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INSTRUCTION MANUAL

SI-OPTISARC

Optical Sarcomere Length Detection System

Serial No._____

www.wpiinc.com

082317

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

Fig. 1–The camera is mounted on your inverted microscope and connected to your computer.

INTRODUCTION

The **SI-H Optical Sarcomere Spacing System** includes a high speed microscope camera, the software necessary to measure and monitor the changing images and record data. It is designed for use with the **SI-CTS Cell Tester**, although a **Cell Tester** is not required for the **Optical Sarcomere Spacing System** to run. The microscope camera, with a optimal frame rate of 500 fps, mounts on your inverted microscope above the **Cell Tester** (if you use one). You may also use the system with slides of muscle tissue viewed using an inverted microscopy. The video images of the cell are fed into the software for analysis. The software is based on the NIH open source, freeware "μManager."

A custom plugin allows the software to monitor sarcomere length. With the plugin, you may:

- Measure striation spacing and sarcomere length changes
- Run sarcomere calculations at 500 fps for the optimal region of interest (ROI) setting
- When used with a Cell Tester, record analog signal proportional to sarcomere length, and muscle parameters like force

Parts List

After unpacking, verify that there is no visible damage to the sensor. Verify that all items are included:
(1) USB3.0 Camera
(1) 2μm Grating for calibration
(1) Software Installation CD
(1) Instruction Manual
(1) Quick Start Guide

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 31 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 31 of this manual.

SYSTEM SETUP

Installing the Software

The WPI system uses the NIH open source µManager program with a custom OptiSarc plugin that is built for the USB3.0 camera. Before setting up the system, install the software.

NOTE: Before you can sucessfully install the software, you must install the JAVA JRE for your operating system. Two files are included on your installation disk. Double click jre-8u131-windows-j586.exe to install the 32-bit version or jre-8u131-windows-x64. exe to install the 64-bit version.

1. Place the install CD into the CD drive on your computer. If the program does not launch automatically, use ***Windows Explorer*** to navigate to the CD drive. Double click the installation file ***Installer.jar*** (Fig. 2). The install program launches (Fig. 3), and all the necessary support programs will be installed, including:
   - WPI specific camera driver for 32-bit or 64-bit systems
   - µManager Environment support file
   - OptiSarc plugin for ImageJ

NOTE: Use 64-bit, when your operating system type is designed for a x64-based processor (most Windows OS7 or later).

TIP: To find the operating system (OS) of your computer, open Windows Explorer, right mouse click on <This PC> and open <Properties>.

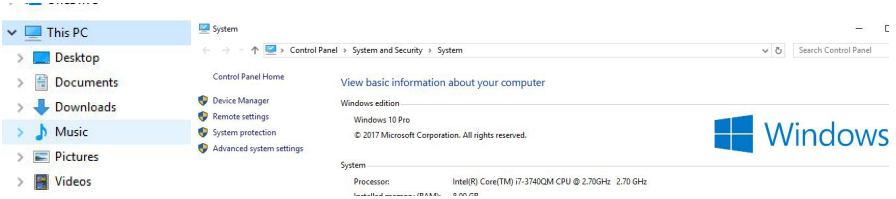


Fig. 2–Right click on This PC and choose Properties to access your system information.

Fig. 3–Double click on the Installer file to initiate the installation process.

2. Choose 32-Bit or 64-Bit from the drop down list, based on the operating system of your computer. Click Next. The Camera Installer appears (Fig. 4).

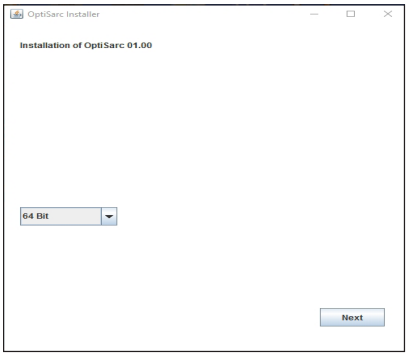


Fig. 4–Choose 32-bit or 64-bit, based on your Operating System.

3. To install the camera software, click ***Install***. The Camera Install Shield Wizard opens. Follow the instruction on screen. When the camera software installation is finished, close the wizard. Click ***Next*** on the ***OptiSarc*** Installer window. The µManger Software Installer appears (Fig. 5).

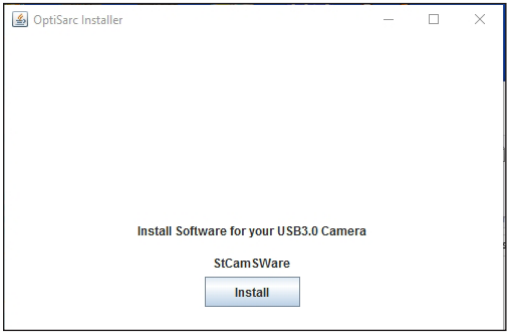


Fig. 5–Click Install to open the camera software installation wizard.

4. To install the µManager software, click ***Install*** and follow the instructions on screen. For best results, do not change the default installation directory. When the installation is complete, µManager opens. Close the µManager program. Then, click ***Next*** on the ***OptiSarc Installer*** window. The ***OptiSarc Plugin installer*** appears (Fig. 6).

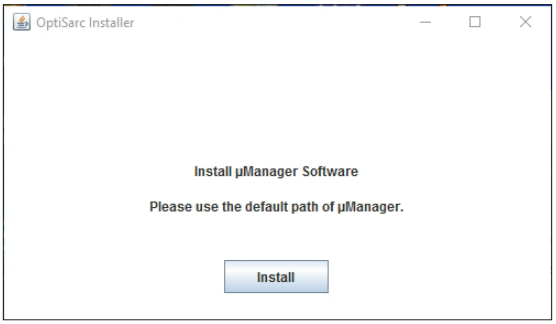


Fig. 6–Click Install to begin the installation of the µManager Software and ImageJ.

5. To install of the ***OptiSarc*** plugin, including the WPI camera driver for µManager, choose the path for the location where the plugin will be installed. This location should be the same place where µManager is installed. Click ***Install***. Then, confirm the installation path (Fig. 7). When the ***OptiSarc*** plugin files are installed in the designated location, a confirmation message appears.

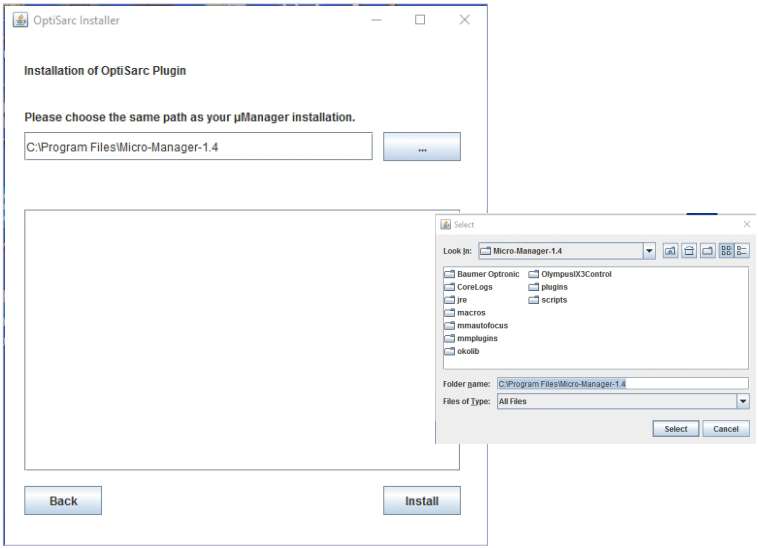


Fig. 7–When the installation of the OptiSarc plugin is complete, a message appears. Browse to the Micro-Manager default directory, if necessary (c:\Program Files\Micro-Manager-1.4).

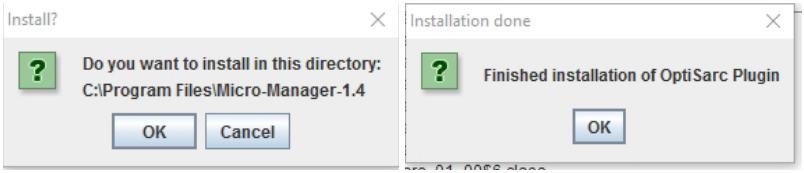


Fig. 8–(Left) Click OK on the installation confirmation message to complete the install.
Fig. 9–(Right) Installation complete message appears.

6. Another message appears indicating that the installation is complete (Fig. 8). Click ***OK***.

Configuring µManager for the USB 3.0 Camera

To use the camera in the µManager environment, you must configure the camera.

1. To configure your camera, plug the camera into a USB 3.0 port on your computer. Then, open the µManager program. The ***µManager Startup Configuration*** window opens (Fig. 9).

2. If you have already configured a camera, you may select it from the drop down list. Otherwise, chose (none) to configure a new camera (Fig. 9).

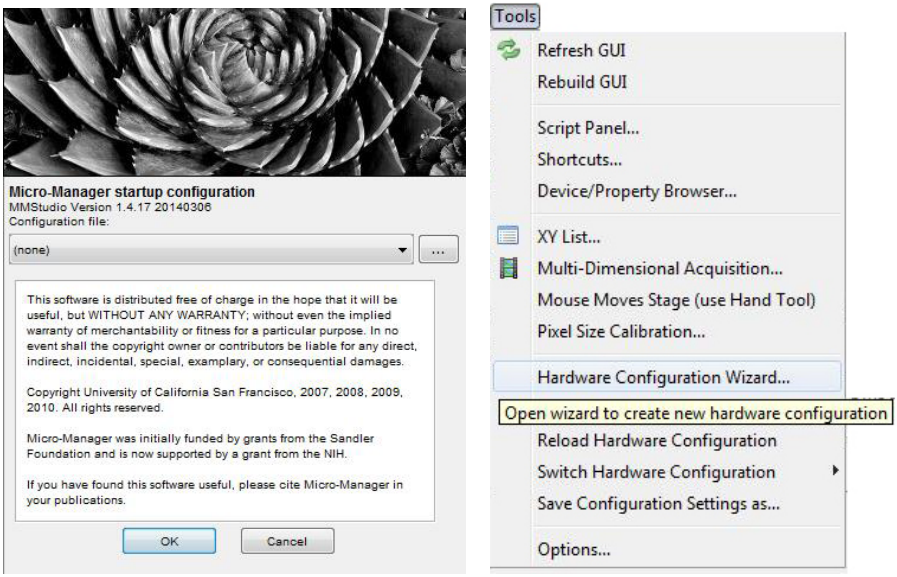


Fig. 10–(Left) µManager Startup Configuration window
Fig. 11–(Right) Access the Hardware Configuration Wizard from the Tools menu.

3. On the µManager main window menu bar, select the ***Tools*** menu and choose ***Hardware Configuration Wizard*** to open the camera configuration (Fig. 10). Click ***Next***. The Hardware Configuration Wizard opens.

4. Choose the ***Create New Configuration*** radio button and click ***Next*** (Fig. 11). A list of available devices appears.

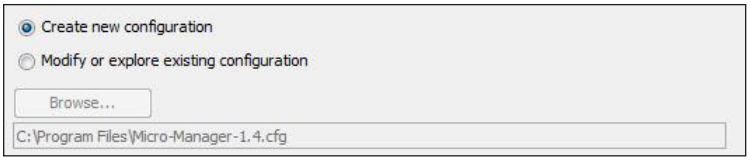


Fig. 12–First select the Create new configuration radio button.

5. To add the camera, search for the Usb3-CAM-HS folder in the list of ***Available Devices***. Open the folder and select ***USB3-CAM-HS | World Precision Instruments camera*** Click ***Add***. (Fig. 12). Click ***Next***.

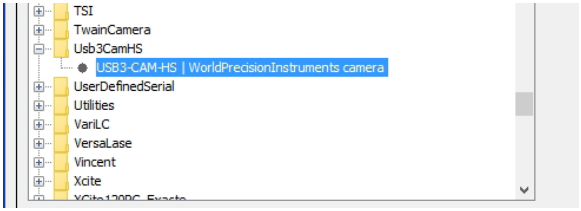


Fig. 13–*Select the USB3CamHS folder to detect your camera device.*

6. Press ***Add*** so that the pop-up window with the selected device appears (Fig. 13), then press ***OK***. The selected camera device will be installed for use (Fig. 14). Click ***Finish*** to save the configuration.

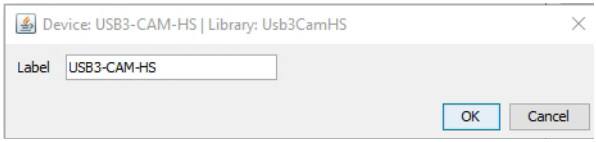


Fig. 14–Label pop-up to indicate the selected camera device.

7. If the USB3-CAM-HS was successfully added, it should be listed in the ***Installed Devices*** list in the upper left corner. Click ***Next*** to finish the configuration. Complete the configuration by using the default values for steps 3–5.

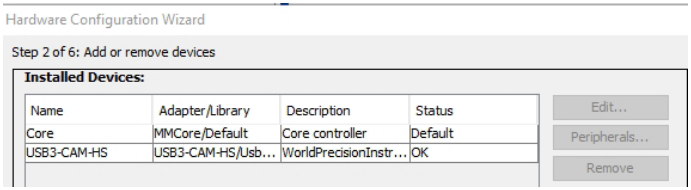


Fig. 15–Indication of successful installation of the selected camera device. The USB3 camera is listed in the Installed Devices section.

NOTE: For information on displaying and acquiring images, see the µManager instruction manual.

Product Activation of OptiSarc

WPI developed the custom plugin for use with the USB 3.0 camera to measure sarcomere length using µManager. When you launch the ***OptiSarc*** plugin for the first time, you are asked to activate the product for your system.

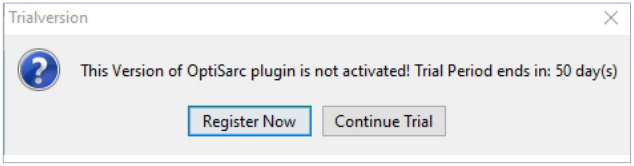


Fig. 16–Product activation is required

1. To register your product press ***Register Now***. The ***Product Registration and Activation*** window opens (Fig. 16).

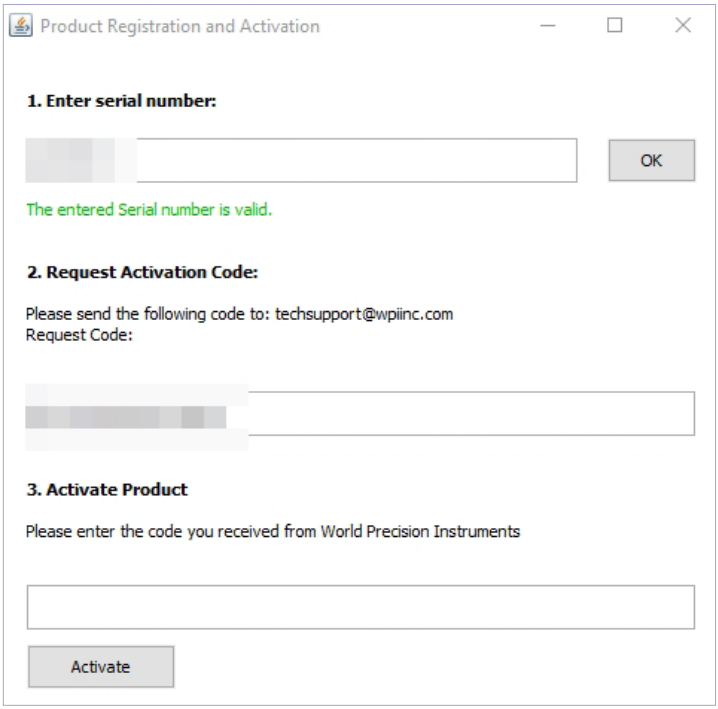


Fig. 17–The Product Registration and Activation window appears.

2. Enter your WPI serial number for the product. Press ***OK*** to validate the serial number. If the serial number is valid, a message appears under the serial number field. A valid Serial number generates a ***Request Activation Code***. Copy the code and send it to WPI at techsupport@wpiinc.com to obtain a product key.

3. When you receive your product key, enter it into the ***Activate Product*** field and press ***Activate***. A confirmation message appears indicating that your software was activated. Then, the ***OptiSarc*** plugin is ready for use.

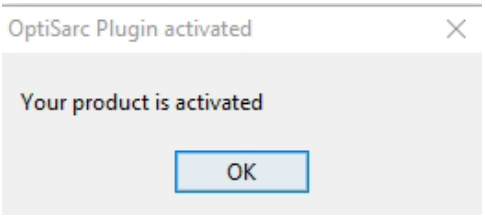


Fig. 18–The confirmation window indicates that the plugin is now activated.

OPERATING INSTRUCTIONS

Quick Start with µManager

NOTE: Please refer to the µManager Instruction Manual for complete direction on the use of µManager. This section is included as a brief overview of using µManager with your USB 3.0 camera.

1. Change the ***Exposure*** time from 10ms to 0.1ms.

Fig. 19–Use these µManager settings when using the USB 3.0 camera.

2. Click on the 

button to show your live image from the USB 3.0 camera. The Snap/Live window appears (Fig. 19).

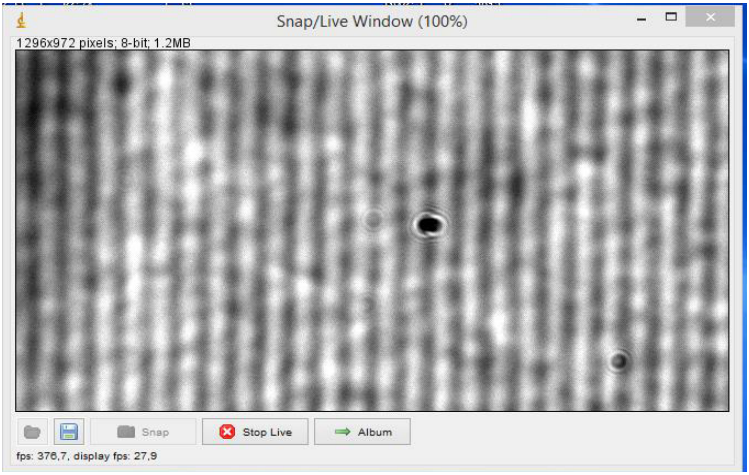


Fig. 20–The live viewing window of the USB 3.0 camera indicates the frame rate (fps). Here the ROI size of 1296x972 leads to 376.7 fps.

3. To increase the fps, change your ROI size in the live image. To do this, select  in the ImageJ window, and then select your desired ROI in the live window. Click on  to activate your selection. Watch as the frame rate changes depending on your ROI size.

   NOTE: Optimal speed of 500 fps is obtained with a ROI size of 296 x 148 pixels. The default ROI size is set by selecting "Set ROI" using the OptiSarc plugin interface.

Using OptiSarc Plugin with ImageJ

When µManager opens, two main windows appears:

- **µManager Main Window**–This is useful for running the camera
- **ImageJ main window**–The OptiSarc plugin for µManager was installed for image processing. You may access the ***OptiSarc*** plugin within ImageJ by selecting the ***Plugins*** menu and choosing ***OptiSarc*** (Fig. 20). This brings up the most recently installed version of the ***OptiSarc*** plugin.

NOTE: For information on using ImageJ, refer to the ImageJ manual.

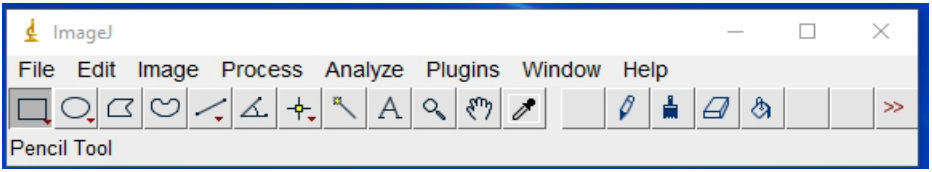


Fig. 21–You can access the OptiSarc Plugin from the ImageJ main window.

1. Edit ImageJ memory buffer in Edit\Options\Memory & Threads, and set the Maximum Memory to 4000 MB (Fig. 21). Restart µManager.

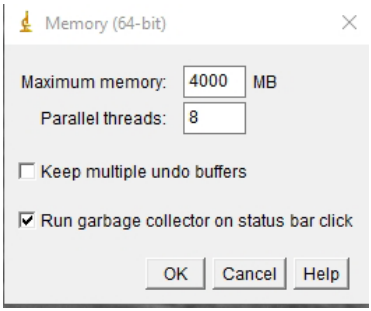


Fig. 22–ImageJ memory buffer is set to 4000 MB.

2. To initiate sarcomere detection, open a sarcomere file (photo or film) or run µManager for live detection of sarcomeres. Choose a region of interest (ROI) where you can easily distinguish the sarcomere striation (light/dark fields). A region of interest length of 10-15 sarcomeres is a reasonable field width.

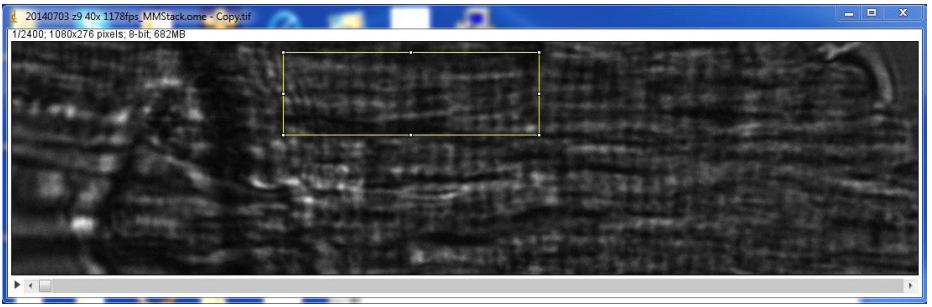


Fig. 23–The ImageJ display of a sarcomere picture was captured with µManager. The yellow rectangle indicates the chosen region of interest. Image courtesy of Dr. Michael Kohlhaas, Universitäts Klinikum des Saarlandes, Homburg/Saar.

TIP: Always open your image in ImageJ or µManager before opening the OptiSarc software, when running image analysis in off-line Mode or on-line Mode with Snap/Live.

3. To access the OptiSarc software select the ***Plugins*** menu from the ImageJ main window menu bar, and choose ***OptiSarc***. The OptiSarc software opens (Fig. 30). The optimal ROI size of 296 x 148 pixels is set by default when opening OptiSarc plugin.
4. Set up the parameters for the plugin and choose the filters you wish to use. See "Adjusting Plugin Parameters" on page 20.

TIP: The FFT frequency filter is easier to adjust for contracting muscle cells.

TIP: For sarcomere length detection, you may simply select the straight line from the ImageJ toolbar as detection ROI and place it over the desired detection position of the image/video. This height limited ROI avoids the detection of image defaults and leads to more reproducible results.

Opening an Image

1. Open an image file or launch ImageJ to detect sarcomere length changes during a contraction. Open ***OptiSarc/OptiSarc 01 00*** to launch the software.

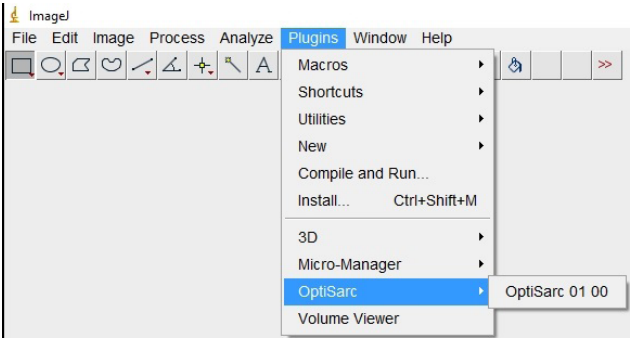


Fig. 24–Open the OptiSarc Plugin.

2. You are prompted to select the region of Interest (ROI), where sarcomere length changes should be detected. The default ROI size and position is displayed and placed in the open Image. This ensures that the ROI is always set on Image Open. Also, the given default ROI size ensures the use of the optimal Sample Frequency of 500 fps, when OptiSarc is used in Live Stream Modus.

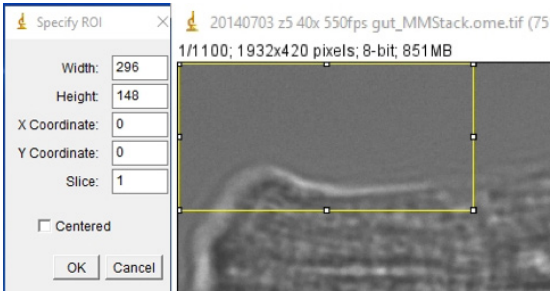


Fig. 25–Specify the ROI size and position or use the default values.

3. For easy detection of the prominent peak press ***Start*** to initiate sarcomere length detection and move the min and max slider so that the prominent peak appears (beside those of the offset at point 0).

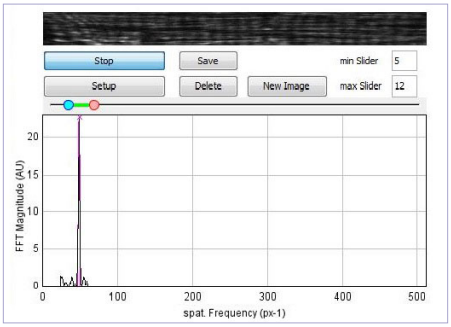


Fig. 26–

You can further limit the slider values to obtain only the prominent peak and the corresponding sub-pixel resolution (magenta line) in a manner that real detection (black line) and sub-pixel resolution (magenta line and cross) will be overlaid. However, this steams the restriction that other images probably need to be readjusted, due to the narrow band-limits of the selection.

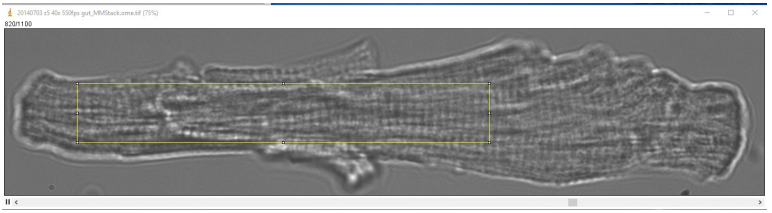


Fig. 27–Sarcomere length change detection spans the ROI over a whole myocyte.

Calibrating the Objective

Before you begin making sarcomere measurements, you must calibrate your microscope eyepiece.

1. Place the 2µm WPI grating (included) on your inverted microscope.
2. Open the image in µManager. For the most accurate calibration measurment, zoom out to make the image as large as possible. Rotate the camera so that the grating lines are nearly vertical.
3. On ImageJ menu bar, select the straight line option from the angle tool (). Draw a horizontal line across your grating.

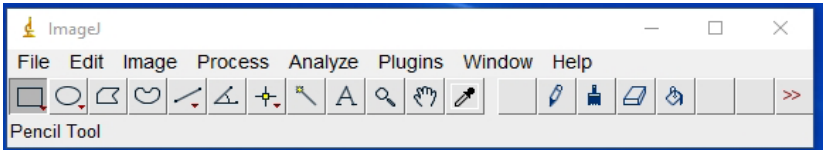


Fig. 28–On the ImageJ window, the Straight Line option of the Angle Tool is selected.

4. Count the grating lines that the ImageJ line crosses. The grating has a spacing of 500 grating lines/mm. So, the grating lines have af 2µm spacing. In Fig. 28, we see 11 grating lines. For accurate calibration there should be at least 10 grating lines. For more information, press ***Help*** on the ***Set Scale*** window (Fig. 29).

Fig. 29–Draw a line in ImageJ across the field of view of the 2µm grating that covers at least 10 grating lines.

5. In ImageJ, select ***Set Scale*** from the ***Analyze*** menu to calculate the scaling factor. The ***Set Scale*** window appears. ImageJ automatically enters into the ***Distance in pixels*** field the distance measurement (in pixels) of the straight line that you drew.

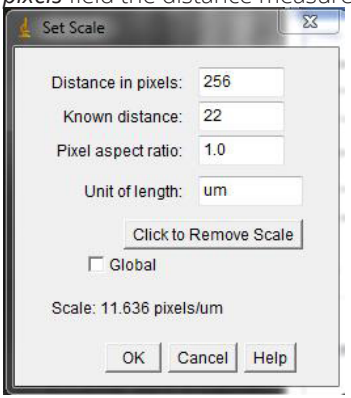


Fig. 30–The Set Scale window is used to calculate the scaling factor. In this example, the known distance is 22µm, and the scaling factor is 11.636 pixels/µm.

6. Since the drawn line crosses 11 2 µm grating lines, we know that the line we drew actually measures 22µm. Enter the actual length of the drawn line into the ***Known Distance*** field. The calculated scale factor is displayed at the bottom of the ***Set Scale*** window (11.636 pixels/µm).

NOTE: If you change the objective during sarcomere measurements, you will need to recalculate the scale factor, because the eyepiece calibration will change.

7. When you are setting the OptiSarc Plugin parameters, enter this scaling factor into the ***Scale (pixels/µm)*** field of the **Setup OptiSarc** window.

OptiSarc Window

This window shows the region of interest, an FFT magnitude chart and a sarcomere length chart. It also has four buttons for controlling the program.

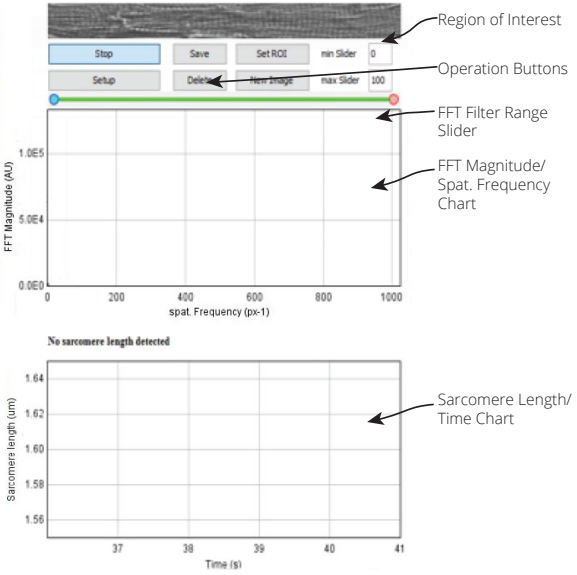


Fig. 31–The OptiSarc plugin shows the region of interest, the FFT magnitude chart and a graph of the sarcomere length v. time.

Region of Interest–The upper image is the display of the highligted region of interest. This image is used to run the calculation of the sarcomere length.

Start / Stop –Use this button to start or stop the data sampling of sarcomere length. The button automatically toggles between Start and Stop.

Save – This button stops the current data sampling and saves the actual sampled data of the temporary data buffer in the file specified in the Setup Menu. Continuing data sampling starts from the actual time point. Press this button to save the measured sarcomere length data. Pressing this button also resets the time buffer for saving data.

Delete – This button stops the current data sampling and clears the sarcomere length – time chart and the corresponding temporary data buffer. All sampled data is lost. This resets the sample time point to zero.

NOTE: Before you save data or delete the buffer, stop the data acquisition.

New Image – This buttons stops the current data sampling and clears the sarcomere length – time chart and the corresponding temporary data buffer, so that all sampled data is lost. It resets the sample time point to zero. It also prompts you to open a file,

to select a new image. It could be an already acquired image or a new image in Snap or Live modus in µManager. Opening a new already acquired image can take several seconds, depending on image size, so that during the opening process a Message pop-up can appear several times indicating that there are no images open. Press ***OK*** when loading of the new image is terminated (loading process can be seen in the ImageJ main window).

Set ROI – This button sets the default ROI size and x-y origin place in the image. If you prefer, you may also set a specific ROI size and placement values, to ensure repeatability of ROI use on images. (See Fig. 24).

NOTE: In general, there is no need to readjust the slider values to obtain the prominent peak, thanks to the overall valid FFT frequency approach in detecting nearly always similar sarcomere length. This makes the FFT frequency approach easy to use while using similar filter parameters to detect the same prominent peak.

NOTE: The best practice is to open a new image is to stop the current data sampling and then press ***New Image***.

NOTE: Use US comma settings for decimal values in Setup Menu (“.” instead of “,”).

FFT Filter Range Slider–When the frequency filter is engaged, this slider is used to filter high and low frequencies in default mode. See “FFT - Frequency” on page 17.

FFT magnitude/Spat. Frequency Chart–The chart in the middle of the window displays the prominent peak of the FFT frequency analyzed signal (black line) and the filtered signal (magenta line and cross)obtained by moving the slider frequency range.

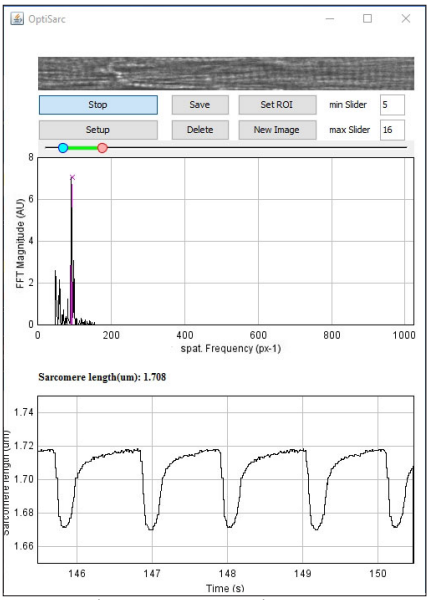


Fig. 32–The FFT magnitude/spat. Frequency Chart shows the peak value detected to filter the sinusoidal pattern out.

FFT - Frequency

The FFT (Frequency Fourier Transform) filter allows you to filter low and high frequencies in the image during the sarcomere length measurement using the range sliders in OptiSarc Main Window. FFT - Frequency filter is the default filter, and it is always active.

1. Press Start on the main plugin window to begin capturing data.
2. Select the filter frequencies by simply moving the range sliders (Fig. 31). The blue slider (left side) can be used to filter low frequencies, and the red slider (right side) can be used to filter high frequencies. Move the slider to optimize the prominent peak detection in the FFT magnitude/spat. Frequency chart.

NOTE: The slider values are visible in the main OptiSarc window. See Fig. 30.

TIP: Move the blue slider (left) to filter out the DC component of the image. Then, move the red slider (right) so that it is close to the blue one. Move the sliders as needed to obtain the highest prominent peak (matches with magenta cross), and the original data set (black line) matches the filtered data set (magenta line). Observe that the sarcomere length detection is enabled when moving the ROI over the image.

Sarcomere Length/Time Chart–The bottom chart displays the measured sarcomere length change over the measured time (Fig. 32).

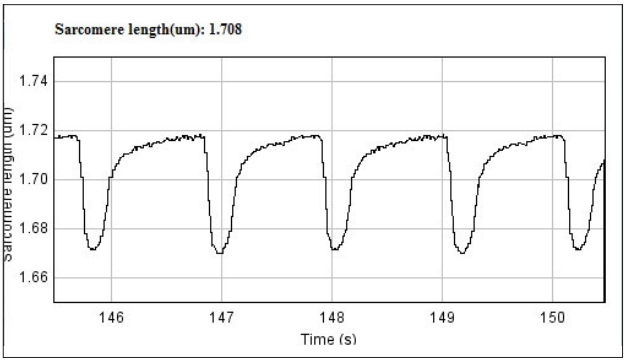


Fig. 33–Sarcomere length change during a ~1 Hz contraction of a cardiac myocyte.

Pre-Filtering Noisy Data

Additional pre-filtering of noisy images can be done by activating the ImageJ genuine FFT band pass filter in the ***Setup*** menu. The functioning of this band pass filter is equal to those described in the ImageJ Manual and can be used either in dynamic mode or static mode. Static mode is more appropriate for improving stable sarcomere length detection in noisy images. The use in dynamic mode is limited due to the time consuming nature of the pre-filtering process, resulting in non-fluent sarcomere length changes. As a pre-filtering option, the ImageJ genuine FFT bandpass filter runs a

preliminary scan over the noisy image before applying the FFT - frequency detection of the prominent peak.

To activate the pre-filtering option, select ***Filter Settings*** in the ***Setup*** menu and adjust your filter parameters to improve image quality. To evaluate changes in image quality, observe how the signal is changing in the Intensity/Sarcomere length chart (upper chart), while changing the filter parameters.

The use of pre-filtering can be canceled by simply opening the ***Setup*** menu and pressing ***Cancel*** (Fig. 35). The default FFT - frequency filtering is then active.

FFT–ImageJ Bandpass Filter

This filter is used to sharpen an image by removing blur caused by things like movement artifacts. Two options for this filter, which are often used together, allow you to filter out the largest structures and/or filter out the smallest structures. When you select the ***FFT-ImageJ*** filter type, the Filter settings button appears. Click the button to open the ***FFT Bandpass Filter*** window (Fig. 34) and set your parameters for the filter. After you set your parameters, click OK. For more information on this filter type, press Help.

Fig. 34–When the FFT ImageJ filter type is selected, the Filter Setting button appears.

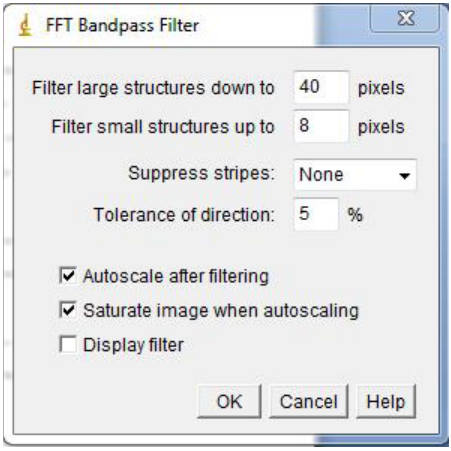


Fig. 35–Use this window to establish the parameters for your filter.

Filter Large Structures Down to–Enter a number of pixels here. Structures comprised of more pixels than this threshold are filtered. This filter corresponds with

the suppression of low frequencies in the image. It is typically chosen to filter out smooth variations like movement artifacts in the background. Values greater than the chosen filter value are suppressed. To suppress movement artifacts, increase this filter value.

Filter Small Structures Up to–Enter a number of pixels here. Structures comprised of fewer pixels than this threshold are filtered. This filter corresponds with the suppression of high frequencies in the image and determines the amount of smoothing. Objects in the image smaller than this size are strongly attenuated. To remove movement artifacts increase the filter value. Resting sharp peak values in the image are also filtered out by increasing this value.

NOTE: Increasing the region of interest size/width may improve the detection quality.

Suppress Stripes–Use this drop down list to choose whether or not to eliminate horizontal or vertical stripes.

Tolerance of Direction–If you use *Suppress Stipes*, you may set a tolerance value. Higher values remove shorter stripes and stripes that are running under an angle with respect to the chosen direction (horizontal or vertical).

Autoscale after Filtering–By selecting this checkbox, you turn on autoscaling. The lowest intensity is set to 0, and the highest intensity is set to 255.

Saturate image when Autoscaling allows some intensities to go into saturation and produces a better visual contrast. Saturate will only produce an effect when autoscaling is enabled.

Display Filter shows the filter that is generated.

Adjusting Plugin Parameters

Before you begin making measurements, you may adjust the plugin parameters. To do this you press Setup. The ***Setup OptiSarc*** window appears.

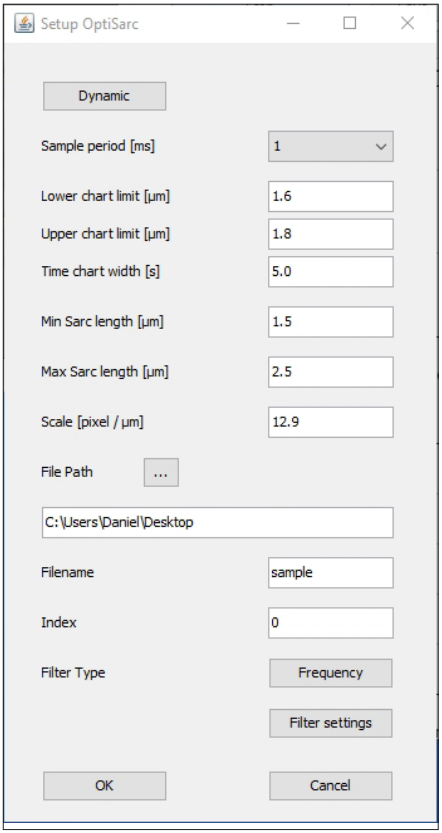


Fig. 36–Setup Menu with use of Filter Type selection. ***Frequency*** *is the default analysis mode, while the additional ImageJ genuine FFT band pass filter can be added by pressing* ***Filter Settings****.*

Static / Dynamic –Press this button to toggle between static mode and dynamic mode. It may be useful to toggle between the modes when you switch from running tests on resting muscle condition (static mode) and contracting muscle condition (dynamic mode). If you are using a large region of interest with imperfections in your image, you will see better results with static mode. You can even use the Min and Max Sarc Length parameters to filter out these image imperfections.

Sample Period (ms)–Select your sample period (in milliseconds) from the drop down list. This defines the waiting period between two measurements.

NOTE: A measurement will not begin until a previous measurement has finished. This could happen if the measurement takes longer than the sample period, which could happen if the region of interest is too large.

Lower Chart Limit (µm)–Set the minimum value in microns of the Y-axis for the *Sarcomere Length/Time Chart*.

Upper Chart Limit (µm)–Set the maximum value in microns for the Y-axis for the *Sarcomere Length/Time Chart*.

Time Chart Width (s)–Set the total width in seconds of the X-axis for ***Sarcomere Length/Time Chart***.

Min Sarc Length (µm) – Set the minimum sarcomere boundary to be detected. A sarcomere length smaller than this threshold is ignored.

Max Sarc Length (µm) – Set the maximum sarcomere boundary to be detected. A sarcomere length larger than this threshold is ignored.

TIP: When sarcomere length is outside the range set by the minimum and maximum thresholds, a question mark displays for the sarcomere length on the OptiSarc Window. To see a value, expand your detection range (Min/Max Sarc Length settings) or select a larger region of interest.

Scale (pixel/µm)–Set the calibrating factor to compare pixels with microns. See "Calibrating the Objective" on page 13.

File Path  –Press this button to open the folder browser and select the location of your saved files. After you select the folder location, the file path appears in the text field. If you need to create a new folder, use Windows Explorer.

File Name–Enter the name for your new data file where the measurement data will be stored. If you do not change the file name here, the existing name will be appended with a number that increments automatically each time measurements are saved.

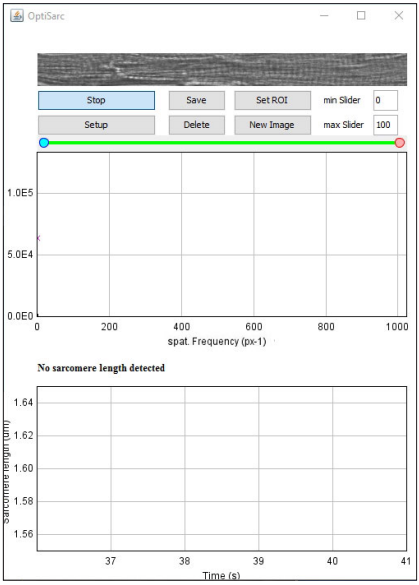
Index–This is the starting number used when the file name is automatically incremented. In the example image, the first file name will be ***sample0***.

NOTE: The index needs to be an integer value.

Filter Type–Filters are used for image processing, and each type has its own settings which are detailed above. The filter type options include:

- ***Bandpass Filter –***This is an ImageJ based FFT image filter (page 18). This is an additional bandpass filter, which may be added to filter out/smooth the image. This additional filtering is more appropriate for use in static mode, as it slow downs the sample frequency.
- ***FFT Frequency–***This filter type is the default filtering, which filters out a selected frequency range (page 17).

Sarcomere Length Detection in Dynamic Mode



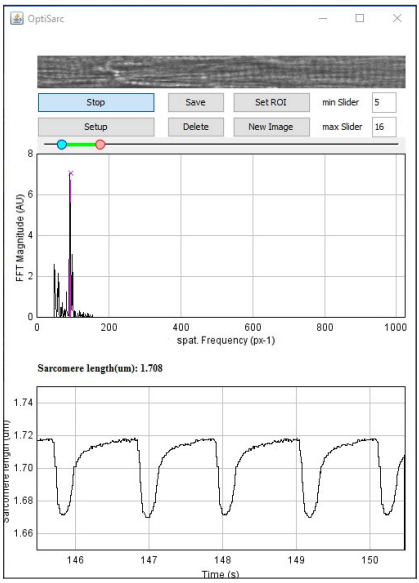


Fig. 37–(Left) No sarcomere length change is detected when the sliders are not set to detect the FFT peak value.
Fig. 38–(Right) Moving the slider detects automatically the FFT peak value and the sarcomere length changes during contraction.

To start sarcomere length detection, press ***Delete***. This clears the current temporary data buffer and restarts data sampling. Current sarcomere length is also displayed as numerical value.

To start the detection of sarcomere length changes during contractions:

1. Select your optimal ROI size of the desired sarcomere area.
2. Run the Live stream image or the acquired film, loaded into ImageJ. In ImageJ you can adjust the play-back sample frequency to match close those of the original film.
3. Adjust the sliders so that the prominent peak of the original data (black line) matches those of the filtered data (magenta cross).
4. Observe the contraction patterns in the Sarcomere length chart (Fig. 37).

NOTE: During sarcomere length detection, the chart may display no sarcomere length change, and the numerical value may indicate that no sarcomere length change was detected. To troubleshoot, check the threshold values (minimum and maximum sarcomere length) in the Setup Menu. The actual detected sarcomere length may be out of the selected limits. Alternatively, check the scale value.

NOTE: The best practice for measuring sarcomere length is to rotate the camera to

align the myocyte axis on the microscope stage as horizontal as possible. Also, avoid rotation of myocytes during contractions. This ensures the most detectable results of sarcomere length changes during contraction. Possible rotation of myocytes can be corrected using ImageJ image processing tools as post image-processing feature.

Sarcomere Length Detection in Static Mode

The initial procedure is like those already described in dynamic mode. The difference of the static mode, selectable in the ***Setup*** Menu, is the fact that the real detected prominent peak is treated. This procedure is most appropriate when analyzing fixed muscle slices, even so, when the USB camera is in Live Mode in µManager. In static mode, the current sarcomere length of the only prominent peak (black cross) from real detection (black line) is displayed as numerical value.

TIP: During sarcomere length detection, the chart might display with no sarcomere length change, and the numerical value would indicate that no sarcomere length was detected. To troubleshoot, check the threshold values (minimum and maximum sarcomere length) in the ***Setup*** menu. The actual detected sarcomere length may be out of the selected limits. Alternatively, you could check the ***Scale*** value.

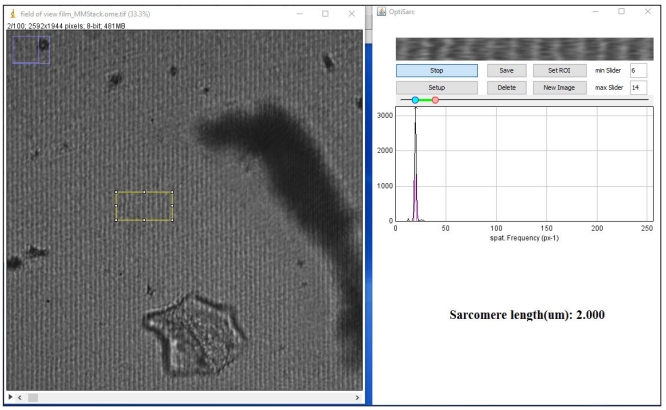


Fig. 39–This is an example of true detection from a grating length of 2 µm.

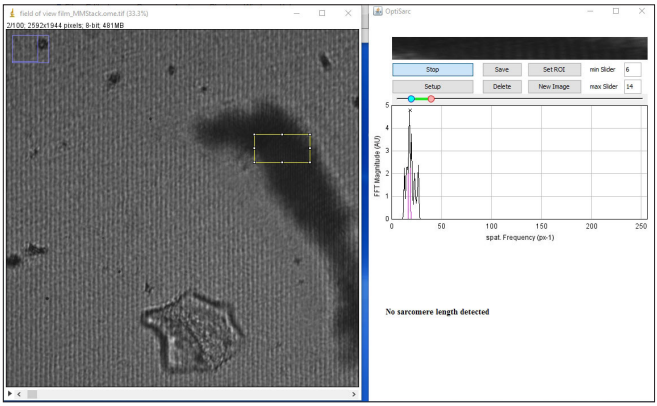


Fig. 40–This is an example of false detection from grating length of 2 µm.

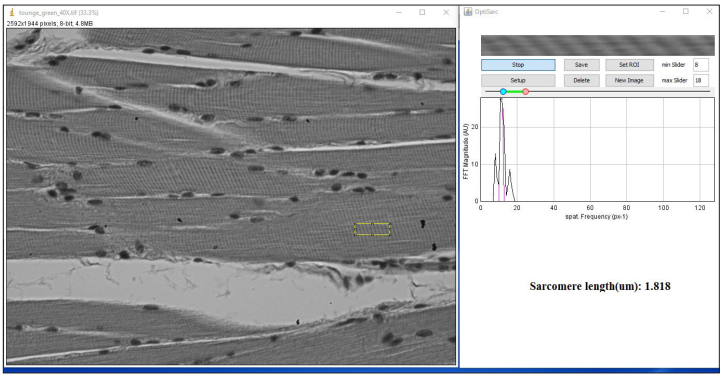


Fig. 41– This shows true sarcomere length detection from microscopic slide of a tongue slice, when ROI is placed over sarcomere striation.

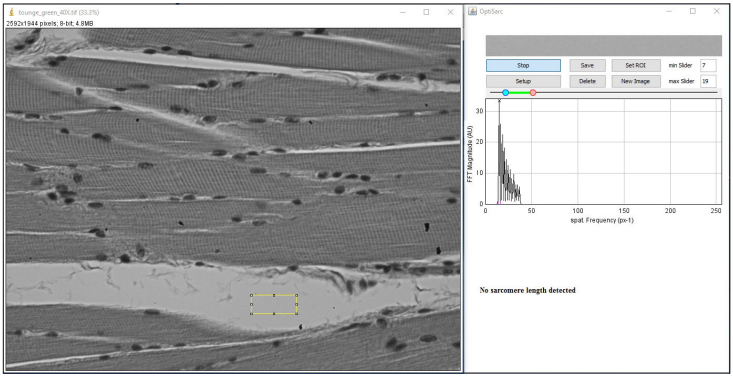


Fig. 42–This shows false sarcomere length detection from microscopic slide of a tongue

slice, when ROI is not placed over sarcomere striation.

TIP: In µManager, the OptiSarc static mode is easier to use in Live mode or Snap mode, but should be preferentially used when slides of muscle slices on a microscope are analyzed, e.g. a still image.

NOTE: The best practice for measuring sarcomere length is to rotate the camera to align the muscle tissue axis on the microscope stage as horizontally as possible.

TROUBLESHOOTING

Issue	Possible Cause	Solution
Program will not zoom out.	Only the ROI from the previous is visible, because it wasn't closed properly.	Open the OptiSarc software. There should be an icon on your desktop. Choose the Options menu and select Settings. When the Settings window opens. Press the Reset button.
Plugin crashed before I even started	No ROI was selected before starting the plugin.	Before making any measurements, you must load an image, a video or the live camera field of view and select a region of interest. Then you can start the OptiSarc software.
The time displays with negative values	Occasionally, you may see negative numbers or spikes on the sarcomere length graph.	Close and restart µManager.

NOTE: If you have a problem/issue that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

This unit conforms to the following specifications:

Camera	5MP, monochromatic, USB3.0
Maximum frame rate	2,106 fps @ 32 x 32
Optimal frame rate	500 fps @ 296 x 148

µManager* developed at Vale laboratory at UCSF, funded by an NIH grant R01-EB007187 from the National Institute of Biomedical Imaging and Bioengineering (NBIB)

*Arthur Edelstein, Nenad Amodaj, Karl Hoover, Ron Vale, and Nico Stuurman (2010), Computer Control of Microscopes Using µManager. Current Protocols in Molecular Biology 14.20.1-14.20.17

APPENDIX A: µMANAGER FUNDAMENTALS

This section is reproduced from the µManager Wiki with permission from µManager. The complete contents of the µManager manual may be found at http://micro-manager.org/wiki/.

Use of µManager for Image Configuration

Snapping single images

To obtain a single image from the camera, press the ***Snap*** button. A display window will pop up with the acquired still image. You can use any of the available ImageJ tools to analyze, save or edit the image. In addition, at the bottom of the window there are shortcut buttons to save the image, enter live mode or send images to album. Each time you press the Snap button, the image in the display window will be updated.

Live image mode

To see a continuously updated, "live" view from the camera, press the ***Live*** button. The images will be displayed in the "Live" window. Pressing this button again, stops live mode. Settings in the Main Window can be changed during live mode and the effects on images in the "Live" window will be immediate.

Acquiring a series of images

With the ***Album*** button, you can collect a series of still images (snaps) in an image series window. The first time you click the ***Album*** button, a new series window will open, with a fresh image obtained from the camera. Every time you click the ***Album*** button thereafter, a new image will be added to the series. Click the ***Save*** button to write all images in the series window to disk.

Memory Settings

It is often necessary to adjust memory settings to optimize µManager performance and prevent errors. This can be a somewhat complicated topic, especially if your computer has limited memory (compared to the size of acquired images) or if you are running the 32-bit version of µManager (which cannot access more than 2 GB of memory, or 4 GB on OS X).

There are two available settings:

- ImageJ memory limit (***ImageJ*** | *Edit* | *Options* | *Memory & Threads...*)
- Sequence buffer size (***Tools*** | *Options*).

These each need to be large enough for what they are used for, but small enough so that everything fits into RAM. They also need to consider some additional constraints.

There are three types of memory used by Micro-Manager:

- General application memory
- Sequence buffer (temporary storage for images during fast acquisitions)
- Image storage (used when acquiring to RAM rather than disk).

The ImageJ memory setting only determines the maximum for the general application

memory, which is not usually used for µManager image storage. (It is used for images opened in ImageJ, though).

Here are some guidelines for how to adjust memory settings:

Generally, you do not need to set the ImageJ memory setting very large, unless you experience "out of memory" errors when processing images using ImageJ and ImageJ plugins.

- For the 64-bit version, 1-4 GB is recommended (but leave several GB of your total RAM for other purposes).
- For the 32-bit version, it is important not to set the ImageJ memory too large. 400-800 MB is usually a good range, and you should not exceed 1.2 GB (a little larger is okay on OS X).
- For the sequence buffer size setting, try first setting the buffer to 20-100 times the size of a single image from your camera. You may get "sequence buffer overflow" (also known as "circular buffer overflow") errors during acquisitions, especially at high frame rates. If so, try increasing the sequence buffer size (for fast streaming of large images, much larger settings can sometimes be useful). You can also use the Sequence Buffer Monitor plugin (***Plugins*** | ***Developer Tools***) to see how much of the sequence buffer is used during a Multi-Dimensional Acquisition.

In general, the total of the sequence buffer size and ImageJ memory setting should not exceed about 80-90% of total available RAM (the free RAM before starting Micro-Manager). Additionally, for the 32-bit version only, the total of the sequence buffer size, ImageJ memory setting, the size of the image datasets you want to acquire to RAM, plus some extra space, must not exceed 2 GB (4 GB on OS X).

Use of µManager for Camera Settings

Camera Settings

A few camera settings found in all systems are directly accessible from the main window:

- Exposure Time: You can set the exposure time of your camera.
- Binning: Apply binning ('pooling' of pixels in both x and y direction).
- Active Shutter: You can change the 'active shutter': that is, the shutter that µManager will open before taking an image and close once the image is made.
- Deactivating Auto Shutter: This behavior of automatically opening and closing the shutter can be defeated by unchecking the 'Auto shutter' checkbox. Doing so will let you open and close the active shutter with the 'Open/Close' button in the Main Window.

Region of Interest (ROI)

Most of the cameras used for microscopy can be configured to image only a Region of Interest (ROI) instead of the full frame. To select an ROI, use the rectangle tool (from the ImageJ window) on the image window. By pressing the ***ROI*** button while

the selection is active, you will apply the current rectangle to the camera. If there is no rectangle placed, then pressing the ***ROI*** button will halve both X and Y dimensions while keeping the same center. Some cameras will internally adjust the rectangle dimensions slightly to fit within specific hardware constraints. To return to the full frame imaging, press the ***Full*** button (right next to the ROI button).

Use of µManager for Recording Images

Multi-dimensional acquisition

To acquire multi-image stacks, press the ***Multi-D Acq***. button to open the Acquisition Control dialog. (This dialog can also be reached through the ***Tools*** | ***Acquisition*** menu entry). µManager allows you to create a stack as multi-channel (wavelength coordinate), multi-frame (time coordinate), multi-slice (Z coordinate), multi-position (XY coordinate) or any combination of these.

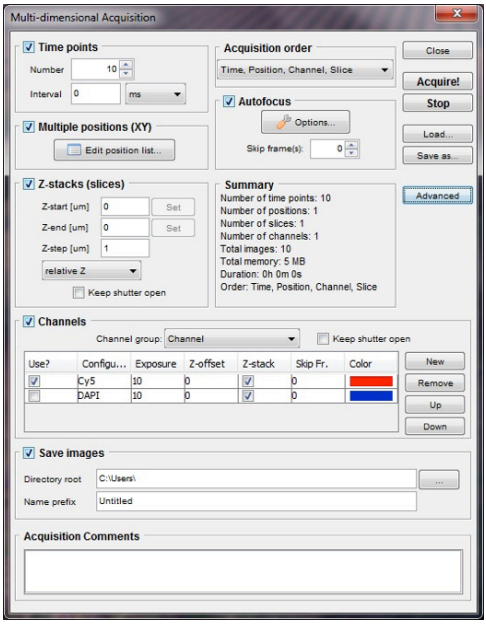


Fig. 43–Acquisition control dialog.

After defining channels, slices and frames by using controls in the dialog, press the ***Acquire*** button. The acquisition starts immediately and an Image Viewer window will open displaying the progress. During and after acquisition you can use controls at the bottom of the 5D-Image window to play-back the sequence, browse channels, slices, frames, or positions, or save the entire image stack to disk.

Time points will allow you to define the number of frames you would like to acquire and the duration of the interval time between each frame. If you would like to acquire

a continuous acquisition, then simple specify the interval time as "0." The rate at which you acquire images will then be limited only by the speed at which your camera will acquire an image (hardware specific) and the exposure time you specify in the Main Window. For more complex time lapse setup click on the ***Advanced*** button in the acquisition control dialogue.

Z Slices can be set either as relative to the current position (you will need to type in the start and end position) or as absolute positions. If absolute positions are selected, the ***Set*** buttons will become active. Clicking these will set the current position to start or end.

Channels can be selected from the active "Channel group." Channel groups are the Configuration Preset groups that are defined in the configuration settings section in Micro-Manager's Main Window. You will need to set the desired exposure time for each channel. You can also set a z-offset for each channel, which can be useful when the main object in one of the channels is in a different focal plane from the other channels. Setting 'Skip Frame' to a number other than 0 will cause the acquisition to 'skip' taking an image in that channel (after taking the first image) for the indicated number of frames. The 5D-Image Viewer will 'fill in' these skipped frames with the previous image. In some situations, it may be desirable to acquire certain channels at lower sampling rates, to reduce photo-toxicity and to save disk space. Clicking inside the "Color" column will open a Color selector that lets you select the color to be used for that channel in the 5D-Image Viewer (You can also change colors later in the Image Viewer).

Acquisition Order lets you choose between carrying out z-stacks with each channel (Slices first) or switching channels at each z-position (Channels first). "Time first" mode will take a complete sequence of frames at a single position before moving on to the next, whereas "Positions first" mode will cycle between all position at each time point, in effect acquiring time lapse sequences at all positions.

Checking the Use ***XY list*** option will cause the acquisition to be executed at each position defined in the Position List. Autofocus options are described below. If the ***Save images*** option is selected, images will be saved to disk continuously during the acquisition. If this option is not selected, images are accumulated only in the 5D-Image window, and once the acquisition is finished, image data can be saved to disk. However, saving files automatically during acquisition secures the acquired data against an unexpected computer failure or accidental closing of image window. Even when saving to disk, some of the acquired images are kept in memory, facilitating fast playback. If such behavior is not desired, check the ***Conserve RAM*** option (***Tools*** | ***Options***).

Fast Time Series Acquisition (Burst)

If you would like to acquire "bursts" of images as fast as possible, Micro-Manager's Multi-Dimensional Acquisition engine is designed to intelligently take care of this. You will need to:

- Set the time interval between frames to zero

- Deactivate Z Stacks
- Choose Time First
- Use no channels or a single channel

Thus, you will have removed any delay that would get in the way of the fast acquisition. This is known as **Burst Mode**.

Files on Disk

µManager can save files in two formats, which referred to as "separate image files" and "Image file stack."

Separate image files

Acquired images are saved to disk as separate TIFF files, each containing a single grayscale image. The file naming convention is ***.img*** prefix followed by frame number, channel name and slice number (img_00000000t_channel_00z.tif). In addition, the folder will contain a file named ***metadata.txt*** that contains the metadata in JSNO format.

Image file stack (in brief)

A TIFF file or group of TIFF files that contain multiple acquired images per a single file. These files conform to the OME-TIFF specification, allowing them to be easily imported into a variety of analysis applications or anything that utilizes the Bio-Formats importer.

Image file stacks are designed to be easily imported into ImageJ without the need for any special reader plugins. A stack file can be dragged onto the ImageJ toolbar and will automatically open as a hyper-stack with the same contrast settings used in Micro-Manager. Any acquisition comments typed into the Multi-Dimensional Acquisition window or the comments tab of the main MM GUI can be viewed by pressing "i" with one of these files open in ImageJ.

By default, one file is created per an XY stage position (up to a maximum of 4 GB per file). In the tools-options menu, this can be changed to save all XY positions in a single file. This is especially useful for acquisitions using a large number XY positions. Since OME-TIFFs require that an identical String of XML metadata be embedded in each file in an acquisition, acquisition that have many XY positions with a small amount of data at each waste space on disk by writing the same String of metadata in each file at the acquisition's conclusion.

NOTE: Writing to ***Image File Stack*** results in faster performance than writing to ***Separate Image Files***, in part because it minimizes the number of system calls to create new files. This can be advantageous in situations where disk write speed is a limiting factor (i.e. writing to a server or collecting data at a high rate).

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

USA
International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258
Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK
1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ
Tel: 44 (0)1462 424700 • Fax: 44 (0)1462 424701 • E-mail: wpiuk@wpi-europe.com

Germany
Zossener Str. 55, 10961 Berlin
Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

China & Hong Kong
WPI Shanghai Trading Co., Ltd.
Rm 25e, No8 Dongfang Rd., Pudong District, Shanghai, 200120 PR China
Tel: +86 21 6888 5517 • E-mail:chinasales@china.wpiinc.com

Brazil
Av. Conselheiro Nébias, 756 sala 2611, Santos-CEP: 11045-002, São Paulo Brazil •
Tel: (013) 406-29703 • E-mail: info@brazil.wpiinc.com

Internet
www.wpiinc.com • www.wpi-europe.com • www.wpiinc.cn