INSTRUCTION MANUAL

Model
900A

Video Sarcomere Length Program

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Program Version: 1.14

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1.0 Introduction

The 900A Video Sarcomere Length (VSL) Measurement System was designed to enable physiology researchers to measure sarcomere length in real-time from a video signal. Video sources can include cameras, VCR output or any other NTSC/PAL video signal. The software can also be used to measure sarcomere length from captured image files that are stored in .jpg format. The system is capable of real-time measurement of sarcomere length at up to 30 images per second; this speed is limited by the NTSC/PAL video standards.

The software also includes a Scope function that provides a scrolling data display of sarcomere length with time. This display can record up to 4 hours of sarcomere length data, measured at up to 30 samples per second. The Scope display can be frozen at any time and the user can then scroll through the sarcomere history. Scope can also log the data to disk.

The VSL software and hardware operates on an ASI 600A Digital Controller PC. The product includes the VSL software, a video capture card that plugs into the PC and a BNC signal cable to connect the video card to the client's camera or video source.

2.0 Specifications
Model: 900A Video Sarcomere Length

Software

Operating System:	Linux-RT
Language:	C
Interface:	GUI (Windows-type), drop down menus
Image Functions:	Brightness, Contrast, Region of Interest (ROI)
Range of Interest (ROI):	Drag out ROI box with mouse, ROI box can be shifted using right mouse button
ROI Display:	Readout in microns or pixels
Image Calibration:	Field of View (FOV) or Calibration Length
Video Display Functions:	Full Image, Range of Interest, Freeze
Calculation Algorithms:	Fast Fourier Transform, Autocorrelation Function / FFT, Autocorrelation Function / Sine-Fit
Calculation Rate:	30 per second maximum (max. set by frame rate of DT3155) Still mode: >100 SL calculations per second (Calculation rate depends on size of ROI and current load on the PC.)

Scope

Display:	Sarcomere Length, Reference Length in real-time
Time base:	10s to 1 hr
Scaling:	Auto, 0.1µm, 0.2µm, 0.5µm, 1.0µm
Sampling Rate:	1, 2, 5, 10, 20, 50, 100 Hz
Data Recording:	Unlimited time, only limited by hard drive size.
Display Freeze:	Display can be frozen and then the previous 4 hours of data can be reviewed

Frame Grabber Card

Type:	Data Translation DT3155
Video Format:	RS-170, RS-330, NTSC (60Hz), CCIR and PAL (50Hz)
Resolution:	640x480 (60Hz), 768x576 (50Hz)
Digitization:	8-bits, 256 gray levels
Aspect Ratio:	1:1, square pixels
Frame Grab Speed:	30 frames per second (60Hz), 25 frames per second (50Hz)
Form:	Half-length PCI bus board (short card)

3.0 Quick Start Guide

3.1 Instrument Setup

The software and camera interface card are pre-installed on the VSL PC. Attach the keyboard, mouse (attach the mouse to the PS2 mouse port not to a USB port) and monitor (use 15 pin analog connector not DVI connector) to the PC. The Linux operating system has been configured for a monitor with a resolution of 1280x1024. Attach an NTSC or PAL composite video signal to the BNC connector on the frame grabber card. This will normally come from a camera attached to your microscope but the video signal could also come from a VCR or other composite video source. Ensure the PC is not powered when making connections. Plug the camera power supply into an appropriate AC power source and into the power connector on the camera.

3.2 Starting the VSL Program

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

Username: asi
Password: asi

Once the VSL PC finishes the login process you will see on the desktop two icons labeled **VSL** and **VSL Still**. The software can be used to calculate sarcomere length from a live video signal or from pre-recorded .pgm or .jpg files. Use the **VSL** icon to start the program analyzing a live video signal from a camera, likewise use the **VSL Still** icon to start the program in still mode.

When the program starts the main window will open showing the current video/still image, the results of the selected calculation method in graphical form beneath the image and several control buttons below the graph (see Figure 1). This window can be repositioned and minimized but its size can not be altered. The maximum image size produced by the camera is 640x480 pixels (NTSC) and is 768x576 pixels (PAL/CCIR). The main screen is fixed at 640x480 pixels and can't be re-sized. On higher resolution monitors the VSL main screen will appear smaller than on lower resolution monitors. We suggest using a screen resolution of 1280x1024 however the program can operate at lower resolutions. We suggest a minimum screen resolution of 1024x768. The Linux desktop and all program windows use normal Windows-type controls for manipulating the display windows.

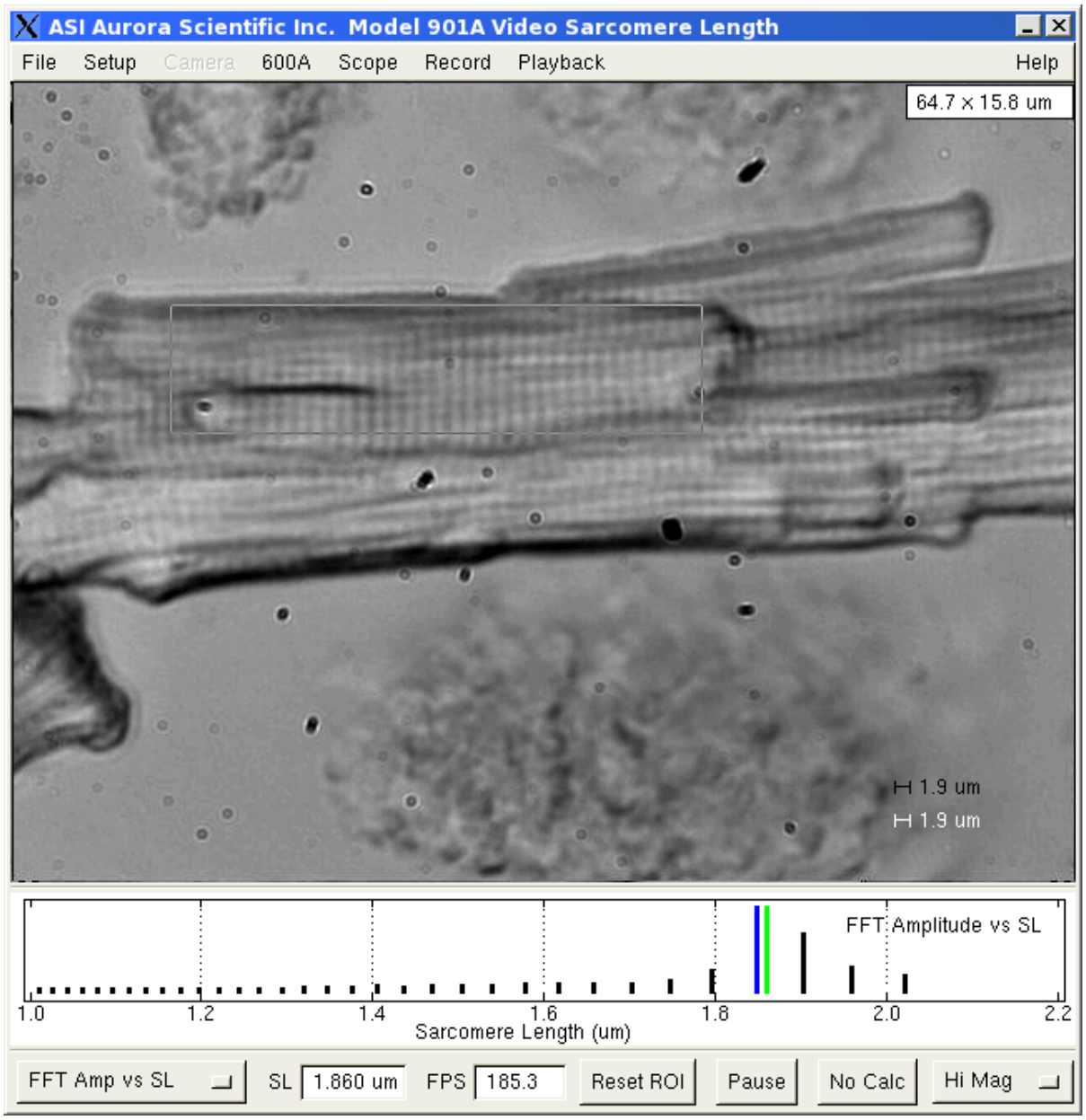

Figure 1 ASI 900A Video Sarcomere Length Main Screen

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

File
Setup
Camera
600A
Scope
Playback
Record
Help

You will note that the Camera and 600A menus are "grayed out" as these features are not available with the VSL program.

Located at the bottom of the main screen are several buttons and readout boxes. The first button controls the graphical display located below the image. The sarcomere length readout (SL) and the calculation rate in frames per second (FPS) readout provide the current SL and the current calculation rate. The Reset ROI button resets the ROI to the full screen. Sarcomere calculations are only made within the ROI. If processing speed is important then choosing a smaller ROI will increase the frames per second up to the limit of the video hardware which is 30FPS for NTSC and 25FPS for CCIR/PAL. The Pause button freezes the image on the current frame. The No Calc button turns off the sarcomere length calculation which provides the maximum frame rate. The Hi Mag/Lo Mag button controls the calibration constant being used to convert pixels to distance in microns (μm).

3.3 Using the VSL Program

Now that that program is up and running we will go through the main steps required to measure sarcomere length. The majority of this description is applicable to both still and video versions of the program. Any differences will be noted.

Assuming that a video signal is present 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 the live video image. The program does not compensate for angled images (non-vertical sarcomeres) so if the image is on an angle the program will overestimate the sarcomere length. This problem can easily be avoided by simply rotating the camera to align the sarcomeres as vertically as possible in the image.

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 chapter 4.0.

Quick Start Guide

1) Turn on the PC, log in, and start the VSL program.
2) Click on the Setup Menu heading which will bring up a separate setup window. The setup window is used to adjust the image contrast and brightness, to calibrate the program and to set display and calculation parameters.
3) Use the Brightness and Contrast sliders to adjust the image if necessary.
4) Set the Video Display to Full Frame.
5) Set the SL Display to Sine Fit.
6) You must calibrate the program before you can produce accurate sarcomere length measurements. There are two calibration methods available in the program and there are two calibration constants that can be set, one for low magnification and one for high magnification. Typically the Hi Mag setting will be used for calculating

sarcomere length while the Lo Mag setting will be used for measuring overall dimensions of the tissue. The following calibration methods are usable for both the Hi and Lo Mag settings.

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 one of the 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, 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 5 pixels. A larger horizontal distance between the two points typically produces a more accurate calibration. Click on the diamond shaped button to the left of the Calibration FOV (um). (The program uses "um" to represent microns). Enter the known horizontal distance between the two points. Press the Calibrate SL (pixels/um) button on the setup screen. 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 Apply button.

b) Calibration Length – this calibration 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 a know line spacing). 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 Length (um) label. Enter the known pattern spacing. 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 9 and the Maximum to about 11. Set the Reference Length (um) to be equal to the pattern spacing. Press the Calibrate SL (pixels/um) button on the setup screen. 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 Calibration Length method is an iterative method that often requires repeated presses on the Calibration SL (pixels/um) button before the solution converges. A simple method of checking for convergence is to press the Calibrate SL 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. 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. If this is the case you may need to manually enter a value in the text box beside the Calibrate SL button and then press the button several times until the solution converges. You are essentially providing the program with a starting point. If you select a starting point too much above the true value then the program may converge to a higher solution or even diverge in which case the calibration number continues to increase with every button press. If you choose a starting point too 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. Press the Apply button to complete the calibration.

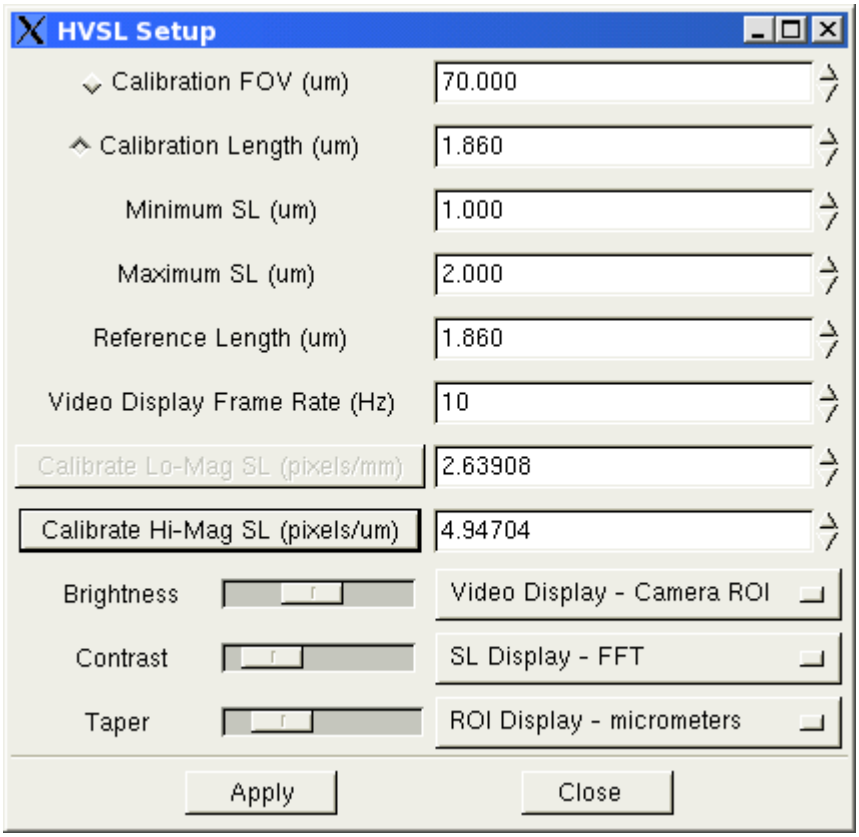

Figure 2 VSL Setup Screen

7) Once calibrated you should set the minimum and maximum SL values to reasonable values that bracket the expected sarcomere length. For example if the resting sarcomere length is 2.5 um then a good choice for the minimum and maximum SL would be 2 and 3 um respectively. Knowledge of the expected range of SL will allow the researcher to ensure the expected range is included between the Minimum and Maximum SL values. Don't choose values that are too far outside of the physiological range of sarcomere lengths. In operation the program will look 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. It is not recommended that you never set the Minimum SL to 0 as

this would allow all the solutions associated with image pixilation to be included in the SL determination.

8) Set the Reference Length (um) to a convenient value within the expected range of sarcomere lengths. Often the Reference Length will be set to the resting SL length or the desired SL for the start of a test.
9) For maximum performance set the Video Display to ROI. In this mode only the image within the ROI is updated on the screen. Improvements in version 1.14 of the program have sped up the calculation and display algorithms to the extent that the PC can display and calculate SL, using the FFT algorithm, on full frame images at the maximum video rate of 30 Hz. When using the ACF-Sine fit algorithm smaller regions of interest will need to be selected to obtain the maximum throughput.
10) A new feature for version 1.14 is the inclusion of a Video Display Frame Rate (Hz) control on the setup menu. By default VSL updates the screen at a rate of 10Hz. This can be changed to higher or lower rates however for live images it makes no sense to run the display faster than the camera 30Hz for NTSC and 25Hz for PAL/CCIR. Higher display rates will slow the program so unless you need higher rates it is advisable to leave the setting at 10Hz.
11) Press the Apply button and then the Close button to close the VSL Setup window.
12) The VSL program should now be producing accurate sarcomere length measurements.
13) The size of the region of interest has an effect on the frame rate. The smaller the region of interest the faster the frame rate. The tradeoff is that the accuracy of the solution decreases with smaller regions of interest. Often a good tradeoff is setting an ROI that is vertically small but horizontally large. The ROI is 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. Improvements in version 1.14 of the program have sped up the calculation and display algorithms to the extent that the PC can display and calculate SL, using the FFT algorithm, on full frame images at the maximum video rate of 30 Hz.
14) A Scope function 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 what the sarcomere length is 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 called of a starting SL of 2.5 um then by using the Scope output you can easily see the sarcomere length change as you adjust the length. Scope shows a green line at the value you have set the Reference Length (on the Setup menu) to. Therefore if you set the Reference Length equal to your starting sarcomere length then it is easy to adjust the actual sarcomere length to be on the green line. The Scope function will store in memory the sarcomere length over a time period of up to 4 hours. The scope window also has a numerical output of the current sarcomere length in the top right corner of the graph. This readout is useful to see what the exact sarcomere length is at the

current time. This is the same value as shown on the bottom of the VSL main screen. Click on Open on the Scope menu to open the Scope window. In addition to buffering up to 4 hours of data Scope can also be used to record data at low sample rates (1 to 20 samples per second). See figure 3 and chapter 5.0 for a more detailed description of Scope.

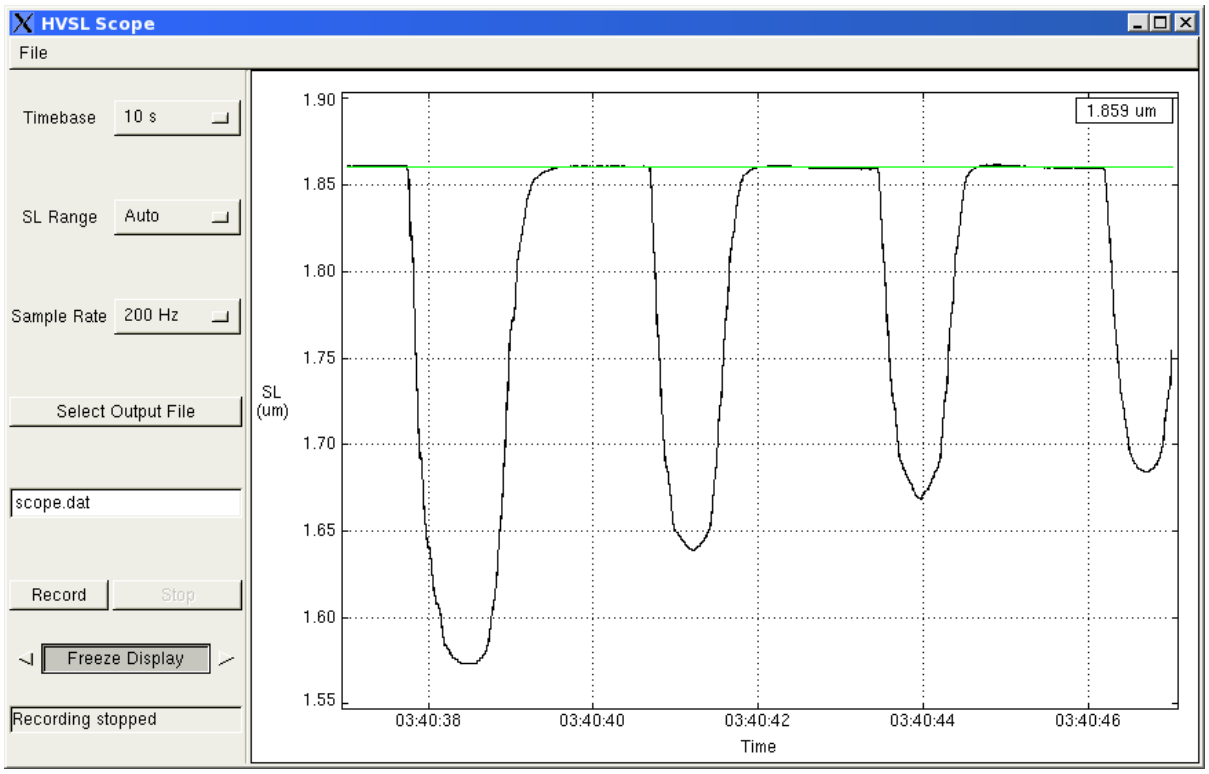

Figure 3 VSL Scope Screen

15) The VSL program can be closed either by clicking Exit on the drop down 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 Log Out. A dialog box will appear where you can select Shut down. Wait for Linux to shut down. When Linux has completed shutting down a "Power down" message will appear on the bottom of the screen. At this point it is safe to switch off the computer.

4.0 Main Program

The VSL 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 the 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.

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, two text readouts and four control buttons located below the graphics window (see Figure 4).

4.1.1 Video Image

The video image portion of the main screen is used to show the current jpg image file or the current video frame from the camera. The video image includes a range 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 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 ROI, the calculation method being used and the load on the PC. The frame rate in live video mode is also limited by the NTSC/PAL video standards which limit the rate to 30 FPS (Fames Per Second) for NTSC and 25 FPS for PAL. The actual frame rate is shown at the bottom of the screen in the FPS text readout.

4.1.2 Sizing and Moving the Range of Interest

A range of interest (ROI) box can be set by the user on the video image. 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 at the bottom of the window. To choose a ROI smaller than the full screen position the cursor at the desired starting point for the ROI, depress 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 (um).

Once an ROI box has been defined the user can reposition the box to any location on the screen by positioning the cursor in the box and pressing and holding the right mouse button. Moving the cursor moves the box with it. The ROI box is fixed at the new location when the mouse button is released. Note: if the Num Lock key is on then the right mouse button will create a new ROI and not move the ROI. Turn off Num Lock to activate the move ROI feature.

Since the sarcomere length calculation is performed only within the ROI the size of the ROI has a dramatic effect on the frame rate. Smaller ROI sizes increase the frame rate. The program is calculating the spacing between vertical bands in the image, for this reason ensure that the ROI extends over a large horizontal distance. The vertical size of the ROI improves averaging. Normally the ROI should be chosen to extend about ¾ of the horizontal distance across the image and about 10% of the vertical distance.

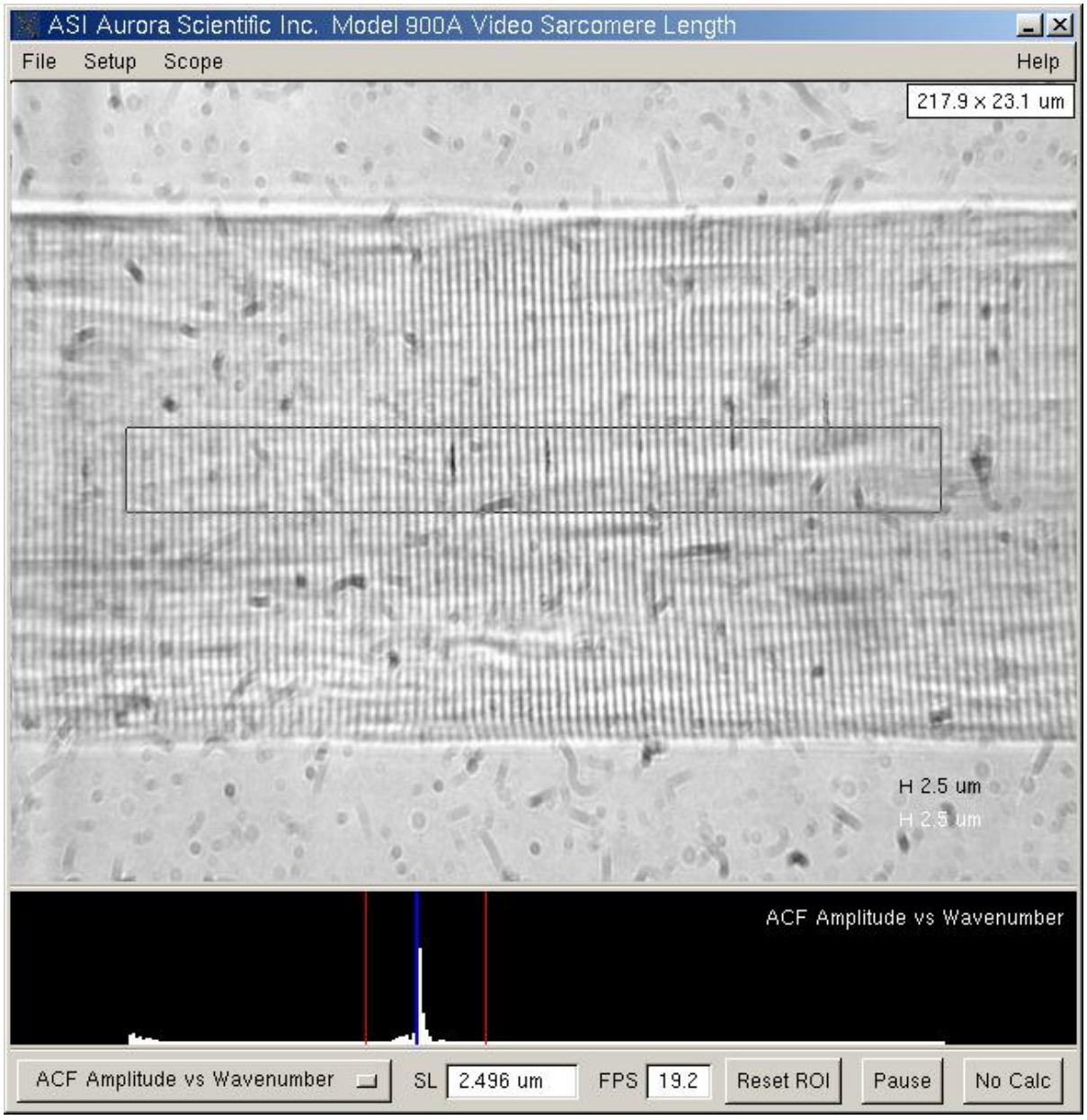

Figure 4 VSL Main Screen showing the Video Image and the ACF Amplitude vs Wavenumber Graph

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 six graphs available, ACF (Filtered), ACF (Unfiltered), FFT Amplitude vs SL, FFT Amplitude vs Wavenumber, ACF Amplitude vs SL and ACF Amplitude vs Wavenumber. The specific graph can be selected by either clicking on the graph which cycles through the available graphs or by using the drop down menu button at the bottom of the screen (see Figure 5).

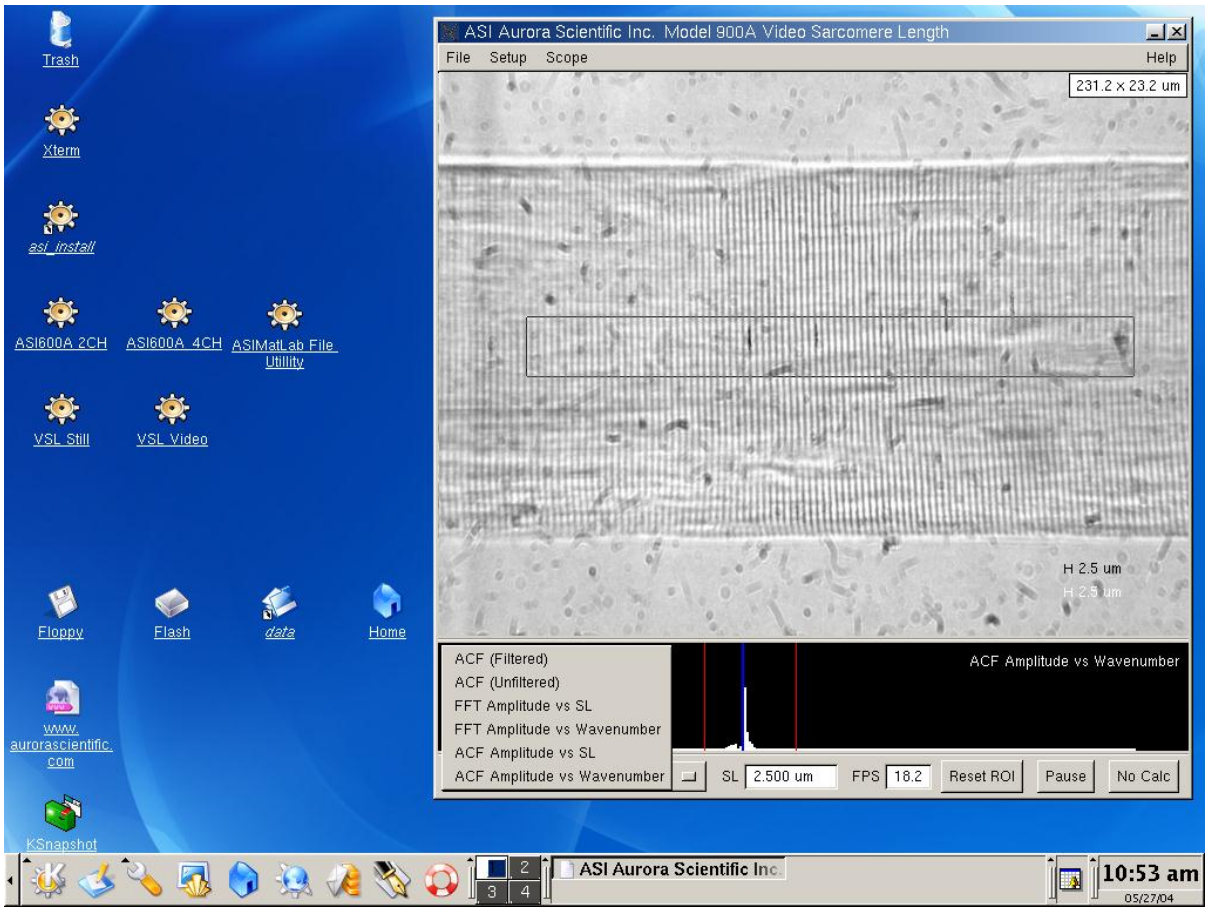

Figure 5 VSL Main Screen Graph Control

The six different graphs are presented in Figures 6 through 11. These graphs are useful for checking on the accuracy of the sarcomere length calculation. See chapter 6 for a detailed explanation of the calculation algorithms.

The ACF filtered and unfiltered graphs (figures 6 and 7) show the autocorrelation function across the ROI in black 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.

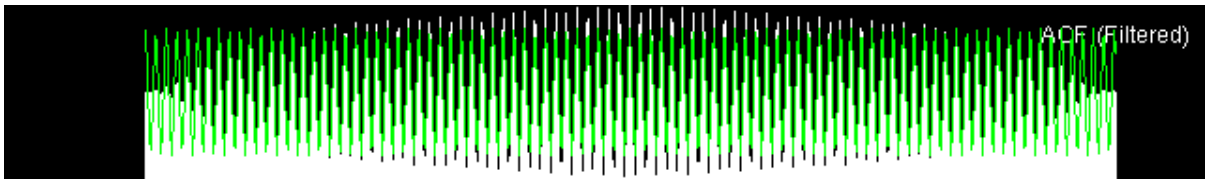

Figure 6 Graph of ACF (Filtered)

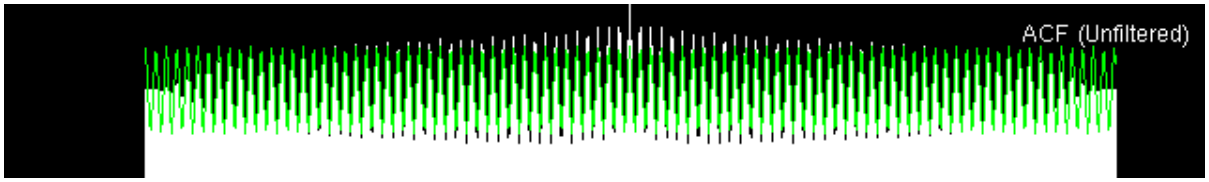

Figure 7 Graph of ACF (Unfiltered)

The FFT Amplitude vs SL and Wavenumber graphs (figures 8 and 9) show the FFT amplitude spectrum calculated across the ROI. The FFT Amplitude vs SL graph shows the peaks of the amplitude spectrum that occur only in the SL length range between Minimum SL and Maximum SL. The black vertical bars on the FFT vs SL graph show the relative amplitude at each sarcomere length. The blue line is the SL that results from the FFT algorithm. The green line represents the peak of a curve fit to the FFT spectrum. By using the peak of a curve fit to the spectrum instead of simply the peak of maximum intensity the resolution of the FFT can be greatly improved. You will notice that the Sine Fit is a continuous function whereas the FFT can only occur at discrete sarcomere lengths that are determined by the number of cycles present in the ROI. More cycles in the ROI produces a finer FFT spectrum. The FFT Amplitude vs Wavenumber graph 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 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 outside of the red bars could be the true sarcomere length if the calibration value is in error.

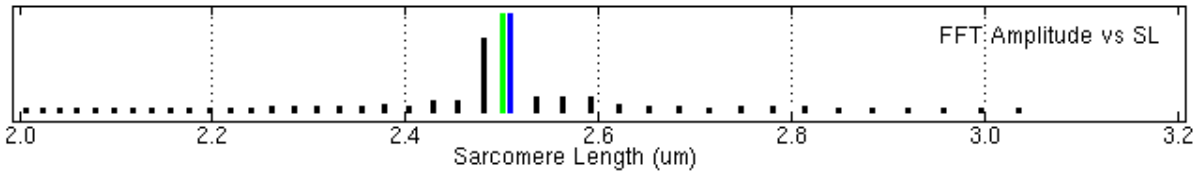

Figure 8 Graph of FFT Amplitude vs SL

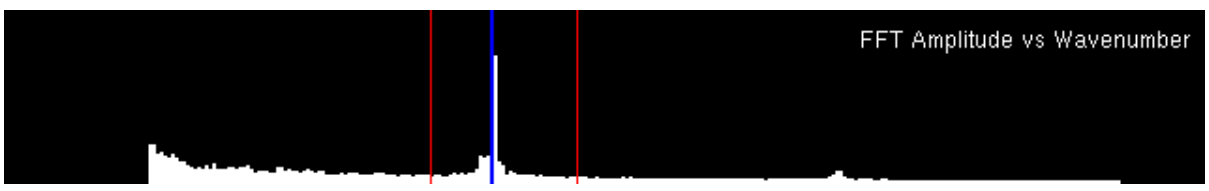

Figure 9 Graph of FFT Amplitude vs Wavenumber

The ACF Amplitude vs SL and Wavenumber graphs (figures 10 and 11) are similar to the FFT graphs but include the algorithm that filters the FFT results using an autocorrelation function. On first inspection you will note that, for the same image, the ACF Amplitude vs SL and Wavenumber graphs have a significantly lower background level which emphasizes the peak in the FFT amplitude spectrum. Chapter 6 provides more details of this algorithm. The ACF is especially useful with poor quality (noisy) images. The red bars indicate the Minimum SL and Maximum SL. The blue line is the highest peak in the FFT and the green line is the Sine Fit solution.

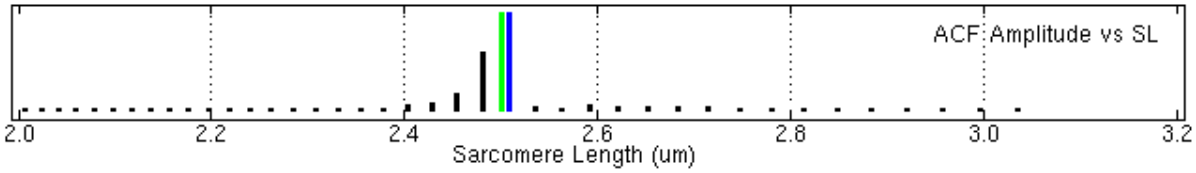

Figure 10 Graph of ACF Amplitude vs SL

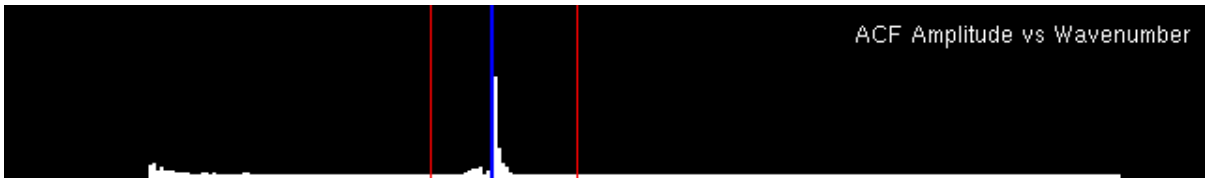

Figure 11 Graph of ACF Amplitude vs Wavenumber

4.1.4 Control Buttons

The main screen includes four control buttons located beneath the graphics window. These buttons are: Graph Display Control, Reset ROI, Pause and No Calc. (see Figure 5).

Graph Display Control Button

This is a drop down menu that allows you to choose the graph to be displayed beneath the image. Details of the different graphs are presented in section 4.1.3.

Reset ROI Button

The Reset ROI button sets the region of interest to the full image size of 640x480 pixels.

Pause Button

The Pause button is used to stop the display (freeze the image). This is especially useful when you have a changing video signal and you want to stop the display to save a particular image. Pressing the button a second time resumes live update of the video image.

No Calc Button

The No Calc button stops all calculations of the sarcomere length which will maximize the frame rate. When this button is pressed the program simply gets data from the frame grabber card and displays it on the screen. This can be useful with quickly changing images that you want to view in almost real time. Pressing the button a second time resumes the calculation of sarcomere length.

Hi Mag/Lo Mag Button

The Hi Mag/Lo Mag button allows the user to quickly change the calibration constant being used for calculations of sarcomere length and also for sizing the tissue. Normally the Hi Mag setting will be used with a higher power microscope objective for measuring sarcomere length and the Lo Mag setting will be used with lower power objectives for measuring overall tissue dimensions. On the setup menu the ROI Display control allows the ROI box to readout in microns or pixels. When set to microns the overall dimensions of the tissue can easily be determined by simply dragging out a box the size of the tissue. Switching between Hi and Lo Mag settings automatically "grays out" the calibration constant that is associated with the Mag setting not in use.

4.1.5 Text Readouts

Two text readouts are located near the bottom of the main screen. The first is the result of the sarcomere length calculation using the calculation method specified on the Setup screen in the box labeled SL Display. The second readout is the current frame rate.

4.2 Menu Structure

The main menu consists of the following eight headings.

File
Setup
Camera
600A
Scope
Playback
Record
Help

The rest of this chapter provides details for the File, Setup and Help menus. Details of the Scope function are presented in chapter 5.

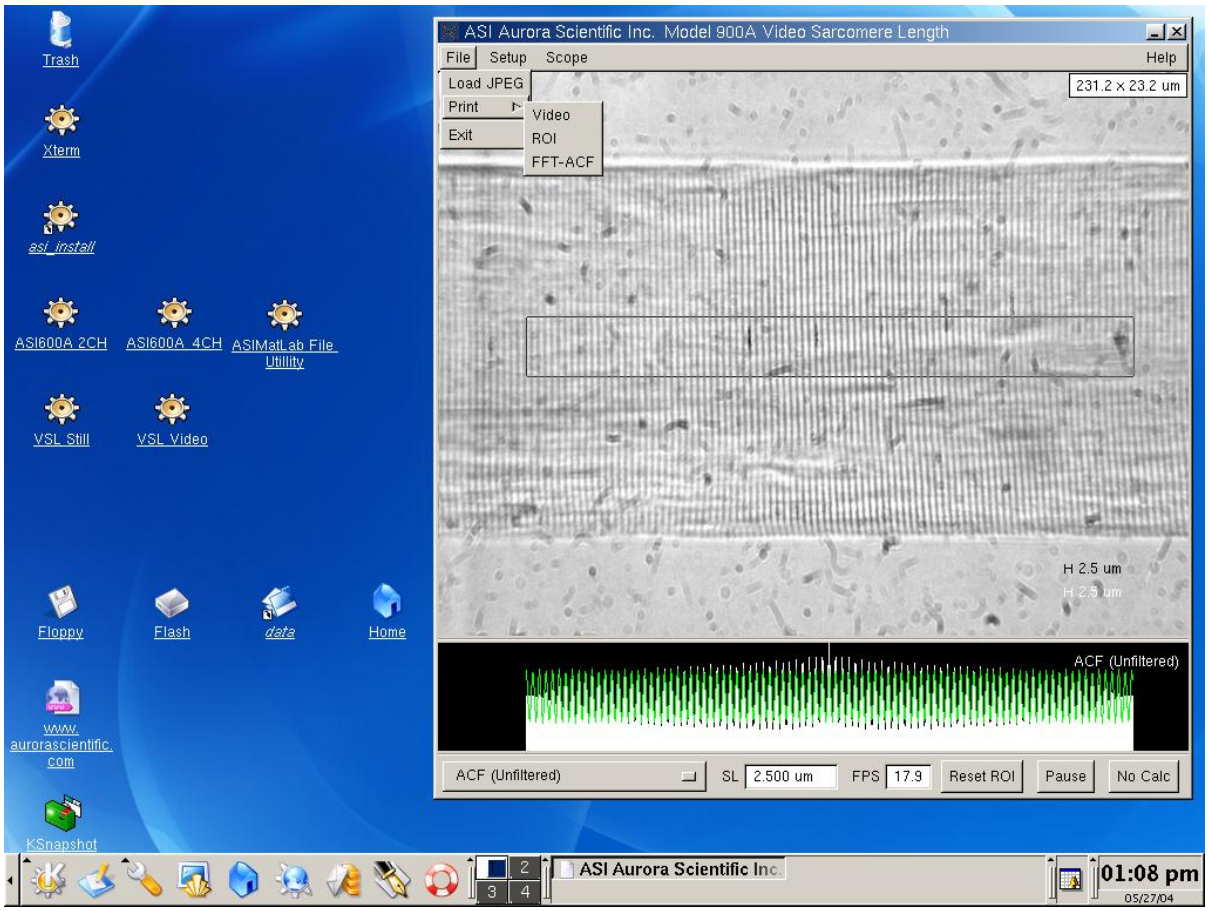

Figure 12 File Menu

4.2.1 File Menu

The File menu contains commands to Load a JPEG image, print either the video portion of the screen or the ROI portion of the screen or the graph portion and 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.

Print

Three options are available, video, ROI and FFT-ACF. The print function opens the XV program and sends the selected portion of the screen to XV. XV can be used to print directly to a printer or can be used to store the image in any number of formats. If the image is stored in JPEG format then the image can be recalled into VSL at a later date for sarcomere length processing.

Exit

The Exit menu item closes the VSL program.

4.2.2 Setup Menu

The Setup menu contains commands to calibrate the image, set the minimum and maximum SL lengths, set the reference length, control brightness and contrast of the image, set a taper on the FFT function as well as control for the video display the SL readout and the units for the ROI display (see figure 13).

Calibration FOV (um)

Clicking the diamond next to the Calibration 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. Using the mouse position the cursor at one of the 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, 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 5 pixels. A larger horizontal distance between the two points typically produces a more accurate calibration. Click on the diamond shaped button to the left of the Calibration FOV (um). Enter the known horizontal distance between the two points. Press the Calibrate SL (pixels/um) button on the setup screen. 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 Apply button to complete the calibration.

Calibration Length (um)

This calibration 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 a know line spacing or an image of a sarcomere pattern with a 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 Calibration Length (um) 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 9 and the Maximum to about 11. Set the Reference Length (um) to be equal to the pattern spacing. Press the Calibrate SL (pixels/um) button on the setup screen. 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 Calibration Length method is an iterative method that often requires repeated presses on the Calibration SL (pixels/um) button before the solution converges. A simple method of checking for convergence is to press the Calibrate SL 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. If this is the case you may need to manually enter a value in the text box beside the Calibrate SL 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 too much above the true value then the program may converge to a higher solution, if you choose a starting point too 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 Apply button to complete the calibration.

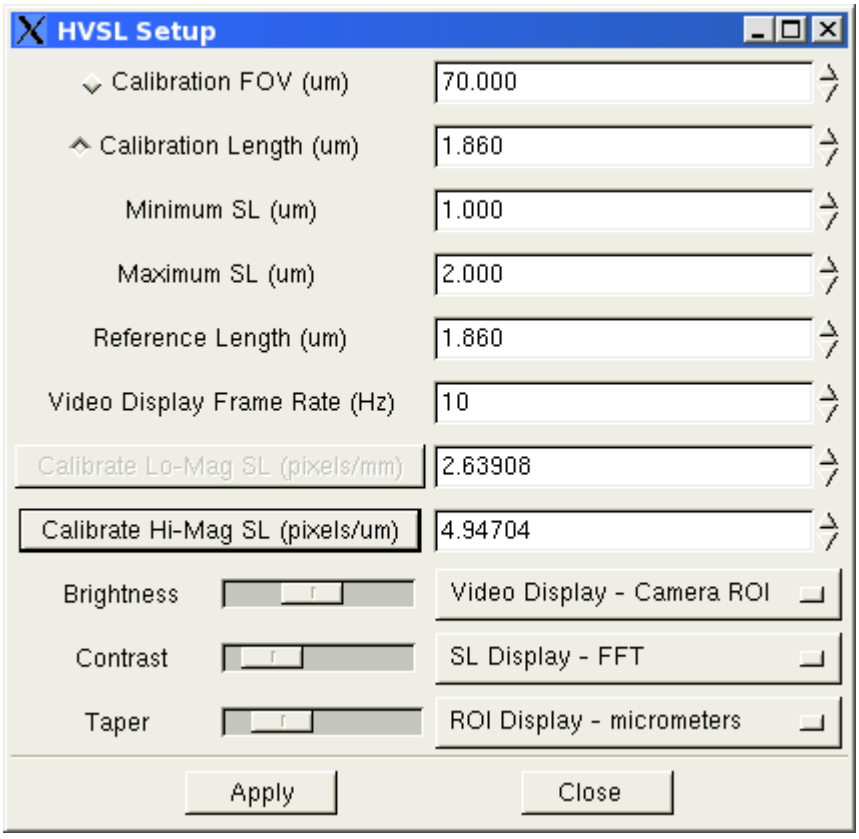

Figure 13 VSL Setup Menu

Minimum SL (um)

This text box allows the user to force the program to calculate a sarcomere length greater than the minimum value entered.

Maximum SL (um)

This text box allows the user to force the program to calculate a sarcomere length less than the maximum value entered.

Reference Length (um)

This text box allows the user to enter a reference length that is displayed as a ruler on the video image and is also displayed as a green line on the Scope graph.

Video Display Frame Rate (Hz)

This text box allows the user to enter a video display rate. The default updates the image on the screen at 10Hz. There is no advantage to setting the display rate faster than the camera FPS rate.

Calibrate Lo-Mag SL (pixels/um)

This button and the accompanying text box are used to calibrate the program (see the discussion labeled Calibration FOV and Calibration Length above) for the Lo Mag setting that is set on the program's main screen. If the calibration constant is known already (from a previous experiment for example) then the calibration value can be directly entered in the text box to the right of the Calibrate SL button.

Calibrate Hi-Mag SL (pixels/um)

This button and the accompanying text box are used to calibrate the program (see the discussion labeled Calibration FOV and Calibration Length above) for the Hi Mag setting that is set on the program's main screen. If the calibration constant is known already (from a previous experiment for example) then the calibration value can be directly entered in the text box to the right of the Calibrate SL button.

Brightness

This slider alters the image brightness.

Contrast

This slider alters the image contrast.

Taper

The taper control sets a window on the FFT calculation to reduce aliasing in Fourier transforms. The taper function is analogous to a Hanning or Hamming window. Movement of the Taper slider to the right increases the effect. The Filtered ACF graph clearly shows the effect of the taper function. Increasing the taper reduces the amplitude of the ACF near both ends of the calculation window. If the various graphs show harmonic peaks of the sarcomere length increasing the taper will often reduce these harmonics.

Video Display

Three options are available, Camera ROI (which is equivalent to full frame), ROI and Freeze. Camera ROI sets the display to the entire video image area (640x480) for each frame. ROI only updates the video image inside the region of interest. Freeze does not update the video image at all but the sarcomere length calculations are still performed on each frame. Freezing the image can often improve the calculation speed since the PC does not have to display each frame.

SL Display

Three options are available, FFT, ACF and Sine Fit. This control sets the calculation algorithm that is used to calculate the sarcomere length. See chapter 6 for a more detailed discussion of the algorithms.

ROI Display

Two options are available pixels and micrometers. This control sets the units displayed for the size of the ROI. This display is in the upper right corner of the video image.

Apply Button

The Apply button is used to apply changes made on the Setup menu to the program. For example if the reference length is changed then this will not be updated on the main screen until either the setup menu is closed or the apply button is pressed.

Close Button

The Close button closes the Setup menu.

4.2.3 Scope Menu

The Scope menu contains two commands that open and close the Scope window. Further details of Scope are presented in Chapter 5.

4.2.4 Record Menu

The Record menu opens the recording window as shown in Figure 14. This window allows the user to stream images to disk at a rate set by the current ROI. Three text entry boxes are provided to allow the Directory, Base filename and the number of frames to be collected to be entered. Pressing the Start button starts collection and logging of frames. The Stop button will stop the collection when pressed. A status bar is shown at the bottom of the Window that provides pertinent information.

Frames are logged to the directory specified using the base name followed by a consecutive number assigned by the program starting with 0. The files are stored in .png format. If you press the Start button repeatedly with the same base name then existing files will be overwritten.

Only the image inside the region of interest (ROI) is logged to disk. If full frame images are desired then press the Reset ROI button at the bottom of the main screen prior to logging images. For maximum logging speed press the No Calc button also located at the bottom of the main screen.

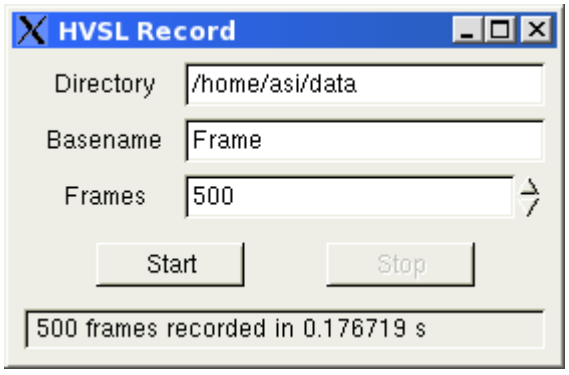

Figure 14 VSL Record Window

4.2.5 Playback Menu

The Playback menu opens the playback window as shown in Figure 15. This window allows the user to read images stored on disk at a rate set by the size of the ROI and the calculation method chosen.

Five text entry boxes are provided to allow the Directory, Base filename, start index for the first file, end index for the last file and the output file name to be entered. Playback can operate in three modes, Loop, Stream and Step. Loop continuously runs through all files between and including the start and end indexes. Stream runs through the specified files once and Step processes one file at a time allowing the user to then step to the next or previous frame. Four control buttons are provided at the bottom of the window, Start, Previous, Next and Stop. With Playback set to Loop or Stream the Previous and Next buttons remain grey. A status bar is shown at the bottom of the Window that provides pertinent information.

Typically playback is used to calculate sarcomere length after a test. Since the test is complete different regions of interest and different calculation methods can be tried on the same images. Sarcomere lengths calculated during playback are stored in a data file specified in the Output File data entry box. A button labeled View plots the contents of the Output File as SL value versus time (see Figure 16).

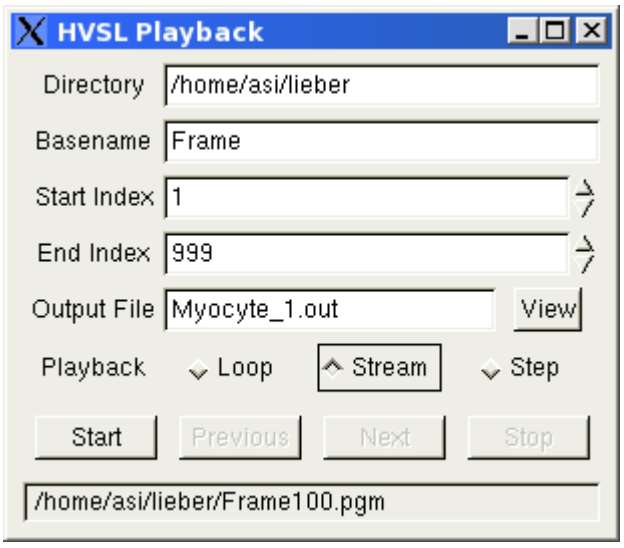

Figure 15 VSL Playback Window

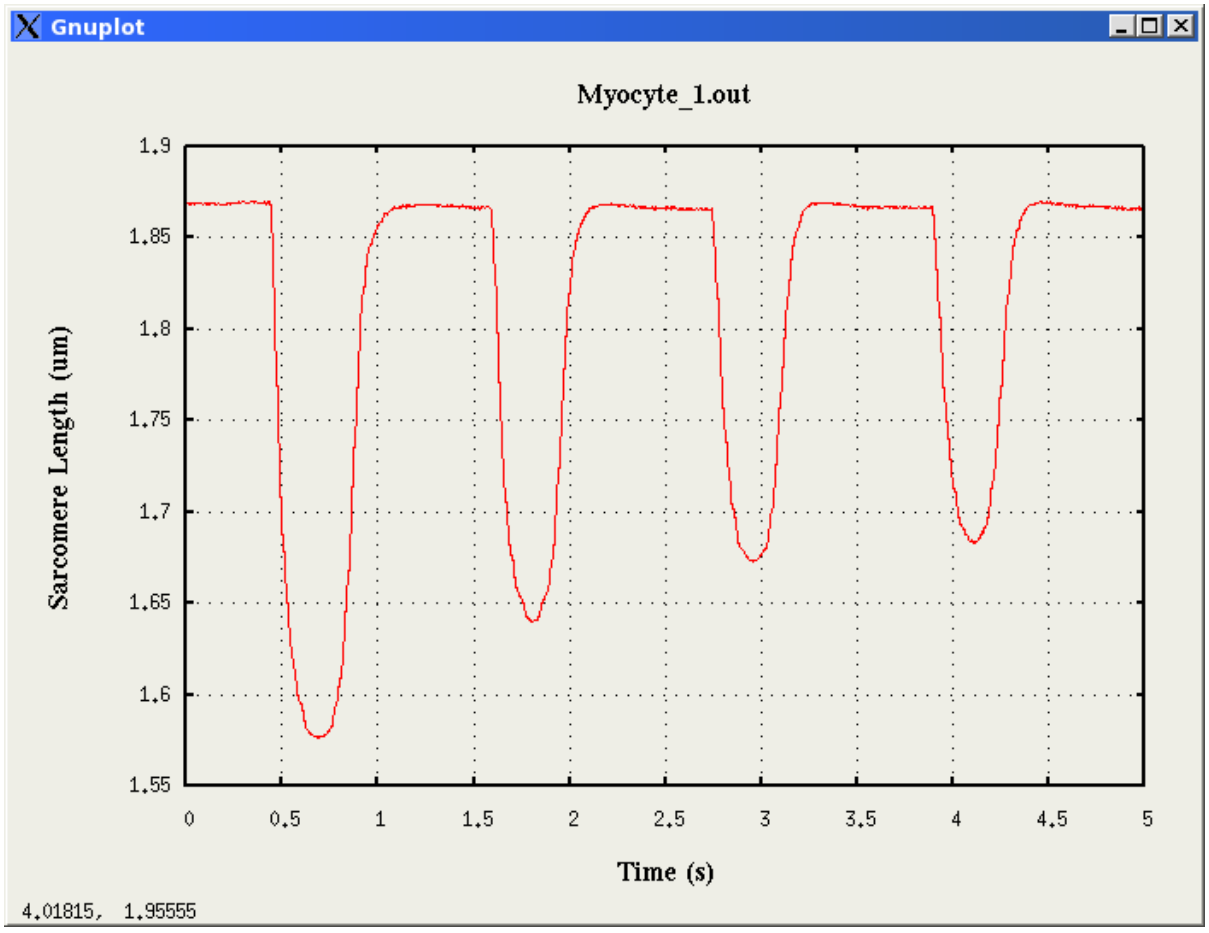

Figure 16 VSL Plot of Sarcomere Length versus Time

4.2.6 Help Menu

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

VSL Help

Opens Adobe Acrobat Reader and displays the VSL help file (this manual) on the screen. Links are included in the document.

About

Provides information about the VSL program which includes the software version number and including contact information for Aurora Scientific Inc.

5.0 Scope

Scope provides an almost 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 4 hours. Scope also includes controls to specify the time base, the SL range and the sample rate. Scope includes a data collection function that allows data to be streamed to disk for as long as the Record function is active. There is no time limit when recording Scope data to a file (other than the size of the hard drive). Scope also includes controls to freeze the display and scroll the display back and forth in time. There is also a data readout located in the upper right corner of the graph. This readout 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. The sample rate for the graph and for storage in a data file is set using the Sample Rate button.

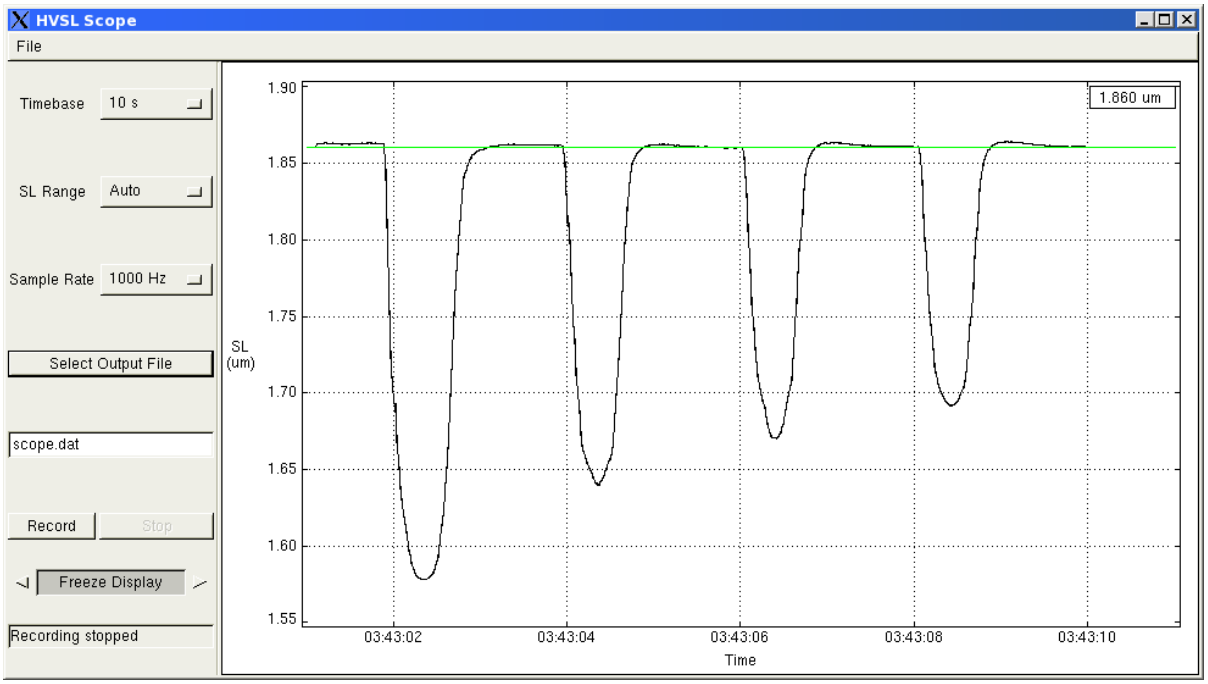

Figure 17 Scope Screen

To Use 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 10s to 60 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 Sample Rate box allows the user to select the data-sampling rate that is written to disk. This control also sets the update rate to the Scope graph.
7) The Select Output File button is used to specify an output data file name and the directory where the file should be stored.
8) As an alternate to (7) the data file name can be typed into the box that shows the current data file name. This box is located below the Select Output File button.
9) Use the Record Button to start recording data to disk.
10) Use the Stop Button to stop the current data recording.
11) 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 4 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.
12) A Status Bar is located under the Freeze Display button that shows status information about Scope including error messages.

6.0 Measurement Algorithms

The Video Sarcomere Length program includes three length calculation methods: Fast Fourier Transform (FFT), autocorrelation function (ACF)/FFT and 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 of the curve fit provides the sarcomere length. The ACF calculates the autocorrelation of each scan line and then calculates an average. For periodic images the result resembles a sine wave. The ACF/FFT method performs an FFT on this average ACF to provide the sarcomere length. The ACF/Sine-fit method fits a sine function to the average ACF to determine sarcomere length.

Of these three methods 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 small number of sarcomeres in the image.

Additionally, both the ACF/FFT and the ACF/Sine-fit algorithm handle 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 calculating the FFT of the ACF function, removing peaks outside of the expected range of sarcomere lengths and then calculating the inverse FFT. The resultant series is a filtered version of the ACF and has most of the noise removed from it. The sine-fit is then performed on this filtered series.

The FFT algorithm has the advantage that the processing time is faster and therefore provides a higher measurement rate than the ACF/FFT and ACF/Sine-fit algorithms.

7.0 Installation of a New Version of the VSL Program

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

We at Aurora Scientific Inc. are striving to make VSL the best low-cost program for online sarcomere length measurement. We are pleased to receive user comments about the program including suggestions for new functions.

New versions of VSL are available by email, from our website at www.AuroraScientific.com or via mail or courier. Once you have received a new version of the program please follow the procedure listed below to upgrade your system.

Note: Linux is case sensitive; ensure that you type the commands as shown below.

1. If the VSL program is running then exit the program.
2. Copy the supplied update file to either a Windows formatted floppy disk or to the USB Memory stick supplied with the PC.
3. Put the floppy disk or memory stick in the ASI PC.
4. Click on the appropriate Floppy or Flash icon on the desktop to open a directory window. Also click on the Home icon to open the home directory window.
5. Drag and drop the update file from the Floppy/Flash to the /home/asi directory.
6. Close the Floppy/Flash and Home directory windows.
7. Click on the *installer* icon on the desktop.
8. Browse and select the upgrade file.
9. 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 “as shipped” root password was “root”.

This completes the update procedure. In order to check that the update has been installed correctly 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.

8.0 Warranty

The 900A VSL program is warranted to be free of defects in materials and workmanship for three years from the date of shipment. Aurora Scientific Inc. will repair or replace, at our option, any part of the 900A system that upon our examination is found to be defective while under warranty. Obligations under this warranty are limited to repair or replacement of the instrument. Aurora Scientific Inc. shall not be liable for any other damages of any kind, including consequential damages, personal injury, or the like. Damage to the system through misuse will void this warranty. Aurora Scientific Inc. pursues a policy of continual product development and improvement therefore we reserve the right to change published specifications without prior notice.

8.1 Contacting Aurora Scientific Inc.

Technical assistance is available by regular mail, email, phone, or fax. Use the information below to contact Aurora Scientific Inc.

Address: Aurora Scientific Inc.
VSL Technical Assistance
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: info@AuroraScientific.com
Web site: www.AuroraScientific.com

9. 0 Terms and Conditions for Returning Equipment

1. Aurora Scientific Inc. **will not** accept any equipment returned without prior authorization in the form of a return material authorization number.

1. **Please call Customer Service at (905) 727-5161 or toll free at 1-877-878-4784 to obtain an RMA#. Please specify the product line.**

2. Please package equipment properly. Goods that are damaged in shipment are the responsibility of the shipper.

3. **Aurora Scientific, Inc. withholds the right to assess charges for the repair or replacement of such damaged goods, regardless of warranty status.**

4. Warranty repairs will be shipped back to the customer via FedEx. If you require or request another form of shipment, the cost of such service is your full responsibility.

5. Aurora Scientific, Inc. **will not** be responsible for any return or replacement **shipping charges** incurred due to an incorrect order placed by the customer.

Return Shipping Address:

Aurora Scientific Inc.
360 Industrial Pkwy. S., Unit 4
Aurora, ON, Canada
L4G 3V7
Attn: RMA Returns

Appendix A – VSL File Structure

Data File Structure

```
VSL Data File
Created: Thu May 27 14:24:53 2004
Sampling Rate: 10 Hz
Comment: Clip = 1 indicates the SL measurement
         was outside the range SLmin:SLmax
*** Sarcomere Length vs Time ***
Time (s)    SL (um)          Clip
0.00        2.499       0
0.10        2.499       0
0.20        2.499       0
0.30        2.499       0
0.40        2.499       0
0.50        2.499       0
0.60        2.499       0
0.70        2.499       0
0.80        2.499       0
0.90        2.499       0
1.00        2.499       0
1.10        2.499       0
1.20        2.499       0
1.30        2.499       0
1.40        2.499       0
1.50        2.499       0
1.60        2.499       0
1.70        2.542       0
1.80        3.000       1
1.90        3.000       1
2.00        3.000       1
2.10        2.633       0
2.20        2.499       0
2.30        2.499       0
2.40        2.500       0
2.50        2.500       0
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