secure, **we strongly suggest that you set a secure user and root password before connecting the 600A to any network**. A secure password should not be a recognizable word, it should be a minimum of 8 characters containing letters (both upper and lower case), numbers and at least one special character (&, ^, $, #, @, !).

Procedure to change the user password.
1. Open an xterm console.
2. Type
   passwd
3. The user will be prompted to enter your current password. By default it is:
   asi
4. The user will be asked to enter a new password. The minimum password length is 5 characters; however we recommend a strong 8 character password. See warning above.
5. Re-enter the password to confirm it.
6. Record the password in a secure location so you do not forget.

Procedure to change the root password (administrator password).
1. Open an xterm console.
2. Login as root. Type
   su
3. The default password is *root*, if it has been changed then enter the current password, otherwise enter
   root
4. Follow steps 2 through 6 of the procedure to change the user password in order to change the root password.
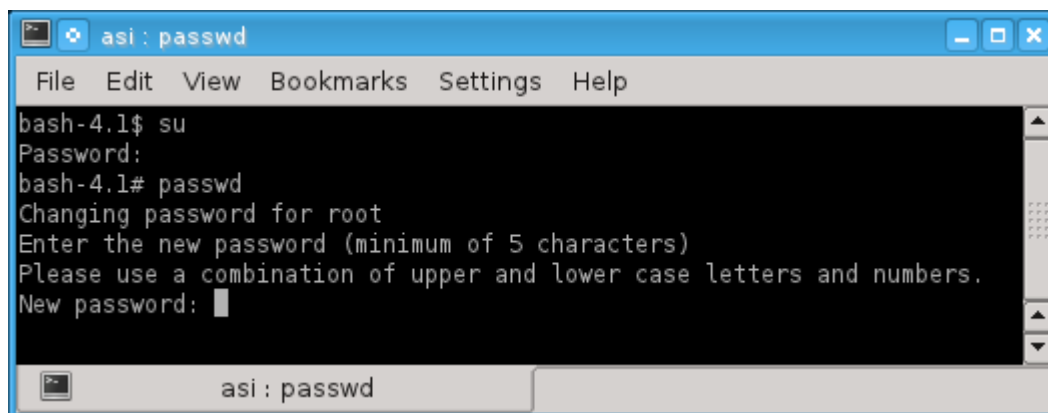
Figure 53 Use passwd to Change the Root Password

Appendix F – Program Start Switches

The program contains several start switches that are read when the program starts and set the program in different operating modes. These start switches can be set by using the properties of the 600A icon. To change the properties right click on the desktop icon and click on Properties. When the Properties window opens click on the Application tab, then click on the Command line and then edit the line to enter the desired start switches. Note: you must leave a space between each start switch if you intend to use multiple switches.

The following is the usage output when starting the program with the –h (help) switch. You can view the start switches by opening an xterm and typing:

Asi600a –h

-2	record 2 channels (the default)
-4	record 4 channels
-i	invert the polarity of Lout
-p	limit Lout to -10:0 volts
-s	enable serial i/o with HVSL for SL measurements
-rt	enable rtpipe i/o with HVSL for SL measurements
-b	enable the 802D Bath Controller commands
-net [asihost]	enable logging and remote control from "asihost"
-master	trigger slaves when 'Start Test' ... is clicked
-slave	respond to triggers issued by masters
-shiftsl	shift SL (in time) to correct for lags in video sl-measurements
-sltodac1 [slmin slmax]	output SL as analog voltage on dac1SL values from slmin:slmax (um) are mapped to -10:+10 volts