

## **INSTRUCTION MANUAL**

### **Models**

**900B Video Sarcomere Length (VSL)**

**901A/B High-Speed Video Sarcomere Length (HVSL)**

Program Version: 3.000

September 10, 2012

### **Aurora Scientific Inc.**

360 Industrial Parkway South, Unit 4

Aurora, Ontario, Canada L4G 3V7

Toll Free: 1-877-878-4784

Telephone: 1-905-727-5161

Facsimile: 1-905-713-6882

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

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



## Table of Contents

Table of Contents .....	1
Table of Figures .....	3
Conventions Used in this Manual .....	4
1.0 Introduction.....	5
1.1 What's New in Version 3.000 .....	6
2.0 Specifications .....	7
Software.....	7
Camera.....	8
3.0 Quick Start Guide .....	10
3.1 Instrument Setup.....	10
3.2 Software Registration and Activation.....	11
3.3 Linux and Desktop Overview .....	13
3.4 Starting the HVSL Program .....	13
3.5 Program Main Window .....	14
3.6 Using the VSL/HVSL Program.....	17
3.6.1 Quick Start Guide.....	18
4.0 Main Program .....	23
4.1 Main Screen.....	23
4.1.1 Video Image .....	23
4.1.2 Sizing and Moving the Region of Interest .....	23
4.1.3 Graph of Calculation Results .....	24
4.1.4 Control Buttons .....	28
4.1.5 Text Readouts.....	30
4.2 Menu Structure .....	30
4.2.1 File Menu .....	31
4.2.2 Edit Menu.....	31
4.2.3 Setup Menu .....	33
4.2.4 600A Menu.....	42
4.2.5 Analysis Menu.....	42
4.2.6 Scope Menu.....	42
4.2.7 Record Menu .....	42
4.2.8 Playback Menu.....	48
4.2.9 Help Menu.....	51
5.0 Analysis.....	52
5.1 File Menu.....	52
5.2 Edit Menu .....	53
5.3 Onscreen Controls .....	53
6.0 Scope.....	57

---

6.1 File Menu.....	58
6.2 Timebase Menu .....	58
6.3 Scaling Menu .....	58
6.4 Logging Menu .....	58
6.5 Quick Start Guide for Scope.....	59
7.0 Measurement Algorithms.....	60
8.0 Software Updates .....	63
9.0 Technical Support, Warranty and Repairs .....	64
9.1 Technical Support.....	64
9.2 Warranty .....	64
9.3 Returning Products to Aurora Scientific Inc. for Repair .....	65
Appendix A – HVSL File Structure.....	67
Data File Structure for Files Created by Record and Playback.....	67
Data File Structure for Files Created by Scope.....	67
Data File Structure for Files Created by Analysis.....	68
Appendix B – Savitsky-Golay Smoothing Filter .....	69
Appendix C – uEye USB CMOS Camera Data Sheet.....	70
Appendix D –Imperx VGA-210L CCD Camera Data Sheet.....	71
Appendix E – Teledyne Dalsa GigE CMOS Camera Data Sheet.....	73
Appendix F – Program Start Switches.....	75
Appendix G – Network Security.....	76

## Table of Figures

Figure 1 ASI Activate Window .....	11
Figure 2 ASI Product Activation Website .....	12
Figure 3 Desktop with HVSL Program Running.....	14
Figure 4 HVSL Main Screen .....	16
Figure 5 HVSL Setup Screen.....	19
Figure 6 HVSL Scope Window .....	22
Figure 7 Graph of FFT Amplitude vs Wavenumber.....	24
Figure 8 Graph of ACF (Filtered).....	25
Figure 9 Graph of ACF (Unfiltered).....	26
Figure 10 Graph of FFT Amplitude vs SL.....	27
Figure 11 Graph of FFT Amplitude vs Wavenumber.....	28
Figure 12 File Drop Down Menu.....	31
Figure 13 Edit Drop Down Menu .....	33
Figure 14 HVSL Setup Menu .....	34
Figure 15 Camera Image Showing Poorly Adjusted Offset .....	38
Figure 16 Camera Image Showing Correctly Adjusted Offset .....	39
Figure 17 SineFit Control Set to 0.50 .....	40
Figure 18 SineFit Control Set to 0.25 .....	41
Figure 19 HVSL Record Window with Duration set by Time .....	43
Figure 20 HVSL Record Window showing Status after Recording Complete .....	44
Figure 21 Record Directory File Dialog .....	44
Figure 22 Record Trial Name File Dialog .....	45
Figure 23 Directory File Structure for Record Function .....	46
Figure 24 Images Directory .....	47
Figure 25 File Error Window.....	47
Figure 26 HVSL Playback Window .....	48
Figure 27 Exported AVI Movie Frame.....	50
Figure 28 Playback Directory File Dialog .....	50
Figure 29 HVSL About Window .....	51
Figure 30 HVSL Analysis Window.....	52
Figure 31 Analysis Display with Data Zoomed.....	54
Figure 32 Output with Filters Set to the Minimum Value .....	55
Figure 33 Output with Filters Set to 4 .....	55
Figure 34 Output with Filters Set to 16 .....	56
Figure 35 Scope Drop Down Menu on the Main Screen.....	57
Figure 36 Scope Window.....	59
Figure 37 SL and Velocity vs Time for FFT SL Algorithm .....	61
Figure 38 SL and Velocity vs Time for ACF/SineFit SL Algorithm .....	62
Figure 39 Use passwd to Change the Root Password.....	76

## 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.

**900B**

This Icon indicates a section specific to VSL, 900B.

**901A**

This Icon indicates a section specific to HVSL with the Imperx camera, 901A.

**901B**

This Icon indicates a section specific to HVSL with the GigE camera, 901B.

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

Throughout this manual the term HVSL (High speed Video Sarcomere Length) will refer to all three models; Video Sarcomere Length (VSL) and both versions of High-speed Video Sarcomere Length (HVSL). When information specific to a particular system is presented then that information will have the appropriate icon clearly shown.

## 1.0 Introduction

This manual explains the proper use and operation of Aurora Scientific Inc.'s line of Video Sarcomere Length (VSL and HVSL) products. The three models covered by this manual each use a different camera and each have a different maximum frame rate. The software is functionally the same for each product. Throughout the manual the term HVSL will refer to all three models; VSL and both versions of High-speed Video Sarcomere Length (HVSL). When information about a specific model is presented then an icon will indicate which model is being discussed. This convention is outlined on page 4 of this manual.

The HVSL systems utilize a standard C-mount camera which is normally attached to an inverted microscope port using an adapter sourced from the microscope manufacturer. 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.

The program uses two independent calibration constants to allow the researcher to easily switch between using a low magnification setting for measuring overall tissue dimensions to a high magnification setting for measuring sarcomere length.

The software includes a Scope window that provides a scrolling data display of sarcomere length with time. This display can buffer up to 2 hours of sarcomere length data. The display can be frozen at any time and the user can then scroll through the sarcomere history (the data buffer). Scope can also log SL data to disk at rates of up to 1000 samples per second.

An Analysis window is provided that plots data as sarcomere length versus time and sarcomere velocity versus time. Also included in the Analysis window is a cursor readout and a zoom function. Filters can be applied to the SL and velocity data as well. Data can be exported from the Analysis window in ASCII text file format.

When installed on a 600A PC, HVSL can interface to the 600A program which can then provide sarcomere length (SL) control. SL data is sent from the HVSL program to the 600A program where SL data can be viewed and also stored with force and length data in the same file using the same time base. Storing the SL data in the 600A data files ensures correct synchronization of the length, force and SL data with respect to time. The 600A program can also trigger HVSL to collect SL and image data. This trigger function is built into the protocol functions of 600A which allows the researcher to precisely control the start of SL data collection with respect to length and force control of the tissue.

The HVSL program can also be triggered by any instrument that can provide a TTL trigger signal. A TTL trigger module (available from Aurora Scientific) is required for this external trigger function.

### **900B**

The 900B VSL system uses a USB CMOS camera that can acquire full frame images (640x480) and calculate sarcomere length at 80 frames per second. When the camera is used with a reduced region of interest the program can acquire and calculate sarcomere length at up to 1000 frames per second.

### 901A

The 901A HVSL system uses a high-speed CCD camera with a Camera link interface card to connect to the computer. It can acquire full frame images (640x480) and calculate sarcomere length at 210 frames per second. When the camera is used with a reduced region of interest the program can acquire and calculate sarcomere length at up to 2250 frames per second.

### 901B

The 901B HVSL system uses a high-speed CMOS camera with a Gigabit Ethernet Connection. It can acquire full frame images (640x480) and calculate sarcomere length at 300 frames per second. When the camera is used with a reduced region of interest the program can acquire and calculate sarcomere length at up to 4690 frames per second.

## 1.1 What's New in Version 3.000

Many improvements and new features have been incorporated in version 3.000 of the HVSL system. A list of improvements and new features follows.

### Tracking

Tracking is a new function that allows the software to track the sarcomeres defined by the initial Region of Interest (ROI). When in operation the ROI moves and changes its horizontal size to keep the same group of sarcomeres in the region of interest. This ensures enhanced accuracy and stability of the SL calculation.

### 901B HVSL with GigE Camera

This new addition to the HVSL family of products provides a large increase in data rate with operation speeds up to 4690 frames per second. To accommodate the increase in data rate and the corresponding increase in processing capability, the HVSL program runs on a six core processor that dedicates two cores to the data acquisition and processing tasks which ensures stable and consistent performance.

### Plot Themes

A dark theme has been included for the Scope and Analysis windows. This dark theme replaces the black lines on white background of the original light theme, with green lines on a black background. This setting allows for more options when displaying your data.

### AVI Movie Exports

The export of high quality AVI videos is now included in the program. When exporting a movie the user can select a trial, frame range and frame rate (fps). The user can also include an overlay that includes the Region of Interest (ROI), calculated sarcomere length, frame number, Time, and Reference Length ruler. More information is available in Section 4.2.8 Playback Menu.





Time base:	1s to 1 hr
SL Range:	Auto, 0.1 $\mu$ m, 0.2 $\mu$ m, 0.5 $\mu$ m, 1.0 $\mu$ m, 2.0 $\mu$ m
Sample Rate:	1, 2, 5, 10, 20, 50, 100, 200, 500, 1000Hz
Select Output File:	Select a file to save SL data.
Record:	Start recording SL data, time limited by available hard drive space.
Stop:	Stop recording SL.
Freeze Display:	Display can be frozen and then the previous 2 hours of data can be reviewed
Cursor Readout:	Displays SL at cursor location.

### **Analysis Window**

### **Plots SL and Velocity vs. time**

Display:	Sarcomere Length, Velocity versus time.
Zoom:	Drag out zoom box, release mouse button to zoom.
Data Scroll:	Scroll bar allows current time window to be scrolled through entire data record.
Data Filter:	Block average filter for SL, Savitsky-Golay Filter for Velocity.
Cursor Readout:	Displays SL, Velocity and Time at cursor location.
Export Data:	Export on screen SL and velocity data to ASCII text file.

### **Record Function**

### **Stores images, calculated SL values or both to disk**

Rate:	Choose the frame rate, dependent on camera model
Duration:	Set as Time (sec) or Frames
Record:	SL, Images or Both
Trigger Mode:	Single shot or repeating.
Trigger:	Manual, 600A or TTL
File Format:	Images: Binary format - .BIN extension SL Data: ASCII text file format - .dat extension

### **Playback Function**

### **Playback data, calculate SL, and create AVI movie files**

Rate:	User set.
Export File Format:	.PNG
Create Image Files:	Converts binary image file to separate .PNG file for each frame
AVI Output:	Collected images can be output in .AVI format
Types:	Step, Stream, Loop

## **Camera**

### **900B**

### **See Appendix C – uEye USB CMOS Camera Data Sheet for camera data sheet**

Model:	IDS (Imaging Development Systems) UI-1225LE
Type:	CMOS
Resolution:	752 x 480 reduced to 640 x 480
Optical Size:	4.512 mm x 2.880 mm
Pixel Size:	6.0 $\mu$ m
Frame Rate:	Full Frame (640x480): 80 frames per second Maximum (Reduced ROI): 1000 frames per second

Lens Mount: C mount, CS-mount, 1/3" format  
Interface: USB 2.0  
Connector: USB mini-B  
Power Supply: Powered through USB connection

**901A**

**See Appendix D –Imperx VGA-210L CCD Camera Data Sheet for camera data sheet**

Model: Imperx VGA-210-LMCN  
Type: CCD  
Resolution: 640 x 480  
Image Area: 5.87 mm x 4.71 mm  
Pixel Size: 7.4  $\mu$ m  
Frame Rate: Full Frame (640x480): 210 frames per second  
Maximum (Reduced ROI): 2200 frames per second  
Shutter Speed: 1/50,000 sec to 1/100 sec  
Lens Mount: C mount, 1/3" format  
Interface: EDT PCI Express DV Camera-Link/SC Card  
PCI 2.3, 33 or 66MHz, 32 bits resolution  
Connectors: Video: MDR-26 pin, standard Camera Link cable  
Strobe: BNC  
Trigger: BNC  
Power Supply: Input: 100-240VAC, 50/60Hz.  
Output: 12VDC @ 4.2W

**901B**

**See Appendix E – Teledyne Dalsa GigE CMOS Camera Data Sheet for camera data sheet**

Model: Teledyne Dalsa Genie HM640  
Type: CMOS  
Resolution: 640 x 480  
Image Area: 4.80 x 3.60 mm  
Pixel Size: 7.4  $\mu$ m  
Frame Rate: Full Frame (640x480): 300 frames per second  
Maximum (Reduced ROI – 16 lines): 4694 frames per second  
Lens Mount: C mount, 1/3" format  
Interface: Standard Gigabit Ethernet Port  
Connectors: Standard or Screw mount RJ-45  
Strobe: BNC  
Trigger: BNC  
Power Supply: Input: 100-240VAC, 50/60Hz.  
Output: 12VDC @ 4.0W

## 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

In most research configurations the camera is attached to an available port on an inverted microscope. Since all of the cameras that are included with VSL/HVSL use a C-mount you may need to purchase an appropriate C-mount port adapter for your model of microscope. In most cases it isn't necessary to match the port image size to the sensor size of the camera (1/3") but if you determine that this is a requirement then an adapter tube with an internal size matching lens may also be required. Contact ASI for assistance with the selection of adapter tubes. Screw the camera onto the port adapter and then insert the adapter into an unused port on the microscope.

#### 900B

The 900B VSL camera uses a USB interface. A 3 m long USB cable is supplied with the camera. This is the only cable that should be used due to issues that can result from using inferior quality USB cables. Plug the USB A connector into an available USB port on the back of the PC. Note: the USB ports found on the front of the PC operate at a slower data rate than those on the back of the PC. For this reason do not plug the camera USB cable into the front panel USB connectors on the PC. Plug the USB mini B connector into the mating port on the camera. The VSL camera is powered through the USB cable so there is no power supply or power cable.

#### 901A

The 901A HVSL camera uses a camera-link cable supplied with the camera. Plug one end of the cable into the camera and the other end into the pre-installed camera-link interface card located in the PC. Plug the camera power supply into an appropriate AC power source and into the power connector on the camera. Strobe and trigger BNC connectors are also provided on the power cable to the camera. Contact ASI for instructions on using these connectors if you need to use them.

#### 901B

The 901B HVSL camera uses a standard RJ-45 Ethernet connection. Plug one end of any Cat 5e or Cat 6 Ethernet cable into the camera, and the other end into the PC. The PC has two Ethernet ports, one on-board and a second on an expansion card. Plug the camera into the port on the expansion card. Plug the camera power supply into an appropriate AC power source and into the power connector on the camera. Strobe and trigger BNC connectors are also provided on the power cable to the camera. Contact ASI for instructions on using these connectors if you need to use them.

Make sure both the PC and the camera are turned off before connecting the cable between them. All required software comes pre-installed on the HVSL or 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.

## 3.2 Software Registration and Activation

The HVSL 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 HVSL 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 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., 017D3. 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., 2Y132-70932-675D7.

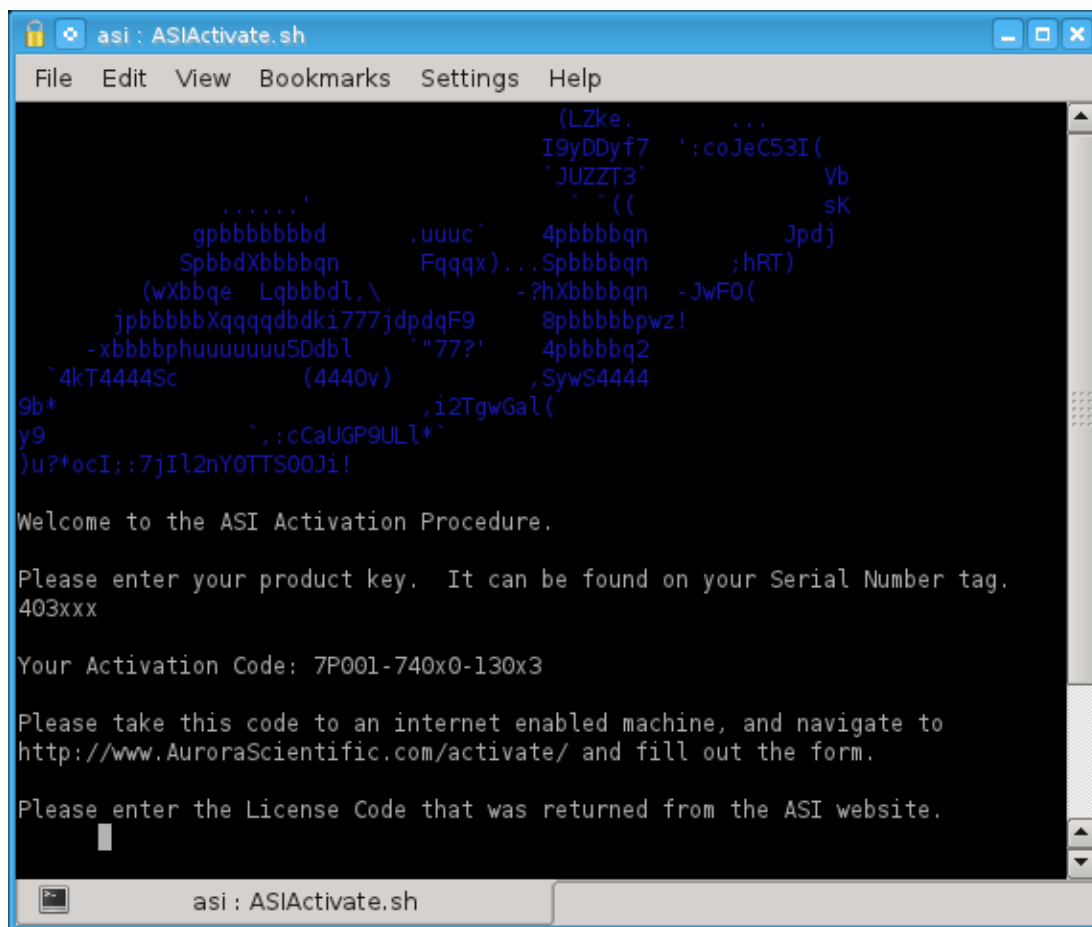


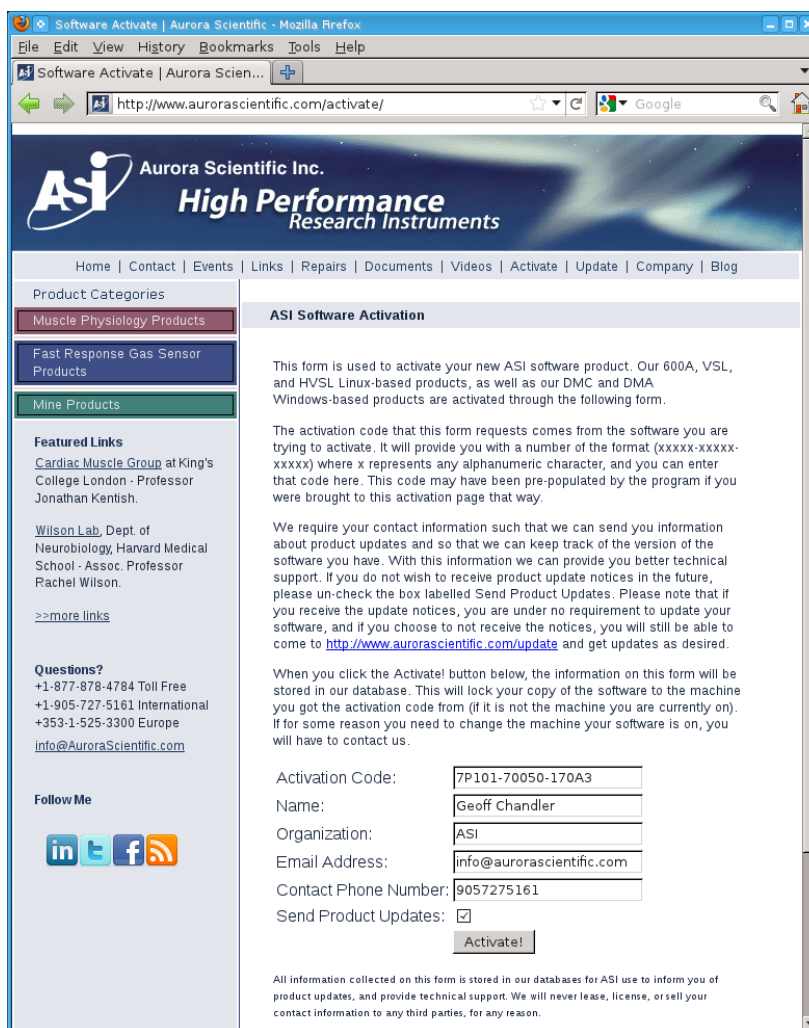
Figure 1 ASI Activate Window

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 2.
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. Click on the desktop icon to start the HVSL 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 901A 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 the process.



**Figure 2 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 **Error! Reference source not found.** The most frequently used icons will be *ASIOg*, *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 opening an *xterm* 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 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 HVSL Program

Once the PC finishes the login process you will see an icon on the Desktop for **VSL/HVSL** (see Figure 3). The software must be licensed before it will run. See section 3.2 Software Registration and Activation for details. Ensure that the camera is powered before starting the HVSL program. The program sends setup information to the camera when the program starts. Improper camera operation may occur if the camera is not powered when the program starts.

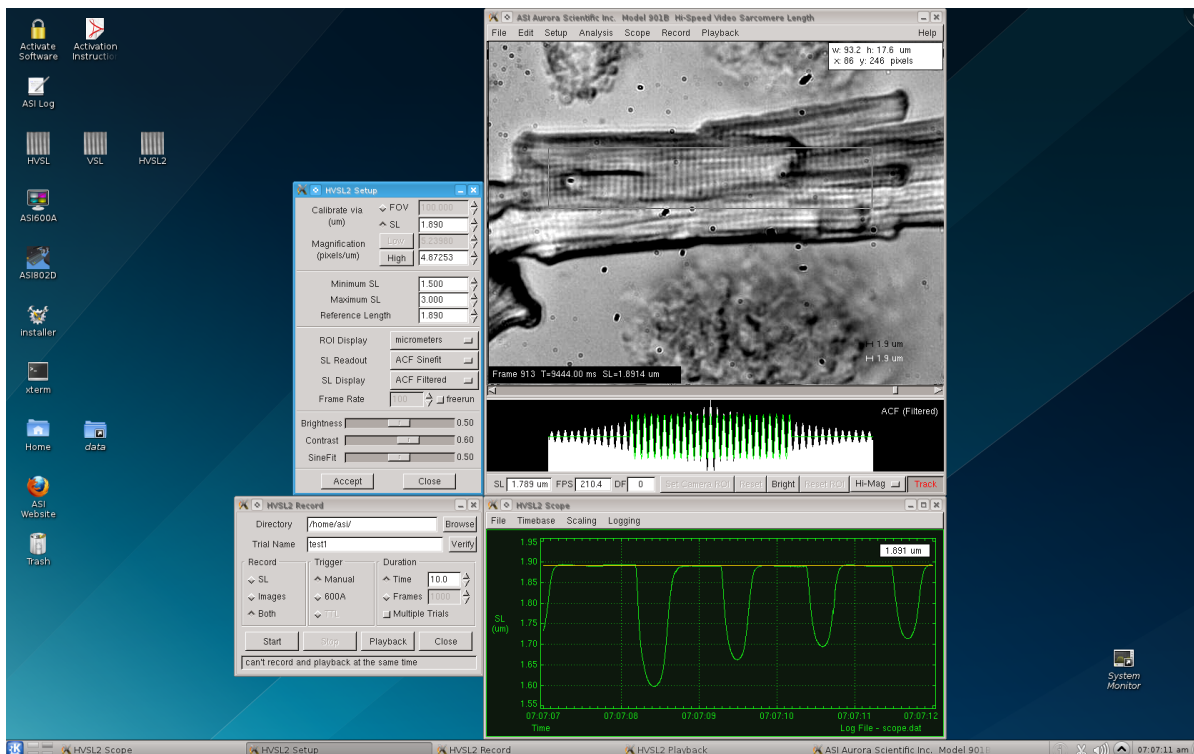


Figure 3 Desktop with HVSL Program Running

### 3.5 Program Main Window

When the program starts the main window will open showing the current video image, the results of the selected calculation method in graphical form beneath the image and several control buttons below the graph (see Figure 4). The main window can be repositioned and minimized but its size cannot be altered since its size is fixed to the camera resolution (640x480). On higher resolution monitors the main window will appear smaller than on lower resolution monitors. We suggest using a minimum PC screen resolution of 1280x1024 pixels however the program can operate at lower screen resolutions. The Linux desktop and all program windows use normal Windows-type controls for manipulating the display windows.

A menu bar is located along the top of the screen that includes the following menu headings.

#### **File Edit Setup Analysis Scope Record Playback Help**

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 Section

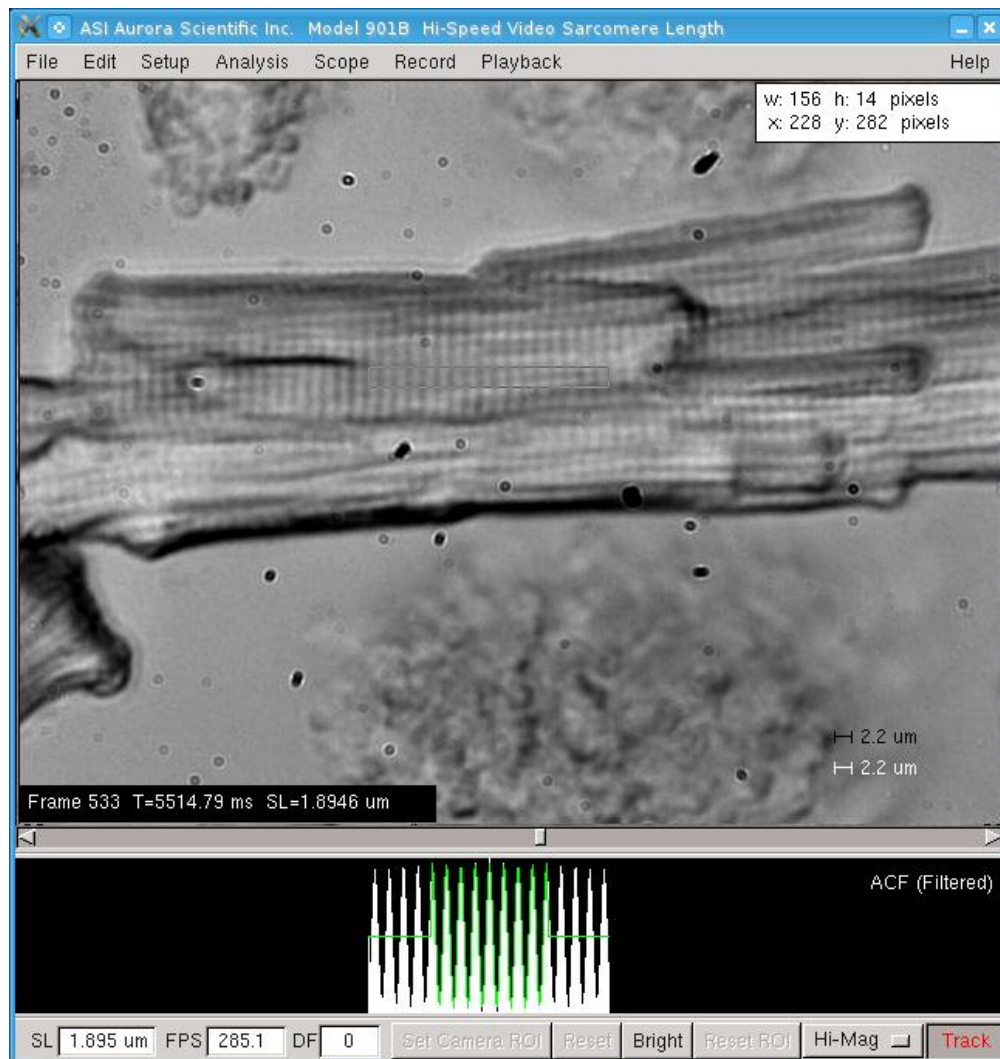
## 4.0 Main Program

The HVSL program.

Located at the bottom of the main screen are several text readouts and buttons. These are, from left to right, sarcomere length readout (SL), frames per second readout (FPS), dropped frames readout (DF), Set Camera ROI (region of interest) button, Reset button, Bright button, Reset ROI button, and a magnification control button (Hi-Mag/Lo-Mag).

The SL text readout displays the sarcomere length of the current frame in microns to three decimal places. This readout updates at the same rate as the screen updates. The update rate is set by a program start switch (see Appendix F – Program Start Switches). The FPS text readout shows the current speed of the camera measured in frames per second (FPS). In most cases the camera runs in a free run mode where the frame rate is determined by the currently defined camera region of interest. For this reason there can be some variation in the frame rate displayed. If you require the frame rate to be a specific fixed value then you can set the frame rate using controls found in the Setup menu. The dropped frames text readout displays the number of frames that have been missed since the last time the camera ROI was set or reset. Dropped frames (DF) occur when the program had not finished calculating the SL of a frame before the next frame became available. In normal operation the DF value should be small or zero. If the DF number becomes large compared to the number of frames processed, then the current camera ROI and Setup settings should reviewed and adjusted to eliminate dropped frames.





**Figure 4 HVSL Main Screen**

The Set Camera ROI button reduces the image size taken by the camera to that defined by the current region of interest (ROI) box. Reducing the camera ROI allows the camera to produce frames at a higher rate. The rate at which data is sent from the camera is fixed which means that when the camera ROI is reduced the amount of data sent from the camera to the PC is reduced which allows the camera to run at a higher frame rate. Thus to increase the calculation speed you must reduce the camera region of interest. The minimum frame rate for 900B/901A/901B is 80/210/300 frames per second respectively. The maximum frame rate for 900B/901A/901B is 1000/2200/4700 frames per second. For more information specific to your model see the Camera heading in Section 2.0 Specifications.

Setting the camera ROI to a reduced size has the dual advantages of eliminating areas of the frame that do not contain sarcomeres while obtaining higher data rates. To reduce the camera region of interest, first use the mouse to create an ROI on the screen by clicking and dragging the mouse and then press the Set Camera ROI button to window down the image coming from the camera.



The frame rate doesn't increase when the horizontal size of the image is reduced; it only increases when the vertical size is reduced.

The Reset button returns the camera ROI to full frame size.

The Bright button automatically adjusts the gain on the video amplifiers in the camera to produce a visible image. As the camera ROI is reduced the shutter is open for a shorter time which results in a darker image. The brightness function compensates for the shorter exposure time by increasing the video gain.



Increased light intensity may be required to obtain good images at the fastest frame rates. The video amplifiers in the camera can compensate for shorter shutter speed but will result in noisy images. Increasing the light intensity will allow the camera to operate at lower gain and produce higher quality (lower noise) data.

The Reset ROI button resets the calculation region of interest (ROI) to the full size of the current camera ROI. Sarcomere calculations are only made within the calculation ROI. Improvements to the calculation algorithm in version 2.0 of the program has resulted in the program being able to calculate SL at the maximum frame rate of the camera even when the calculation ROI is set to the full size of the camera ROI. This means that the user no longer needs to use small calculation ROIs to obtain high speed data. Choose the calculation ROI to include the area where you want to calculate SL. We recommend that to improve accuracy the calculation region of interest should be sized to only include areas of the image that contain a sarcomere pattern. Try to avoid including areas of background within the calculation region of interest.

The Hi-Mag/Lo-Mag button is used to change the calibration constant in use by the program. If you intend to operate the program at different optical magnifications then you must first calibrate the program for each of the two magnifications. Whenever you change the optical magnification of the microscope you must switch the program's calibration constant using the Hi-Mag/Lo-Mag button.

### 3.6 Using the VSL/HVSL Program

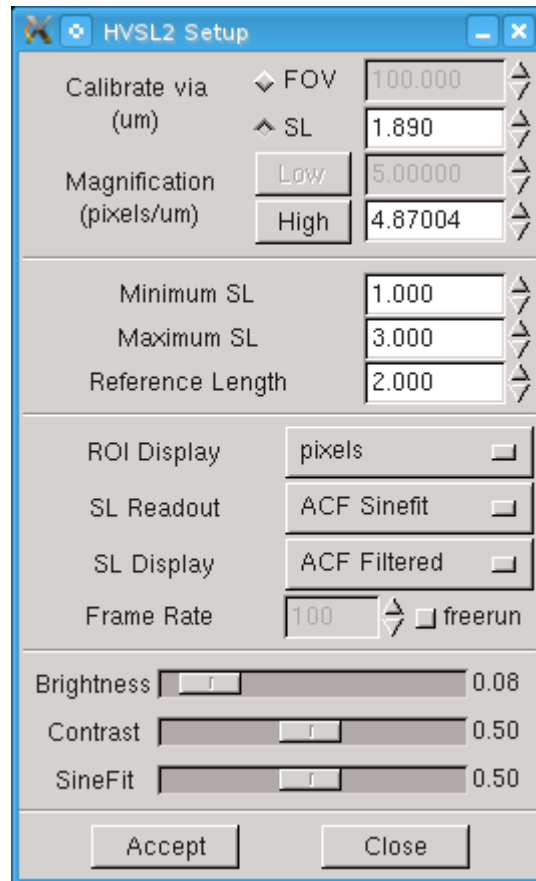
Assuming that the camera is connected to the microscope and to the PC and that the lighting is sufficient, then the image should be updating on the screen as soon as the program is started. Try moving the tissue a small amount to confirm that you are seeing a live video image. The program does not compensate for angled images (non-vertical sarcomeres) so if the sarcomeres in the image are on an angle the program will overestimate the sarcomere length. This problem can easily be solved by simply rotating the camera to align the sarcomeres to be as vertical as possible in the image. We recommend that you loosen the port adapter locking screws and rotate the port adapter/camera to align the sarcomere pattern as opposed to unscrewing the camera from the port adapter to align the image. Re-tighten the port adapter locking screws when done. You can use the Scope function to monitor the SL as you rotate the port adapter/camera. You will observe that the SL is at a minimum when the sarcomere pattern

is vertical on the screen. Once the sarcomere image is vertical, rotation of the camera in either direction will result in an increase in the currently calculated SL.

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 complete details about using the program are provided in chapters 4, 5 and 6.

### 3.6.1 Quick Start Guide

- 1) Turn on the PC, log in, turn on the camera and then start the VSL/HVSL program. Note: the VSL camera is powered by the USB cable so there is no need to turn the camera on.
- 2) Once the program starts click on the Setup Menu (see Figure 5) which will open the setup window. The setup window is used to calibrate the program, to adjust the image contrast and brightness, and to set display and calculation parameters.
- 3) Use the Brightness and Contrast sliders to adjust the image if necessary.
- 4) You must calibrate the program before you can produce accurate sarcomere length measurements. There are two calibration methods available in the program and two magnification scale factors. Normally the Hi-Mag setting is used for measuring SL whereas the Lo-Mag setting is used for measuring the overall dimensions of the tissue. The following instructions should be followed to calibrate the program for both high and low magnification settings. Although the instructions below only refer to the Hi-Mag setting the Lo-Mag calibration is done in the exact same manner. Ensure you switch the program to the correct magnification setting before proceeding with the calibration. To change between the two settings press the Hi-Mag/Lo-Mag button located in the bottom right corner of the main window. As you press this button the program will cycle between the two magnification scale factor settings. You will notice the Hi-Mag SL and Lo-Mag SL buttons located on the Setup window will grey and un-grey as the magnification setting is changed. The two calibration methods, field of view and calibration length, are explained below.
  - a) Field of View (FOV) – this calibration method assumes that you know the exact horizontal distance between two points in the image. Using the mouse position the cursor at the first known point and press the left mouse button. While keeping the mouse button depressed drag out a region of interest (ROI) box that extends horizontally to the second known point. Since the calibration calculation is done on the horizontal distance, the vertical extent of the ROI box doesn't matter. However it is good practice to ensure that the vertical ROI box size is at least 20 pixels high. A larger horizontal distance between the two points produces a more accurate calibration. Click on the diamond shaped button to the left of the Calibration via FOV ( $\mu\text{m}$ ) label. Enter the known horizontal distance between the two points. Press either the High or Low Magnification SL (pixels/ $\mu\text{m}$ ) button on the setup screen. The program will calculate the number of pixels/ $\mu\text{m}$  in the image and display this value in the text box to the right of the button. Press the Accept button. For best program performance the calibration value should be greater than 4 pixels/ $\mu\text{m}$ . Values significantly less than this will affect the accuracy of the measured SL. If the calibration value is too low then increase the optical magnification of the microscope and recalibrate the program.



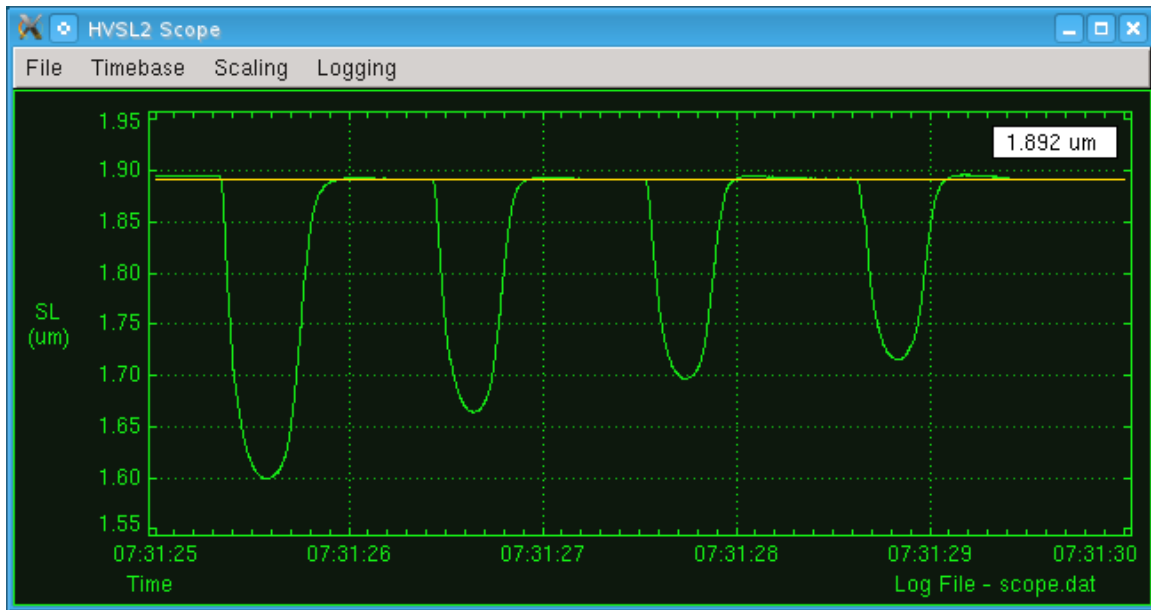
**Figure 5 HVSL Setup Screen**

- b) Calibration Length – this calibration method assumes that there is an image present that contains a repeating pattern of a known spacing (an example of this would be a calibration grating). The program calculates the pattern spacing and uses the known distance to calibrate the system. Using the mouse, position the cursor at a point within the repeating pattern. Press the left mouse button and, while holding the button down, drag out a region of interest (ROI) box that extends horizontally and vertically across a large portion of the pattern. The most accurate results will be obtained with the largest ROI that remains fully within the pattern. Click on the diamond shaped button to the left of the Calibration via SL ( $\mu\text{m}$ ) label. Enter the known pattern spacing. If the pattern spacing is outside of the range set by Minimum SL ( $\mu\text{m}$ ) and Maximum SL ( $\mu\text{m}$ ) then change these values to contain the pattern spacing value, i.e., if the pattern spacing is  $10\ \mu\text{m}$  then the Minimum SL ( $\mu\text{m}$ ) should be set to about 9 and the Maximum to about 11. Set the Reference Length ( $\mu\text{m}$ ) to be equal to the pattern spacing. Press either the High or Low Magnification SL (pixels/ $\mu\text{m}$ ) button on the setup screen. The program will calculate the number of pixels/ $\mu\text{m}$  in the image and display this value in the text box to the right of the button. The program will also place a labeled reference bar in the lower right corner of the image. The Calibration Length method is an iterative method that often requires repeated presses on the High or Low Magnification SL (pixels/ $\mu\text{m}$ ) button before the solution converges. A simple method for checking convergence is to press the High or Low

Magnification SL button several times while noting the value displayed in the text box to the right of the button. When the numbers no longer change the correct solution is usually found. Under some circumstances the program can converge to the wrong solution or diverge (never stop changing the calibration factor). A simple way of checking for an accurate solution is to compare the length of the reference bar shown in the bottom right of the main screen with the pattern spacing visible in the image. If the reference bar is not the same length as the distance between the lines in the pattern then the program has converged to an incorrect solution. Likewise if the reference bar continually changes length with each press of the High or Low Magnification SL button then the solution is diverging. In either case you may need to manually enter a value in the text box beside the High or Low Magnification SL (pixels/ $\mu\text{m}$ ) button and then press the button several times until the solution converges. When you enter a value you are essentially providing the program with a starting point for the calculation. If you select a starting point much greater than the true value the program may diverge or even converge to a higher solution, if you choose a starting point much less than the true value the program may converge to a lower solution. A few tries and some experience will usually allow you to converge to the correct solution within a few button presses. Press the Accept button to complete the calibration. For best program performance the calibration value should be greater than 4 pixels/ $\mu\text{m}$ . Values significantly less than this will affect the accuracy of the measured SL. If the calibration value is too low then increase the optical magnification of the microscope and recalibrate the program.

- 5) Once calibrated you should set the minimum and maximum SL values to reasonable values that bracket the expected sarcomere length. Assuming a sarcomere length of 2.5  $\mu\text{m}$ , good choices of the minimum and maximum would be 2 and 3  $\mu\text{m}$  or 1.5 and 3.5  $\mu\text{m}$ . Don't choose values that are too far outside of the physiological range of sarcomere lengths. The program looks for the most dominant length that occurs within the range of the maximum and minimum SL. If you set the range too large or too small the program may not calculate the correct sarcomere length.
- 6) Set the Reference Length ( $\mu\text{m}$ ) to a convenient value within the expected range of sarcomere lengths. The Reference Length is plotted on the Scope display as a horizontal green line. Most researchers set the Reference Length to be equal to the desired resting SL of the tissue.
- 7) The calculation method should now be set using the SL Readout button. Two choices are available; FFT and ACF SineFit. Details of these methods are provided in chapter 7. The fastest and most accurate method is ACF SineFit. The particular requirements of your experiment will determine the best method to choose. Changes made in version 2.00 of the program have significantly increased the speed of all of the calculation methods and there is no longer a need to select a particular method in order to obtain maximum performance. We recommend using the ACF SineFit calculation method as this provides the most accurate and fastest SL measurements.
- 8) Press the Accept button. It is often most convenient to leave the Setup window open during tests as this allows setup parameters to be easily changed to improve performance. Note: setup parameters only take effect after the Accept button has been pressed or the setup menu has been closed.

- 9) The HVSL program should now be producing accurate sarcomere length measurements.
- 10) A Region of Interest (ROI) can be set by positioning the cursor at a starting point and then pressing the left mouse button and dragging a box out. When the mouse button is released the ROI is set. If the Num Lock key is off then the right mouse button can be used to reposition a defined ROI. Place the cursor within the ROI, press and hold the right mouse button, move the ROI around within the image using the mouse. Release the button to fix the ROI in the new position. Note: if the Num Lock is on then the right and left mouse buttons will both create a new ROI. The arrow buttons on the keyboard can also be used to move the ROI.
- 11) A Camera ROI can be set by clicking on the Set Camera ROI button on the bottom of the main screen. When a Camera ROI is set the camera only sends back the image data from that region. This allows the camera to increase its frame rate.
- 12) Once a Camera ROI is set then a calculation ROI can be set within the Camera ROI. The sarcomere length is only calculated on the image inside the calculation ROI. A major increase in performance for version 2.00 of the program now means that the program can calculate SL faster than the camera frame rate regardless of the size of the ROI. Therefore the only reason to create a smaller calculation ROI than the camera ROI is to highlight an area of the tissue for measurement. The SL calculation is more accurate when the calculation ROI only contains sarcomeres, therefore keep the calculation ROI within the tissue image and don't include large areas of the image outside of the borders of the tissue.
- 13) A Scope function (see Figure 6) is available that provides a real-time scrolling output of the sarcomere length on a separate window that resembles an oscilloscope or a strip-chart recorder. Scope is very useful at providing a long-term record of the sarcomere length and it is especially useful at the beginning of a test protocol that requires setting the sarcomere length to a given value. If for example your test protocol calls for a starting sarcomere length of  $1.89\mu\text{m}$  then by using the Scope output you can easily see the current sarcomere length and track the change in SL as you adjust the overall tissue length. Scope places a green line at the Reference Length value (set using the Setup menu). Therefore if you set the Reference Length equal to the desired initial sarcomere length then it is easy to adjust the actual sarcomere length to be on the green line. The Scope function also provides a means of reviewing the past history of the sarcomere length because it stores up to 2 hours of SL data in a buffer which can be reviewed by using the controls provided on the Scope window. After 2 hours the oldest data will be overwritten. The scope window also has a numerical output in the top right corner of the graph that shows the sarcomere length at the cursor location. This readout is useful to see what the exact sarcomere length was at any given time. Click on Open on the Scope menu to open the Scope window. In addition to buffering up to 2 hours of data Scope can also be used to record data at sample rates from 1 to 1000 samples per second. See chapter 5.0 for a more detailed description of Scope.



**Figure 6 HVSL Scope Window**

- 14) An Analysis window is available that allows SL and Velocity data to be viewed. Filters are available to smooth the data. Zoom and scroll controls are also provided. SL data collected with the Record menu, calculated through the Playback menu or recorded with Scope can be viewed in Analysis. Data from the Analysis window can also be exported to an ASCII text file.
- 15) The HVSL 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.
- 16) 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

The HVSL 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 and minimized but not re-sized. In the upper right corner of the window are buttons for minimizing and closing the window. Clicking anywhere on the window will bring the focus to the window. Most of these controls are similar to those found on Windows or Apple operating systems.

### Program Start Switches

HVSL can be started with various switches that enable different features of the program. Normally these settings will not need to change from the default however they are discussed in Appendix F – Program Start Switches.

## 4.1 Main Screen

The main screen consists of a menu bar at the top, a 640x480 pixel video image area located in the centre, a graph showing the results of the sarcomere calculation beneath the image, three text readouts and five control buttons located below the graphics window (see Figure 7).

### 4.1.1 Video Image

The video image portion of the main screen displays the current live video frame from the camera or, if using the Playback function, the current playback frame. The video image includes a region of interest (ROI) box that can be set by the user, two labeled reference length rulers, one in black and the other in white and the size and location of the ROI either in pixels or microns. The video image is continually updated and runs at a frame rate dependent on the size of the Camera ROI. The actual frame rate is shown at the bottom of the screen in the FPS (frames per second) text readout.

### 4.1.2 Sizing and Moving the Region of Interest

A region of interest (ROI) box can be set by the user within the video image area. The program calculates the sarcomere length only within this ROI box. The ROI can be set to the full screen by pressing the Reset ROI button located at the bottom of the window. To choose an ROI that is smaller than the full screen, position the cursor at the desired starting point for the ROI, click and hold the left mouse button and drag out the ROI box until it is the desired size. The size of the ROI is displayed in the top right corner of the video image and changes as you drag out the ROI box. The size readout can be set to display size in pixels or in microns ( $\mu\text{m}$ ).

Once an ROI box has been defined the user can reposition the box to any location within the image. This is accomplished by simply using the up, down, left and right arrow keys to navigate around the image. Alternatively the user can right click and drag the ROI box around the image, the location is set when the right mouse button is released.



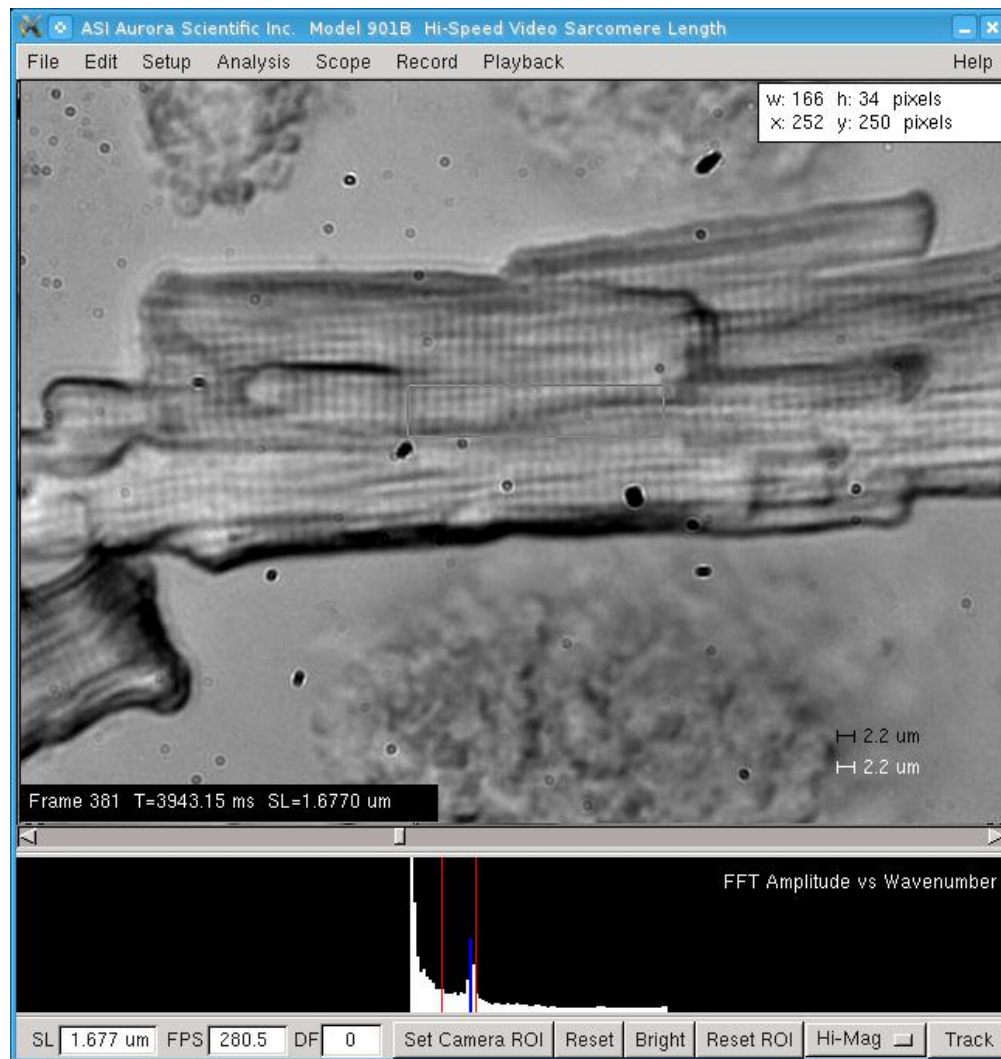
If the Num Lock key is on, the right mouse button will create a new ROI instead of moving the existing ROI.



The sarcomere length calculation is performed only within the ROI. The resolution of the SL measurement is affected by the number of sarcomere bands within the ROI, therefore to obtain the best resolution ensure that the ROI extends over a large horizontal distance. Increasing the vertical size of the ROI will increase the amount of averaging and produce more accurate and quieter signals.



Normally the ROI should be chosen to stay within the sarcomere pattern and to exclude attachments and areas with no tissue in them. There is no advantage to having the program calculate SL on a portion of the image that doesn't contain sarcomeres.

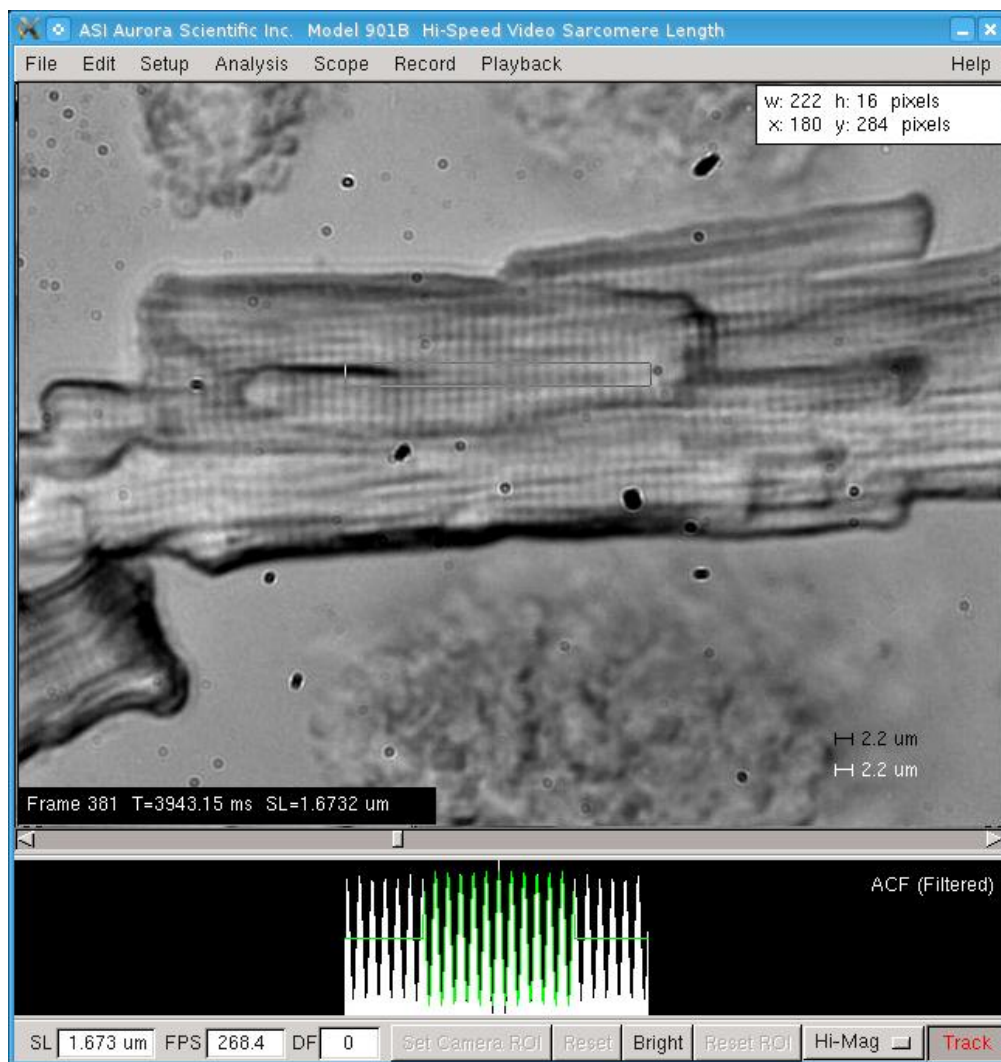


**Figure 7 Graph of FFT Amplitude vs Wavenumber**

### 4.1.3 Graph of Calculation Results

The main screen includes a graph beneath the video image that displays the results of the sarcomere length calculation. There are four graphs available, ACF (Filtered), ACF (Unfiltered), FFT Amplitude vs. SL and FFT Amplitude vs. Wavenumber. The specific graph can be selected by either clicking on the graph itself; this action causes the display to cycle through the available graphs, or by picking the desired graph using the controls found on the Setup window.

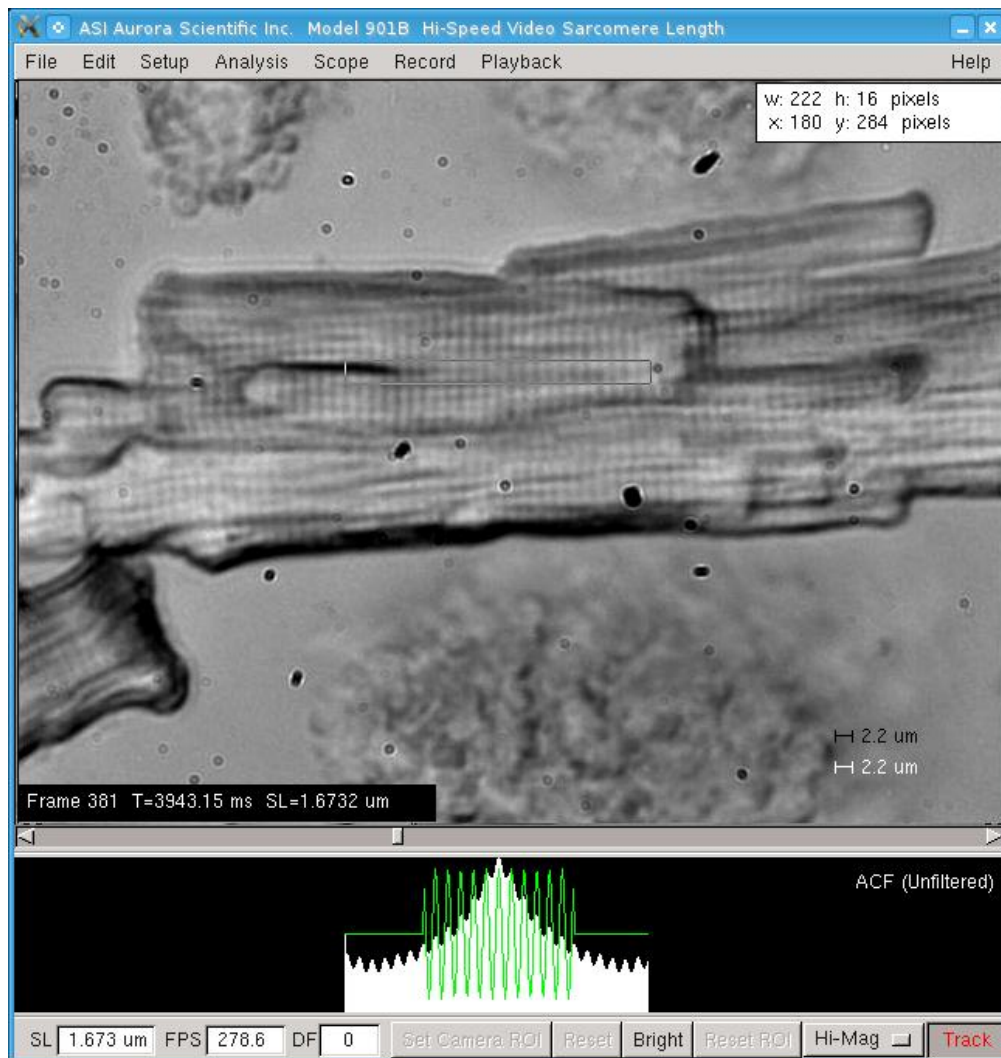
The four different graphs are presented in Figure 8 through Figure 11. These graphs are useful for checking on the accuracy of the sarcomere length calculation. Note the graphs only cover the horizontal extent of the currently defined region of interest. No calculations are made outside of the ROI so nothing is shown in the graph area for these regions. See chapter 7.0 Measurement Algorithms for a detailed explanation of the calculation algorithms.



**Figure 8 Graph of ACF (Filtered)**

The ACF filtered and unfiltered graphs (Figure 8 and Figure 9) show the autocorrelation function across the ROI in black and white and the sine fit to the ACF in green. These graphs should be examined for significant differences between the ACF and the sine fit which could indicate problems with the ROI size or position or problems with the calibration constant. Also the wavelength of the sine fit should be equal to the observed sarcomere spacing on the image. Note that the program defaults to calculating the sine fit only for the central 50% of the ACF. There is virtually no improvement in measurement accuracy for sine fits over more than 50% of the ACF. This is why the green sine wave is only fit to the central portion of the ACF in Figure 8. Calculating more than 50% of the ACF only slows the program down with no measurable improvement in SL accuracy. In many cases an accurate SL can be determined with the sine fit

set to 30% of the ACF. The amount of the ACF function that is fit with the sine wave is set using the control found on the Setup window.

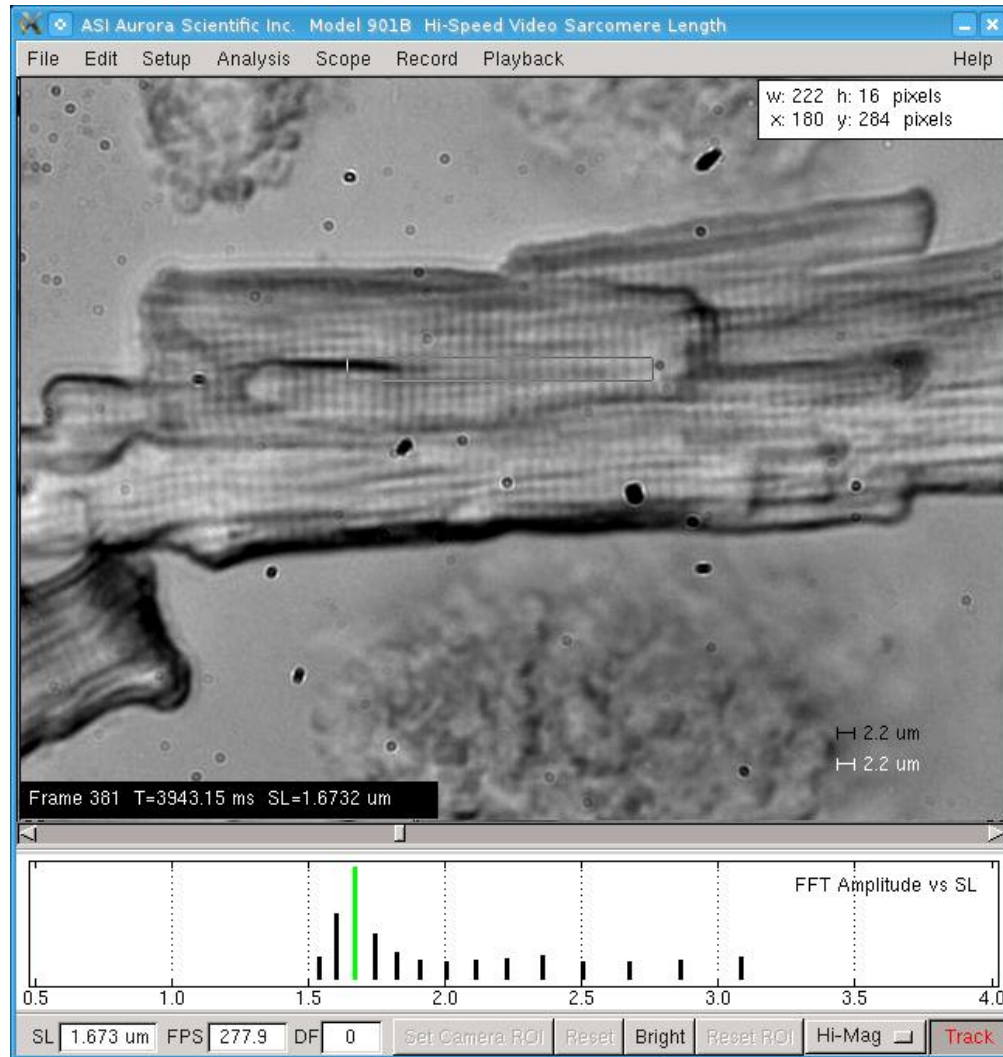


**Figure 9 Graph of ACF (Unfiltered)**

Figure 9 shows the unfiltered ACF function and the green sine fit to the function. The sine fit is more obvious in this figure due to the nature of the unfiltered signal. Note an autocorrelation function is a symmetric function and this symmetry can clearly be seen in Figure 9.

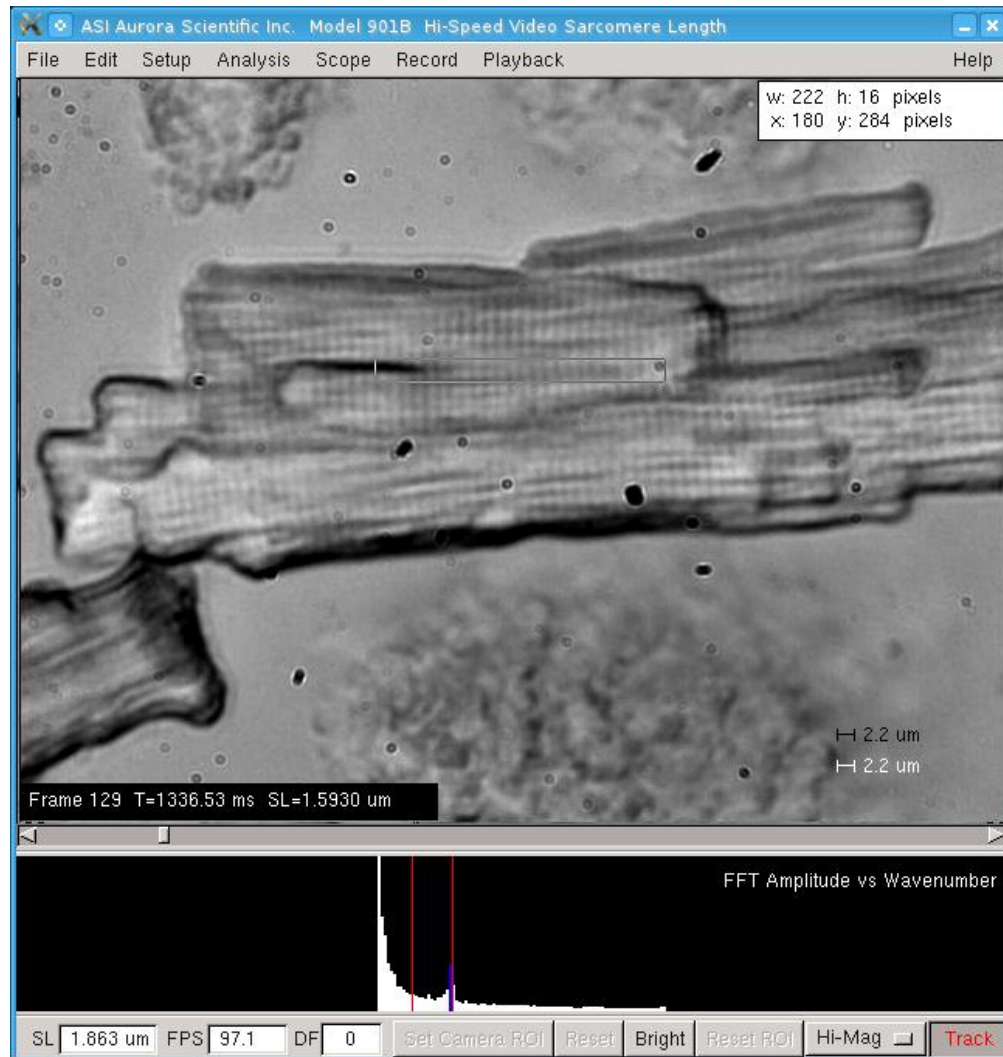
The FFT Amplitude versus SL and versus Wavenumber graphs (Figure 10 and Figure 11) show the FFT amplitude spectrum calculated across the ROI. The FFT Amplitude versus SL graph shows the peaks of the amplitude spectrum that occur only in the SL length range between the values set by the Minimum SL and Maximum SL parameters. The black vertical bars on the FFT versus SL graph show the relative amplitude at each sarcomere length. The blue line denotes the peak wavenumber/SL determined from the FFT spectrum. The green line is the wavenumber/SL value that results from a curve fit to the FFT spectrum. By fitting a curve to the FFT spectrum and then calculating the peak of the curve we can improve the sarcomere length resolution. Without this curve fit the FFT resolution is related to the number of cycles present in the ROI. More cycles in the ROI produces a finer FFT spectrum and a more accurate SL

measurement. Likewise fewer sarcomeres in the image will result in a coarser FFT spectrum. Note that the FFT function can't calculate values between the frequency bins which are represented by the vertical black bars seen on the FFT Amplitude vs SL graph.



**Figure 10 Graph of FFT Amplitude vs SL**

The FFT Amplitude vs Wavenumber graph (Figure 11) shows the entire FFT spectrum across all wave numbers (Wavenumber is the inverse of wavelength (SL)). The vertical red bars represent the Maximum and Minimum SL values that have been entered on the Setup window. You will often notice peaks outside of the red bars. Some of these are due to the pixel spacing, some due to other repetitive features in the image and some are harmonics of the sarcomere length. A large peak just outside of the red bars could be the true sarcomere length if the true SL value is outside of the range set by the maximum and minimum SL values set in the Setup menu. Another reason for a strong peak being outside of the maximum/minimum SL range is because of an error in calibrating the system.



**Figure 11 Graph of FFT Amplitude vs Wavenumber**

#### 4.1.4 Control Buttons

The main screen includes five control buttons located beneath the graphics window. These buttons are: Set Camera ROI, Reset, Bright, Reset ROI and Hi-Mag/Lo-Mag. (see Figure 11).

##### Set Camera ROI Button

The Set Camera ROI allows the camera's region of interest (ROI) to be changed. Reducing the camera ROI causes the camera to send data only from the camera ROI. This reduces the amount of data per frame coming from the camera which allows the camera frame rate to be increased. This allows the frame rate to increase by up to approximately a factor of 10 compared to a full frame image when the ROI is reduced sufficiently. For information specific to your model see the Camera heading in Section 2.0 Specifications.

To adjust the camera ROI first position the cursor at a point in the image that you would like to include in the reduced ROI. Hold the left mouse button down and drag out an ROI box with the desired horizontal and vertical extent then let go of the mouse button. Once the ROI is set simply press the Set Camera ROI button and the camera image will be windowed down to the

size of the ROI. Note that no data will come from regions outside of the camera ROI and therefore everything outside of the camera ROI will be black. Note that the frame rate increases with decreasing ROI. Since the horizontal extent of the camera ROI doesn't affect the frame rate we suggest you select a camera ROI that is the full width of the frame (640 pixels). After the camera ROI is set you can then use the calculation ROI windowing function to select a calculation region of interest within the camera region of interest. It is best if the calculation ROI only includes visible sarcomeres and not areas without visible sarcomeres, such as tendon, attachments and the like, or areas outside of the tissue boundaries.

### **Reset Button**

The Reset button resets the camera to the maximum size of 640x480 pixels.

### **Bright Button**

The Bright button adjusts the gain on the video amplifiers in the camera to produce a visible image. As the camera ROI is reduced the shutter is open for a shorter time which results in a darker image. The brightness function compensates for this by increasing the video gain. Whenever the camera ROI is changed the bright function is executed. Under some circumstances you may need to execute the Bright function a second time, in this case simply press the Bright button. Note the brightness can also be adjusted manually using the control found on the Setup window. If the image remains dark after repeated presses of the Bright button then the microscope illumination must be increased. Note: if the image is completely white then this indicates the video gain is too high. For this case pressing the Bright button will reduce the gain and produce a visible image.

### **Reset ROI Button**

The Reset ROI button sets the calculation region of interest to the full size of the Camera ROI. Sarcomere calculations are only made within the ROI. Improvements to the calculation algorithm in version 2.0 of the program now mean that the program can calculate SL at the maximum frame rate of the camera even with the ROI set to the full frame size. Choose the calculation ROI to include the area of interest where you want to calculate SL. The algorithm works best when the calculation ROI only includes areas of the image that contain a sarcomere pattern.

### **Hi-Mag/Lo-Mag Button**

The Hi-Mag/Lo-Mag button is used to toggle between Hi and Lo magnification calibration constants. In most cases the researcher will use the low magnification setting for making overall measurements of the tissue and the high magnification setting for measuring SL. Once the image has been calibrated for two different magnifications you can simple switch between the two calibration constants by pressing the Hi-Mag/Lo-Mag button.



The Hi-Mag/Lo-Mag button doesn't change the actual magnification of the image it only changes the calibration constant in use by the program. You must use the microscope to set the actual optical magnification.

Although the program is capable of calculating SL at low optical magnification the results are not as accurate. We strongly suggest you use an optical magnification that provides a minimum of 4 pixels per micron for the calibration constant. For most inverted microscopes this means using a 40X objective for imaging/measuring SL and a lower magnification (10X or 20X) objective when measuring tissue dimensions.

## **Tracking**

Tracking dynamically changes the size and location of the calculation ROI to keep a defined group of sarcomeres in the ROI. By performing the SL calculation on the same sarcomeres throughout an experiment the consistency, accuracy and stability of the SL measurements is ensured. When Tracking is enabled you will see the ROI move with the tissue as it moves, you will also see the ROI box stretching and compressing as the SL changes. With Tracking turned off the ROI is fixed within the image window. For this case when the tissue translates the sarcomeres move through the ROI and when the sarcomere length changes the number of sarcomeres within the ROI changes.

### **4.1.5 Text Readouts**

Three text readouts are located near the bottom of the main screen. The first, labeled SL, is the sarcomere length of the most recently obtained image. This sarcomere length is obtained using the calculation method specified on the Setup screen in the box labeled SL Display.

The second readout, labeled FPS, is the current frame rate which is the actual number of frames per second being processed by the camera.

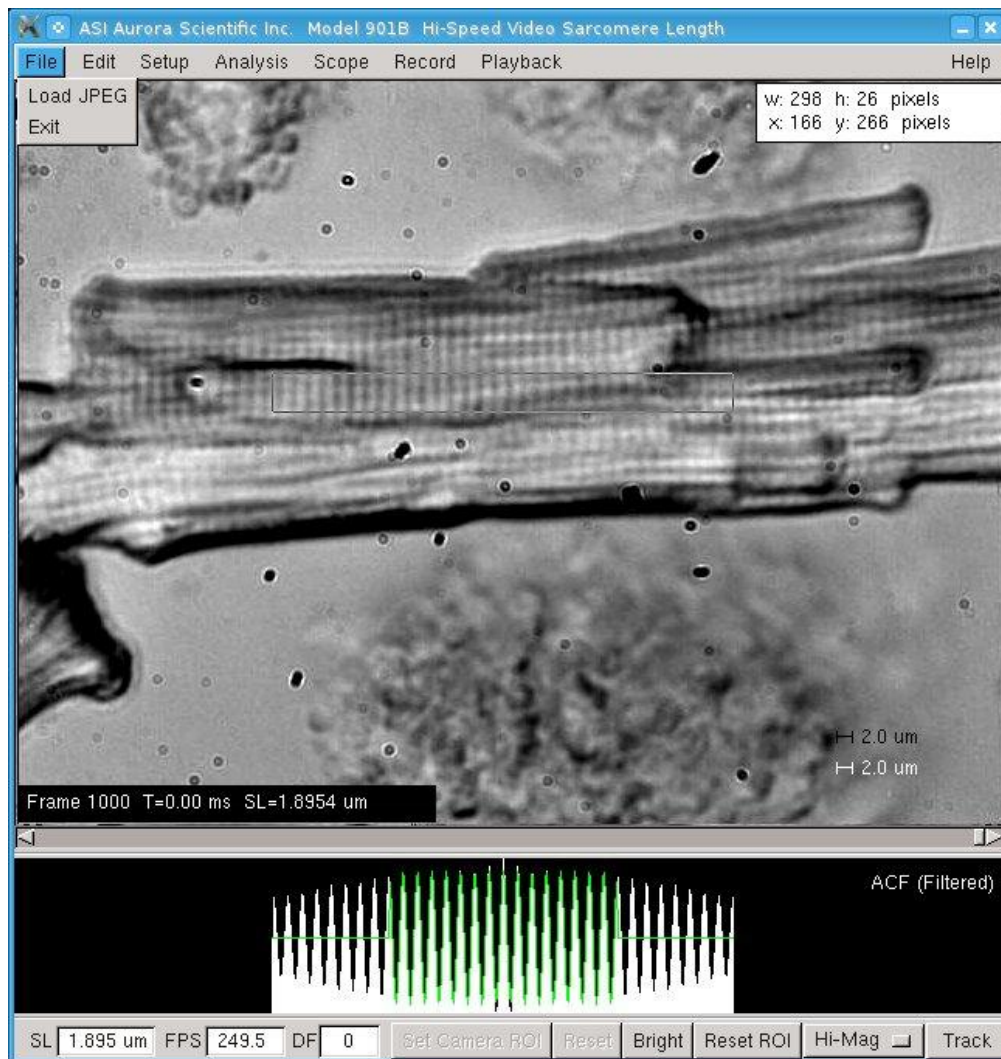
The third text readout, labeled DF, shows the number of dropped frames. Dropped frames occur when there is a timing issue between the program and the camera. In most cases the number of dropped frames should be quite small compared with the total number of frames processed.

## **4.2 Menu Structure**

The main menu consists of the following headings.

**File Edit Setup Analysis Scope Record Playback Help**

The rest of this chapter provides details for the File, Edit, Setup, Record, Playback and Help menus. Details of the Analysis and Scope functions are presented in chapter 5.0 Analysis and chapter 6.0 Scope.



**Figure 12 File Drop Down Menu**

### 4.2.1 File Menu

The File menu contains commands to Load a JPEG image and to Exit the program, See Figure 12.

#### Load JPEG – Still Version Only

Opens a data file dialog box that allows the user to select a JPEG file to read into the program.

#### Exit

The Exit menu item closes the VSL/HVSL program.

### 4.2.2 Edit Menu

The Edit menu contains commands to control what is seen on the main screen and to change the zoom settings of the displayed image (see Figure 13).



### **Display Controls**

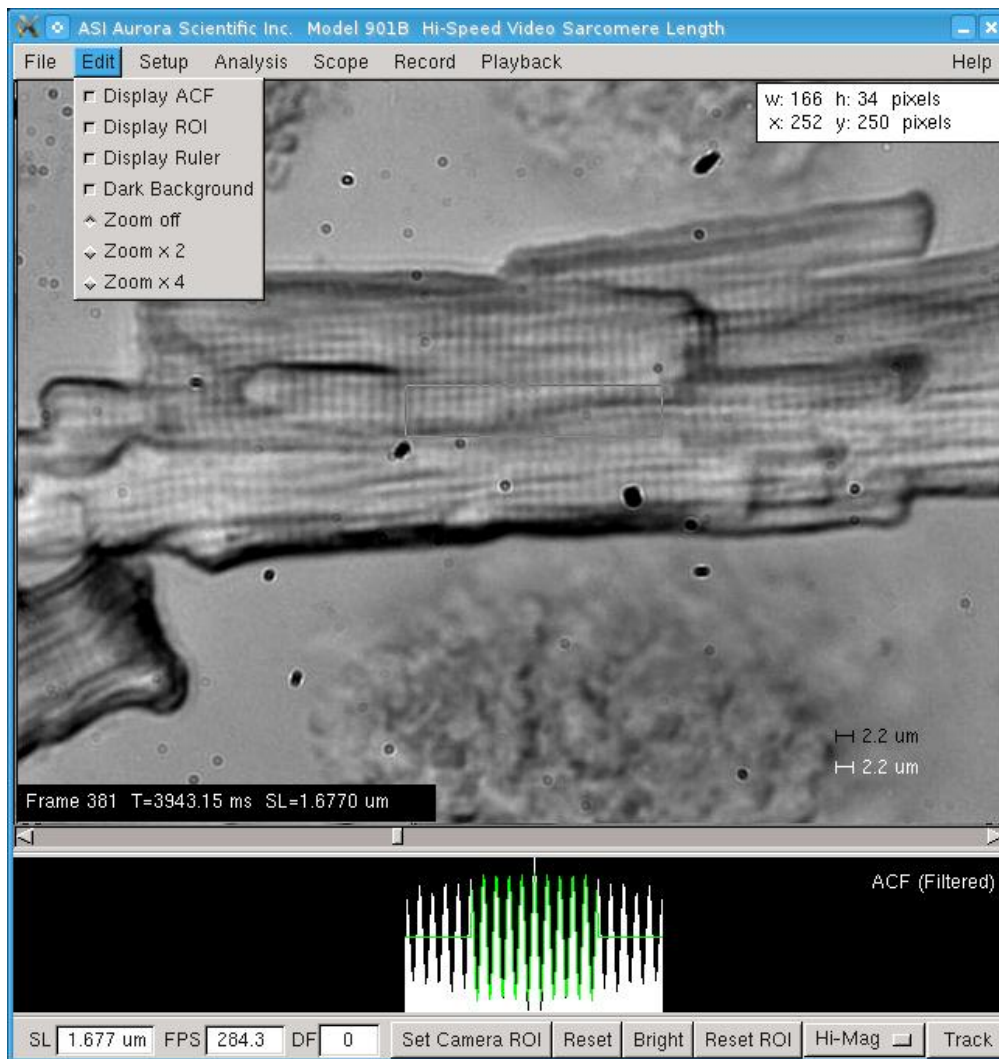
Three display controls are provided: Display ACF, Display ROI and Display Ruler. The Display ACF control turns the graph at the bottom of the main window on and off. The Display ROI control changes the display so that only the image within the ROI gets updated the rest of the image is static. The Display Ruler control toggles the display of the ruler at the bottom right corner of the image window. The length of the ruler is set on the setup window.

### **Zoom Controls**

Three zoom controls are provided that allow the image to be viewed full size (Zoom off), magnified by a factor of 2 (Zoom x 2) and magnified by a factor of 4 (Zoom x 4). The zoom function is a software control only; no settings are changed on the camera. When the image is zoomed a small image of the full frame appears in the upper left corner of the image window and a box appears in this image that shows the size and location of the currently displayed image. The left mouse button can be used to move the zoomed image around within the full image.

### **Dark Background**

A dark theme has been included for all plots. When the dark theme is activated it replaces the black lines on white background, of the original light theme, with green lines on a black background. This setting allows for more options when displaying your data.



**Figure 13 Edit Drop Down Menu**

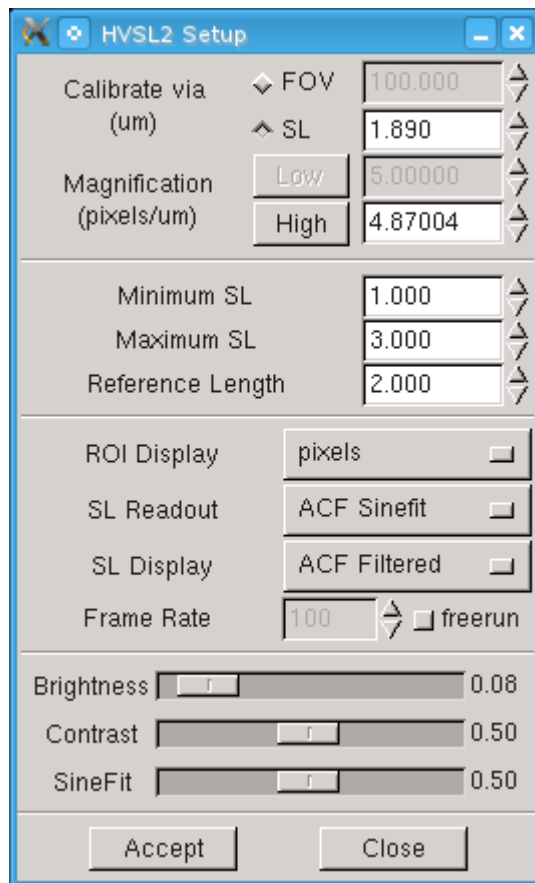
### 4.2.3 Setup Menu

The Setup menu control opens the Setup window. The Setup window contains controls to calibrate the image, to set display and calculation parameters and to control camera settings (see Figure 14).

#### Calibrate via FOV

Clicking the diamond next to the Calibrate via FOV sets the calibration method to Field of View (FOV). This calibration method assumes that you know the exact horizontal distance between two points in the image. Use the mouse to position the cursor at one of the known points and press the left mouse button. While keeping the mouse button depressed drag out a region of interest (ROI) box that extends horizontally to the second known point. Since the calibration calculation is done on the horizontal distance only, the vertical extent of the ROI box doesn't matter. It is good practice to ensure that the vertical ROI box is at least 20 pixels high. A larger horizontal distance between the two points produces a more accurate calibration. Enter the known horizontal distance between the two points in the box to the right of Calibration via FOV (um). Press the High or Low Magnification (pixels/um) button on the setup screen for the magnification currently set. The program will calculate the number of pixels/um in the image

and display this value in the text box to the right of the button. Press the Accept button to complete the calibration.



**Figure 14 HVSL Setup Menu**

### Calibrate via SL

The Calibrate via SL method assumes that there is an image present that contains a repeating pattern of a known spacing (examples of this would be a calibration grating with known line spacing or an image of a sarcomere pattern with known spacing). The program calculates the pattern spacing and uses the entered length to calibrate the system. Using the mouse position the cursor at a point within the repeating pattern. Press the left mouse button and, while holding the button down, drag out a region of interest (ROI) box that extends horizontally and vertically across a large portion of the pattern. The most accurate results will be obtained with the largest ROI that remains fully within the pattern. Click on the diamond shaped button to the left of the Calibrate via SL label. Enter the known pattern spacing (length) in the text box to the right of the label. If the pattern spacing is outside of the range set by Minimum SL (um) and Maximum SL (um) then change these values to contain the pattern spacing value, i.e., if the pattern spacing is 10 um then the Minimum SL (um) should be set to about 8 and the Maximum to about 12. Set the Reference Length (um) to be equal to the pattern spacing. Press the appropriate High or Low Magnification (pixels/um) button on the setup screen depending on the current setting of the Hi-Mag/Lo-Mag button. Note that only the button that corresponds to the current magnification setting will be visible, the other button will be “greyed” out. The program will calculate the number of pixels/um in the image and display this value in the text

box to the right of the button. The program will also place a labeled reference bar in the lower right corner of the image.

The Calibrate via SL method is iterative and the researcher will be required to press the High or Low Magnification (pixels/um) button several times before the solution converges. A simple method of checking for convergence is to press the High or Low Magnification (pixels/um) button several times while noting the value displayed in the text box to the right of the button. When the numbers are no longer changing the correct solution is usually found. However under some circumstances the program can converge to the wrong solution. A simple way of checking for this is to compare the length of the reference bar with the pattern spacing. If the bar is not the same length as the distance between the patterns then the program has converged to an incorrect solution. In this case you may need to manually enter a value in the text box beside the High or Low Magnification (pixels/um) button and then press the button several times until the solution converges. By entering a value you are essentially providing the program with a starting point. If you select a starting point much higher than the true value then the program may converge to a higher solution, if you choose a starting point much lower than the true value the program may converge to a lower solution. A few tries and some experience will usually allow you to converge to the correct solution within a few button presses. Ensure that you compare the length of the Reference Length ruler on the image with the actual spacing. This provides a simple check on the accuracy of the solution. Press the Accept button to complete the calibration.



Many of the settings on the Setup window can be modified using the keyboard arrow keys. Left and right arrows can be used to move sliders like Brightness. Up and down arrows can be used to modify text boxes that have spin buttons. Click on the setting of interest and then use the arrow keys.

### **Magnification (pixels/ $\mu\text{m}$ )**

The two buttons labeled High and Low represent the calibration constants for each magnification. The purpose of having two magnification calibration constants is to allow the researcher to use a low magnification value when looking at the entire fibre and measuring the tissue dimensions, and then switch to a high magnification constant when measuring sarcomere length. These two buttons and their accompanying text boxes are used to calibrate the program when the image is at high/low magnification. The calibration constant can only be modified for the magnification that is currently in use; this will be the active text box while the unused magnification setting will be inactive and greyed out. To change magnifications, click the Hi-Mag/Lo-Mag button in the bottom-right corner of the main HVSL window. This will open a drop down menu to select either high or low magnification.

If the calibration constant is known (from a previous experiment for example) then the calibration value can be entered in the active text box to the right of the button. Alternatively the calibration constant can be found by selecting one of the two calibration methods (see Calibration via Length and Calibration via FOV above).



The calibration constants depend only on the optical characteristics of the microscope. Once you have determined the high and low magnification calibration constants they will remain the same as long as no changes are made to the objective or the microscope.

### **Minimum SL ( $\mu\text{m}$ )**

The minimum SL should be set to a value less than the minimum physiological length expected. This value provides a lower bound to the calculation algorithm.

### Maximum SL ( $\mu\text{m}$ )

The maximum SL should be set to a value greater than the maximum physiological length expected. This value provides an upper bound to the calculation algorithm.

### Reference Length ( $\mu\text{m}$ )

The Reference Length value is displayed as a ruler on the video image and is also displayed as a green line on the Scope graph. In most cases the researcher will set the Reference Length to be the relaxed SL or starting SL.

### Frame Rate (fps)

The camera can operate in free run or fixed frame rate modes. The Frame Rate textbox and radio button control the mode and the frame rate. The radio button allows the user to switch the camera between free run and fixed frame rate mode; the label to the right of the button will change from fixed to free run to reflect the current mode.

In free run mode the camera operates at the highest possible frame rate which is dependent on the camera ROI. When in free run mode, the text box is disabled because the camera determines the frame rate. Fixed mode allows the camera to run at a user selected rate that must be less than the free run rate. For example, assume that due to the Camera ROI settings the current frame rate is 537 fps, the user can switch the mode to fixed rate and specify the camera rate at 500 fps. The user cannot specify a frame rate greater than the 537 fps free run rate.

### ROI Display

Two options are available for the ROI display, pixels and micrometers. This control sets the units displayed for the size of the ROI. The size of the ROI is displayed in the upper right corner of the video image. The coordinates of the bottom left hand corner of the ROI are also shown in the ROI Display; these coordinates are always shown in pixels.

### SL Readout

Two options are available for the SL Readout; ACF Sinefit and FFT. This control sets the calculation algorithm that is used to calculate the sarcomere length. See Section 7.0 Measurement Algorithms for a discussion of the two algorithms.

The ACF Sinefit method calculates sarcomere length by calculating an autocorrelation function on each line of pixels in the calculation region of interest. An average ACF is then calculated and a sine wave is fit to this result. The wavelength of this sine fit is the sarcomere length.

The FFT method calculates a fast Fourier transform (FFT) of each line of pixels and then calculates an average FFT. A curve is fit to the FFT spectrum in order to calculate the peak of the spectrum. This peak value is then used to calculate the sarcomere length.



The ACF Sinefit method is significantly faster and more accurate than the FFT method and we suggest leaving this control set to ACF Sinefit at all times.

### SL Display

The SL display control sets the type of graph displayed on the main screen underneath the image area. Four options are available, FFT vs Wavenumber, FFT vs SL, ACF (Unfiltered) and ACF (Filtered). The type of graph displayed can also be controlled from the main window by repeatedly left clicking on the graph. Each click cycles the graph type.

## Brightness

This Brightness slider alters the image brightness. When connected to a camera the brightness slider controls the gain on the video amplifier inside the camera. When the camera ROI is set to smaller regions of interest the camera runs faster and the shutter time is decreased. This results in the image getting darker. To compensate for this the gain on the video amplifier can be increased using the Brightness control.

When the program is used in still mode or when used to play back stored images then the brightness slider alters the brightness of the image in software. For still images a setting of 0.50 corresponds to no software control of brightness. Numbers less than 0.50 will darken the image while values greater than 0.50 will lighten the image.

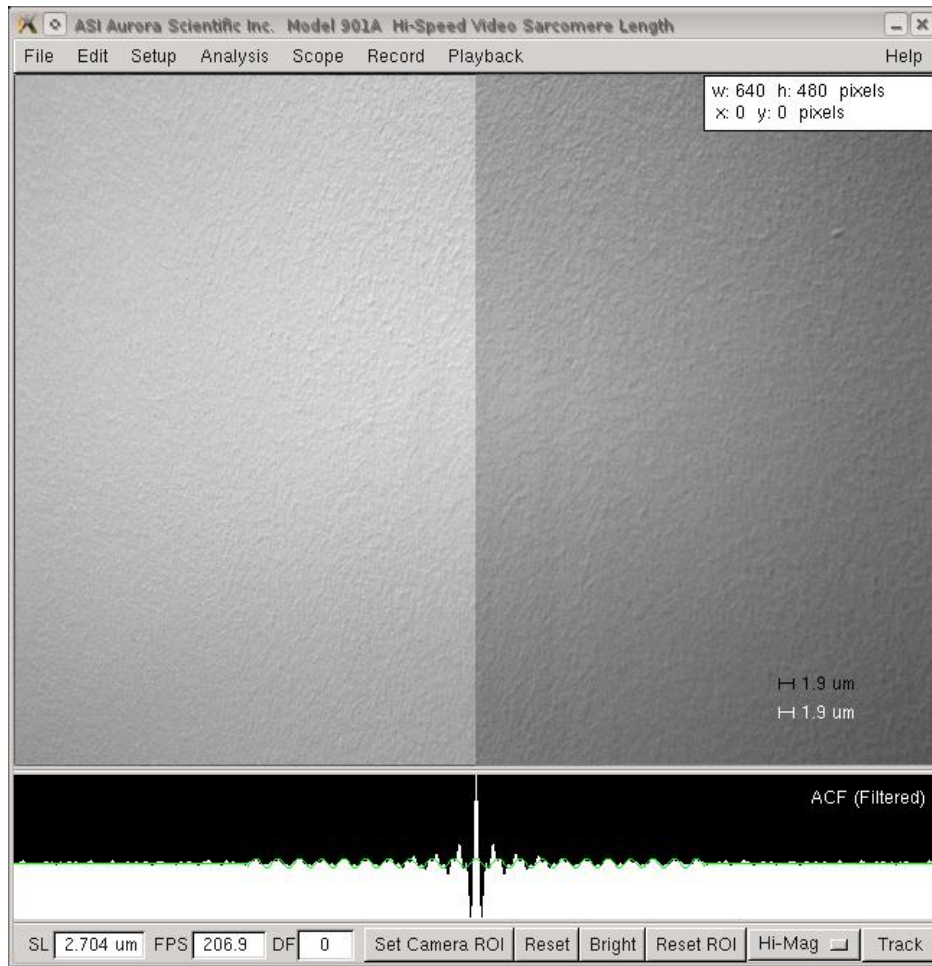
## Contrast

The Contrast slider alters the image contrast.

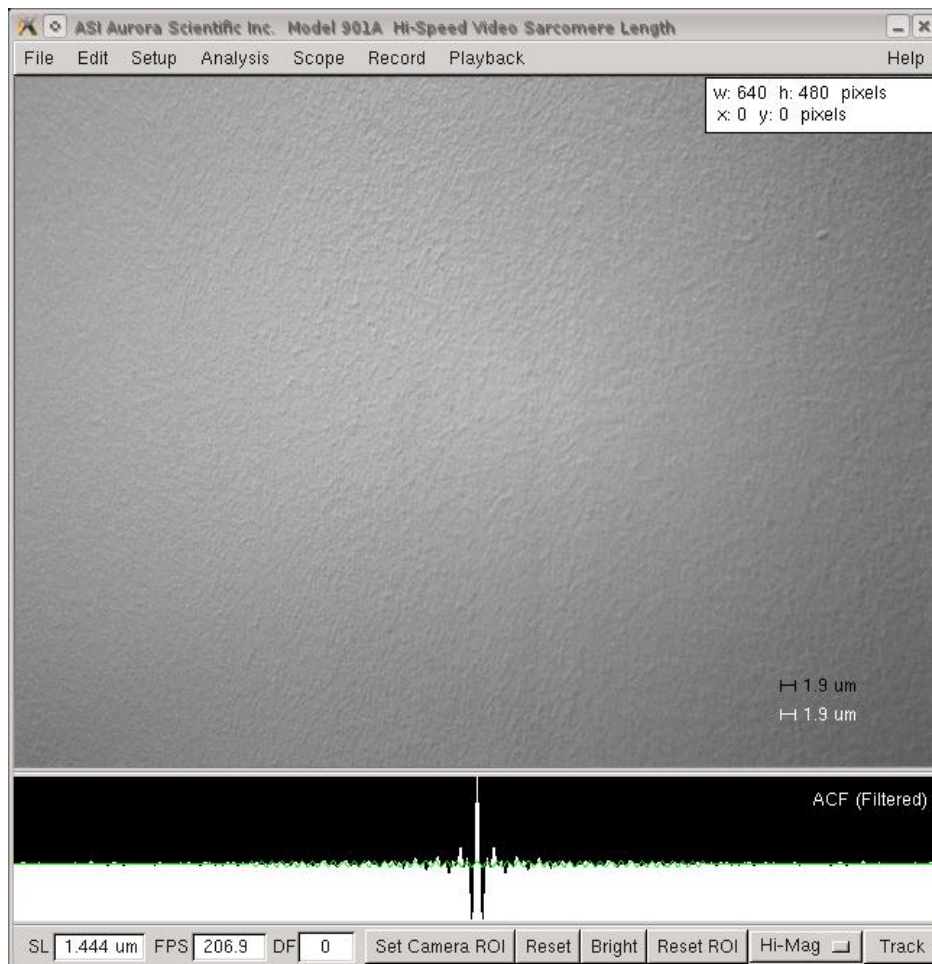
**901A**

### L-Offset/R-Offset

The HVSL camera contains two video amplifiers one for the left side of the image and the other for the right side. In some cases one side of the image can appear brighter than the other. In this case use the Offset sliders to adjust the background brightness of each side of the image. There is a separate slider for adjusting the brightness offset for each side of the image called L-Offset and R-Offset. See Figure 15 for an example of an image where the offset requires adjustment. Figure 16 is the same image after offset adjustment. The slider can be moved with the mouse or using the right and left arrow keys. Note the arrow keys only work after you have clicked on the slider bar.



**Figure 15 Camera Image Showing Poorly Adjusted Offset**

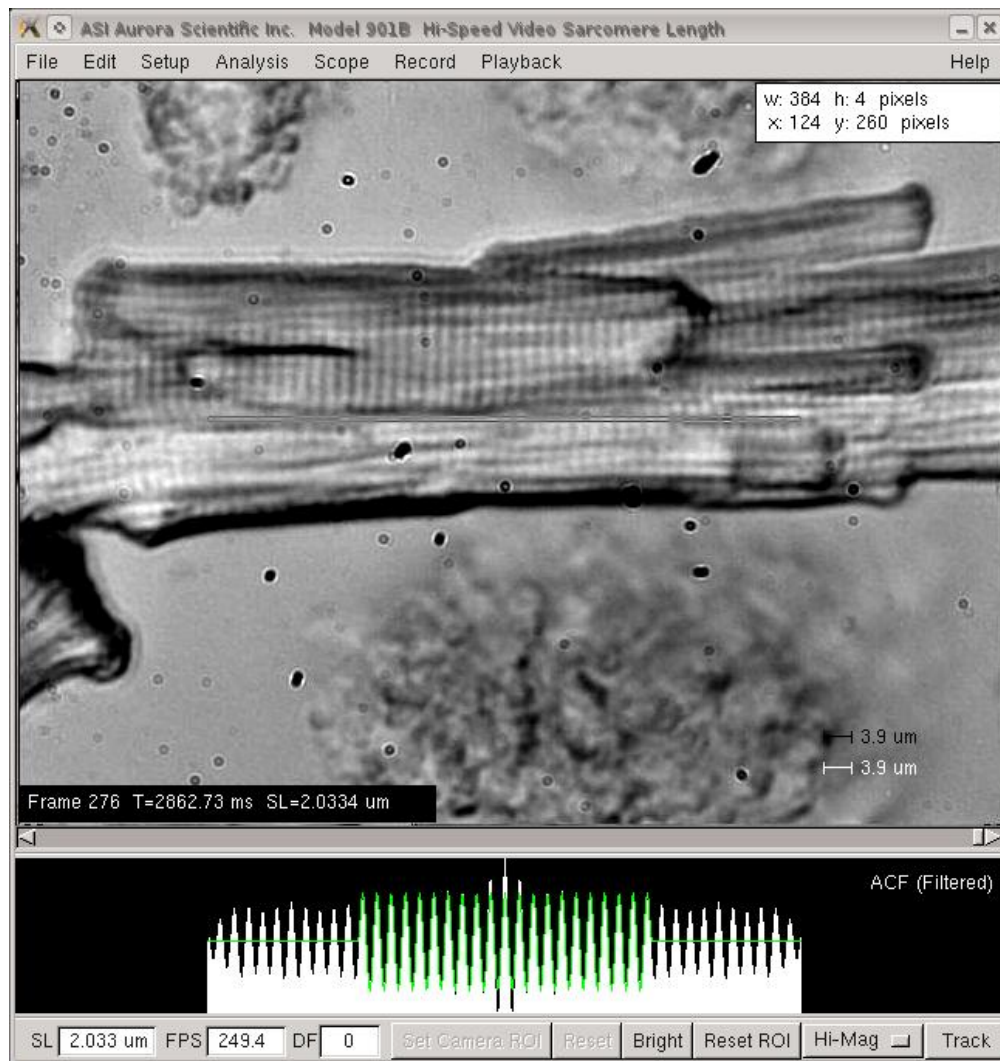


**Figure 16 Camera Image Showing Correctly Adjusted Offset**

### SineFit

The SineFit control sets the number of cycles of the ACF function that the sine wave will be fit to. Decreasing the SineFit value will speed computation with a loss of accuracy. Likewise increasing the SineFit value will slow calculation speed with some improvement in accuracy. A value in the range of 0.25 to 0.50 will normally produce the optimum combination of speed and accuracy. Movement of the SineFit slider to the right increases the effect. The effect of the SineFit slider can easily be seen if the ACF (filtered) graph is shown in the bottom panel of the main window. See Figure 17 and Figure 18 for comparison of the effects of the SineFit slider set to 0.50 and to 0.25 respectively.





**Figure 17 SineFit Control Set to 0.50**

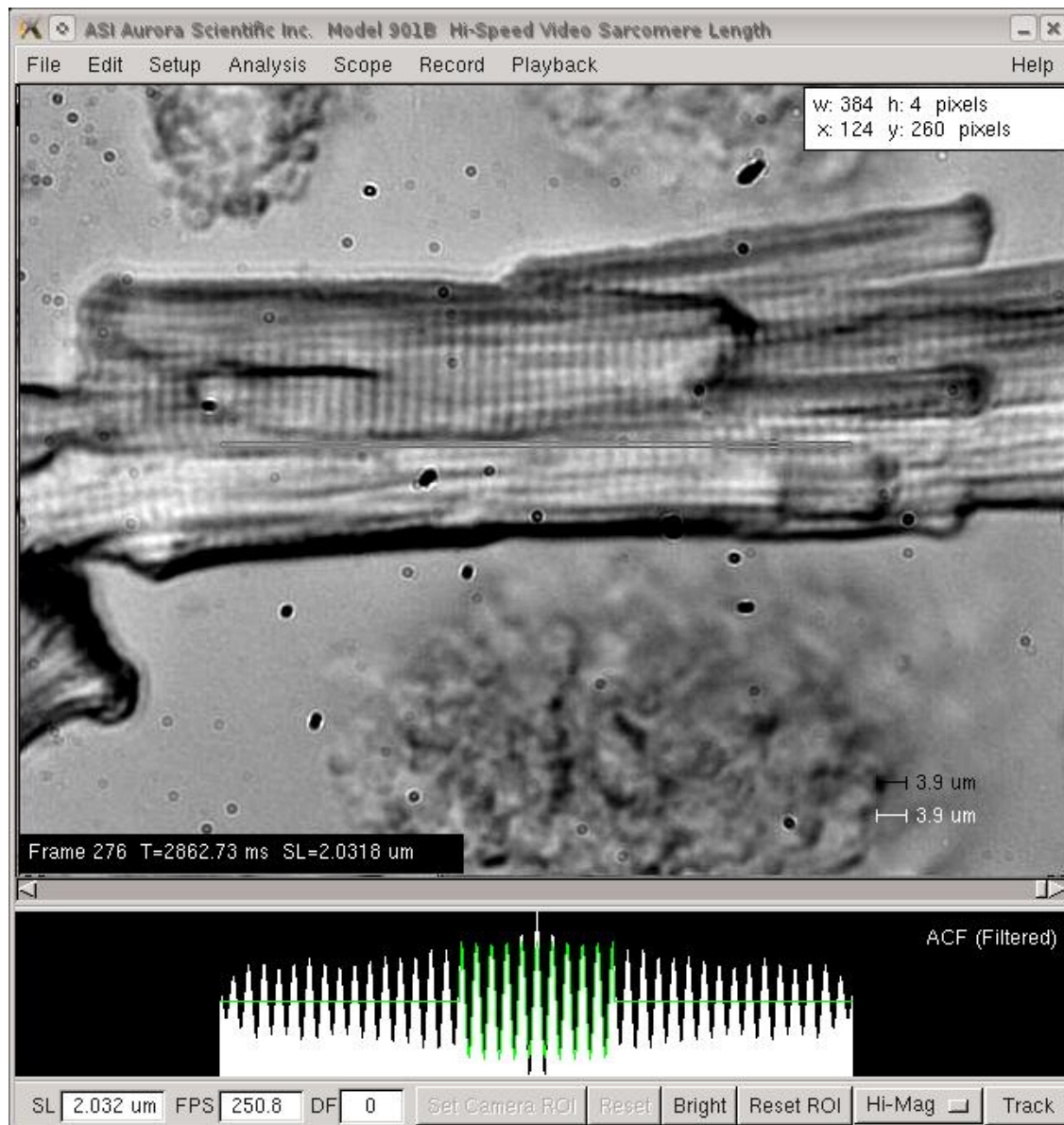


Figure 18 SineFit Control Set to 0.25

### 901A Factory Button

This button restores the camera to the factory settings.

### Accept Button

Accepts changes made on the Setup menu and updates the program. For example if the reference length is changed on the Setup window this value will not be updated on the main screen until either the Accept button is pressed or the setup menu is closed.

### Close Button

Closes the Setup menu window.

#### 4.2.4 600A Menu

The 600A menu allows communication to be established between the HVSL program and the ASI model 600A Digital Controller program. Once communication is established with the 600A calculated sarcomere length data from HVSL will be stored in the 600A data files along with tissue length and force data.

Data is transferred between the two programs with no delay. Thus 600A can time stamp each SL measurement with the time that it is received. Note: this program link does not transfer image data between the two programs. All image data is stored and manipulated using the HVSL program.

600A includes protocol commands that can be used to trigger HVSL to store images and/or SL data. Thus a researcher can use 600A to control the entire experiment including the control of tissue overall length, force and sarcomere length.

#### 4.2.5 Analysis Menu

The Analysis menu contains two commands that open and close the Analysis window. Further details of Analysis are presented in chapter 5.0 Analysis.

#### 4.2.6 Scope Menu

The Scope menu contains two commands that open and close the Scope window. Further details of Scope are presented in chapter 6.0 Scope.

#### 4.2.7 Record Menu

The Record menu item opens the VSL Record window as shown in Figure 19. This window allows the user to record images and/or SL data to disk. The rate at which the data is recorded is set in the Setup menu.

The data file directory (Directory) and name (Trial Name) are set using the two text boxes at the top of the window. The directory can be entered manually or the Browse button can be used to open a file explorer and then the desired directory can be found.

The Duration of the trial is set either in terms of Time or Frames. Clicking the radio button to the left of the desired option sets that text box active and disables the other textbox. By default whenever the Record menu is opened the Time textbox will be active. Enter the desired time or number of frames in the active textbox.

The Record box includes radio buttons to control what is saved to disk with the options being; SL data, Images or Both. The default setting is Both.

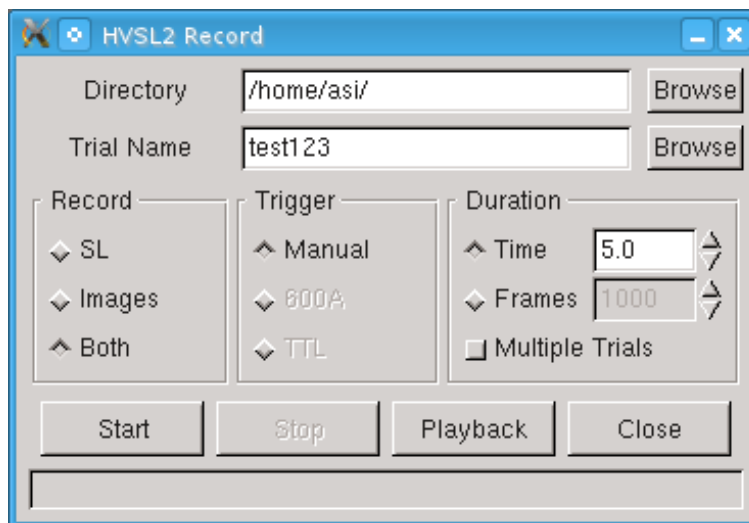
The Trigger box determines the type of trigger that will start the recording and its source. The square button next to the Trigger word toggles the trigger mode to be either single or multiple trigger. In single trigger mode only a single trial is stored regardless of the number of trigger signals received by the program. In multiple trigger mode the program stores data for each trigger it receives and automatically generates file names for each individual trigger.

The trigger source can be set to Manual, 600A or TTL. In Figure 19 you will note that both 600A and TTL are greyed out meaning they are not available. This is because the available triggers depend on the switches used when starting the program. Manual trigger is the default and it is always available. Starting the program with the *-rt* switch will enable real time piping of data to 600A and also allows triggering of HVSL by the 600A program. When this switch is

set the 600A trigger source will become un-greyed. Starting the program with the `-ttl` switch will enable the TTL trigger source. An optional TTL trigger module can be purchased from ASI if you intend to use this type of trigger.



VSL can also be started with both switches active (i.e., `-rt -ttl`).



**Figure 19 HVSL Record Window with Duration set by Time**

Below the setup part of the Record window are the action buttons Start, Stop, Playback and Close.

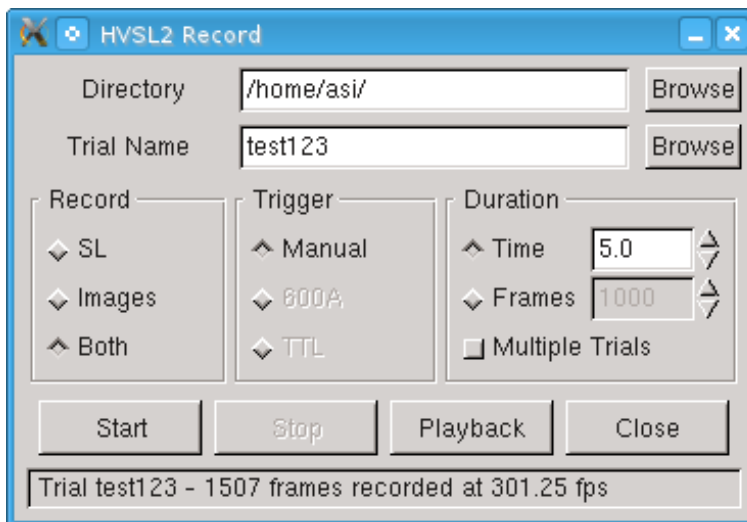
Pressing the Start button starts data collection and logging of data/frames based on the Trigger type. If the Trigger type is set to manual then the recording will start as soon as the Start button is pressed. If the Trigger type is set to 600A or TTL then when the Start button is pressed the program will start looking for a trigger signal. As soon as the trigger signal is received the recording will start.

The Stop button stops data collection when it is pressed.

The Playback button opens the Playback window and automatically transfers the Directory, Trial Name and number of frames for the most recent data collected to the Playback window. The Playback button provides the convenience of allowing you to quickly review the most recently collected data without the need to enter any file information.

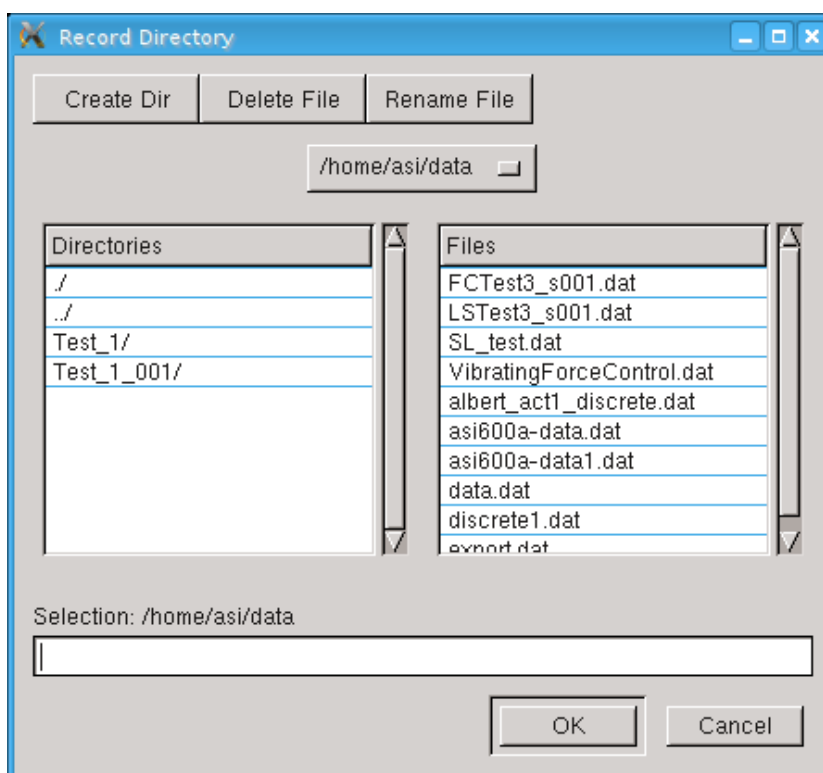
The Close button closes the Record window.

At the bottom of the Record window is a status bar (Figure 20) that provides pertinent information about each recording.



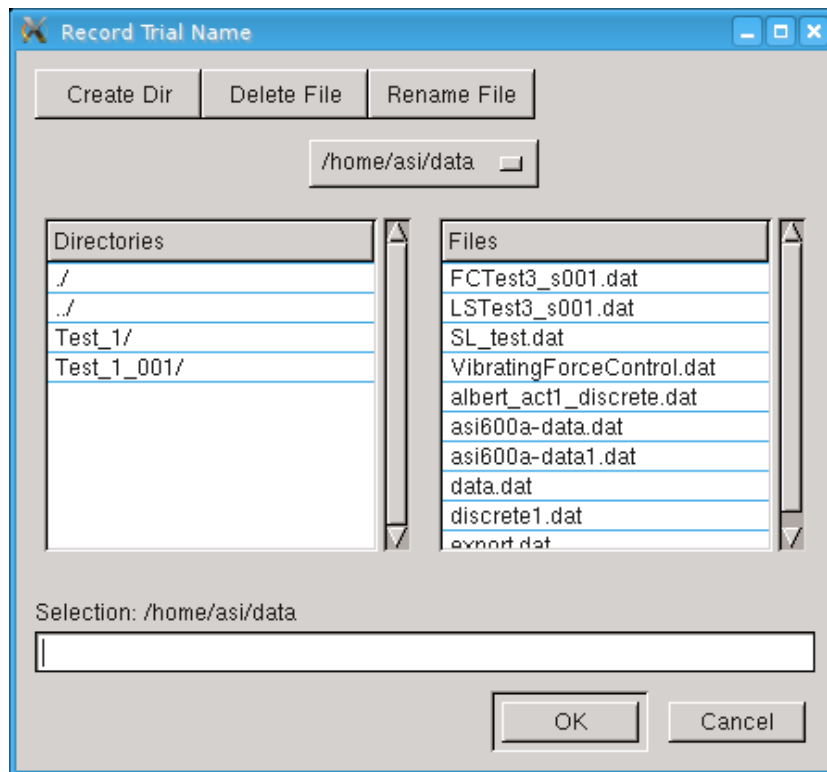
**Figure 20 HVSL Record Window showing Status after Recording Complete**

A Browse button is located to the right of both the Directory and the Trial Name text entry boxes. Pressing the Directory Browse button opens a file dialog windows as shown in Figure 21.



**Figure 21 Record Directory File Dialog**

The Browse button next to the Trial Name text box opens a file dialog window that allows the Trial Name to be specified as is shown in Figure 22.



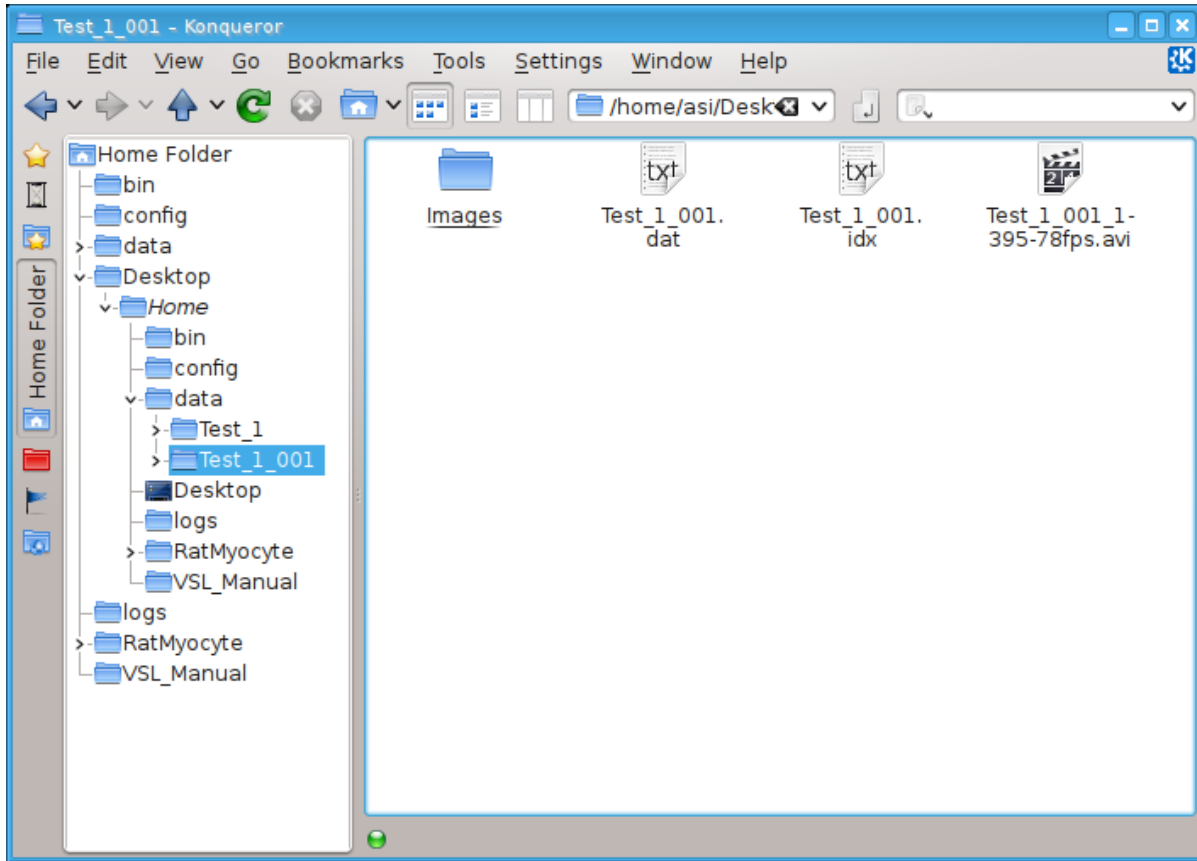
**Figure 22 Record Trial Name File Dialog**

The Record function creates a separate directory for each recording. These directories are sub-directories of the directory listed in the Directory line of the Record window. The sub-directory has the same name as the Trial Name. SL data files and index files are placed in this sub-directory. When the Record button is set to SL or to Both then the program will produce an SL data file (structure of the file is presented in Appendix A) that has the Trial Name followed by the extension **.dat**. When logging images to disk the program also creates an index file with the Trial Name followed by the extension **.idx**. This index file contains the frame (image) number and the time when the image was captured. Do not delete the idx files as they are required to use the Playback function. Images are stored in a sub-directory of the trial directory called Images. Image files are named using the Trial Name followed by an underscore ( \_ ) and then followed by a consecutive number assigned by the program starting with 001. All image files are stored in a single binary file with a **.bin** extension. In the example shown in Figure 23 the directory structure will be as follows.

Main Directory - */Home/asi/data*

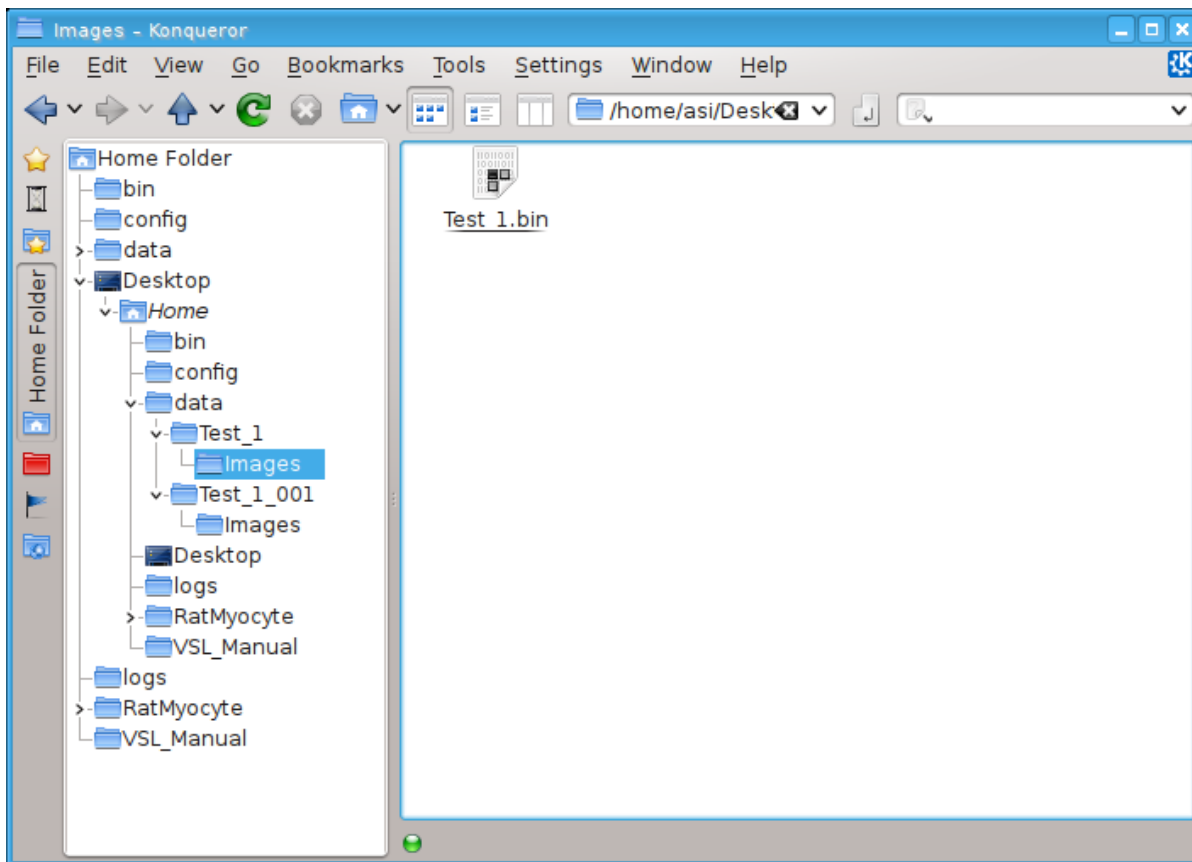
Index File, SL Data file and AVI Directory - */Home/asi/data/Test\_1\_001* (see Figure 23)

Image File Directory - */Home/asi/data/Test\_1\_001/Images* (see Figure 24)



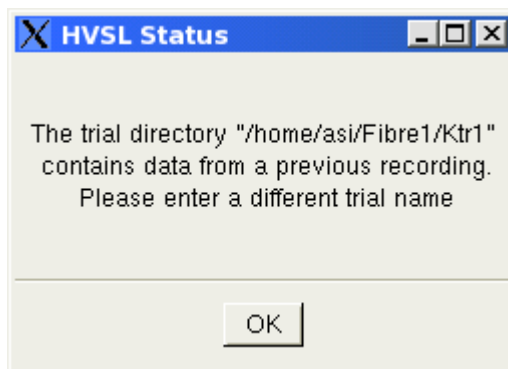
**Figure 23 Directory File Structure for Record Function**

If the Playback menu is used to create an AVI file then this file will also be stored in the sub-directory for that particular test (*/Home/asi/data/Test\_1\_001* as shown in Figure 23).



**Figure 24 Images Directory**

If you press the Start button and the Trial Name has already been used then the program will prompt you to change the Trial Name (see Figure 25). Existing data/image files will not be overwritten.



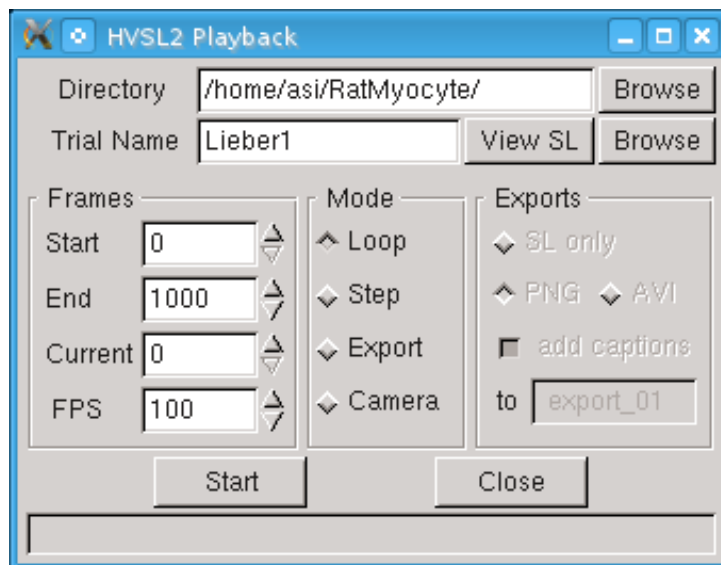
**Figure 25 File Error Window**

Note that recorded images only contain the image data from inside the region of interest (ROI). If full frame images are desired then use the Camera control buttons as follows. Press Reset to reset the camera ROI to the full 640x480 image size and then press Reset ROI to make the calculation ROI the full size of the image.



## 4.2.8 Playback Menu

The Playback menu opens the playback window as shown in Figure 26. This window allows the user to playback image data previously stored to disk. Playback also allows users to calculate the SL with or without using the Tracking feature.



**Figure 26 HVSL Playback Window**

At the top of the window there are text boxes for the user to enter the Directory and the Trial Name they wish to playback. Alternatively each textbox has a browse button which opens a file explorer and the user can select each folder. The trial should be the folder containing the trial, not a specific file within it.

There is also a View SL button that opens the SL data file associated with the original trial in the Analysis window. To view an exported SL file, open it from within Analysis.

### Frames

On the left side of the window there is a heading labeled Frames, these controls allow the user to specify the range of frames to playback, the current frame, and the Frame Rate (frames per second).

The range of frames is from the Start frame to the End frame, inclusive. These values are limited by the actual first frame and last frame of data collected, meaning the user cannot ask HVSL to play back a frame that does not exist.

The current frame displays the frame currently displayed during playback. Additionally the current frame can be changed while in Step mode, to step through the frames starting at any desired the frame.

FPS is the Frame Rate at which the images are played back in Loop mode. The frame rate is limited by the Calculation Method, Tracking, Calculation ROI and Camera ROI. The playback will run as fast as possible, limited by the specified Frame Rate. See Section 7.0 Measurement Algorithms for details on Calculation Methods.

## Mode

In the middle of the window there is a heading labeled Mode, this segment of controls allow the user to select the playback mode. Playback can operate in four modes, Loop, Stream and Step, and Camera.

Loop mode continuously runs through all frames between and including the start and end indexes at the specified Frame Rate (See Frames heading above for details).

Step mode allows the user to step through each individual frame. This is accomplished using the arrow buttons to navigate frames as well as the textbox labeled Current under the Frames heading. This box accepts any integer value between the Start and End frames and will display that frame. The user can switch between Step and Loop modes by pressing Stop, changing the mode and then pressing Start. The playback continues at the current frame when switching modes between Loop and Step.

The Camera mode switches the program back to streaming live data from the camera. This allows the user to easily switch between playback and live images without having to close the Playback window. This is a new feature of version 3.000.

The current selected mode will continue until the stop button is pressed or the mode is changed. Closing the playback window will return the camera to streaming live images.

## Exporting Data

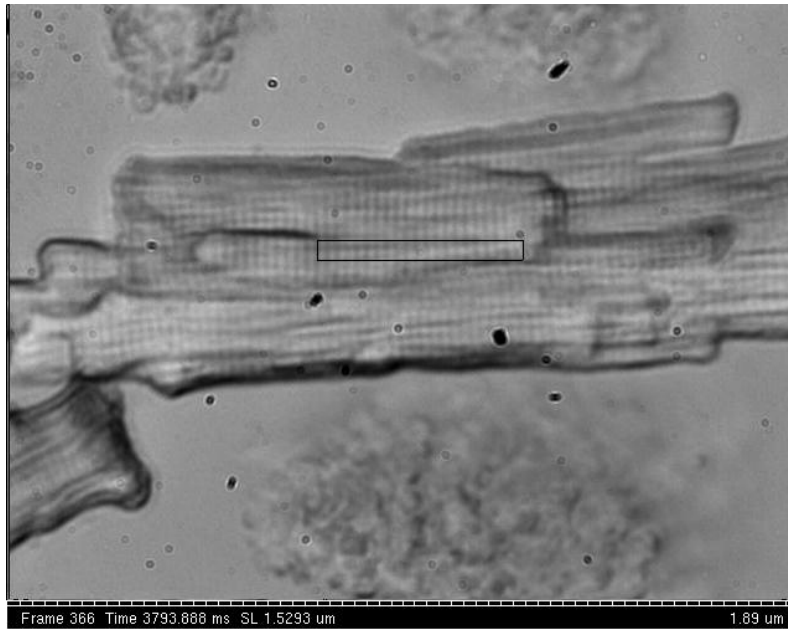
On the right side of the window there is a heading labeled Exports, this segment of controls allow the user to export PNG images, AVI movies, or SL data files. Buttons allow the user to select which option to export.

To export from HVSL, press the button corresponding to the desired format. When exporting PNG images or AVI movies, there is an option to add captions to the export. This adds a display at the bottom of the frame displaying the frame number, time, sarcomere length and a ruler displaying the reference length. These captions are shown at the bottom of Figure 27. The captions option also displays the currently selected calculation ROI in the exported file which allows the researcher to see the area where the SL calculation was performed.

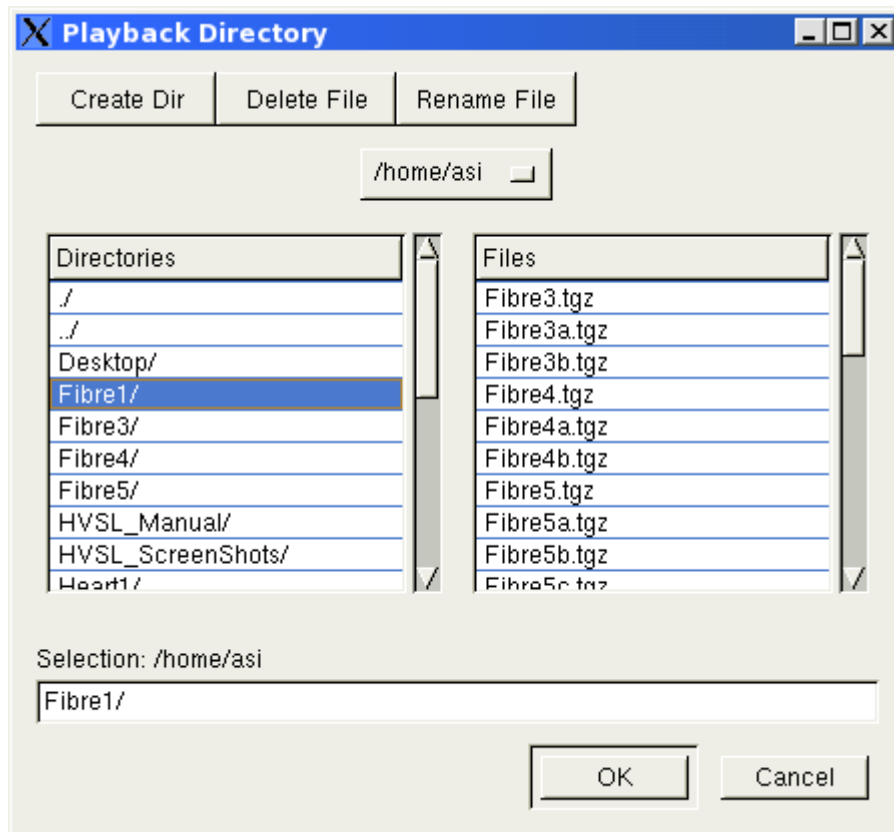
Exported data is created in a folder inside the main trial folder that is set when recording the data. The folder is automatically named in the format of export\_xx, starting with 001 and auto incrementing thereafter.



To view AVI movies on the Linux PC use the MPlayer program. If MPlayer does not start automatically then right click on the file and select Open With, then select MPlayer.



**Figure 27 Exported AVI Movie Frame**



**Figure 28 Playback Directory File Dialog**

## 4.2.9 Help Menu

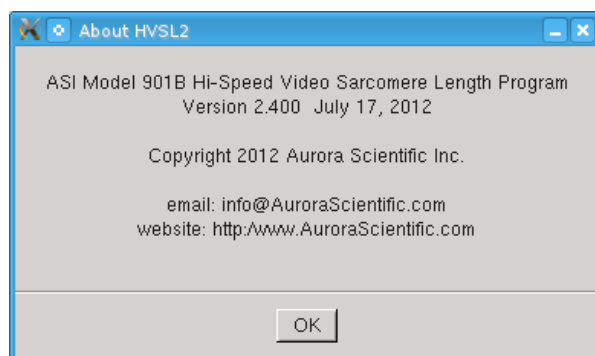
The Help menu contains two commands: Help and About.

### Help

Pressing this menu item will opens Adobe Acrobat Reader and displays the HVSL help file (this manual) on the screen. Links are included in the document.

### About

Provides information about the HVSL program including: software version number and contact information for Aurora Scientific Inc. (see Figure 29).



**Figure 29 HVSL About Window**

## 5.0 Analysis

The Analysis window provides a limited set of analysis, graphics and storage functions. Two graphs are shown on the Analysis screen. The top graph is SL ( $\mu\text{m}$ ) versus Time (s) and the bottom graph is Velocity V ( $\mu\text{m/s}$ ) versus Time (s).

The Analysis menu bar has File and Edit drop-down menu items. The purpose and function of these controls is discussed in Sections 5.1 File Menu and 5.2 Edit Menu respectively.

Analysis also includes controls to set two filters (SL Filter and V Filter) for SL and Velocity respectively, zoom controls and a button to toggle the Digital Readout on/off. A horizontal scroll bar located at the bottom of the window allows the user to scroll through the data using the currently defined time range. All of these controls are discussed in detail in Section 5.3.

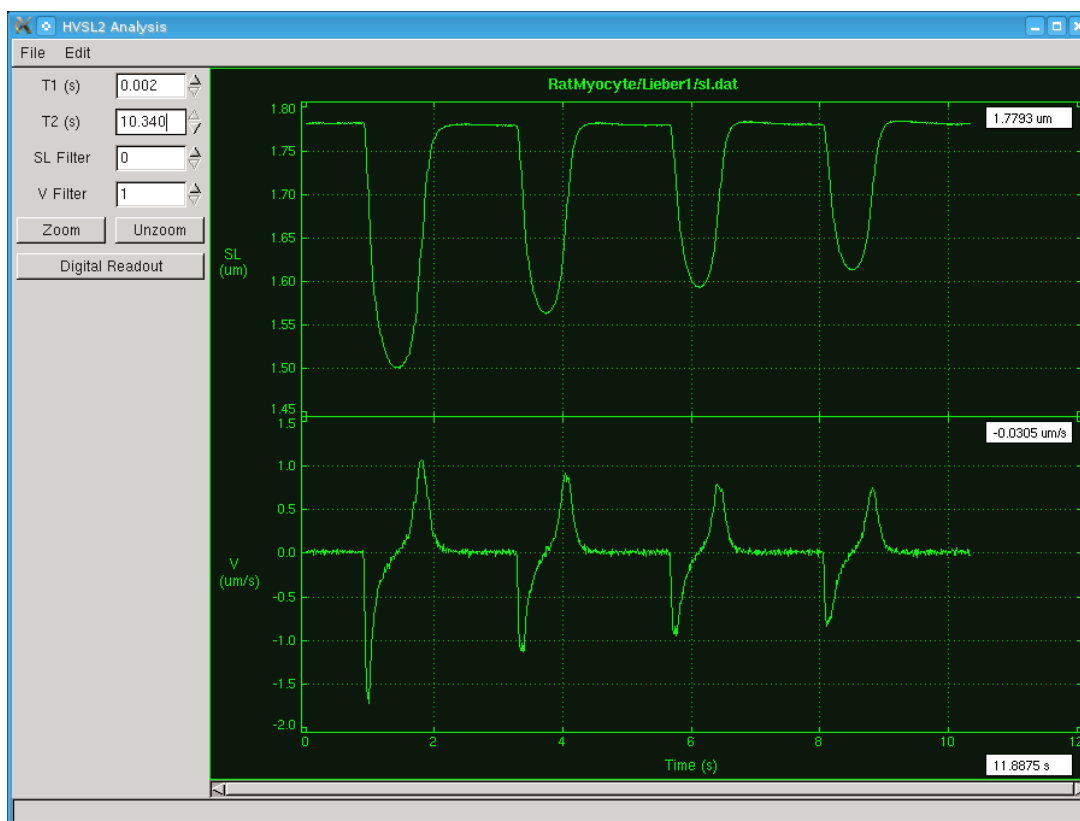


Figure 30 HVSL Analysis Window

### 5.1 File Menu

#### Open

File>Open allows the user to open an SL data file stored on the PC. Analysis can open data files created through the Record menu, generated using the Playback menu or files stored using Scope.

## Save

File>Save allows the currently displayed data on the Analysis screen to be written to a file for use in another program such Excel, MatLab, etc. The file format is shown in Appendix A.

## Close

File>Close will close the Analysis window.

## 5.2 Edit Menu

### Show Controls

Edit>Show Controls allows the user to hide or show the graph controls normally seen on the left side of the Analysis window. Hiding these controls allows the Analysis plotting area to be larger thus optimizing the use of the available area on the PC monitor.

## 5.3 Onscreen Controls

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

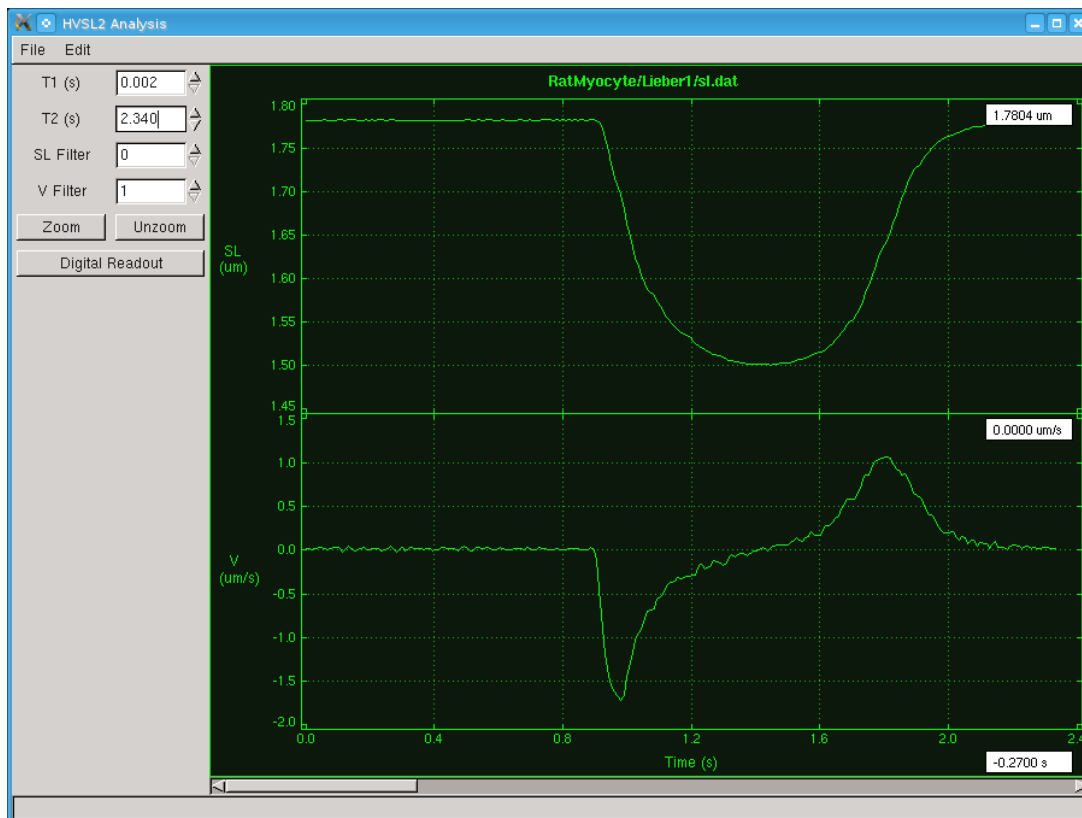
### Zoom Controls

Zoom controls are used to look at a desired time interval of data in more detail. Two methods are available for this control, the simplest is to click and drag from the start of the desired region, to the end of it. This can be done with either a right or left click. The vertical size of the region is not important as this is scaled automatically to fit the data on the plot. The other zoom method is to enter times in the two text boxes at the left side of the window labeled T1 and T2. These specify the start and end time of the desired section of data. Enter values into these boxes and then click the zoom button to expand the plot, see Figure 31. To return the graph to show the entire data set simply press the Unzoom button, see Figure 30.



Both the SL and the Velocity plots are zoomed to the same extent. As a result a zoom region can be specified on either graph which will affect both graphs.

Whenever the graphs are zoomed a horizontal scroll bar will show at the bottom of the window. This bar can be used to scroll through the entire data record using the currently defined time base. The size and location of the scroll bar along the length of the graph provides the user with an indication of the current time base size as a fraction of the total data record and its position within the data record. The scroll bar can be repositioned by clicking on the bar and dragging it side to side or by clicking on the arrows located at the ends of the scroll bar.



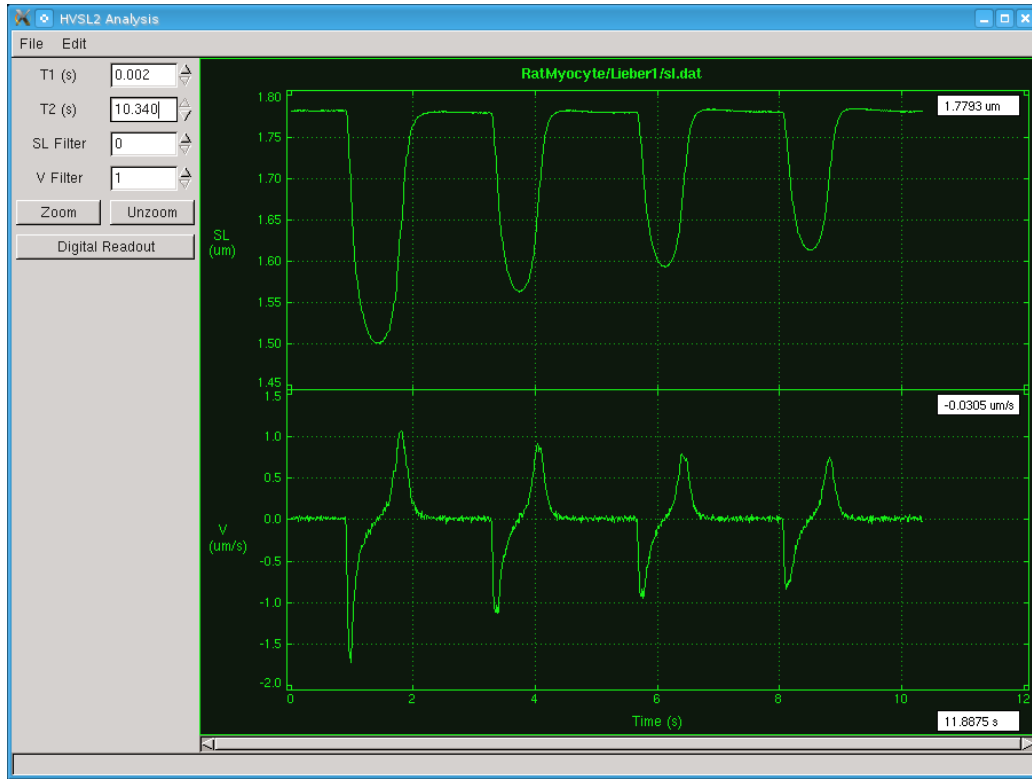
**Figure 31 Analysis Display with Data Zoomed**

### Filter Controls

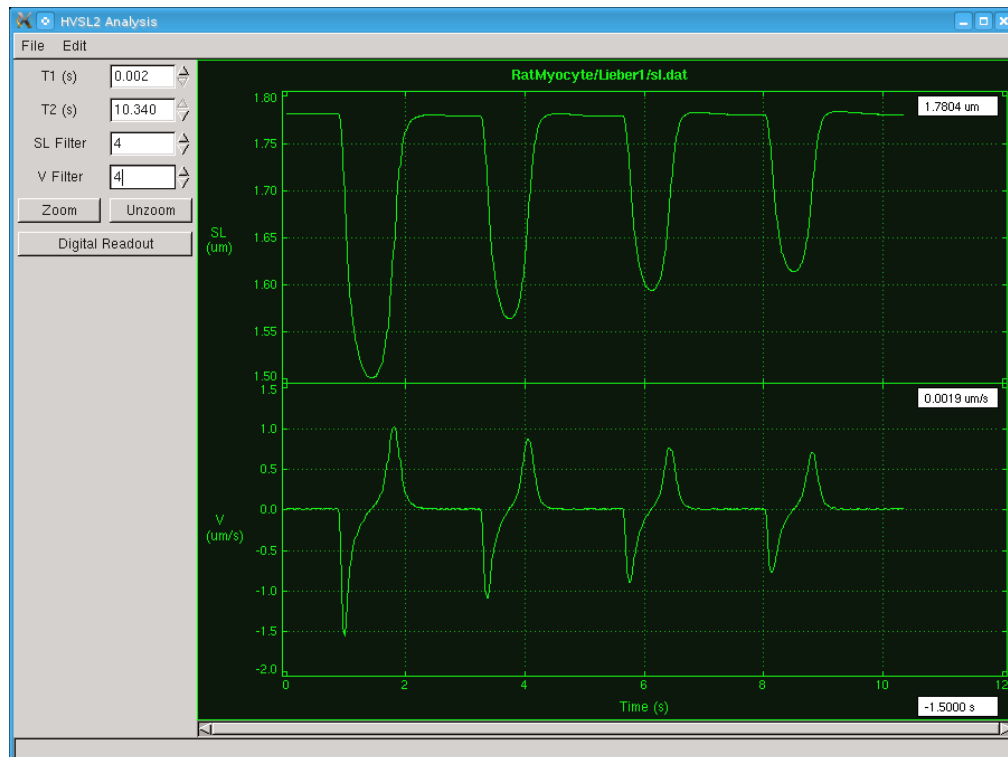
Two filters are provided one for the SL data and the other for the Velocity data. The range of filter values is from 0 to 16 for SL and from 1 to 16 for Velocity. The degree of each filter can either be typed into the data entry box or the adjacent up/down arrows can be used to increase/decrease the filter respectively.

The SL filter is a simple block average filter that averages the current point with  $n$  points greater than and  $n$  points less than the current point. Where  $n$  is the number specified in the SL Filter text box. For example if  $n$  is 4 (as shown in Figure 33) at each time point the value will be the average of the SL at the current time with the SL at the 4 next higher times and the SL at the 4 next lower times. In other words, for this example, at each time the SL will be an average of 9 data points.

Velocity is calculated as the derivative of SL with respect to time. Calculated derivatives calculated from recorded data can be very noisy. To reduce the noise a Savitsky-Golay smoothing filter is built into the calculation of Velocity.



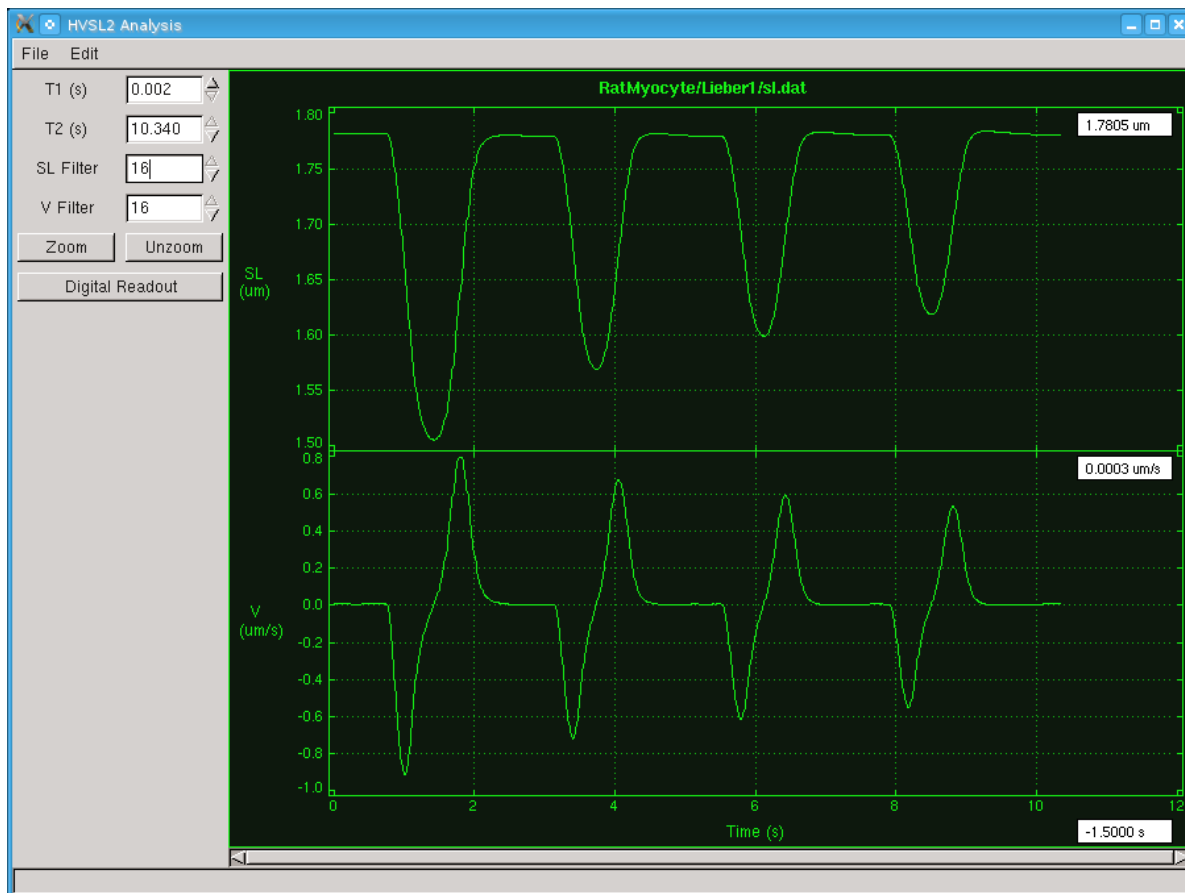
**Figure 32 Output with Filters Set to the Minimum Value**



**Figure 33 Output with Filters Set to 4**



A graphic visualization of the effect of the two filters can be seen in Figure 32, Figure 33 and Figure 34. Note the much smoother SL and velocity data when the filter length is increased. Details of the Savitsky-Golay filter are provided in Appendix B.



**Figure 34 Output with Filters Set to 16**

### Digital Readout

The Digital Readout button toggles the display of the digital readout for each graph and for the time axis. A readout of Y-axis values is presented in the top right corner of each graph. 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, SL and Velocity at the cursor location.

## 6.0 Scope

The Scope window provides a real-time output of sarcomere length vs. time 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 sarcomere length over the past 2 hours.

To open the Scope window click on the Scope menu item on the main window and then click on the Open button that appears on the drop down menu, see Figure 35.

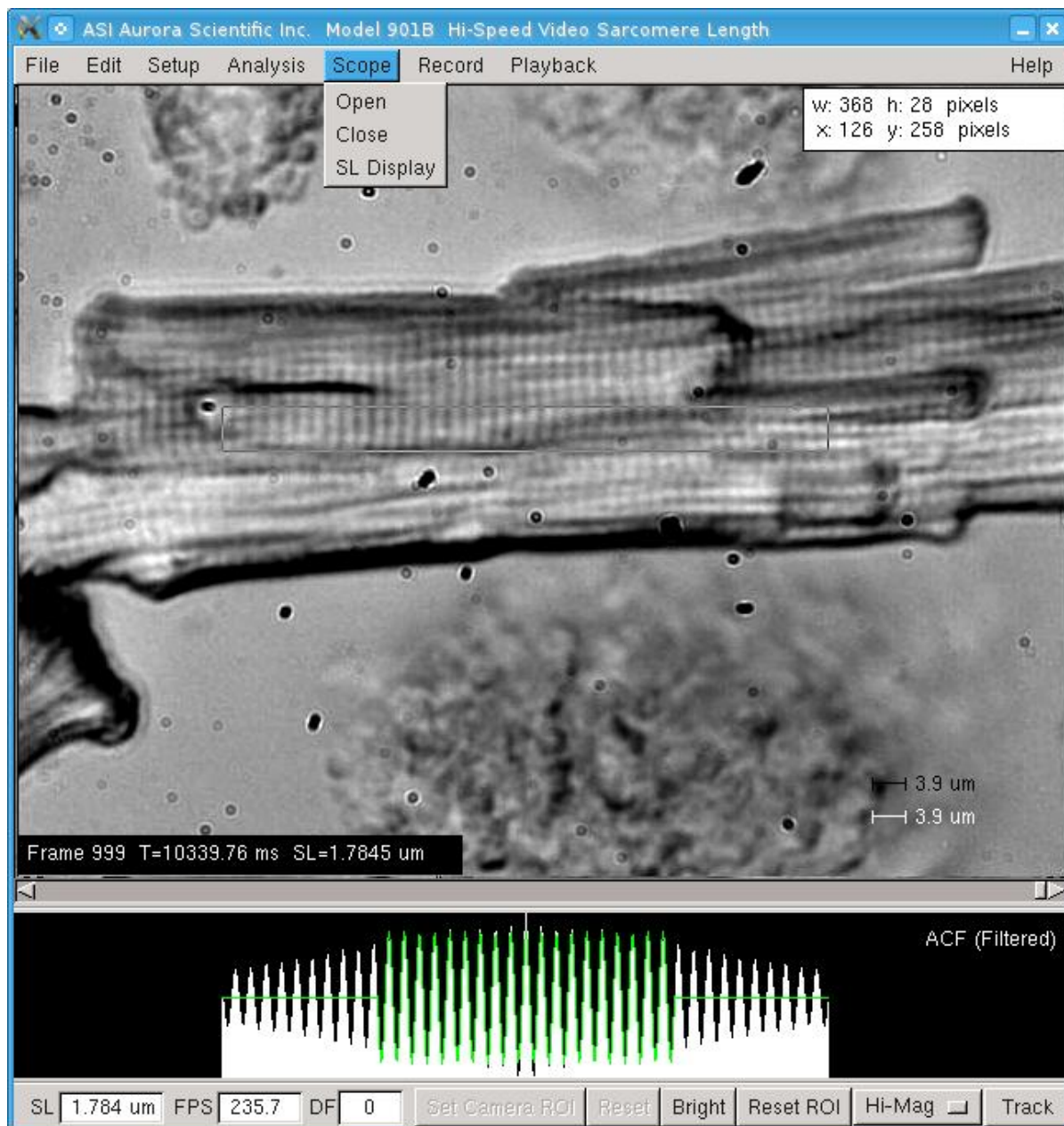


Figure 35 Scope Drop Down Menu on the Main Screen

Three items appear on the Scope drop down menu: Open, Close and SL Display. Open and Close will open and close the Scope window. The SL Display button opens a separate window that shows the current SL value as very large numerals that can be seen from a distance.



The SL Display item opens an SL readout large enough to be seen across the lab and is very useful for the situation where the SL PC and monitor are not located adjacent to the experiment.

## 6.1 File Menu

### Log File

This menu item opens a file explorer to select a location for the data log file.

### Close

Closes the Scope window.

## 6.2 Timebase Menu

### Timebase

The Time Base sets the horizontal time base for the scope plot, the amount of time displayed on the screen. The time base can be set to 1s, 2s, 5s, 10s, 20s, 30s, 1min, 2min, 5min, 10min, 20min, or 30min

### Freeze

The Freeze display button stops the display and provides controls to scroll the display back in time. Scope continues to run in the background and no data is lost while the display is frozen. As soon as the Freeze Display button is pressed a second time the window will update with the most recent data and start scrolling again.

A cursor readout is located in the upper right corner of the graph which shows the most recent sarcomere length calculated and plotted. When the Freeze Display button is pressed the readout shows the sarcomere length that corresponds to the current location of the mouse pointer on the graph. This provides an easy method for the researcher to check the SL value at any point in time.

## 6.3 Scaling Menu

The Scaling drop down menu sets the vertical data range of the plot. The valid choices are: Auto, Full Range, 0.1  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 0.5  $\mu\text{m}$ , 1  $\mu\text{m}$ , or 2  $\mu\text{m}$ . When set to Auto the vertical axis of the Scope display (SL) is automatically scaled to the range of the data. Setting the range to Full Range will display a range from min SL to max SL, these values are specified in the Setup menu. The other range settings fix the display range of SL to the selected value.

## 6.4 Logging Menu

### Start

Starts logging the Scope data to the data log file which is set in the file menu.

### Stop

Stops logging data to the data log file.

## View

Opens the data log file and displays it in analysis.

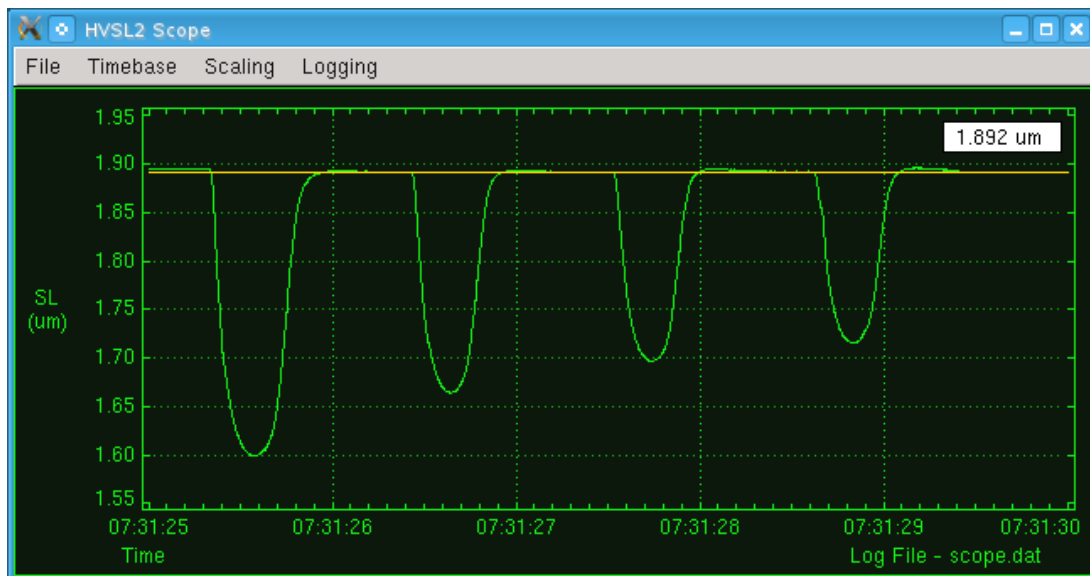


Figure 36 Scope Window

## 6.5 Quick Start Guide for Scope

- 1) Start the VSL 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 1s to 30 min.
- 5) Select SL Range to set the vertical scale to Auto or various fixed scales. Auto will automatically scale the data to fit the screen. Fixed allows you to set the vertical limits of the sarcomere length around the reference value.
- 6) The Log File button is used to specify an output data file name and the directory where the file should be stored.
- 7) Use the Start Button in the Logging menu to start recording data to disk.
- 8) Use the Stop Button to stop the current data recording.
- 9) Use the Freeze Display and Arrow Buttons to first freeze the display and then use the left or right arrow buttons to scroll through all data measured in the past 2 hours (or since the program was started). 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 would be in the Scope display buffer. You could then freeze the scope display as soon as you start Scope and be able to scroll back in time through the data that was stored in the buffer since the main program started.

## 7.0 Measurement Algorithms

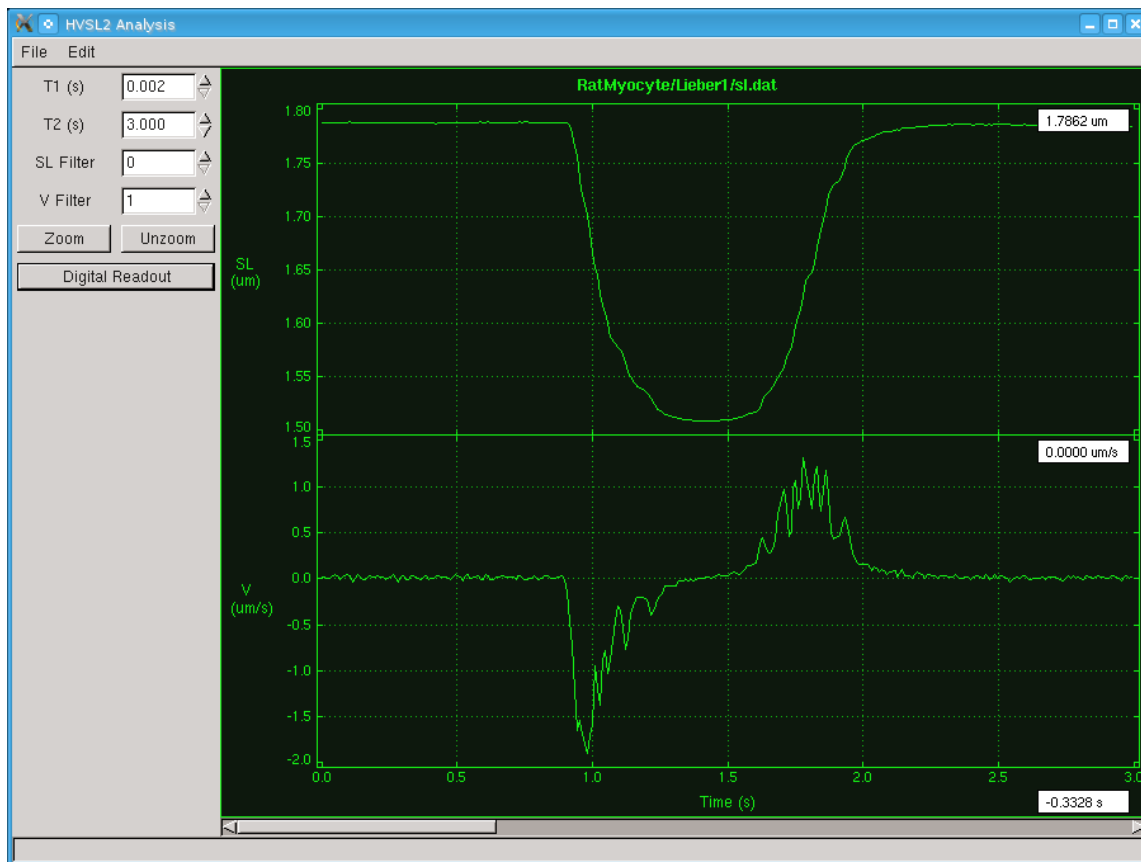
Each of the sarcomere length programs, VSL, HVSL and HVSL2 use the same measurement algorithms. Two calculation methods are available: Fast Fourier Transform (FFT), autocorrelation function/Sine-fit (ACF/Sine-fit).

The FFT method calculates an FFT for each scan line in the Region of Interest (ROI). An average spectrum is then computed and a curve is fit to the spectrum. The peak value of the curve fit function provides the sarcomere length.

The ACF/Sine-fit method calculates an autocorrelation of each scan line and then calculates an average ACF. For periodic images the result resembles a sine wave. A sine function is then fit to the average ACF to determine the sarcomere length.

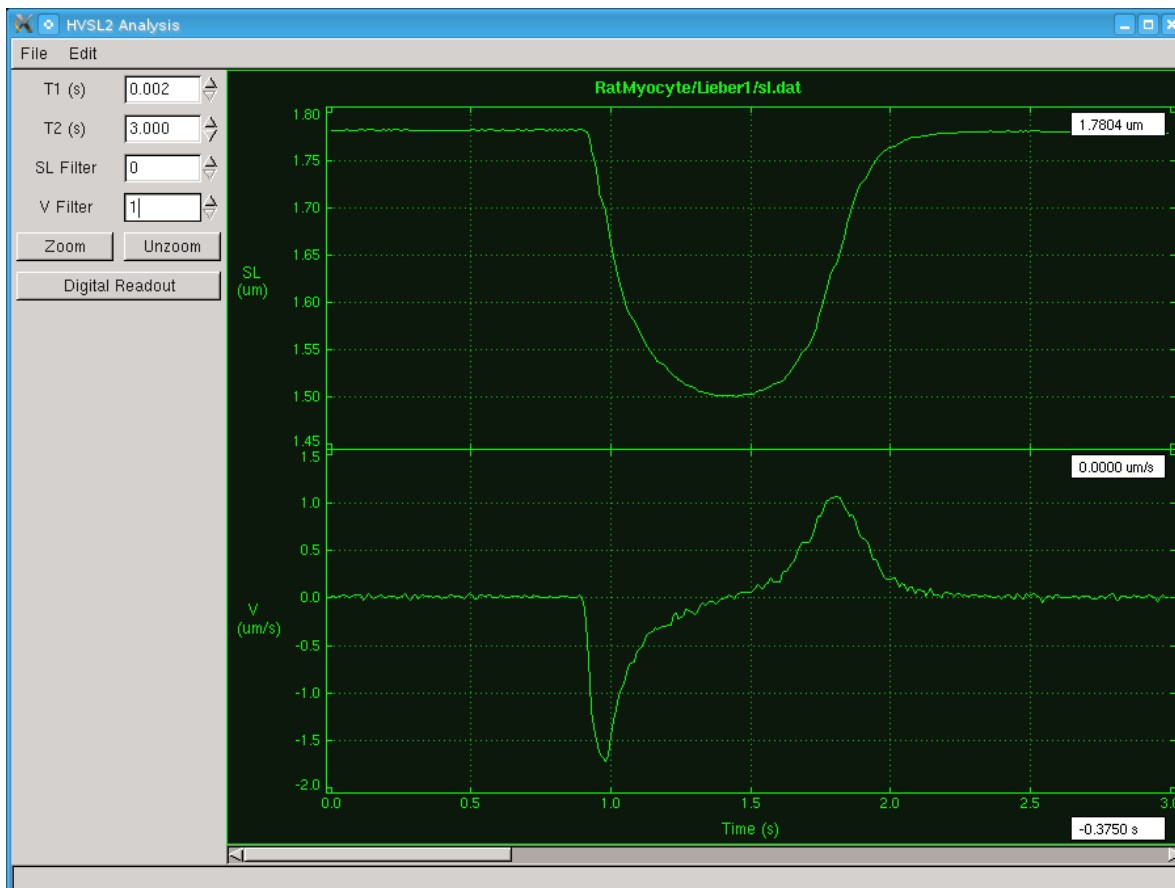
The ACF/Sine-fit provides the most robust and accurate method of determining sarcomere length. With this technique the measurement resolution is not dependent on the size of the ROI or on the number of cycles in the ROI. Traditional FFT methods produce a coarse measurement of sarcomere length when the optical magnification and ROI result in a small number of sarcomeres present in the image. This is not the case with the ACF/Sine-fit method which maintains length resolution even with small sample size or a small number of sarcomeres in the image.

The FFT method is included because some researchers have published using the FFT method and this was the first method developed for the program. In an effort to improve the FFT measurement accuracy we fit a parabolic curve to the amplitude spectrum of the FFT and then calculate the peak of this curve fit. The SL value is then calculated as the peak of the curve fit. The curve fit can introduce errors in the SL determination as is seen in Figure 37. This data was recorded from contractions of a rat myocyte. The curve fit tends to move quickly from one peak of the FFT spectrum to the next and then dwells on the peak. The result is an SL output that appears to change rapidly then plateau and then change rapidly again. The velocity trace clearly shows this behaviour with multiple velocity peaks during the contraction and relaxation phases.



**Figure 37 SL and Velocity vs Time for FFT SL Algorithm**

Figure 38 shows the SL and Velocity for the same rat myocyte contraction when an ACF/SineFit algorithm is used to determine the SL. Comparison of the figures clearly shows that the ACF/SineFit algorithm does not include these curve fitting errors present with the FFT method. The same images were used to calculate both traces and the same filter values were used for both figures.



**Figure 38 SL and Velocity vs Time for ACF/SineFit SL Algorithm**

The ACF/Sine-fit algorithm handles noisy or poor images better than the FFT method. This is due to the nature of the autocorrelation function which emphasizes repetitive structure in the image. The ACF/Sine-Fit algorithm further improves performance with noisy signals due to our technique of using an FFT and an inverse FFT as a filter on the ACF function. The filter performs a fast Fourier transform on the ACF function and then removes all frequencies outside of the expected SL range. An inverse FFT is then calculated with the resultant series being a filtered version of the original ACF. The sine-fit is then performed on this filtered series.

In older versions of the HVSL program the FFT algorithm had the advantage that the processing time was faster and provided a higher measurement rate than the ACF/SineFit algorithm. Improvements made in Version 2.0 of the program have greatly increased the computational speed of the ACF/SineFit algorithm to the point where the ACF/Sine-fit method is faster than the FFT method.



The ACF/sine-fit method should be used because of its greater accuracy and higher computational speed than that available with the FFT method.

## 8.0 Software Updates

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

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

New versions of HVSL 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 HVSL 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 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.



## 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](mailto:TechSupport@AuroraScientific.com)  
Web site: [www.AuroraScientific.com](http://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 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:**

<b>Canada, USA, Asia</b>	<b>Europe</b>
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 – HVSL File Structure

### Data File Structure for Files Created by Record and Playback

HVSL Data File

Created: Mon July 16 18:10:15 2012

Comment: Clip = 1 indicates the SL measurement  
was outside the range SLmin:SLmax

\*\*\* Sarcomere Length vs Time \*\*\*

Frame	Time (s)	SL (um)	Clip
00000	0.0003	1.88969	0
00001	0.0047	1.89007	0
00002	0.0087	1.88981	0
00003	0.0127	1.88987	0
00004	0.0168	1.89003	0
00005	0.0208	1.88974	0
00006	0.0254	1.89021	0
00007	0.0302	1.88971	0
00008	0.0349	1.89007	0
00009	0.0396	1.88977	0
00010	0.0443	1.88989	0
00011	0.0513	1.88999	0
00012	0.0560	1.88975	0
00013	0.0599	1.89017	0
00014	0.0639	1.88969	0
00015	0.0681	1.89010	0

- 
- 
- 

### Data File Structure for Files Created by Scope

HVSL Data File

Created: Mon July 16 18:09:19 2012

Sampling Rate: 200 Hz

Comment: Clip = 1 indicates the SL measurement  
was outside the range SLmin:SLmax

\*\*\* Sarcomere Length vs Time \*\*\*

Time (s)	SL (um)	Clip
0.0000	1.89227	0
0.0050	1.89238	0
0.0100	1.89238	0
0.0150	1.89223	0
0.0200	1.89235	0
0.0250	1.89216	0
0.0300	1.89220	0
0.0350	1.89252	0
0.0400	1.89209	0
0.0450	1.89220	0
0.0500	1.89245	0
0.0550	1.89217	0
0.0600	1.89221	0
0.0650	1.89239	0
0.0700	1.89245	0

-

## Data File Structure for Files Created by Analysis

HVSL Analysis File  
Created: Mon July 16 18:10:15 2012  
Original HVSL Data File: RatMyocyte/Lieber1/sl.dat  
SL Filter Length: 5  
Velocity Filter Length: 5

\*\*\* Sarcomere Length and Velocity vs Time \*\*\*

Time (s)	SL (um)	Vel (um/s)
2.7685	1.89233	0.00118
2.7777	1.89235	-0.00024
2.7869	1.89240	0.00112
2.7960	1.89239	0.00092
2.8056	1.89243	0.00043
2.8147	1.89239	0.00119
2.8240	1.89237	-0.00118
2.8445	1.89238	-0.00011
2.8537	1.89243	0.00229
2.8627	1.89249	0.00380
2.8719	1.89252	0.00494
2.8810	1.89253	0.00304
2.8901	1.89252	-0.00004
2.8992	1.89246	-0.00034
2.9087	1.89242	-0.00376
2.9179	1.89236	-0.00517
2.9270	1.89232	-0.00757

- 
- 
-

## Appendix B – Savitsky-Golay Smoothing Filter

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

savgol: Savitsky-Golay smoothing filter coefficients.

Usage:  $c = \text{savgol } fp \ d$

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 – uEye USB CMOS Camera Data Sheet



### USB UI-1225LE

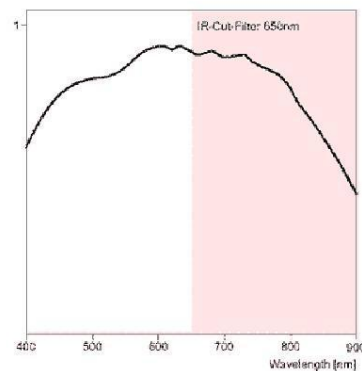
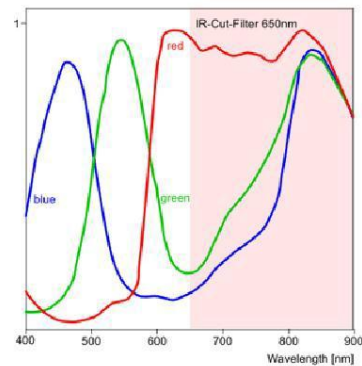
The UI-1225LE is an extremely compact camera with modern Aptina CMOS sensor in Wide VGA resolution (752x480 pixels). Through the use of the widespread USB 2.0 technology the camera can be interfaced with a vast variety of systems without problems. The light-weight housing of the UI-1225LE features a C/CS lens mount with adjustable flange back distance.



### Specification

#### The characteristics at a glance

Interface	USB
Lens Mount	C-Mount CS-Mount
Sensor Technology	CMOS (Aptina)
Model Description (color)	UI-1225LE-C
Model Description (mono)	UI-1225LE-M
Resolution (h x v)	752 x 480
Resolution Depth	8bit (10bit ADC)
Resolution Category / Pixel Class	Wide VGA
Sensor Size	1/3"
Shutter	Global
max. fps in Freerun Mode	87 fps
Exposure Time in Freerun Mode	80µs-5.5s
Exposure Time in Trigger Mode	80µs-5.5s
AOI Modes	horizontal + vertical
Binning Modes	horizontal + vertical
Subsampling Modes	---
I/O - Strobe	-
I/O - Trigger	-
I/O - RS-232	-
I/O - GPIO	-
I/O - I2C	-
HDR (High Dynamic Range)	-
Sensor Model (m/c)	MT9V032STM / MT9V032STC
Pixelpitch in µm	6.00
Optical Size	4.512 x 2.880 mm
Protection Classes	IP30
Dimensions H/W/L	44.00 mm, 44.00 mm, 25.40 mm
Mass	32.00 g
Power Supply	USB



## Appendix D –Imperx VGA-210L CCD Camera Data Sheet



VGA 8/10/12 BIT, 210 FPS, PROGRAMMABLE DIGITAL CAMERA



IPX-VGA90-L  
IPX-VGA120-L  
IPX-VGA210-L

### Features

- 640 x 480 pixels @ 210 fps
- 8/10/12 bit data, Base Camera Link
- Single or Dual tap operation
- RS232 serial communication
- 32 bit RISC processor
- Horizontal and vertical binning
- Highly programmable:
  - resolution • frame rate
  - electronic shutter • long integration
  - external trigger • pre-exposure
  - fast triggering • double exposure
  - strobe output • gain and offset
  - area of interest • user LUT
- Dynamic Transfer Function correction
- Dynamic S/N correction
- Temperature monitor
- Field upgradeable:
  - software • firmware • user LUT
- Automatic Iris Control - optional

The **IPX-VGA210-L** is an advanced, high-resolution progressive scan, fully programmable and field upgradeable CCD camera, built around KODAK's KAI-0340 interline transfer CCD. The camera provides 640 x 480 resolution and delivers 210 frames per second at full resolution. The camera image processing engine is based on a 1 million gate FPGA and 32 bit RISC processor, featuring programmable resolution, AOI, binning, triggering, shutter, long integration, transfer function correction and temperature monitor.

### Applications

- Medical and Scientific Imaging
- Machine Vision and Metrology
- Microscopy
- Remote Sensing
- Surveillance

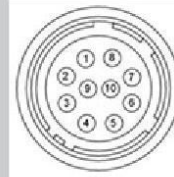




## Specifications for IPX-VGA90-L, IPX-VGA120-L, IPX-VGA 210-L

<b>Active Image Pixels</b>	640 (H) x 480 (V)
<b>Active Image Area</b>	5.87 mm x 4.71 mm (0.231" x 0.185")
<b>Pixel size</b>	7.4 um
<b>Video Output</b>	Digital, 8/10/12 bit, one or two outputs
<b>Camera Interface</b>	Base Camera Link
<b>Data Clock</b>	40,000 MHz
<b>Resolution</b>	640 x 480 pixels max
<b>Frame Rate - VGA210</b>	210 fps (dual) / 120 fps (single), up to 3000 fps
<b>Frame Rate - VGA90/120</b>	VGA90 - 90 fps (single), VGA120 - 120 fps (single)
<b>Shutter Speed</b>	1/50000 sec to 1/100 sec
<b>Long Integration</b>	1/100 sec to 10 sec
<b>Gamma Correction</b>	G=1.0, G=0.45, User defined LUT
<b>Black Level Offset</b>	256 levels/output
<b>Video Gain</b>	6-40 dB, 1024 steps/output
<b>External Trigger</b>	Asynchronous
<b>Hardware Trigger</b>	External, level sensitive, 3.3V - 5.0V, 10mA., optically isolated, 0 to 30 msec pre-exposure
<b>Software Trigger</b>	Frame-grabber via CC1, programmable pre-exposure
<b>S/N Ratio</b>	58 dB min
<b>Strobe Output</b>	Active HIGH, for external light source
<b>Lens Mount</b>	C mount, 1/3" format
<b>Environmental</b>	Operating: 0° - +50° C, Storage: -10° - +65° C
<b>Min. Illumination</b>	1.0 Lux, f=1.4, no IR cut filter, no shutter

### Power Connector



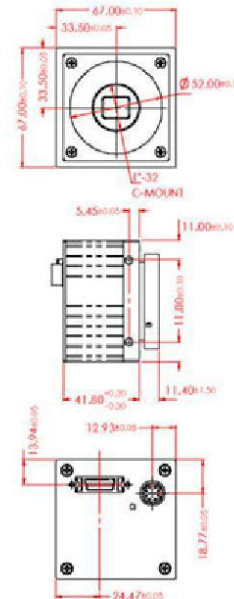
Rear Camera View

- 1 Trigger IN -
- 2 Trigger IN +
- 3 -12 VDC
- 4 -12 VDC
- 5 +12 VDC
- 6 +12 VDC
- 7 Strobe OUT -
- 8 Strobe OUT +
- 9 Auto Iris +
- 10 Auto Iris -

### Power Requirements

- 12V DC, (10V min, 15V max)
- 350 mA steady, 1.0 A inrush
- 4.2 W constant power
- Connector: Hirose HR 10A-10R-10P

### Mechanical Dimensions



Ordering: **IPX-VGA210-LMCI**

**210** - 210 fps

**120** - 120 fps

**90** - 90 fps

Camera Family

L - LYNX Family

Sensor Type

M - Monochrome

C - Color

Options

blank - none

I - Auto Iris

Camera Family

Lens Mount

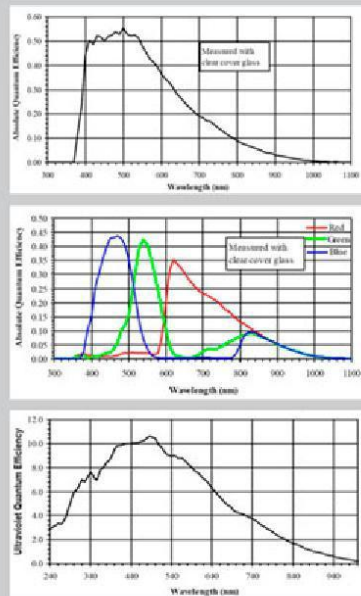
blank - "C" mount

O - Open frame

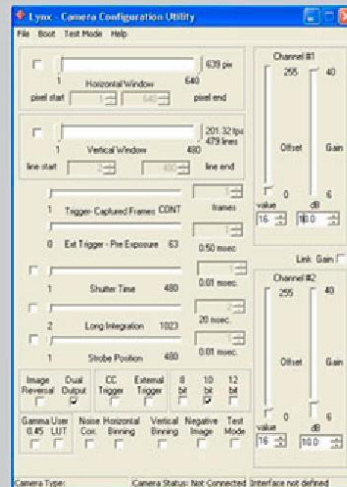
U - UV sensitive (no glass)

S - Special - user filter

### Spectral Response



### Configuration Utility



- CameraLink protocol compliant
- Control all camera features via:
  - our intuitive, easy-to-use GUI, or
  - simple ASCII commands

For specific details and ordering information, consult the camera user's manual or contact us at [sales@imperx.com](mailto:sales@imperx.com).

Copyright © 2004, Imperx, Inc. Product information subject to change without notice.

Rev. 1.0, 11/08/04



Imperx Incorporated • 6421 Congress Avenue • Boca Raton, FL 33487 • USA  
 Phone: 1-561-989-0006 • 1-866-849-1662 • Fax: 1-561-989-0045  
[www.imperx.com](http://www.imperx.com) • [sales@imperx.com](mailto:sales@imperx.com)

## Appendix E – Teledyne Dalsa GigE CMOS Camera Data Sheet

### Genie HM640

Area Scan Monochrome Cameras



Genie HM640

#### Overview

### Extreme high speed acquisition with uncompromised image quality.

The Genie HM640 offers unprecedented high speed image acquisition with uncompromised signal-to-noise ratio, significantly optimizing the opportunity for increased yield. DALSA's proprietary sensor technology allows for image capture rates of 300 fps in VGA resolution while maintaining exceptional image quality. The Genie HM640's gigabit Ethernet interface takes full advantage of this Genie's high frame rate by transmitting data over standard CAT-5e and CAT-6 cables to distances of up to 100 m. Like all Genies, the HM640 is based on the AIA (Automated Imaging Association) GigE Vision Standard to directly link the camera to a PC.



#### Key Features

- Uses standard PC and server Ethernet ports & hardware
- Facilitates cable lengths up to 100 m (CAT-5e or CAT-6)
- Simplified set-up with field proven Sopera Essential software featuring CamExpert
- Engineered to accommodate industrial environment with a Ruggedize, screw mount, RJ-45 connector
- Small compact form factor

#### Programmability

- Achieve 300 fps in full resolution
- Higher frame rates achievable in partial scan mode
- Global electronic shutter with exposure control
- Programmable LUT
- On-board flat-field correction
- 1000x antiblooming

#### Typical Applications

- Electronics manufacturing inspection
- Industrial metrology
- Intelligent traffic systems

#### Specifications

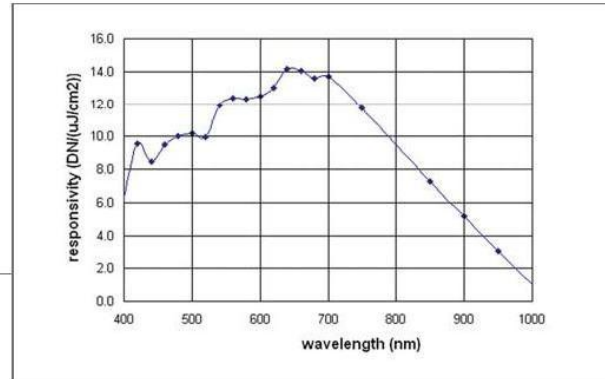
Active Resolution	640 x 480
Frame Rate	300 fps
Pixel Size	7.4 $\mu$ m
Data Format	8-bits
Exposure Control	Programmable, or via External Trigger
Dynamic Range	48 dB
Nominal Gain Range	0 dB to +12 dB
Output	Gigabit Ethernet, also supports 100 Mbps
I/O Ports	2 opto-isolated input, 2 opto-isolated output
Lens Mount	C or CS-mount / Right-Angle option is also available
Size	44 mm x 29 mm x 67 mm (including lens adapter)
Mass	-115 g (without lens)
Operating Temp	0°-45°C
Power Supply	12 to 24V
Power Dissipation	4W
Data Connector	Standard or Screw mount RJ-45
Power and I/O	Hirose 12-Pin
Software Platform	DALSA Sopera LT
GigE Vision Compliant	Yes
Regulatory Compliance	FCC Class A, CE, RoHs 2002/95/EC
Part Number	CR-GM00-H640x



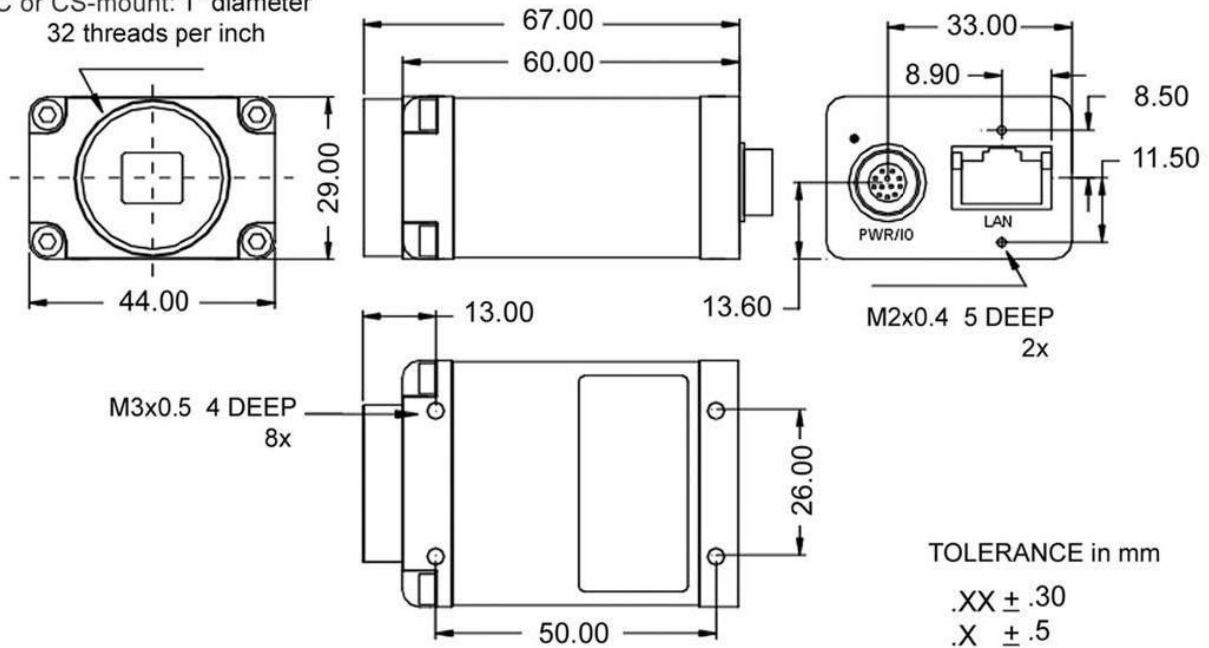
## Genie HM640

Area Scan Monochrome Cameras

All Genie cameras feature value added functionality designed specifically for imaging and machine vision applications. All features are easily accessible with DALSA's advanced software tools. These tools deliver superior image capture, performance, and control.



C or CS-mount: 1" diameter  
 32 threads per inch



[www.dalsa.com](http://www.dalsa.com)

**Americas**  
 Boston, USA  
 Tel: +1 978-670-2000  
[sales.americas@dalsa.com](mailto:sales.americas@dalsa.com)

**Europe**  
 Munich, Germany  
 Tel: +49 8142-46770  
[sales.europe@dalsa.com](mailto:sales.europe@dalsa.com)

**Asia Pacific**  
 Tokyo, Japan  
 +81 3-5960-6353  
[sales.asia@dalsa.com](mailto:sales.asia@dalsa.com)

DALSA is an international leader in digital imaging and semiconductors and has its corporate offices in Waterloo, Ontario, Canada.

All trademarks are registered by their respective companies. DALSA reserves the right to make changes at any time without notice. © DALSA 2008. genie\_hm640\_050708



## Appendix F – Program Start Switches

The HVSL 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 VSL/HVSL 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:

`vsl -h` or `hvsl -h`

usage: hvsl [options]

options:

`-h` print this message

`-nv` no video (playback images only)

`-rt` enable memory-pipe communication with a 600A

`-ttl` use the parallel port ack- pin for TTL triggers

`-fps N` set the video display rate to N frames/second (N = 10:60 default=30)

`-l x` set the SL in the test image to x micrometers

`-b C` uEye camera: set the pixel clock rate to C MHz (default C=40)

`-usbdac sl1 sl2 v1 v2`

output SL as an analog voltage via a 903 D/A converter.

Sarcomere lengths in the range `sl=sl1:sl2` um are converted (and clipped) to voltages `v=v1:v2` volts, where  $0 < v < 2.50$ .

Example: `hvsl -usbdac 1.0 3.0 0.1 2.40`

- scale the usb DAC output so that SL values in the range 1 to 3 um are converted to voltages from 0.1 to 2.4 volts.

`-net asihost`

enable remote logging. The PC named "asihost" must be running the asilog program in order to see log information.

The default settings for each of the Desktop icons are as follows:

**900B**

`vsl -rt -b 40`

**901A**

`hvsl -rt`

**901B**

`hvsl -rt`

## Appendix G – Network Security



Many users will elect to connect the VSL/HVSL 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 VSL/HVSL 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.

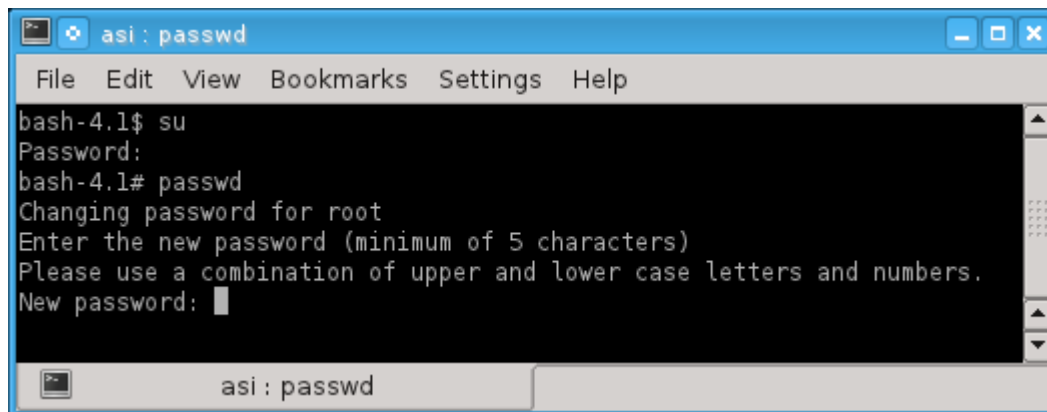


Figure 39 Use passwd to Change the Root Password