Aurora Scientific Inc.

INSTRUCTION MANUAL

Model
950A FluoroTrack

Program Version: 3.100

May 30, 2013

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Conventions Used in this Manual

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

 This icon indicates an important point to remember.

Italicized Text   Indicates a command to be typed exactly as shown.

1.0 Introduction

This manual explains the proper use and operation of Aurora Scientific Inc.'s (ASI) FluoroTrack software program. FluoroTrack was developed to allow researchers to control and take data from an Ion Optix fluorescence system.

The FluoroTrack system consists of the following equipment: 950A FluoroTrack software, 960A FluoroDAQ Data Acquisition Controller, 961A HyperSwitch Dual Excitation Light Source, 962A Cell Framing Adapter, 963A Photomultiplier Subsystem, and 964-x Microscope Components – x=microscope manufacturer.

The 950A FluoroTrack system must be used in conjunction with an ASI 600A Digital Controller. The 600A is a PC-based data acquisition and control system that operates under a real-time Linux operating system. 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. Further information about 600A can be obtained by reading the 600A software manual.

Under normal operation the researcher will also require an ASI model 900B (VSL) or 901B (HVSL) video sarcomere length system. VSL/HVSL consists of a software package that runs on the 600A PC and a camera that captures high speed images of the tissue being tested. The VLS/HVSL camera mounts to the IonOptix C-mount adapter included with the 962A Cell Framing Adapter. The Cell Framing Adapter is, in turn, attached to an unused port on an inverted microscope. The video is streamed to the PC where advanced algorithms are used to accurately calculate sarcomere length (SL) even with poor image quality and relatively small calculation regions of interest. In addition to real-time sarcomere length measurements, the software can stream images to disk and can later calculate SL from these captured image files. Further information about VSL/HVSL can be obtained by reading the 900B/901B software manual.

The 950A FluoroTrack software controls the IonOptix equipment and collects the fluorescence signals from the photomultiplier. Test protocols written using the 600A Protocol Editor control the entire experiment including controlling the stimulator, high speed length controller, apparatus, fluorescence equipment, sarcomere length measurement and all data collection from these various instruments plus data collection from a force transducer. This integration of all equipment used in the experiment and the storage of all collected data into a single, synchronized, time stamped, data file provides a level of integration seldom found with advanced research equipment.

The software includes a Scope window that provides a scrolling data display of selected data which includes: the available fluorescence data (numerator, denominator, ratio, Calcium), Length, Force and sarcomere length (SL) all presented with respect to time. This display can buffer up to 2 hours of data and when the display is frozen the user can then scroll through the data collected to that time. Scope also logs all data to disk which provides a backup of all collected data.

An Analysis window is provided that allows twitch-based analysis to be performed. All of the data streams can be analyzed to provide average twitch data and statistics. Data can be exported from the Analysis window in ASCII text file format.

Because 950A is integrated with 600A and with VSL/HVSL all experiment data is also stored in 600A data files which provides a single data file containing time, force, length, SL, and fluorescence data. Storing all of the data in the 600A data files ensures correct synchronization of the length, force, SL and fluorescence data with respect to time.

2.0 Specifications

Model: **950A FluoroTrack**

Software

Operating System
Type:	Linux - RTAI real-time
Kernel:	2.6.37.6-rt
Distribution:	Slackware 13.37

ASI950A Software
Version:	3.100
RTAD Version:	3.10
Language:	C
Interface:	GUI (Windows-type), drop down menus

Main Window   **Display Real Time Data, Control Experiment**

Menu
File:	New Session, Quit
Edit:	Display Settings, Show Controls
Setup:	Open, Close
Tags:	View, Edit (F1-F9)
Analysis:	Open, Close
Stimulus:	Open, Close
600A:	Protocol, Setup, Main
Help:	Quick Start, ASI950A Help, FluoroDAQ Help, About

Edit
Display Settings:	Opens Display settings window which controls channels plotted on main window
Show Controls:	Toggles the Control pane of the main display on or off

Setup Window   **Set operating modes, Calcium conversion, Background**

Detected Mode:	Shows operating mode of FluoroDAQ Controller
	1 – Dual Excitation Interpolated (Fura)
	2 – Dual-Excitation Interleaved (Fura)
	3 – Single Excitation Dual Emission (Indo)
	4 – Single Excitation Single Emission (Fluo)
Previous Mode:	Shows previous mode of program
Calculate [Ca2+]:	Yes, No
Kd – dissociation constant (nM):	Text entry box
Rmin – ratio at Zero Ca2+:	Calculated quantity from Calculate Factors button
Rmax – ratio at saturated Ca2+:	Calculated quantity from Calculate Factors button
Beta – ratio of Den (0)/Den (Cs):	Calculated quantity from Calculate Factors button
Formula:	$[Ca2+] = Kd * Beta * (R-Rmin) / (Rmax-R)$

Record Background:	Button which records Numerator and Denominator background
Record Zero [Ca2+]:	Button which records Numerator and Denominator at zero [Ca2+]
Record Saturated [Ca2+]:	Button which records Numerator and Denominator at saturated [Ca2+]
Calculate Factors:	Button to calculate all factors

Tags

| View: | Shows the Tags currently set |
| Edit (F1-F9): | Edit the Tag that is automatically created when an F key is pressed |

Analysis Window   **Displays Records and Performs Twitch Analysis**

Menu

File:	Save Data, Load Data, Close
Setup:	Open, Close
View:	Time Series – Single Record
	Transients – Single Record
	Transients – All
	History

View

Time Series – Single Record:	Displays the time history of the selected Record
Transients – Single Record:	Displays the transients of the selected Record
Transients – All:	Displays all transients
History:	Displays experiment history in terms of Records

Setup Window   **Set Twitch Analysis Options**

Trace Display:	Controls channels displayed on Time Series and Transients graphs. Controls relative size of each channel
Twitch Length (%):	Sets amount of data to be used before and after the stimulation pulse during twitch analysis
	Pre-trigger: 0-100%
	Post-trigger: 0-100%
	Baseline: 0-Pre-trigger %
Average Twitch Filters:	Savitsky-Golay smoothing filter controls, 1-16
Backgrnd:	Check box: use background data from the Record specified in the Rec text box, displays the values in N and D text boxes
	No check: use background typed into N and D text boxes
Average:	Controls which averages are calculated and shown on the right side of the screen. Turns dX/dt and Stats displays on/off.
Select Parameters:	Selects the parameters to be included in the Stats section. Parameters are: baseline, peak, peak ht, peak %, peak ht %, max dX/dt, min dX/dt, T dX/dt max, T dX/dt min, T peak, T 10%

rise, T 50% rise, T 90% rise, dT 10-90%, T 90% fall, T 50% fall, T 10% fall, dT 90-10%.

Stimulus Window	**Manual Stimulation Control**
Frequency:	Set the stimulation pulse frequency in units of Hz or beats per minute (bpm)
Pulse Width:	Set the pulse width of the stimulation pulses in ms
Start/Stop:	Starts or Stops the manual stimulation

600A

Protocol:	Opens 600A Protocol window, click on this item when the Protocol window is open will close it
Setup:	Opens 600A Setup window, click on this item when the Setup window is open will close it
Main:	Opens 600A Main window, click on this item when the Main window is open will close it

Help

Quick Start:	Opens a .pdf version of the quick start guide for 950A on-screen
950A Help:	Opens a .pdf version of this manual on-screen
FluoroDAQ Help:	Opens a .pdf version of the FluoroDAQ 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 start guide will help you get your system up and running in as little time as possible. The guide includes both setup and explanation of basic software features.

3.1 Instrument Setup

Follow the hardware setup instructions provided by IonOptix and setup the fluorescence equipment. Likewise follow the setup instructions for other equipment used in the experiment such as the length controller, force transducer, stimulator, 600A Digital Controller and VSL/HVSL sarcomere measurement system.

3.1.1 Attaching the VSL/HVSL Camera to the Cell Framing Adapter

Attach the VSL/HVSL camera to the end of the IonOptix Cell Framing Adapter (CFA) using the following procedure.

1) Loosen the thumbscrew holding the C-mount adapter to the CFA and then remove the C-mount adapter (see Figure 1).
2) Screw the C-mount adapter onto the camera (see Figure 2).
3) Slide the camera through the camera fork (the camera fork is specific to the camera model you have) that is attached to the end of the Cell Framing Adapter and once the C-mount adapter is fully inserted then tighten the thumbscrew (see Figure 3).
Tighten the thumbscrew until you feel resistance and then loosen it one full turn.
This allows the camera to be rotated along with the Cell Framing Adapter.
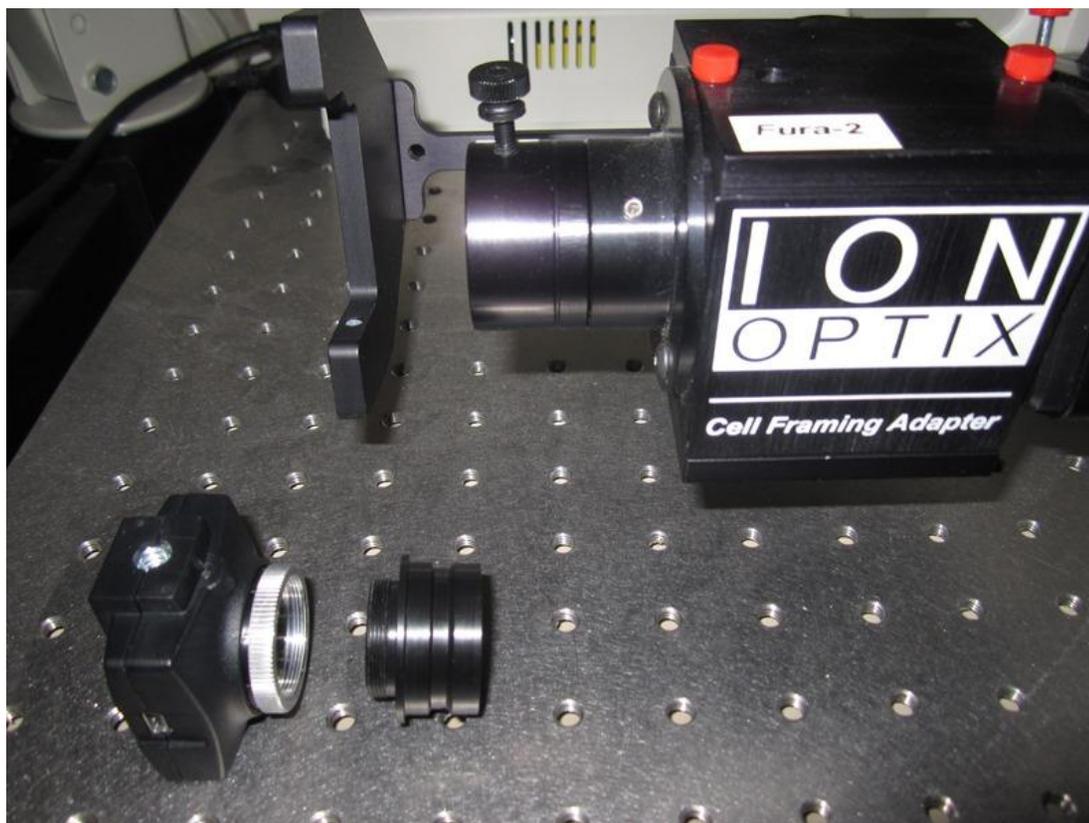
Figure 1 Camera, C-mount and Cell Framing Adapter (note thumbscrew on top)

Figure 2 C-Mount attached to camera

Figure 3 Camera attached to Cell Framing Adapter

3.1.2 Signal Connections

Using the supplied BNC-BNC cables make all of the signal connections between the FluoroDAQ controller and the 604A Signal Interface as follows:

Table 1 FluoroDAQ/604A Signal Interface Connections

Connection	Function	604A Signal Interface (front panel)	FluoroDAQ (back panel)
1	Numerator	Aux1	Path 1 Out
2	Denominator	Aux2	Path Out 2
3	FluoroDAQ Trigger	TrgOut1	Trig In
4	Operating Mode	TrgIn1	Mode 1 Out
5	Operating Mode	TrgIn2	Mode 2 Out

3.1.3 Stimulator Connections

Using the supplied BNC-BNC cables connect the stimulator to the apparatus and to the 604A Signal Interface as follows:

Table 2 Stimulator/604A Signal Interface Connections

Connection	Function	604A Signal Interface (front panel)	701C Stimulator (front panel)	Apparatus
1	Stimulator Trigger	Stimulator	External Trigger	No Connection
2	Stimulator Output	No Connection	Pulses Out	Connect to electrode connector

3.1.4 PC Startup

The 950A FluoroTrack software comes pre-installed on the 600A PC. Attach the keyboard, mouse and monitor (we suggest you attach the monitor using the DVI connector for improved performance) to the PC. The Linux operating system will determine the native resolution of the monitor and automatically set the video resolution to that value. It is therefore important that you turn on the monitor before turning on the PC. If desired you can run two

monitors with the 600A PC, please contact Aurora Scientific Inc. for more information about this option.

3.2 Software Registration and Activation

The FluoroTrack program must be licensed before it will run on your new PC. The following procedure should take about 5 minutes to complete.

You will need:

- PC with ASI software to be activated
- PC with an internet connection (does not have to be the 600A PC)
- 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 4 (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., 017J3. Ensure you enter the Product Key and not the serial number.
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., 7P001-740x0-130x3.
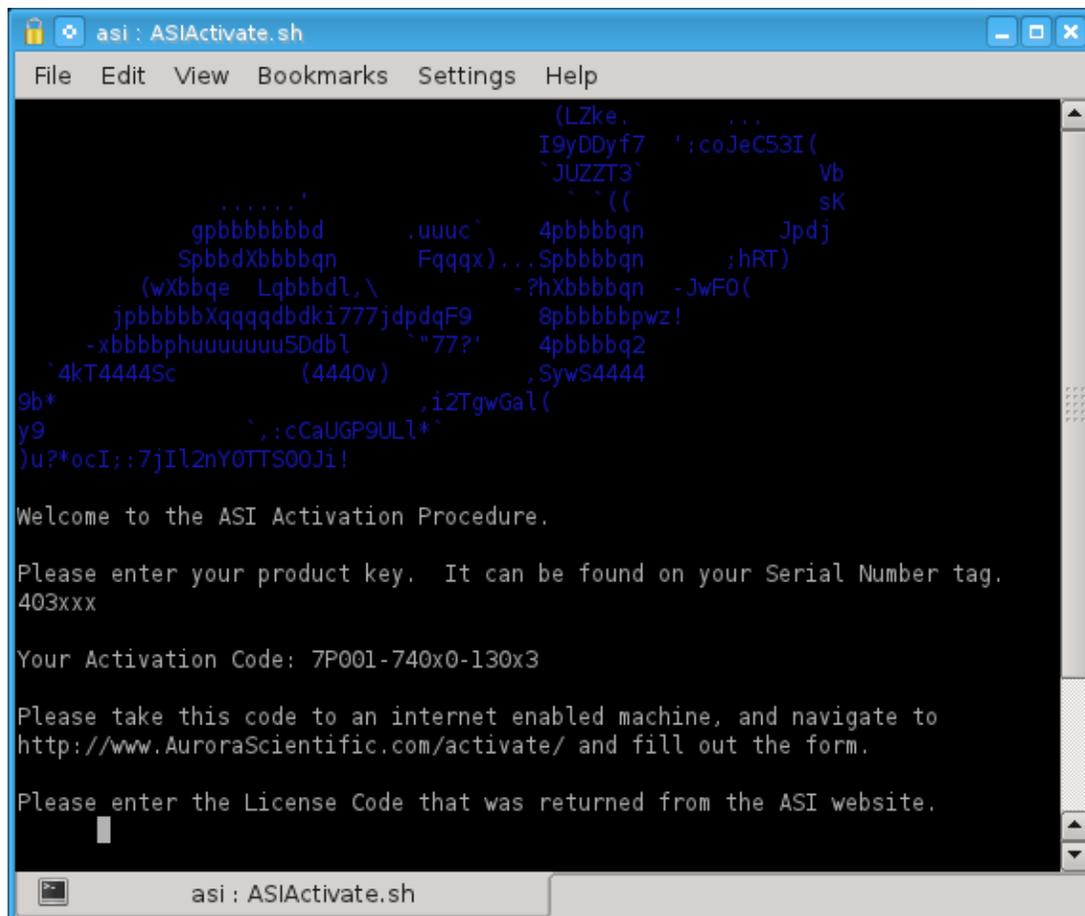
Figure 4 ASI Activate Window

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 5.

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, then enter your new password.)

8. Repeat as required for additional ASI software. For example, if you have a PC with both 600A and 901B software, you must complete this procedure twice.

9. Click on the desktop icon to start 600A and then click on the 950A menu item to start the FluoroTrack program. If the program starts then the license was installed correctly.

Should you encounter any issues with this procedure, please contact ASI by phone or email, and we will be happy to help you with the process.
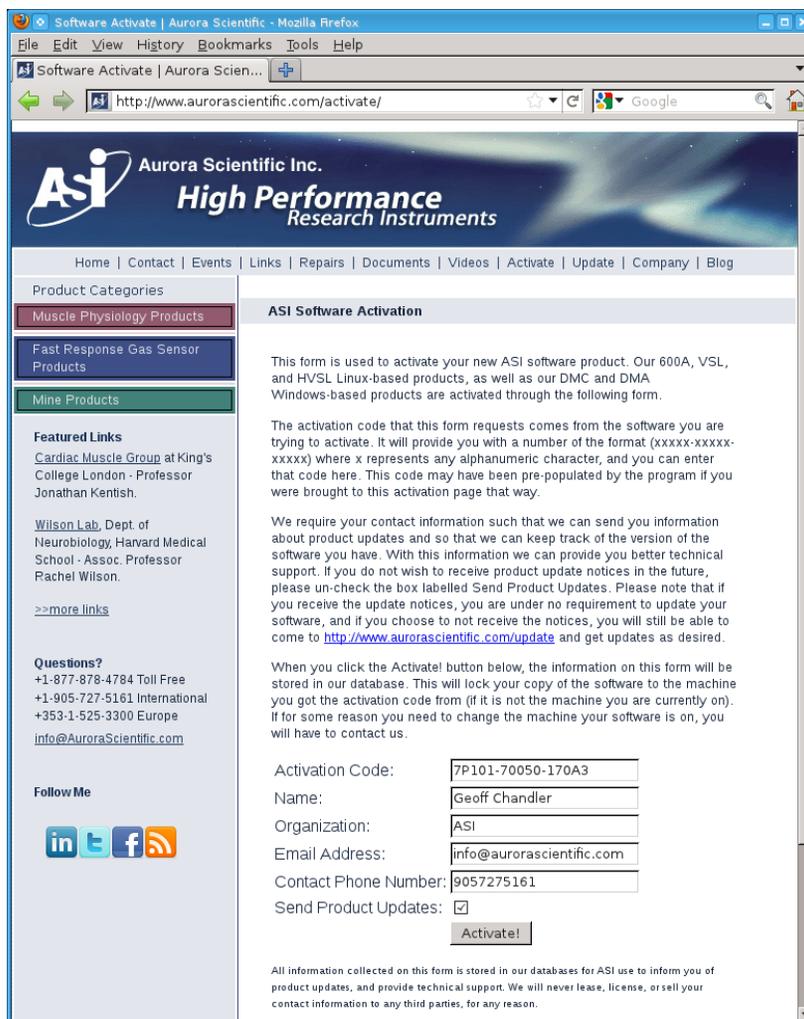
Figure 5 ASI Product Activation Website

3.3 Linux and Desktop Overview

Once all components of the system have been connected together then turn on the monitor and 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 6. The most frequently used icons will be *600A*, *VSL*, *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 and then typing the following in the xterm:

```
        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 600A/950A/HVSL PC to a network. Since the PC was shipped with no login password and the root password is not secure be sure to set strong passwords before connecting the PC to a network. A strong password should not be a recognizable word, it should be a minimum of 6 characters containing letters, numbers and at least one special character (&, ^, $, #, @, !).

3.4 Starting the FluoroTrack Program

In most applications the researcher will be using three software programs during the experiment, these are: 600A Digital Controller, 900B/901B VSL/HVSL and 950A FluoroTrack. Once the PC finishes the login process you will see an icon on the Desktop for **600A** and for **VSL** or **HVSL** (depending on which system you own), see Figure 6. You will not see an icon for 950A FluoroTrack because it is a module installed within the 600A program. Make sure you license 600A, VSL/HVSL and FluoroTrack before continuing (see section 3.2 Software Registration and Activation for details).

Start the 600A program by clicking on the 600A icon. Setup 600A according to the instructions found in the 600A manual. Before starting VSL/HVSL make sure that the camera is powered. The program sends setup information to the camera when the program starts so improper camera operation may occur if the camera is not powered when the program starts. Click on the VSL or HVSL icon to start the program. Setup the VSL/HVSL program according to the instructions found in the VSL/HVSL manual.

FluoroTrack is a module installed within 600A so you will notice on the top menu bar of the 600A program a 950A menu item, click on this menu to start the 950A FluoroTrack program.
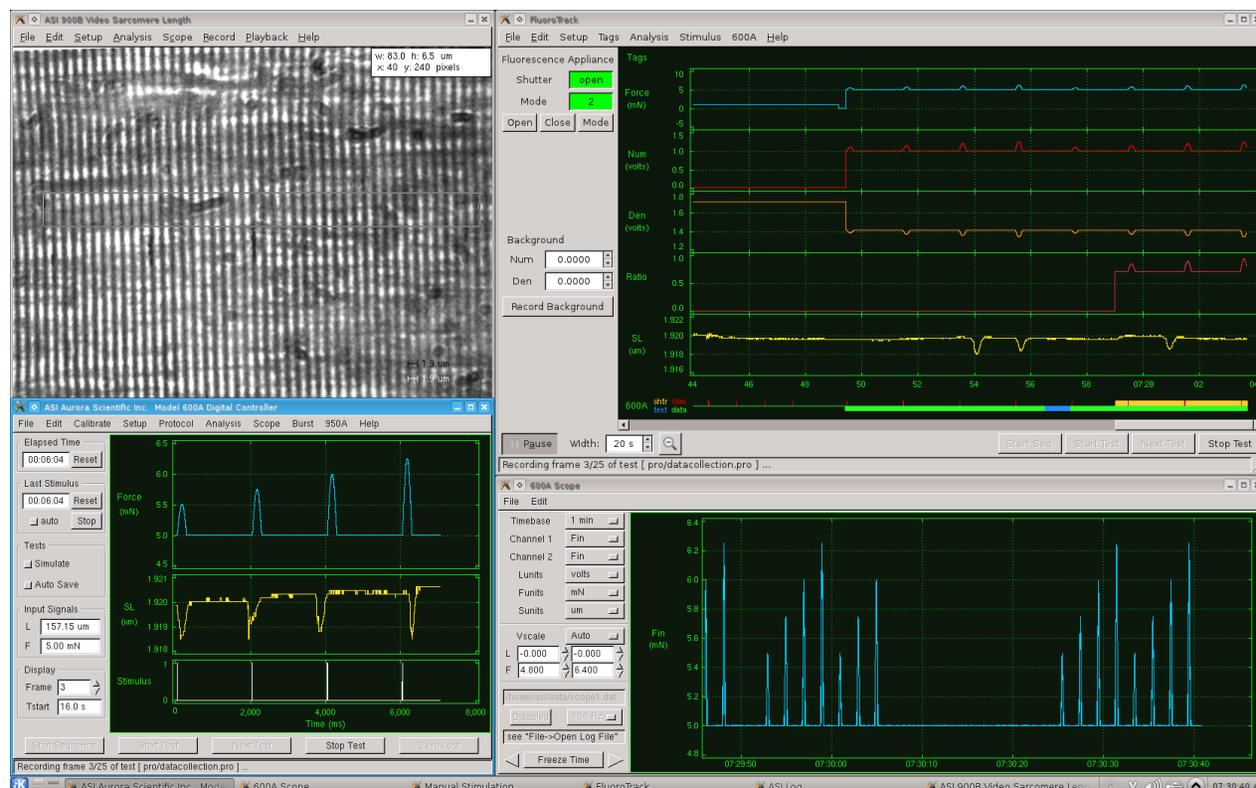
Figure 6 Desktop with 600A, FluoroTrack and VSL Programs Running

3.5 FluoroTrack Program Main Window

When the FluoroTrack program starts the main window will open showing the FluoroTrack scope screen and the controls for the IonOptix FluoroDAQ fluorescence controller (see Figure 7). The main window is separated into four main sections: the top menu bar, the left side control pane, the lower control buttons above a status information line and finally the Scope graph area. The main window can be repositioned, minimized and resized as per normal Windows type controls. When the program is closed it remembers the size and location of the main window and will start next time at the same size and in the same location. Because three programs must be displayed simultaneously you may want to use a dual monitor setup. The 600A PC is capable of driving two monitors; please contact ASI for instructions on setting up Linux for display on two monitors. The Linux desktop and all program windows use normal Windows-type controls for manipulating the display windows.

The menu bar located along the top of the screen includes the following headings (shown in bold) with the drop down menu items listed below.

File	**Edit**	**Setup**	**Tags**	**Analysis**	**Stimulus**	**600A**	**Help**
New Session	Display Settings	Open	View	Open	Open	Protocol	Quick Start
Quit	Show Controls	Close	Edit (F1-F9)	Close	Close	Setup	ASI950A Help
						Main	FluoroDAQ Help
							About

Clicking on any menu item opens a drop-down menu that lists the commands available under the selected heading. A detailed description of each command is provided in Chapter 4.0 Main Program.

Figure 7 FluoroTrack Main Screen

The left side control pane includes controls for the FluoroDAQ fluorescence controller. These include Status and Mode readouts, control buttons to turn the UV light source on or off and to set the operating mode. In the middle on the left is a section for recording and showing the current background settings for the Numerator and Denominator signals. The Record Background button records the current background levels of the Numerator and Denominator inputs. These are automatically stored by the program and the applied to all data displayed after the Record Background button is pressed. If the researcher knows the background signals (measured in volts) then they can also type these known values in the Num and Den text boxes. At the bottom of the left control pane are display controls that allow the time base for the Scope section to be set and a Pause/Run button. Pressing the Pause button allows a researcher to stop the live scope output and then scroll back through the data for up to 4 hours.

The lower control buttons and the status information line provide the main experiment control buttons from the 600A program (you can start a test from 600A or from 950A; the button does the same job regardless of where it is pressed). The control buttons include Start Sequence, Start Test, Next Test and Stop Test. The 600A control buttons are all explained in the 600A

Instruction Manual. At the bottom of the screen is a status line which provides information about what the program is doing at any given time.

The Scope display provides a real-time scrolling display of the data channels along with a Tags bar at the top of the screen and a 600A graphical status indicator at the bottom of the screen. Clicking on Edit>Display Settings will open the Display Settings window. This window is shown in Figure 8 and allows the channels to be selected, the colour of the each channel to be set, the relative size of each channel, the background colour scheme as well as a control button which will set the channel colours the same as they are currently defined in the 600A program.
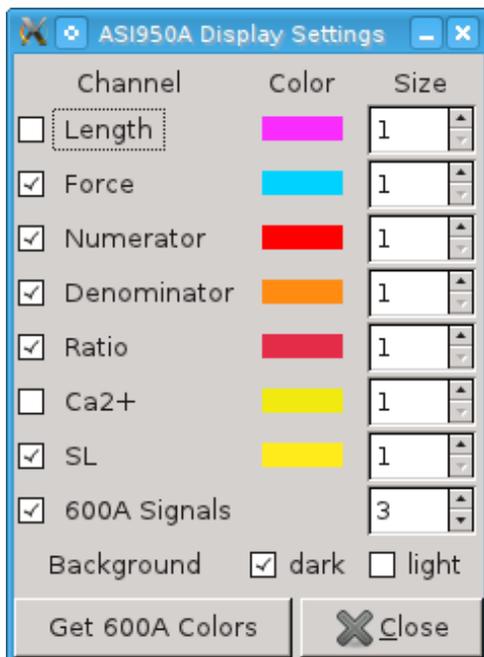
Figure 8 FluoroTrack Display Settings Screen

The Tags bar at the top of the main screen area provides a graphical indication of where the researcher has tagged the data (see Figure 9).

Figure 9 FluoroTrack Main Screen with Tags

There are two methods of inserting a tag into the data, either left click the mouse with the pointer on the Tags bar which creates a tag at that point in time and opens the Tag Editor, as can be seen in Figure 10, or press one of the functions keys from F1 to F9. Pressing a function key creates a tag and automatically populates the text box with Tag information that the researcher has previously assigned to that particular F key. Text can be assigned to function keys by opening the FluoroTrack Tags screen by clicking on Tags>Edit Tags (F1-F9) as is shown in Figure 11.

Figure 10 FluoroTrack Main Screen with Tag Editor Open
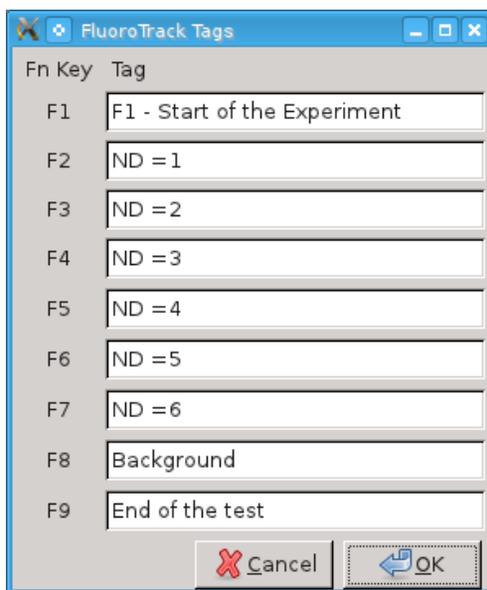
Figure 11 FluoroTrack Tags Window

At any time the researcher can view all tags created since the program was started. This is done by clicking Tags>View from the main menu. The View Tags window opens and shows the tags created along with their creation time. Clicking on any tag line will cause the main screen display to be paused and then the display will jump to the time that the selected tag was created. Clicking on any of the headings will sort the Tags by that heading. See Figure 12 for the View Tags window.

Figure 12 FluoroTrack Tags Window

The 600A/950A graphical status bar located at the bottom of the Scope window provides a simple method of determining exactly what the 600A and 950A programs are doing at any time. Refer to Figure 13 which shows the 600A Signals display. The display is separated into two sections; the first is above the horizontal green line and the second below the line. Above the line you will notice red vertical lines and yellow sections. The red lines show the time at when a stimulation pulse (stim) was produced. The red lines are plotted with a width proportional to the stimulation pulse width. When the time base of the graph doesn't allow the width to be shown then the red lines will be one pixel wide which ensures that the stimulation signals can be seen even for the shortest pulses. The yellow areas show where the UV light source was turned on, i.e., shutter (shtr) open. Ratio and Calcium Fluorescence data is only collected while the shutter is open. Below the line the display can be either green or blue. Blue indicates when a protocol is running on the 600A (test) and green indicates when data collection is occurring (data).
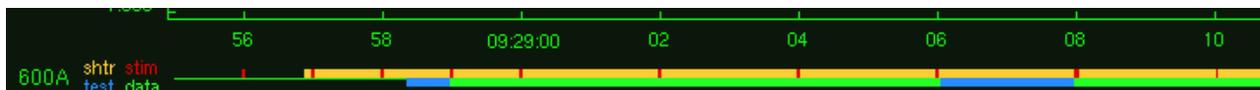
Figure 13 600A Signals Display

3.6 Quick Start Example – Papillary muscle Force-frequency with calcium

This example will describe the steps required to perform force-frequency with calcium measurements in intact papillary muscle fibers. One example of a study similar to this is provided in

Charged residue alterations in the inner-core domain and carboxy-terminus of α-tropomyosin differentially affect mouse cardiac muscle contractility
Robert D Gaffin, Carl W Tong, David C Zawieja, Timothy E Hewett, Raisa Klevitsky, Jeffrey Robbins, Mariappan Muthuchamy, J Physiol. 2004 December 15; 561(Pt 3): 777–791.

The experiment involves mounting a right ventricular papillary muscle between a force transducer and length controller in a temperature controlled bath. The muscle is electrically stimulated and the resulting force and calcium transients are measured. The test is repeated at different stimulation frequencies to obtain the force-frequency relationship with calcium measurement.

3.6.1 Equipment Required

1. 1500A Small Intact Muscle Test System
   Consisting of:
   - 322C High Speed Length Controller
   - 400A Force Transducer
   - 600A Digital Controller
   - 701C High Current Field Stimulator
   - 801C Small Intact Muscle Apparatus
2. VSL/HVSL Sarcomere Length System
3. Fluorescence Photometry System
   Consisting of:
   - 950A FluoroTrack Software
   - 960A FluoroDAQ Data Acquisition Controller
   - 961A HyperSwitch Dual Excitation Light Source
   - 962A Cell Framing Adapter
   - 963A Photomultiplier Subsystem
   - 964-x Microscope Components – x specifies microscope manufacturer
4. Inverted Microscope
5. Vibration Isolation Table
6. Misc. Lab Equipment – glassware, pump, suction, tools, etc.

3.6.2 Method

For the purposes of this method we will assume that all of the instruments are set up correctly, all signal connections are made and the setup of 600A and VSL/HVSL has been done. Details of setup of 600A and VSL/HVSL can be found in their respective instruction manuals.

3.6.3 Experiment/Software Setup

1) It is strongly recommended that you power the instruments on in the following order:
   - Cairn Xenon Power Supply
   - 960A FluoroDAQ - ensure the PMT switch on the front of the FluoroDAQ is OFF and then turn on the FluoroDAQ
   - 961A HyperSwitch
   - 600A PC
   - 322C High Speed Length Controller
   - 400A Force Transducer
   - 701C High Current Field Stimulator

- 825A TEC Controller

2) Log into the 600A PC.

3) Turn on the camera and then start the VSL/HVSL program. Ensure that the program is calibrated correctly for the objective that you will be using to image sarcomere length (normally a 40X objective is required for accurate SL measurement) also calibrate the low magnification setting of the program for either a 10X or 20X objective that is used for observing the tissue, measuring tissue dimensions, etc.).

4) Start the 600A program. Use Edit>Display Settings to choose the channels you would like to view on the 600A main window. You can also setup specific colours for each channel. We recommend that you view Force In, SL and Stim on the 600A main screen.

5) Open the 600A Scope screen and choose the channels to observe in real-time. We recommend that you view Force In. Scope provides a scrolling display of the selected data channels and is an excellent tool for observing the long term trends in data. For example it is very easy to use Scope to see force changes with time that are related to stimulation frequency or other control variables.

6) On the 600A menu bar click on the 950A heading which will open the FluoroTrack program. Click on Edit>Display Settings and choose the channels you would like to observe on the 950A main screen. Please be aware that you can view all of the 600A channels through 950A as well as the fluorescence channels and sarcomere length. Since 950A also includes many of the experiment control buttons you could choose to minimize the 600A main screen and just run the experiment using the 950A main screen. For now we will assume that both main screens from 600A and 950A will be on the Desktop and therefore we recommend that you view Force In, Numerator, Denominator, Ratio and SL on the main screen of 950A. You will note that you can automatically set the channel colours of the 950A display to be the same as 600A. This is recommended as you will then be able to easily recognize a channel on either main window simply by its colour.

7) Open the Manual Stimulation controls by clicking on Stimulus>Open. The control window will be as is shown in Figure 14.

8) Set the Frequency to 60 bpm (beats per minute) or to 1.0 Hz (the program automatically converts between the two units, so use the units of most convenience). Set the Pulse Width to 6.0 ms (or whatever you determine is best for your experimental setup).
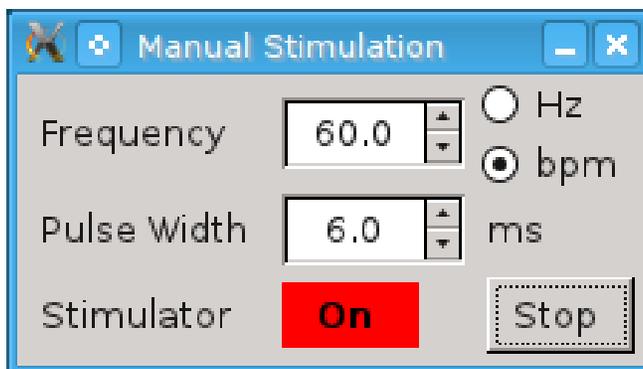
Figure 14 Manual Stimulation Window

3.6.4 Tissue Setup

1) Start superfusion through the 801C bath and then mount the papillary muscle in the 801C bath. The valve and the ventricular regions should be attached to the force transducer and the lever system. Refer to the 801C instruction manual for information about mounting methods.

2) With the tissue slack, record the zero level force (Record Fin) using the 600A Setup window. This sets the zero level for the force transducer. You can open the 600A Setup window either from 600A or from 950A. From 950A you would click 600A>Setup on the main menu.

3) Start stimulation pulses to pace the tissue by clicking on the Start button on the Manual Stimulation window.

4) Adjust the bundle length until you observe force twitches on the 600A Scope screen.

5) Optimize the bundle length and stimulation current or voltage (depending on the operating mode of the 701C Stimulator) to obtain maximum force twitches.

6) With the optimum length set use VSL/HVSL on Low Magnification to measure the tissue length and cross section dimensions. Record the reference length in the 600A Setup window and also press the Record Lin button to record the length datum.

3.6.5 Fluorescence Photometry Equipment Setup

1) Observe the tissue using VSL/HVSL and set the microscope to a minimum of 40X objective in order to see the sarcomere pattern. In some cases sarcomeres will not be visible and if this is the case then no SL data can be collected. If sarcomeres are visible then use the MyoHandle on the Cell Framing Adapter (CFA) to rotate the camera so that the sarcomeres are vertical as shown on the VSL/HVSL screen.

2) Use the horizontal and vertical aperture adjustments on the CFA to set a region over which SL and Calcium measurements will be taken. You should ensure that the aperture is set so that there is only tissue within the aperture.

3) On the 950A menu bar open the Setup window (Figure 15).   Use the Selected Mode drop down control to set the operating mode of the program the same as what you have set on the FluoroDAQ controller. In most cases you will use the Dual Excitation Interleaved (FURA) mode.

4) Turn off the room lights and then turn on the PMT (switch located on the front panel of the FluoroDAQ instrument – see FluoroDAQ instruction manual).

5) Open the shutter by clicking on the Shutter Open button located on the main window of the FluoroTrack program.

6) You should now be observing Numerator, Denominator and Ratio signals on the main window of FluoroTrack. At this point FURA-2 has not been loaded into the tissue so the Numerator and Denominator signals should not change as the tissue contracts. If a change in Numerator and/or Denominator is seen during contractions then adjust the size and location of the aperture until these signals stay constant during a contraction. Any changes seen at this point will most likely be due to the tissue moving out of the aperture and causing a change in background.
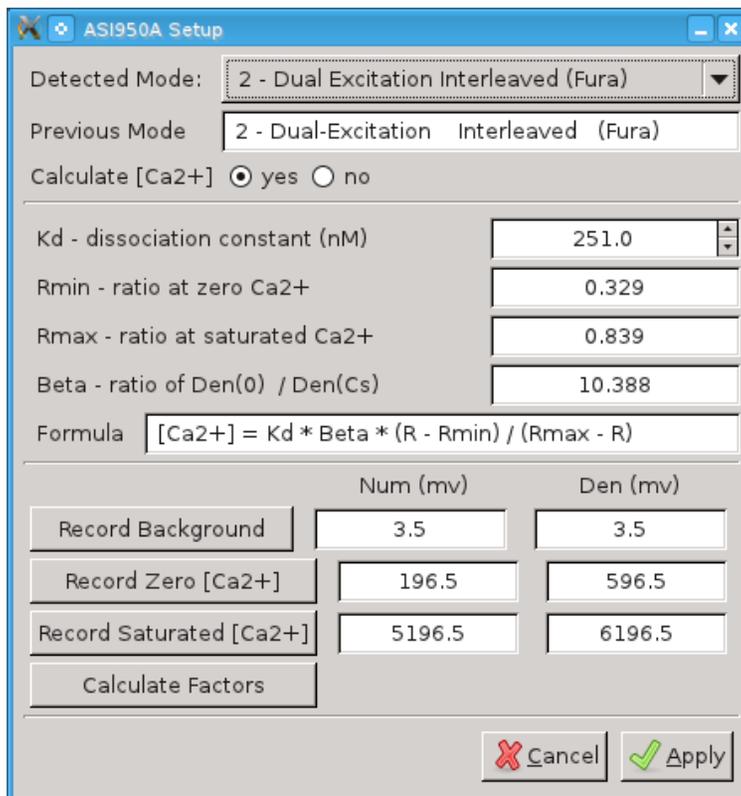
Figure 15 Setup Window

7) If the Numerator or Denominator signal is too large you may need to change the Filter Flipper on the HyperSwitch to switch in a neutral density (ND) filter which will reduce the amount of light being seen by the PMT. You may want to record fluorescence data for each of the ND filter settings so that you can see the effect of the full range of the available filters. Note: Flipper position 1 corresponds to no ND filter and the filter increases with increasing Flipper number. Flipper position 6 corresponds to ND 1.3 which filters 95% of the light (5% transmission).

8) Once you have selected the appropriate ND filter then record the background fluorescence signal by clicking on Record Background on the main window. Note: background must be collected when there is no FURA loaded in the tissue.

9) Close the shutter by clicking Close on the FluoroTrack main window. This closes the HyperSwitch shutter and stops the calculation of Ratio.

10) Shut off the PMT. It is a best practice to only power on the PMT when you are taking measurements. Leaving it off at all other times will increase lifetime and prevent possible damage caused by bright light.

3.6.6 FURA-2 Loading

1) Using the method described in the referenced paper load the tissue with FURA-2 dye. This procedure often takes 1 to 1.5 hours. Many researchers decrease the stimulation frequency during loading. This can be easily done by using the Frequency control on the Manual Stimulation window.

3.6.7 Controlling the Collection of Data

There are two methods of controlling the fluorescence photometry equipment and taking Calcium measurements. 1) manual method which can only be used if you do not need any of the 600A protocol control functions, 2) 600A control method which must be used if you want to use 600A protocol control functions or if you want to automate the experiment. 600A protocol control functions allow the researcher to control the length, force or SL of the tissue during an experiment. They also include functions for controlling the stimulator, for changing baths on an 802D apparatus and for controlling the 950A and the VSL/HVSL programs. 600A protocol functions also allow you to automate much of the experiment.

In this example experiment we want to collect Force, Numerator, Denominator and SL data at several different stimulation frequencies. The experiment will involve setting a desired stimulation frequency, waiting for the force to stabilize, collecting data from 40 twitches and then repeating these steps several times for each of the desired frequencies. The noisy nature of fluorescence signals can be greatly improved by collecting many twitches and averaging them to produce an average twitch with much lower noise. For this example we will collect data at 1, 1.5, 2, 2.5 and 3 Hz. We will not be controlling tissue length nor do we need to control the VSL/HVSL program. Therefore either of the two data collection methods could be used.

3.6.7.1 Manual Method of Collecting Data

The 950A automatically logs data to a file whenever the program is open. This file can hold a maximum of 4 hours of data and if the program is open for more than 4 hours a new data file will be automatically created every 4 hours. Alternatively the user can initiate a new data file at any time by clicking File>New Session on the FluoroTrack main menu. However, ratio data is only collected whenever the shutter is open. The manual method involves opening and closing the shutter whenever you want to collect data. Each Open/Close will generate a "record" in the data file. Records are the method used to separate different recordings. The manual method uses the following procedure.

1) Use the Manual Stimulation control and set the stimulation frequency to 1 Hz.

2) Monitor the Force In signal using the Scope function of 600A. Set the time base for Scope long enough that you can see 2 or more minutes on the screen at a time. Whenever you change the stimulation frequency you will typically see a change in the peak force. This change often occurs over a 1 to 2 minute time period. The procedure involves waiting for the peak force to stabilize and then going on to the next step.

3) Ensure the room lights are off and then switch on the PMT using the switch on the front panel of the FluoroDAQ controller.

4) Press the Open button on the 950A main window which will open the HyperSwitch shutter and start the collection of data.

5) Count the number of twitches and when you reach 40 press the Close shutter button on the 950A main window. Alternatively you can simply time how long the shutter is open, at 1 Hz you will require 40 seconds, at 1.5 Hz you will require 27 seconds, at 2 Hz 20 seconds and so forth.

6) Repeat steps (1) through (5) for each of the stimulation frequencies in your experiment. For this example you will repeat the steps at frequencies of 1.5, 2, 2.5 and 3 Hz.

7) When the experiment is complete set the stimulation frequency back to 1 Hz.

8) Proceed to section 3.6.8 for measuring the fluorescence background after the test.

3.6.7.2 600A Protocol Method of Collecting Data

1) During the FURA-2 loading you will have plenty of time to load the experimental protocols required during the experiment. These protocols are included in Appendix B at the end of this manual. You will also find the protocol files in the /pro directory on the 600A PC.

2) To use 600A to control the experiment and collect data you will require a series of protocols that set the different stimulation frequencies required and control the HyperSwitch and collect data. These protocols are put together in a sequence file which controls the execution of each individual protocol.

3) Click on Protocol>Open on the 600A main menu or on 600A>Protocol on the 950A main menu to open the 600A Protocol window.

4) Click on the Sequencer Tab at the top of the 600A Protocol window and then click on Load and pick the sequence called: Force_Freq_950A.seq. The sequence is shown below as Figure 16.

5) Examination of the sequence file shows that it consists of a series of protocols that first set the stimulation parameters and then collect 40 twitches of data. At the end of the sequence the stimulation frequency is set back to 1 Hz. Manual timing is used after each of the stimulation setup protocols. Once the stimulation protocol executes the Next Test button found at the bottom of the 950A main window will un-grey. When the researcher wants to start data collection they simply have to press the Next Test button and the next protocol will execute. This allows the researcher to monitor peak force and choose when to start data collection. After each of the data collection protocols the sequence timing is automatic which means that the next stimulation frequency will be automatically set as soon as the current data collection finishes. The time can easily be changed to a fixed wait time between setting a new frequency and collecting the twitches if the researcher wanted to configure the test that way.
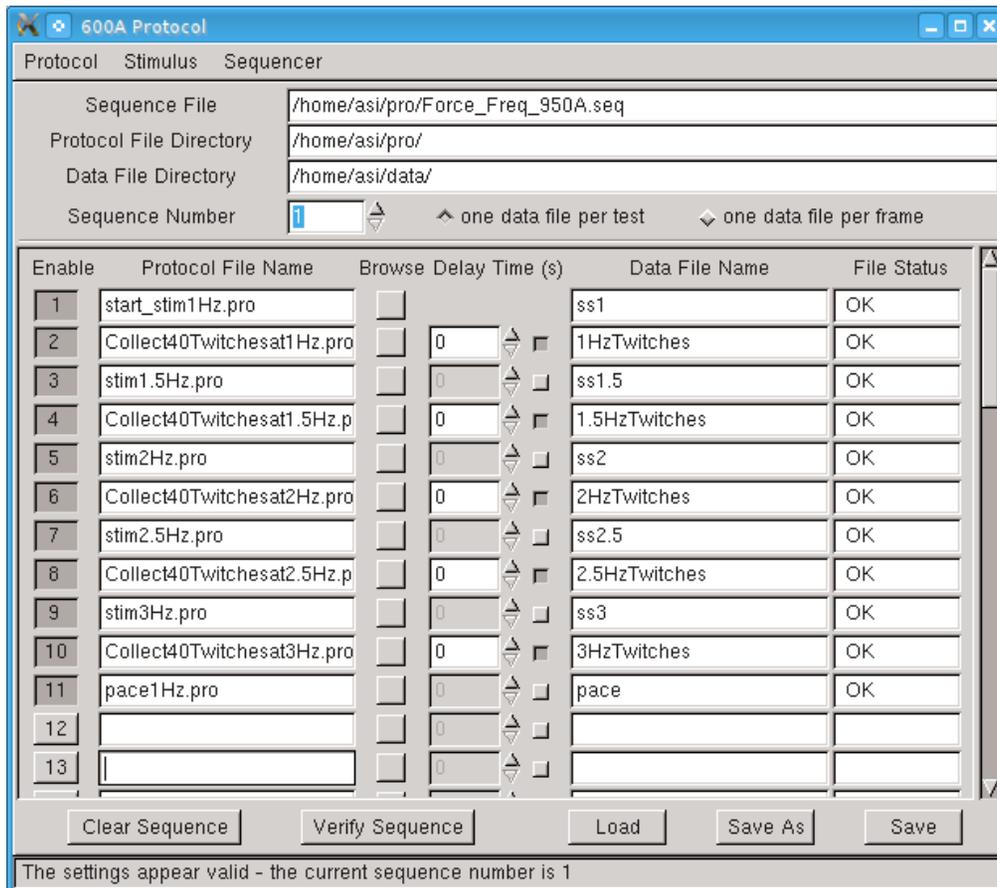
Figure 16 600A Sequencer Window

6) Each protocol listed in the sequence file is printed in Appendix B and is provided in the /pro directory on the 600A PC. A typical stimulation protocol is shown in Figure 17 and the Stimulation Parameters are shown in Figure 18. A new 600A control function has been created called Sync-Stimulus. This function must be the first function called by the protocol and it causes the start of the protocol to coincide exactly with the next stimulus pulse produced. Sync-Stimulus can be used with any protocol when stimulation is already occurring. The function will also synchronize a new stimulation profile with one that is already occurring; this avoids the problem of generating stimulation pulses that are out of sync which can disturb the pacing of heart tissue. As can be seen in Figure 18, all of the stimulus profiles required by the experiment have been programmed; to change the stimulation frequency it is then a simple matter of calling a different Stimulus profile number.
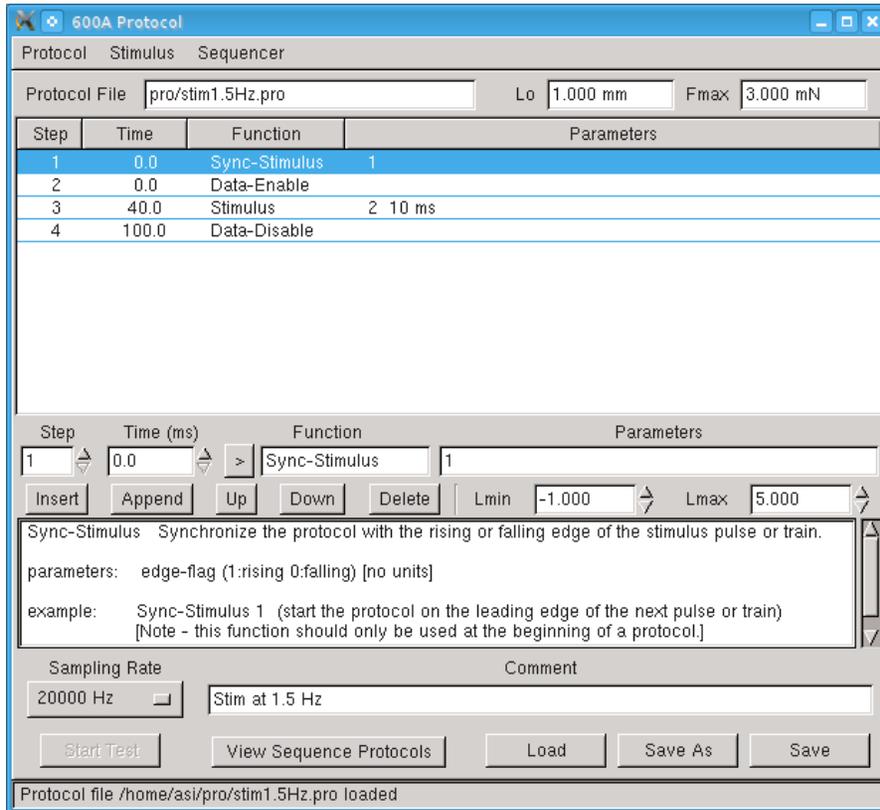
Figure 17 600A Stimulation Protocol
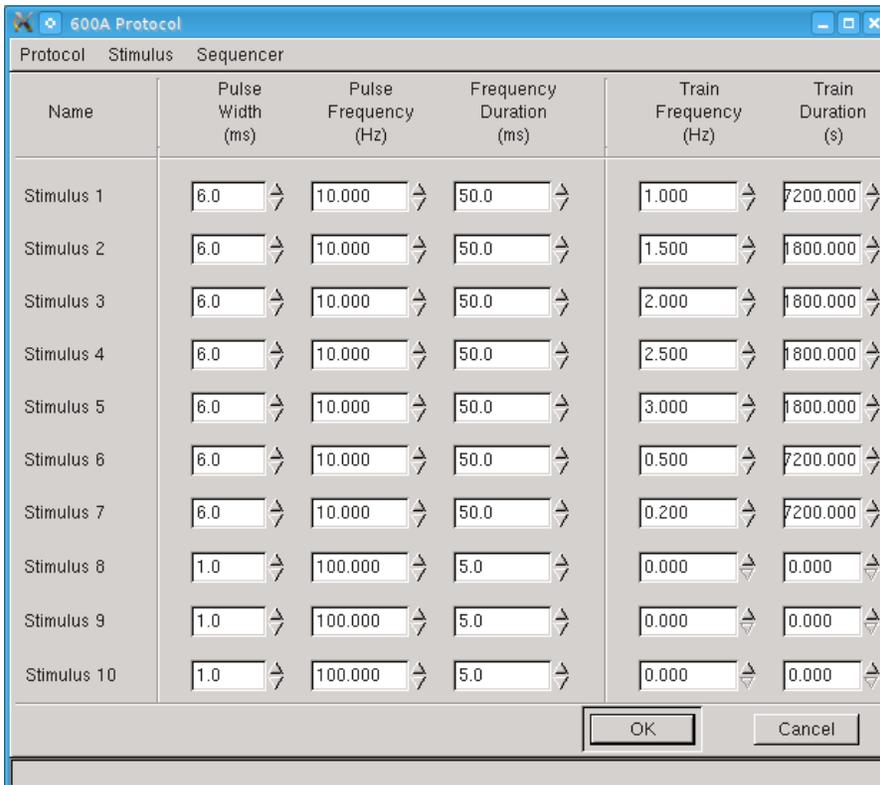
Figure 18 600A Stimulus Window

7) The test protocol that collects the data and controls the 950A is shown in Figure 19. This protocol starts with a Sync-Stimulus command and contains a new function, FLA-Trigger, which controls the 950A fluorescence system. The FLA-Trigger command opens and closes the HyperSwitch shutter based on the parameters used in the command. The FLA-Trigger function uses two parameters, the first controls the shutter and the second sets a delay time. A 0 in the shutter parameter closes the shutter while a 1 opens the shutter. The delay time operates like other delays for 600A functions and allows the user to open/close the shutter at a time after the time when the function is issued. This allows synchronization of the shutter control with other functions such as length, force or SL controls.

8) Once the sequence has been loaded it is a simple matter to start the execution of the sequence by click the Start Sequence button on the 950A main window. Each of the protocols will execute with the start of all of the collection protocols requiring the user to click the Next Test button when they want to collect fluorescence data. Note: the FluoroDAQ does not control the PMT power so you must remember to turn on the PMT before starting the sequence. Since the total execution time of the sequence is only 10-15 minutes it is reasonable to leave the PMT on for the entire sequence. However, if desired, you could turn on the PMT prior to each click of the Next Test button and then turn it off as soon as you see the frequency change to the next value.

9) During execution of the sequence protocols data will automatically be collected and placed in the files that you specified on the Sequencer window. These are 600A data files which also contain all of the 600A data and SL. Note however that data will also have been collected by the 950A program and will be available in the log file for that session.

10) After the sequence completes proceed to section 3.6.8 for instructions on measuring the background after the test completes.
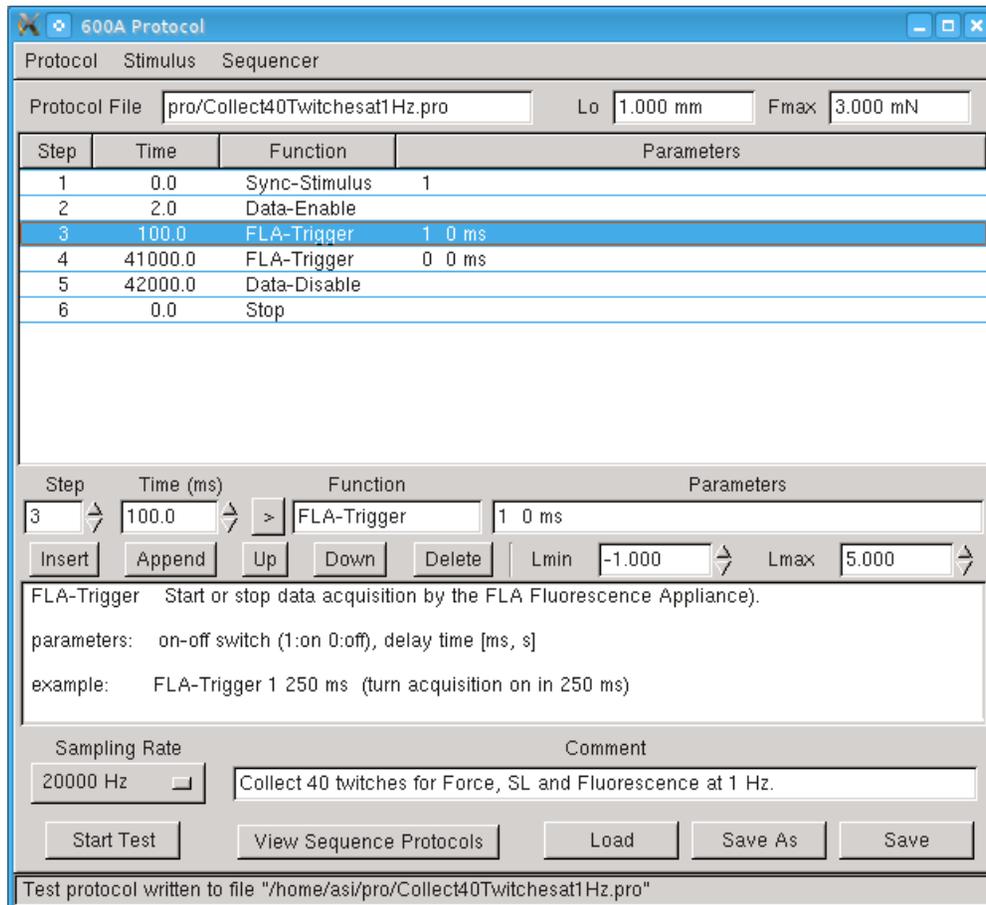
Figure 19 600A Twitch Data Collection Protocol

3.6.8 Measuring Fluorescence Background after the Test

1) After the test is complete many researchers use a solution of manganese (Mn^{2+}) to quench the FURA-2 present in the tissue. Turn on the PMT and then open the shutter to monitor the Numerator and Denominator signals during the quenching process. This allows you to determine the point at which the FURA-2 is completely quenched.

2) Once this level is reached the fluorescence background is measured by clicking on the Record Background button. Usually this final background is the one used during analysis instead of the background obtained before adding FURA-2.

3.6.9 Analysis

FluoroTrack includes powerful twitch analysis functionality. Each time the shutter is opened a "record" is created. Records are closed when the shutter is closed. All closed records can be viewed and analyzed either during or after the experiment. Use the following procedure to perform twitch analysis.

1) Click on Analysis>Open on the main 950A menu bar. This will open the Twitch Analysis window and display the View Records screen as is shown in Figure 20.

2) The Analysis window includes a top menu bar which provides the following headings (shown in bold) and the drop down items shown below.

File	**Setup**	**View**
Save Data	Open	Time Series – Single Record
Load Data	Close	Transients – Single Record
Close		Transients – All
		History
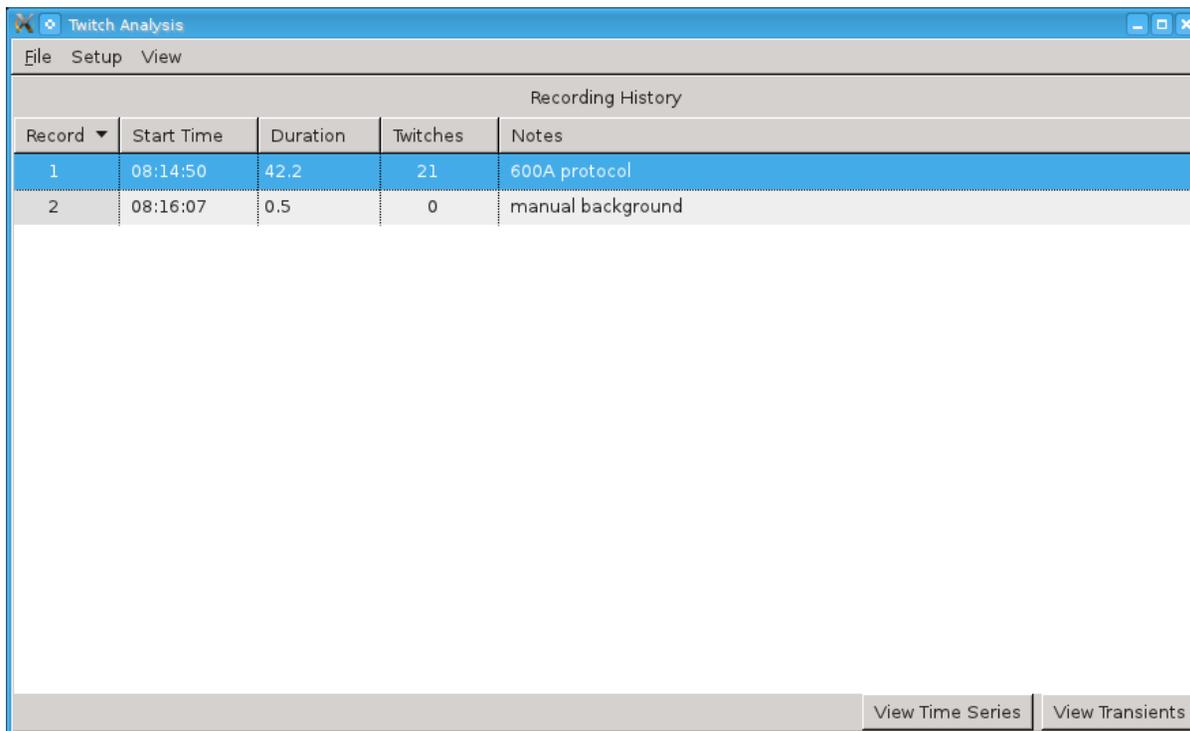
Figure 20 Analysis - Recording History Window

3) Assuming that you have just complete the collection of data then all of the records associated with your experiment should be shown on the View Records screen. Click on the record of interest and then click on either the View Time Series or View Transients button located in the bottom right corner of the window.

4) The View Time Series graph, see Figure 21, will show the signals obtained versus time and this will be the same as you saw on the main 950A screen. You can zoom the data record by left clicking the mouse at a point of interest and then, while holding the button, drag out a box to enclose the data you are interested in viewing. The screen will automatically zoom to the region you have selected. When the data has been zoomed a scroll bar will appear below the graph which allows you to scroll through the data using the currently defined time base. Pressing the magnifying glass symbol will un-zoom the graph. Also note that Tags are shown on the Time Series at the time when they were created.
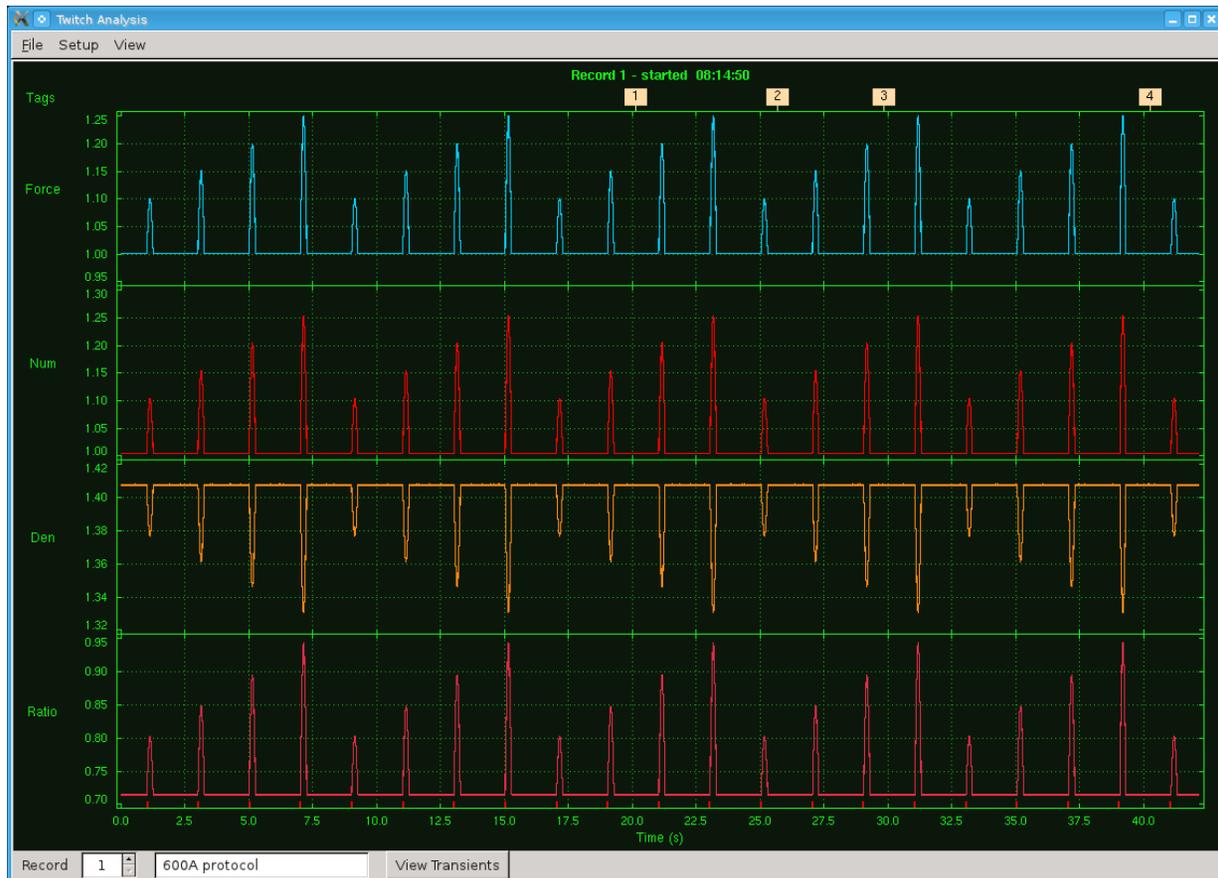
Figure 21 Analysis – View Time Series window

5) From either the Recording History window or from the Time Series window you can
click on the View Transients button and a graph showing each transient (twitch) will be
shown. In this case the x axis is twitch number as is shown in Figure 22. The View
Transients display is where the analysis takes place and where the results of the analysis
are shown. The window is split into two sections; the left side shows each twitch plotted
against twitch number, the right side shows the average of the twitches that are shown on
the left side of the window.

6) The relative size of the two sections can be changed by clicking and holding the left
mouse button on the vertical bar between the two sections and then dragging the bar back
and forth.

7) The twitch analysis process involves summing all of the displayed twitches and
calculating an average twitch. This averaging process greatly reduces the noise on the
signal. Noise reduces as you increase the number of twitches included in the average.
Experience shows that an average of about 40 twitches provides a good result, however
the particular circumstances of your experiment will determine the best number to use. It
is important to understand that the average Ratio value is computed from the average
Numerator and average Denominator values and not from averaging ratios calculated
from each individual twitch. Any noise on the Denominator signal tends to create much
larger noise levels on the Ratio signal, therefore it is important to calculate Ratio from a
quiet signal and thus average Ratio = average Numerator / average Denominator.
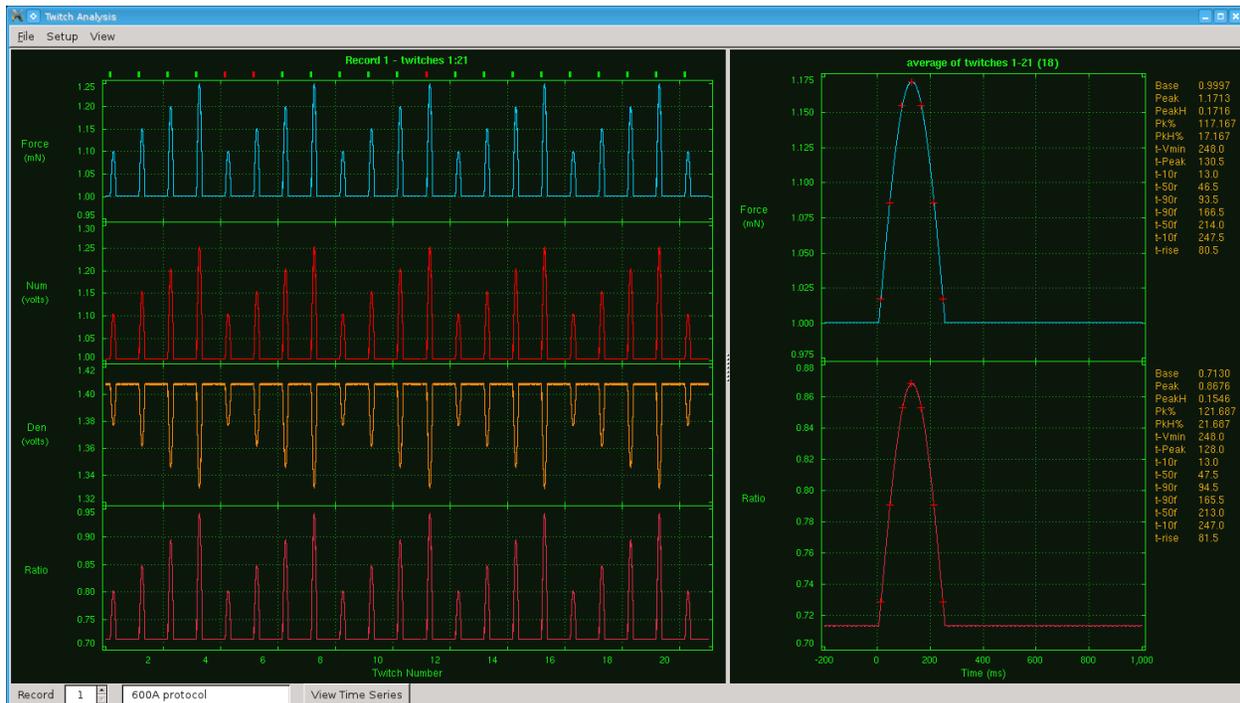
Figure 22 Analysis - View Transients window

8) The Twitch Analysis Setup menu item opens the Setup window which is used to control the Twitch Analysis process. See Figure 23 for details of the Setup window.

9) The Setup window is separated into 6 sections: Trace Display, Twitch Length (%), Average Twitch Filters, Backgrnd, Average, and Select Parameters.

10) The Trace Display section provides controls to determine which data channels (signals) are displayed on both the View Time Series and View Transients windows. Controls are also provided to set the relative size of each data channel.

11) The Twitch Length (%) section provides controls that set the amount of data to be used for each twitch during the analysis. The pre-trigger value sets the amount of data before the stimulation pulse. This value is set as a percentage of the total time between stimulation pulses. The post-trigger value sets the amount of data after the stimulation pulse. This value is also set as a percentage of the total time between stimulation pulses. The baseline value controls how much of the data before the stimulation pulse should be used for calculating the baseline value for the twitch. This is also set as a percentage of the total time but is internally limited to a maximum equal to the pre-trigger percent.

12) The Average Twitch Filters section allows the user to filter the raw signals and the derivatives of these signals using a Savitsky-Golay smoothing filter. The order of the filter is set in the text boxes next to each channel label.

13) The Backgrnd section allows you to manually or automatically set the background for the Numerator and Denominator signals. If these values are known then you can simply type the baseline values in the N and D text boxes. When you click on Apply then all data is recalculated using these background values. An alternative method for setting the background is to choose a background record out of the available records. If, for example, you took background as your second record (as is shown in Figure 20) you can click the checkbox beside the Backgrnd label and then choose the appropriate record number using the spin button next to the Rec label. The N and D values for that record

will automatically be placed in the N and D text boxes and all data will be recalculated using these values.

14) The Average section allows the user to specify which channels are averaged and shown on the right side of the window. The four available channels are listed along with a dX/dt button and a Stats button. Selecting the dX/dt button will display the derivative of all of the selected channels. Selecting the Stats button will print the selected statistics to the right of each average graph (as is shown in Figure 22)

15) The final section is the Select Parameters section which allows the user to specify which statistics are to be calculated and displayed.
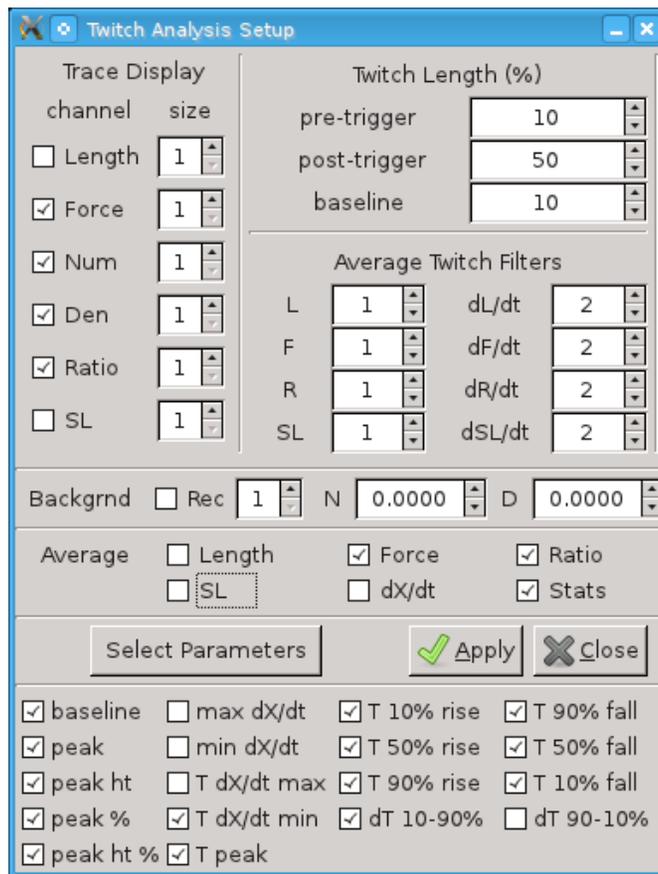
Figure 23 Analysis - Setup window

16) Click on the Export button shown at the bottom of the Time Series and Transients windows to export the data to a file. The export function will export the data as is shown in the current display. If you are on View Time Series and you have zoomed in on a section of data and chosen, for example Force, Numerator, Denominator and Ratio then when you export the data you will get a data file with the time series including only these channels for the time period currently being shown. The Export button on the Time Series window only exports the time series data. In order to export transient data you must click the Export button located at the bottom of the Transients window. When you click Export from the Transients window the program will create three files: the first which will be the twitches currently displayed for the channels selected, the second will be the average signals for the channels selected and the third file will be the statistics for the channels and parameters currently selected. You can import these exported files into

Excel or most other programs. The data file format is ASCII delimited data and typical files are shown in Appendix A.

3.6.10 Program Shutdown

The 950A program should normally be closed using File>Quit and then the 600A and VSL/HVSL programs can be closed in the same manner.

In order to shut down the PC you must first log out and then tell Linux to shut down the PC. Never simply power down the PC since this can result in file corruption. To log out click on the *K* icon at the left end of the task bar at the bottom of the desktop (this is analogous to the Start button for Windows) and select Shutdown. A dialog box will appear where you can select Shut down. The PC will be powered off automatically when Linux finishes its shut down procedure.

4.0 Main Program

To be completed.

5.0 Twitch Analysis

To be completed.

6.0 Software Updates

This chapter provides a brief description of the procedure required to update to a newer version of the 950A program. Please be aware that 950A is a subset of 600A so there will often be companion upgrades of 600A and 950A.

Aurora Scientific Inc. is striving to make 950A the best program for real time Calcium measurement. We are pleased to receive user comments about the program including suggestions for new functions.

New versions of 950A FluoroTrack are available from our update website, via email or by courier.

http://www.AuroraScientific.com/update

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/950A programs running. Close the programs 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 and then click on the desired update to download it.
4. Once the file is downloaded close all open programs.
5. Copy the update file to the */home/asi* directory.
6. Click on the **installer** icon on the desktop.
7. Browse and select the upgrade 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. The root password was set to "root" when the system was shipped. Please note that when entering the password nothing is shown on the screen. Therefore carefully type in the password and then press Enter.
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.

7.0 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.

7.1 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

7.2 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.

7.3 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 Canada if you purchased the instrument from Aurora Scientific or USA 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 – 950A File Structure

Data File Structure for Time Series Files Created by Analysis>Export

```
# FluoroTrack Time Series File
# Created: Fri May 31 06:46:27 2013
#
# Record 3
# Start Time: Fri May 31 06:45:40 2013
# Duration:    32.80 seconds
# Transients: 17
# Comments:   600A protocol
# Controller Mode: 2
# Sampling Rate: 2000 Hz.
# Number of Scans: 65669
# Background Num : 0.000000
# Background Den : 0.000000
#
# Output Time Range (s): 0.000000 32.834500
#
# Time        Force       Num         Den         Ratio
# (s)         (mN)        (volts)     (volts)
0.00000    4.998780   1.003327   1.378235   0.727979
0.00050    4.998475   1.003296   1.378052   0.728054
0.00100    4.998780   1.003296   1.378174   0.727989
0.00150    4.998169   1.003327   1.378296   0.727947
0.00200    4.998627   1.003266   1.378082   0.728016
0.00250    4.998627   1.003449   1.378266   0.728052
0.00300    4.998475   1.003418   1.378143   0.728094
0.00350    4.998627   1.003418   1.378418   0.727949
0.00400    4.999085   1.003388   1.378204   0.728040
0.00450    4.998780   1.003418   1.378204   0.728062
0.00500    4.998627   1.003388   1.378327   0.727975
0.00550    4.998322   1.003388   1.378174   0.728056
0.00600    4.998322   1.003296   1.378113   0.728022
.
.
.
```

Data File Structure for Transient Series Files Created by Analysis>Export

```
# FluoroTrack Transient Series File
# Created: Fri May 31 06:46:47 2013
#
# Record 3
# Start Time: Fri May 31 06:45:40 2013
# Record Length: 32.80 seconds
# Transients: 12 - 28
# Transient Length: 2000 ms
# Pre-Trigger: 500 ms
# Post-Trigger: 1500 ms
# Baseline: -200 - 0 ms
# Comments:   600A protocol
# Controller Mode: 2
# Sampling Rate: 2000 Hz.
# Background Num : 0.000000
# Background Den : 0.000000
#
# Time        Force       Num         Den         Ratio
# (s)         (mN)        (volts)     (volts)
#
# Transient Number 12
# Stimulus at t = 0.7091 seconds
```

```
#
-0.50000      4.998627      1.003418      1.378113      0.728110
-0.49950      4.998475      1.003357      1.378174      0.728034
-0.49900      4.998780      1.003357      1.378174      0.728034
-0.49850      4.998322      1.003388      1.378113      0.728088
-0.49800      4.998475      1.003266      1.378174      0.727967
-0.49750      4.998169      1.003357      1.378113      0.728066
-0.49700      4.998475      1.003327      1.378021      0.728092
.
.
.
-0.00450      4.998322      1.003296      1.378296      0.727925
-0.00400      4.998169      1.003204      1.378296      0.727858
-0.00350      4.998169      1.003204      1.378174      0.727923
-0.00300      4.998627      1.003266      1.378327      0.727887
-0.00250      4.998017      1.003235      1.378143      0.727961
-0.00200      4.998322      1.003204      1.378204      0.727907
-0.00150      4.998017      1.003357      1.378143      0.728050
-0.00100      4.997712      1.003357      1.378143      0.728050
-0.00050      4.997712      1.003296      1.378052      0.728054
0.00000       4.997712      1.003266      1.378204      0.727951
0.00050       4.998475      1.003266      1.378113      0.728000
0.00100       4.998017      1.003327      1.378082      0.728060
0.00150       4.998627      1.003266      1.378266      0.727919
0.00200       4.998017      1.003388      1.378082      0.728104
0.00250       4.998627      1.003266      1.377960      0.728080
0.00300       4.998627      1.003327      1.378143      0.728028
0.00350       4.998322      1.003327      1.378021      0.728092
0.00400       4.998475      1.003327      1.378143      0.728028
0.00450       4.998169      1.003357      1.378082      0.728082
.
.
.
1.49700       4.998475      1.003357      1.378388      0.727921
1.49750       4.998322      1.003327      1.378174      0.728012
1.49800       4.998017      1.003327      1.378113      0.728044
1.49850       4.998169      1.003327      1.378113      0.728044
1.49900       4.998627      1.003266      1.378143      0.727983
1.49950       4.998322      1.003357      1.378021      0.728114
#
# Transient Number 13
# Stimulus at t = 2.7090 seconds
#
-0.50000      4.998322      1.003296      1.378204      0.727973
-0.49950      4.998780      1.003357      1.378174      0.728034
-0.49900      4.998322      1.003357      1.378021      0.728114
-0.49850      4.998627      1.003296      1.378052      0.728054
-0.49800      4.998475      1.003357      1.378113      0.728066
-0.49750      4.998322      1.003204      1.377899      0.728068
-0.49700      4.998322      1.003296      1.378052      0.728054
.
.
.
```

Data File Structure for Average Transient File Created by Analysis>Export

```
# FluoroTrack Average Transient File
# Created: Fri May 31 06:46:47 2013
#
# Record 3
# Start Time: Fri May 31 06:45:40 2013
# Record Length: 32.80 seconds
# Transients: 12 - 28
```

```
# Transient Length: 2000 ms
# Pre-Trigger: 500 ms
# Post-Trigger: 1500 ms
# Baseline: -200 - 0 ms
# Comments:   600A protocol
# Controller Mode: 2
# Sampling Rate: 2000 Hz.
# Background Num : 0.000000
# Background Den : 0.000000
#
# Time          Force         Ratio
# (s)           (mN)
-0.50000    4.998338    0.728038
-0.49950    4.998338    0.728038
-0.49900    4.998360    0.728042
-0.49850    4.998322    0.728036
-0.49800    4.998316    0.728031
-0.49750    4.998297    0.728028
-0.49700    4.998274    0.728026
.
.
.
-0.00250    4.998255    0.728013
-0.00200    4.998239    0.728028
-0.00150    4.998249    0.728038
-0.00100    4.998274    0.728057
-0.00050    4.998284    0.728044
0.00000     4.998322    0.728046
0.00050     4.998332    0.728038
0.00100     4.998344    0.728042
0.00150     4.998309    0.728037
0.00200     4.998309    0.728028
0.00250     4.998281    0.728026
.
.
.
1.49500     4.998370    0.728021
1.49550     4.998376    0.728016
1.49600     4.998357    0.728028
1.49650     4.998363    0.728039
1.49700     4.998367    0.728049
1.49750     4.998373    0.728045
1.49800     4.998360    0.728035
1.49850     4.998351    0.728031
1.49900     4.998338    0.728032
1.49950     4.998338    0.728032
```

Data File Structure for Statistics File Created by Analysis>Export

```
# FluoroTrack Statistics File
# Created: Fri May 31 06:46:48 2013
#
# Record 3
# Start Time: Fri May 31 06:45:40 2013
# Record Length: 32.80 seconds
# Transients: 12 - 28
# Transient Length: 2000 ms
# Pre-Trigger: 500 ms
# Post-Trigger: 1500 ms
# Baseline: -200 - 0 ms
# Comments:   600A protocol
# Controller Mode: 2
```

```
# Sampling Rate: 2000 Hz.
# Background Num : 0.000000
# Background Den : 0.000000
#
                    Force       Ratio
 Baseline           4.9984      0.7280
 Peak               5.8704      0.8893
 Peak Height        0.8720      0.1612
 Peak%              117.4457    122.1461
 Peak Height%       17.4457     22.1461
 Maximim dR/dt      11.2375     2.0275
 Minimum dR/dt      -11.1993    -2.0083
 Time to Max dR/dt  12.0000     12.0000
 Time to Min dR/dt  248.0000    248.0000
 Time to Peak       130.5000    131.5000
 Time to 10% Rise   13.0000     13.0000
 Time to 50% Rise   46.5000     47.5000
 Time to 90% Rise   93.5000     94.5000
 Time to 90% Fall   166.5000    165.5000
 Time to 50% Fall   213.5000    212.5000
 Time to 10% Fall   247.5000    247.0000
 10-90% Rise Time   80.5000     81.5000
 90-10% Fall Time   81.0000     81.5000
```

Appendix B – 600A Test Protocols

These test protocols are used in the Quick Start Guide for the Force-Frequency with Calcium experiment.

Sequence File: Force_Freq_950A.seq

```
ASI 600A Test Protocol Sequence File
Created: Wed May 22 12:58:49 2013
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 stim1Hz.pro ss1
Protocol 2: 1 0 0 1 Collect40Twitchesat1Hz.pro 1HzTwitches
Protocol 3: 1 0 0 0 stim1.5Hz.pro ss1.5
Protocol 4: 1 0 0 1 Collect40Twitchesat1.5Hz.pro 1.5HzTwitches
Protocol 5: 1 0 0 0 stim2Hz.pro ss2
Protocol 6: 1 0 0 1 Collect40Twitchesat2Hz.pro 2HzTwitches
Protocol 7: 1 0 0 0 stim2.5Hz.pro ss2.5
Protocol 8: 1 0 0 1 Collect40Twitchesat2.5Hz.pro 2.5HzTwitches
Protocol 9: 1 0 0 0 stim3Hz.pro ss3
Protocol 10: 1 0 0 1 Collect40Twitchesat3Hz.pro 3HzTwitches
Protocol 11: 1 0 0 0 pace1Hz.pro pace
```

Protocol File: stim1Hz.pro

```
ASI 600A Test Protocol File
Created: Thu Apr 18 08:31:14 2013
A/D Sampling Rate: 20000 Hz
Comment: Stim at 1 Hz
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 06: 6.0 ms 0.500 Hz 1000000.0 ms 0.000 Hz 0.000 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        Sync-Stimulus       1
     0.0        Data-Enable
     1.0        Stimulus            1 0 ms
   100.0        Data-Disable
     0.0        Stop
```

Protocol File: Collect40Twitchesat1Hz.pro

```
ASI 600A Test Protocol File
Created: Tue Apr 16 03:00:12 2013
A/D Sampling Rate: 20000 Hz
Comment: Collect 40 twitches for Force, SL and Fluorescence at 1 Hz.
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
```

```
Stimulus 06: 6.0 ms 0.500 Hz 1000000.0 ms 0.000 Hz 0.000 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       Sync-Stimulus       1
     0.0       Data-Enable
   100.0       FLA-Trigger         1 0 ms
   101.0       950A-Trigger        0 40 0 ms
 41000.0       FLA-Trigger         0 0 ms
 42000.0       Data-Disable
     0.0       Stop
```

Protocol File: stim1.5Hz.pro

```
ASI 600A Test Protocol File
Created: Thu Apr 18 08:31:14 2013
A/D Sampling Rate: 20000 Hz
Comment: Stim at 1.5 Hz
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 06: 6.0 ms 0.500 Hz 1000000.0 ms 0.000 Hz 0.000 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       Sync-Stimulus       1
     0.0       Data-Enable
     1.0       Stimulus            2 0 ms
   100.0       Data-Disable
     0.0       Stop
```

Protocol File: Collect40Twitchesat1.5Hz.pro

```
ASI 600A Test Protocol File
Created: Tue Apr 16 02:48:39 2013
A/D Sampling Rate: 20000 Hz
Comment: Collect 40 twitches for Force, SL and Fluorescence at 1.5 Hz.
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 06: 6.0 ms 0.200 Hz 1000000.0 ms 0.000 Hz 0.000 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       Sync-Stimulus       1
     0.0       Data-Enable
   100.0       FLA-Trigger         1 0 ms
   101.0       950A-Trigger        0 40 0 ms
 28000.0       FLA-Trigger         0 0 ms
```

```
 29000.0    Data-Disable
     0.0    Stop
```

Protocol File: stim2Hz.pro

```
ASI 600A Test Protocol File
Created: Thu Apr 18 08:31:14 2013
A/D Sampling Rate: 20000 Hz
Comment: Stim at 2 Hz
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 06: 6.0 ms 0.500 Hz 1000000.0 ms 0.000 Hz 0.000 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     Sync-Stimulus        1
     0.0     Data-Enable
     1.0     Stimulus             3 0 ms
   100.0     Data-Disable
     0.0     Stop
```

Protocol File: Collect40Twitchesat2Hz.pro

```
ASI 600A Test Protocol File
Created: Tue Apr 16 02:49:11 2013
A/D Sampling Rate: 20000 Hz
Comment: Collect 40 twitches for Force, SL and Fluorescence at 2 Hz.
Minimum Length: -1.000 (Lo)
Maximum Length: 5.000 (Lo)
PD Deadband:    0.000000 mN
Stimulus 01: 6.0 ms 1.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 02: 6.0 ms 1.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 03: 6.0 ms 2.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 04: 6.0 ms 2.500 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 05: 6.0 ms 3.000 Hz 1000000.0 ms 0.000 Hz 0.000 s
Stimulus 06: 6.0 ms 0.200 Hz 1000000.0 ms 0.000 Hz 0.000 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     Sync-Stimulus        1
     0.0     Data-Enable
   100.0     FLA-Trigger          1 0 ms
   101.0     950A-Trigger         0 40 0 ms
 21000.0     FLA-Trigger          0 0 ms
 22000.0     Data-Disable
     0.0     Stop
```

Appendix C – Program Start Switches

The 950A program is called by 600A whenever the 950A menu item is clicked on. There are no user changeable start switches when starting 950A in this manner. However, when using the 950A program in playback mode there are several start switches that can be used to start the program. These start switches can be set by using the properties of the 950A Playback 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 asi950a –h as shown in Figure 24.
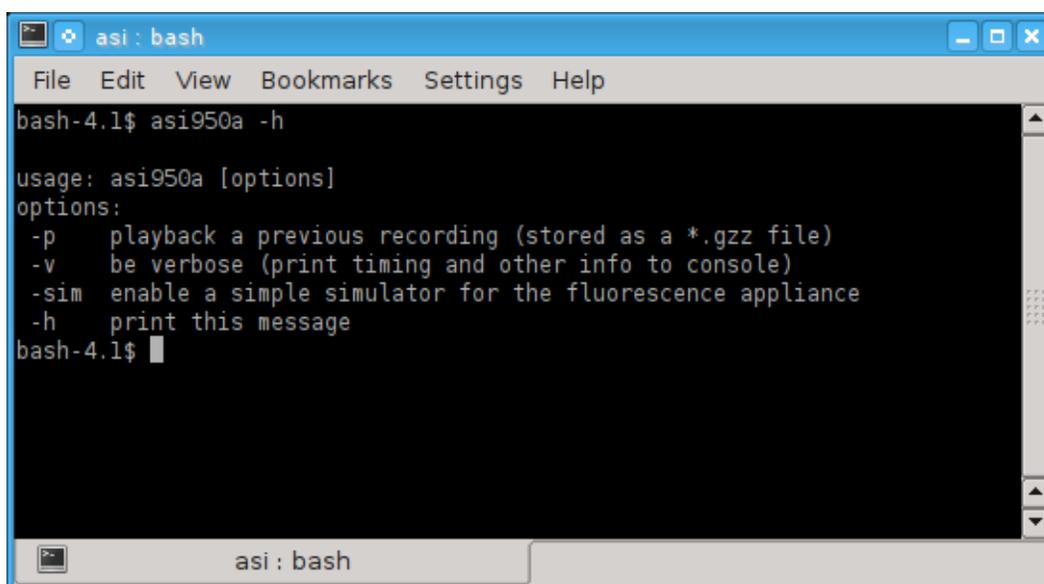
Figure 24 950A Start Switches

The default settings for the 950A Playback icon are as follows:

asi950A –p

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Appendix D – Network Security

⚠️ Many users will elect to connect the 600A PC to a network. Because the PC 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 PC 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.
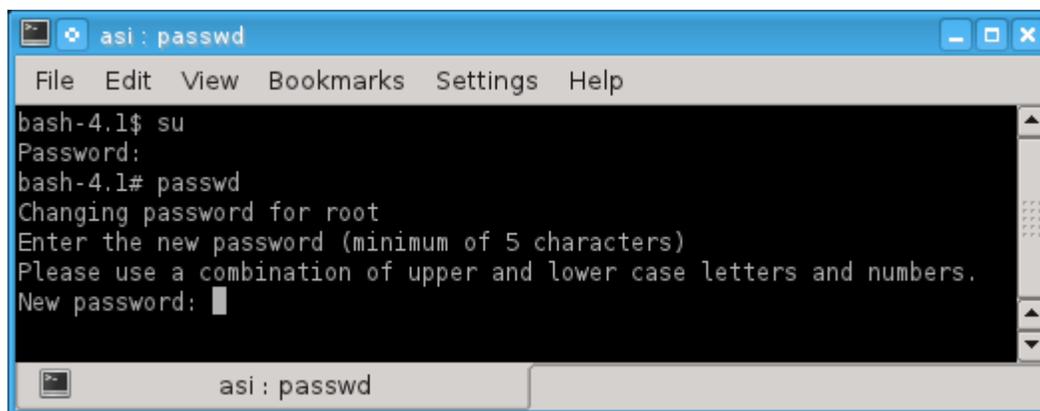
Figure 25 Use passwd to Change the Root Password