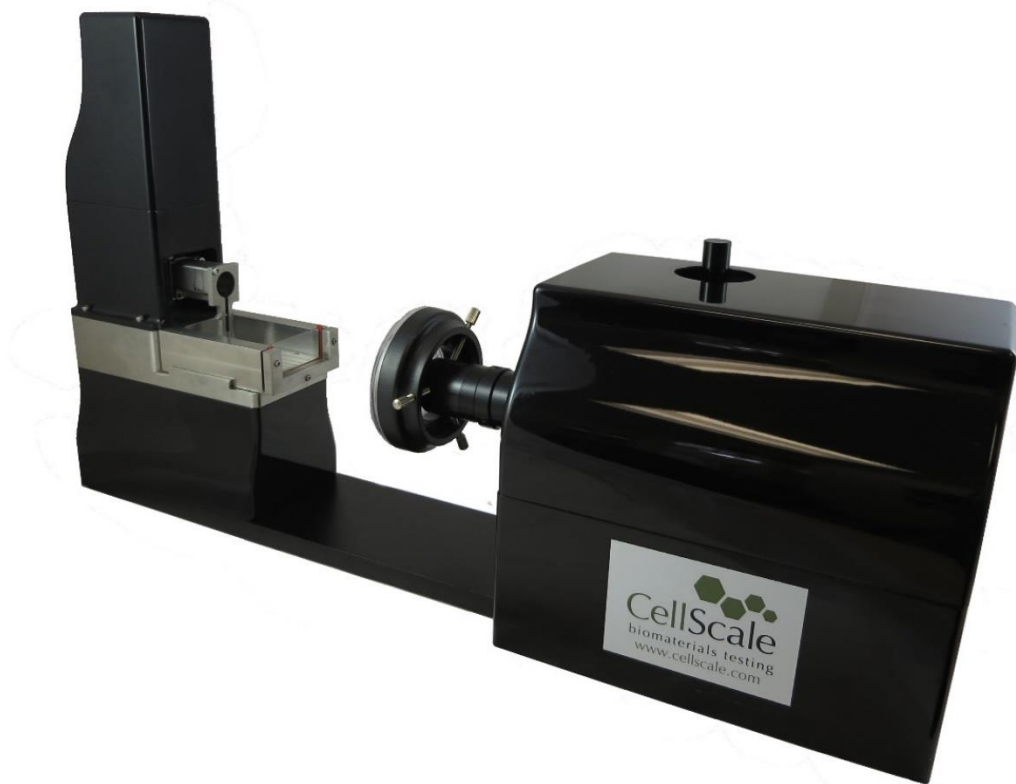




# MicroSquisher

Micro-scale Tension-Compression Test System

*User Manual*  
*version 3.5*



## *Mechanical measurement and analysis of biomedical materials*

CellScale provides scientific and medical researchers with turn-key systems for measuring the mechanical properties of biomaterials.

Our focus is soft tissue – including skin, ligaments, blood vessels, heart valves, sclera, membranes and scaffolds. We provide user-friendly software, easy-to-use patented attachment systems, and effective data analysis tools.

Our foundation was laid at one of the world's leading research institutions – The University of Waterloo. We understand research and aim to provide effective solutions at a reasonable price.

Explore our web site [www.cellscale.com](http://www.cellscale.com) or contact us to learn more about our measurement systems.

© 2016 CellScale. All rights reserved. This material may not be reproduced, displayed, modified or distributed without the express prior written permission of the copyright holder. For permission, contact CellScale Biomaterials Testing at [info@cellscale.com](mailto:info@cellscale.com).



Sales and Support  
CellScale Biomaterials Testing  
564 Weber St N, Unit 11, Waterloo, Ontario  
Canada N2L 5C6  
Phone: 519.342.6870

# Table of Contents

1. General Information .....	1
System Assembly .....	1
Connections to Supply .....	1
Safety Warnings.....	1
Manual Operating Controls .....	1
General Maintenance.....	1
Approvals and Certification .....	2
2. Parts .....	3
Tools .....	3
Basic Components .....	3
3. Testing Terminology.....	4
Multiphase Test Cycles.....	4
Phases, Cycles, and Test Sequences .....	4
Test Phases: The Smallest Unit of Testing.....	6
Control Modes.....	7
Control Functions .....	7
4. System Overview .....	8
Microbeam .....	8
Actuators .....	10
Imaging System .....	10
Fluid Bath .....	10
Software Overview .....	12
Output Files and Data Structures .....	14
5. Setting Up & Starting a Test.....	15
Overview .....	15
Step 1: Start a New Test.....	16
Step 2: Modify Testing Parameters (optional) .....	17
Step 3: Specify the Microbeam Properties (Occasional) .....	20
Step 4: Load the Specimen.....	21
Step 5: Set the Optics Zoom Level .....	22
Step 6: Adjust the Initial Size Crosshairs .....	22
Step 7: Select a Tracking Point .....	23
Step 8: Execute the Test.....	24
Step 9: Terminate the Test Prematurely (optional).....	24
6. Additional Settings .....	25
Camera Shutter and Gain .....	25
Temperature .....	26
Configuring the Real-Time Graphs .....	27

Data Output Settings.....	28
Advanced Settings .....	29
7. Reviewing Test Results.....	30
Overview .....	30
Selecting Images .....	31
Image Playback Options .....	31
Data Overlay .....	32
Exporting Images and Movies .....	33
Image Tracing.....	34
Exporting Traced Data .....	36
8. System Calibration and Advanced Tools .....	37
Piezo Calibration .....	37
Optics Calibration.....	38
Tuning the Temperature Controller.....	39
Snap Image.....	39
9. Mounting a MicroBeam .....	40
10. Force Calculation Formulas .....	46
11. Appendix A: System Setup .....	47
Unpack Main Unit, Controllers, and Accessories .....	47
Remove Packing Material from the Main Unit Cables .....	48
Remove Plastic Cap and Foam from the Piezo Stack.....	49
Attach Spacer to Vertical Piezo Stage.....	50
Set Test Chamber on the Chamber Holder .....	51
Place Temperature Sensor in the Test Chamber .....	51
Remove Lens Cap and Mount the Ring Light on the Lens Assembly .....	52
Connect Cables .....	53
System Calibration Check.....	58
12. Appendix B: Computer Setup .....	59
13. Appendix C: Shear Testing .....	65

# 1. General Information

<b>Electrical Input to Power Supply</b>	100-240VAC, 50-60Hz
<b>Input Current Rating of Power Supply</b>	0.6 Amp
<b>Electrical Output from Power Supply</b>	20 VDC
<b>Output Current Rating of Power Supply</b>	2.0 Amp
<b>Environmental Conditions</b>	Maximum Operating Temperature 25°C 20% - 80% Relative Humidity
<b>Installation Category</b>	Category II
<b>Pollution Degree</b>	Degree II
<b>Data Connections</b>	1 – USB for camera/PC communication 1 – USB for actuator controller/PC communication 1 – USB for position sensor/PC communication (additional USB port required for 2-axis systems) 1 – USB for temperature controller/PC communication USB hubs can be used for computers that have insufficient ports. The temperature controller can be hubbed on a USB port with computer peripherals. All other devices require their own USB port.

## ***System Assembly***

Please see instructions in Appendix A for details on system assembly. See Appendix B for details on setting up the host computer.

## ***Connections to Supply***

Connect power cords to properly grounded 100-240VAC power source to ensure safe operation. Ensure that the power cords are easily accessible at all times. The use of an Uninterruptible Power Supply (UPS) is recommended to protect against data loss.

## ***Safety Warnings***

This equipment must be used in accordance with the procedures outlined in this manual to prevent injury and/or damage.

## ***Manual Operating Controls***

There are a few manual controls on the MicroSquisher main unit. The camera assembly contains X, Y and Z adjustment stages to position and focus the camera view and the camera lens has a zoom ring which can be set by the user. In addition, the camera brightness can be adjusted using the camera iris ring at the base of the lens.

## ***General Maintenance***

Clean the system as needed with mild soap and water or alcohol based cleaning solutions.

## ***Approvals and Certification***

We declare that the CellScale MicroSquisher complies with the requirements of the Electromagnetic Compatibility Directive, 2004/108/EC by demonstrating compliance with the following standards to the extent applicable:

EN 55011: 2008 Class A

EN 61000-3-3: 2008

EN 61000-4-2: 2009

EN 61000-4-3: 2006 (+A1:2008 +A2:2010)

EN 61000-4-4: 2005 (+A1: 2010)

EN 61000-4-5: 2006

EN 61000-4-6: 2009

EN 61000-4-8: 2010

EN 61000-4-11: 2004

## **2. Parts**

### ***Tools***

Small flat head screwdriver

SAE hex wrenches: 0.050", 5/64", 7/64", 3/16", 1/16"

Metric hex wrenches: 2.5mm, 5mm

Rectangular flat wrench

### ***Basic Components***

Main Assembly

Temperature Controller

Piezo Stage Controller

Position Sensor Controller (with LED power supply)

### 3. Testing Terminology

The MicroSquisher is designed to apply compressive loads to small test specimens 50 to 2000  $\mu\text{m}$  in size. Typical specimens include biological tissue samples, scaffolds, cell aggregates, or hydrogel microspheres.

#### ***Multiphase Test Cycles***

In order to properly characterize and test a specimen, it is often necessary to load it to different degrees and at different rates. There are three main reasons for doing this:

***Preconditioning*** - The goal of preconditioning is to restore a specimen to its physiological or *in vivo* state. During the process of specimen storage and preparation, a specimen may swell, dry out, have its material fibers realign, or its molecules reorganize. It may take multiple preconditioning cycles for a specimen to be restored to its natural state.

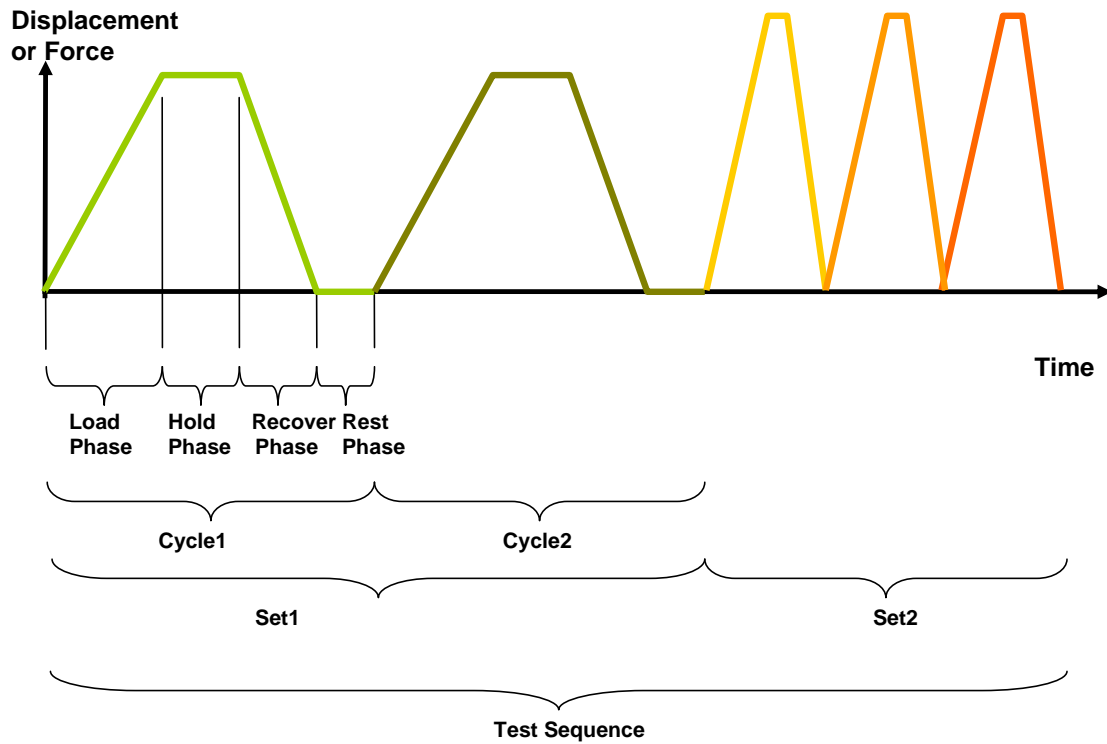
***Reproducing Physiological Conditions During Testing*** - By applying various loads and load rates, natural expansion and contraction of a specimen can be reproduced (for example, the pulse pressure in an organ). In as much as the physiological conditions can be recreated, the specimen can be tested in a more realistic state.

***Varying Test Conditions*** - Variable loads and rates allow you to create a variety of test profiles to best study your specimen.

#### ***Phases, Cycles, and Test Sequences***

As the following diagram demonstrates, each application and release of load on the specimen is called a ***test cycle***. The same test cycle can be repeated multiple times to achieve a certain goal (preconditioning, physiological conditioning, or testing); this is called a ***test set***. Finally, a ***test sequence*** is made up of multiple test sets.





The above example describes the following:

- The entire diagram presents a full *test sequence*.
- Within that sequence, there are two *test sets*.
- Within the first set, two identical *test cycles* are implemented to bring the sample to a satisfactory in vivo state.
- Finally, the second set is made up of three cycles.

## Test Phases: The Smallest Unit of Testing

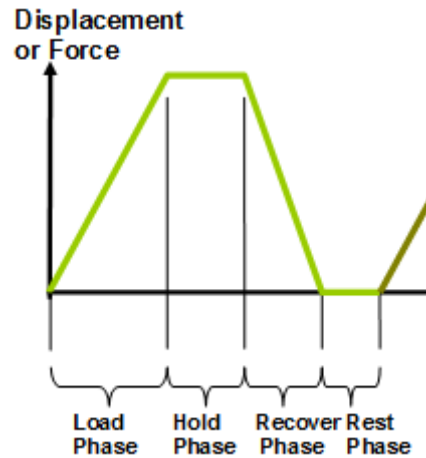
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

**Loading** - During the load phase, a deformation is applied to the specimen. The deformation can be specified either in terms of force applied or displacement achieved.

**Holding** - The deformation can be held for a given duration. The duration for which it is held is dependent on the nature of the testing.

**Recovering** - The recovery phase is the time during which the force being applied to the specimen is removed. The duration of the recovery time is configurable and dependent on the nature of the testing.

**Resting** - Finally, the rest phase is the time between the end of one cycle and the beginning of the next. Some tests may specify a short recover time, while others may specify a longer time. The duration is configurable and dependent on the nature of the testing.



## Control Modes

There are two control modes which define the basic approach to a given test: displacement control and force control:

Under **displacement control**, the displacement of the specimen is predefined. The MicroSquisher compresses the specimen until the predefined displacement is achieved. The force required to achieve the displacement is an output of the test.

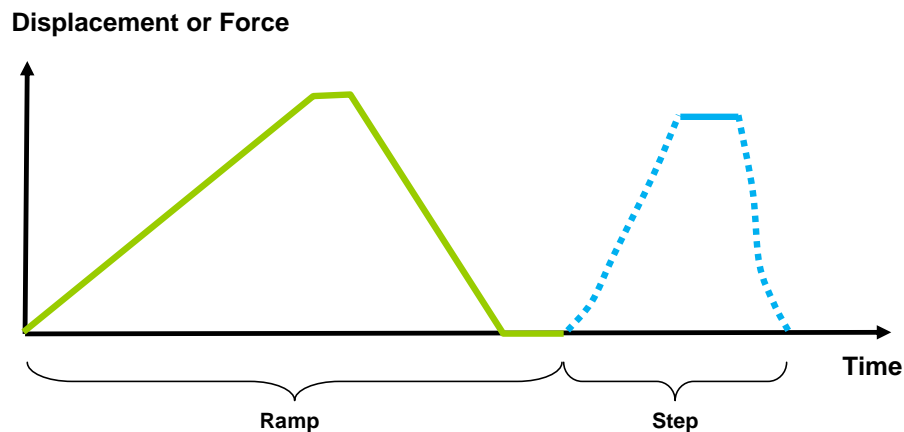
Under **force control**, the force applied to the specimen is predefined. The MicroSquisher compresses the specimen until the predefined force is achieved. The displacement required to achieve the force is an output of the test.

## Control Functions

The MicroSquisher makes it possible to test specimens under several control functions with Displacement or Force control modes:

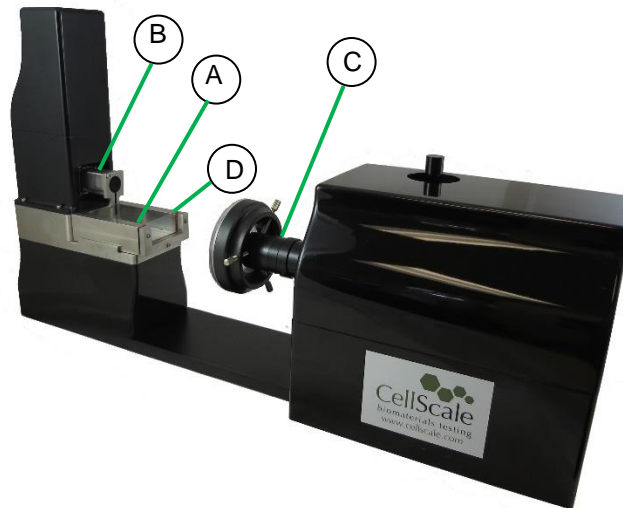
The **ramp function** applies the displacement at a constant nominal rate. This is equivalent to a constant engineering or nominal strain rate.

The **step function** achieves and maintains the desired force as quickly as possible. The amount of time it takes to achieve the desired force depends on the material being tested and the control settings.



## 4. System Overview

The MicroSquisher is a mechanical test device that can be used to test the properties of a variety of tissues and materials.



- A Microbeam.
- B Actuators.
- C Imaging system.
- D Fluid Bath (Test Chamber)

The main components of the device are the load application microbeam, the actuators, the imaging system, and the fluid bath/test chamber.

### ***Microbeam***

The microbeam is rigidly fixed to the vertical actuator (which is under closed loop control). It has a flexible shaft that is typically between 30mm and 50mm long and can be made from a length of metal, glass, or plastic with a circular or rectangular cross section. Circular tungsten beams are supplied with the system. At the end of the shaft is a rigidly fixed compression plate. This plate is typically made from a small square of micro-cut stainless steel and is adhered to the end of the shaft. Cyanoacrylate and adhesive accelerator is provided for this purpose.

The microbeam is used to apply deformations to the test specimen while measuring the applied forces. The deformation is determined by measuring the difference between the current tip position and the initial tip position. These measurements are obtained in real time at a frequency of 5 Hz by image tracking techniques. The applied force is determined by calculating the deflection of the microbeam (the difference between the displacement of the tip and the displacement of the actuator). The displacement of the actuator is obtained directly from a position feedback device (displacement sensor) mounted to the actuator.

Because the microbeam is used for 2 purposes (apply deformation and measure forces), an iterative control scheme is necessary to achieve the desired result. For example, if a tip displacement of 100 $\mu$ m is desired, it is unknown at the start of the test how much actuator travel will be required to move the tip by this amount since some of the stage travel will result in tip displacement and some will result in microbeam bending.

The microbeam can have a variety of constructions and must be tailored to the force ranges that are to be measured. If the microbeam is too stiff, then the beam deflection will be too small. This has a negative influence on the force resolution. This is a result of the position resolution of the base and tip displacement measurements, which are both approximately 0.1 $\mu$ m. For example, if the microbeam only deflects 2  $\mu$ m during the test, then the force resolution is only 1/20 of the maximum force.

Conversely, if the microbeam is too flexible, then the deflection will be too large. This can have a negative influence on the reaction time of the device and on the parallelism of the compression plates. With more deflection of the microbeam, the actuator must travel further to achieve the desired tip displacement. Since the velocity of the actuator is limited to about 2mm/s, this can cause unwanted delays. Also, as the beam deflects, the top compression plate can become slightly less parallel to the lower compression plate. In a test with a moderate microbeam deflection of 100µm, this effect is very small, since the beam is typically over 30mm long. In a test with a large microbeam deflection of 2mm, this effect becomes more significant.

The table below shows a list of the microbeams that come with the MicroSquisher system and the appropriate maximum forces for each diameter. A chapter containing details of the force calculations can be found later in this manual.

Diameter (µm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (µN)	0.012	0.038	0.191	0.604	3.056	9.658	34.521
Recommended Max Force (µN)	4	11	57	181	917	2,897	10,536



*Microbeam with Platen*

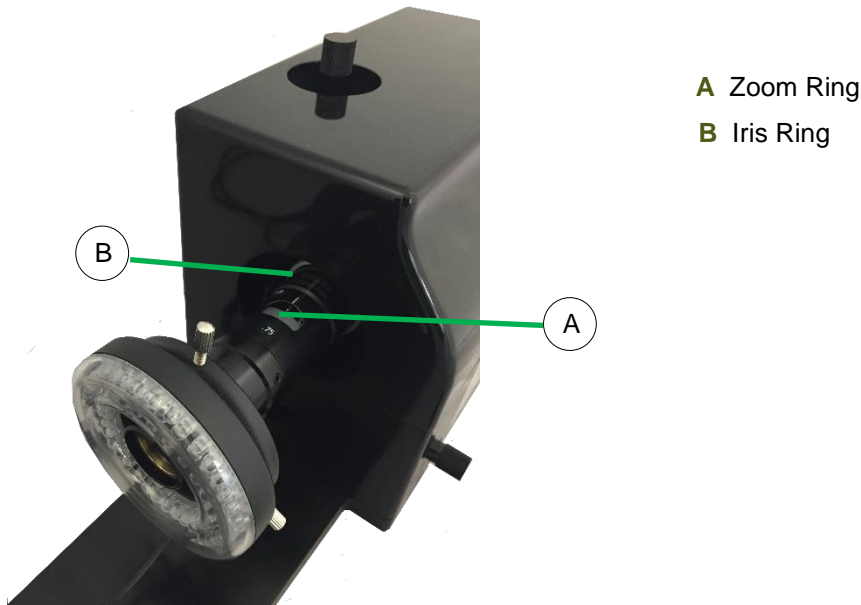
## ***Actuators***

There are 2 actuation systems on the MicroSquisher. The first system is comprised of the 2 piezo motor driven actuators that are used to position the microbeam in plane before the start of the test. The actuators are controlled by the user, using on-screen controls in an open loop jog mode. There is no absolute measurement of how far they have been moved since feedback is based on user interpretation of the live on-screen image.

The second actuator system is the closed-loop, piezo motor actuator that is used to move the microbeam vertically. It is driven in a user-controlled jog mode during setup and then under computer control during the test (specially modified systems are also available to track horizontal displacement as well, in which case the horizontal actuator is also under closed loop control). The actuator used for this purpose is identical to the other 2 axes, but the system also incorporates a position feedback device. This displacement sensor has 0.1 $\mu$ m resolution and allows for the software to determine the displacement of the actuator relative to the start of the test. This motion constitutes the movement of the base of the microbeam. The displacement of the base and tip of the microbeam are needed in order to determine the deflection of (and hence the force applied by) the microbeam.

## ***Imaging System***

The horizontally-oriented imaging system is comprised of a USB digital camera, a zoom lens, and an XYZ stage assembly to allow position adjustment and focusing. The monochrome camera has a resolution of 1280 X 960 pixels and is capable of matching the control frequency of 5Hz. The zoom optics allow for a field of view ranging from approximately 0.8mm to 5.6mm.



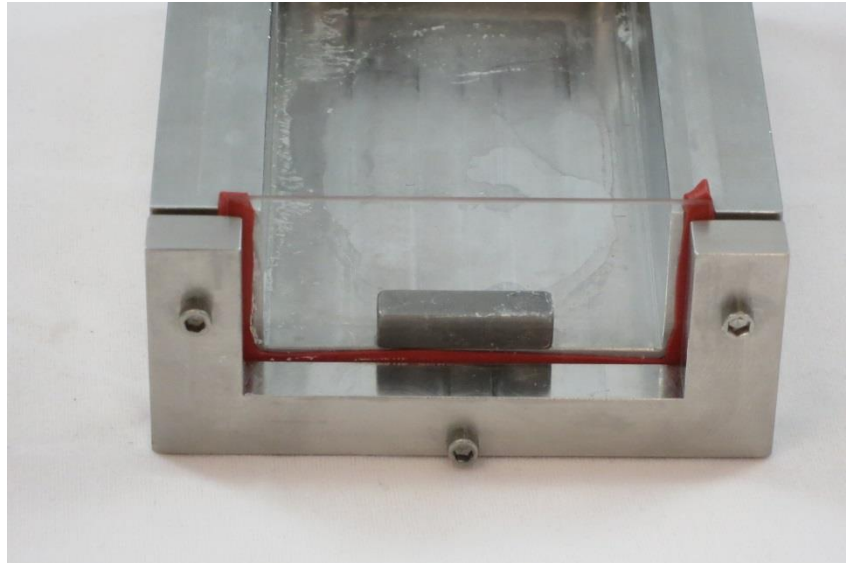
## **Fluid Bath**

The fluid bath contains approximately 45mL of fluid and is heated by a resistance foil heater in the base of the bath. The fluid temperature is controlled by a Thorlabs TC200 temperature controller that communicates with the PC via a USB. The temperature of the bath is measured by a solid state temperature probe with a temperature resolution of 0.1°C. To function normally, the software requires that the controller be turned on. Provided that the unit is on, the SquisherJoy software will log the actual temperature of the fluid. To enable the controller to heat the fluid, the “ENABLE” button must be pressed to illuminate the “HEATER ON” light on the temperature controller.

### **MicroSquisher Tip: Temperature Probe**

*The temperature controller works best if the temperature probe tip is completely submerged and **not** touching the side of the fluid chamber.*

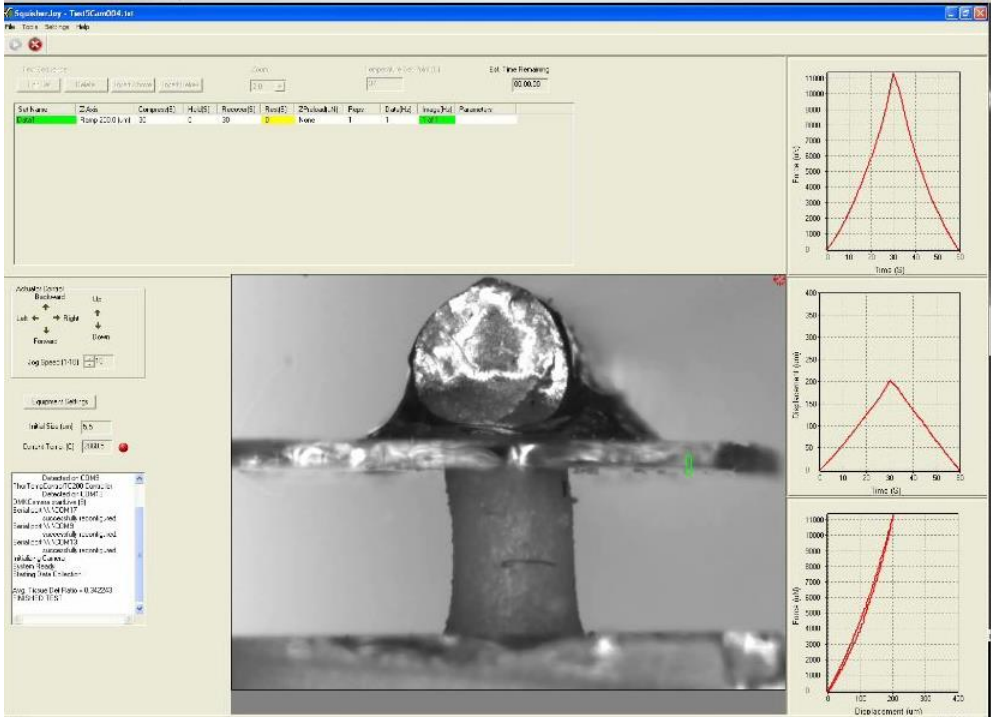
At the front of the bath is a glass window (microscope slide), which allows for imaging of the test and the lower compression plate. The compression plate is typically made of a stainless steel block and is flush with the front window.



# Software Overview

The software included with the MicroSquisher system is called SquisherJoy. It is divided into two modules: a data collection module and a review and analysis module. The data collection module is used to set test parameters, enable specimen loading and testing, and monitor test progress. The screen layout for this module is shown below:

Test Parameters



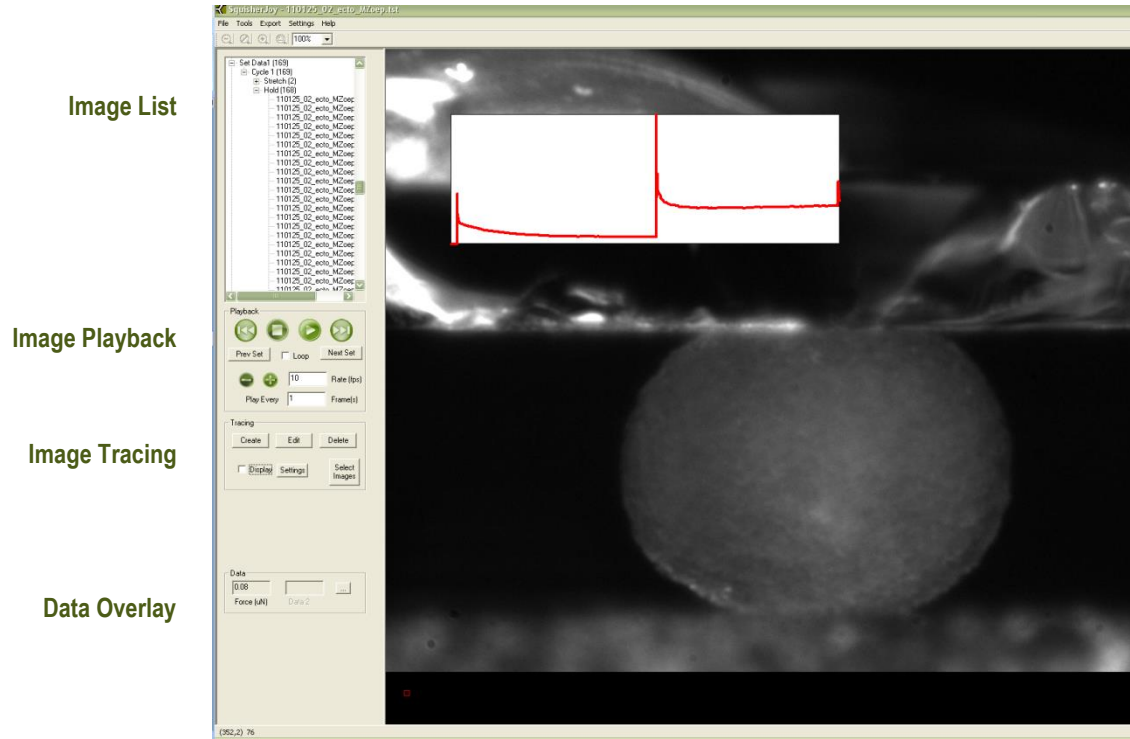
Live Output

Live Video

Live Graphs



The review and analysis module is used to playback accelerated or decelerated test images, perform image analysis and tracking, and output movie files for presentation purposes. The screen layout for this module is shown below:



## Output Files and Data Structures

For each test, the MicroSquisher creates and saves three file types. The following table describes the five file types for a project named "Sample1". Output from this test would be found in a "Sample1" output directory (a sub directory of the user specified data directory).

File Type	Description
.tst file	The Sample1.tst file that contains the exact protocol and settings used for the test.
.csv file	The Sample1Data.csv file that contains comma separated numerical data such as time, force and displacement values.
.jpg files	Captured images such as Sample1.0001000.jpg, which would correspond to an image captured at 1000 milliseconds from the start of the test.

Each test folder will also contain 2 subfolders. The "Logs" subfolder contains a text file of the content of the text dialog portion of the main screen. It is useful as reference to troubleshoot problems with your system, should they occur.

The "Tracking" subfolder is initially empty. If tracking is done on any of the images in the test folder using the "Analyze and Review" software module, there will be data files that contain the tracking information stored in this directory.

While using the "Analyze and Review" software module, you may create additional data files such as text files (\*.csv) containing tracking results, images with force data or tracking results overlaid (\*.wmf), or movie files (\*.avi). These additional files can be stored in the test directory or elsewhere on your computer's hard drive without interfering with the software application (once created, they cannot be opened by the SquisherJoy application).

# 5. Setting Up & Starting a Test

## ***Overview***

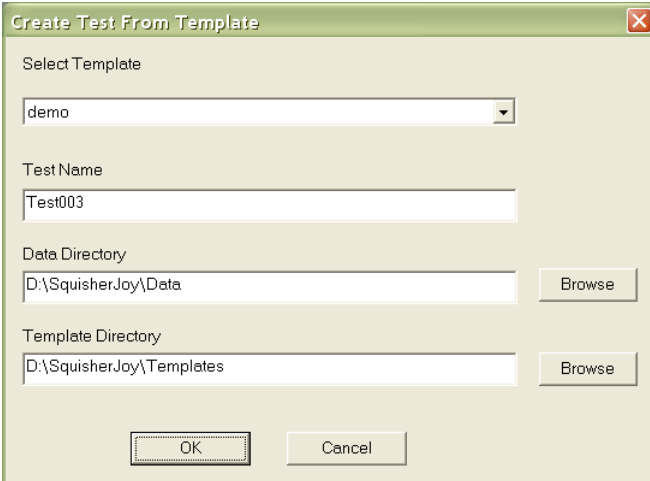
Setting up and running a new test is a simple process made up of both mandatory and optional steps. The following list presents all of the steps, while the rest of this chapter describes each of the steps in detail.

- Step 1: Start a New Test
- Step 2: Modify the Test Parameters (optional)
- Step 3: Specify the Microbeam properties (occasional)
- Step 4: Load the Specimen
- Step 5: Set the Optics Zoom Level
- Step 6: Adjust the Initial Size Crosshairs
- Step 7: Select a Tracking Point
- Step 8: Execute the Test
- Step 9: Terminate the Test Prematurely (optional)

## Step 1: Start a New Test

Launch the SquisherJoy software, and then select Collect New from the File menu. In the Create Test From Template dialog, perform the following steps:

1. Select a template that matches the type of test you wish to perform. See the *SquisherJoy Tip* on how to select and use a template. You can modify the template parameters in Step 2, below.
2. Name your test. The dialog will have a default test name. You can use the default or rename the test. Each time you start a new test, the default name will continue to increment the number at the end of the name.
3. If desired, you can change the location of the output data. The location of your output data and images is determined by specifying a Test Name and Data Directory. The template and data directories are user specific (computer login name). Each system user can store their files to a different default location. The system uses the last location selected as the default.



The screenshot shows a dialog box titled "Create Test From Template". It has a close button in the top right corner. The dialog contains the following fields and controls:

- Select Template:** A dropdown menu with "demo" selected.
- Test Name:** A text input field containing "Test003".
- Data Directory:** A text input field containing "D:\SquisherJoy\Data" and a "Browse" button to its right.
- Template Directory:** A text input field containing "D:\SquisherJoy\Templates" and a "Browse" button to its right.
- Buttons:** "OK" and "Cancel" buttons at the bottom.

### **SquisherJoy Tip: Selecting and Using Templates**

*How to select a template: Designing an appropriate test sequence is an art that depends on both the type of material being tested and the specific material properties you are interested in measuring. When first testing a new material, you should expect to have to experiment with the settings until the test yields meaningful data.*

*The system comes loaded with example templates to help you get started. Selecting a template does not lock you into a specific test sequence or protocol – rather a template defines a test sequence and settings, all of which can be changed before a test is run. Once you have developed a test sequence and settings that are appropriate for the material you are testing, you should save these settings using “Save As Template” from the file menu. You can then select your template the next time you initiate a test.*

## Step 2: Modify Testing Parameters (optional)

You can select and modify test parameter sets by clicking on their row in the Test Parameter Specification table and then pressing the Edit Set button (or by double clicking on their row).

Test Sequence				Zoom	Temperature Set Point (C)					Total Time
<input type="button" value="Edit Set"/> <input type="button" value="Delete"/> <input type="button" value="Insert Above"/> <input type="button" value="Insert Below"/>				1.0	37					00:02:00
Set Name	Axis	Magnitude	Load(S)	Hold(S)	Recover(S)	Rest(S)	Reps	Data(Hz)	Image(Hz)	Parameters
Data1	Z-Compress	Ramp 200.0 (um)	20	10	20	0	1	5	1	
Data2	Z-Compress	Ramp 100.0 (um)	10	0	20	5	2	5	1	

When you do so, the Set Parameter Editor Dialog will appear.

Note that displacements are specified in either % strain, or  $\mu\text{m}$ . Force loads are specified in  $\mu\text{N}$ , and durations are specified in seconds.

The following table describes each of the parameters.

Set Parameter Editor
✕

Name:  (Example: "Squish1", "TestData1")

Axis

Control Axis:

Control Mode:

Control Function:

Compress Magnitude:

Tolerance (um):

Correct Overshoot

Cycle

Loading Duration (S):   Do Not Load
 Repetitions:

Hold Duration (S): 
Data Output Frequency (Hz):

Recovery Duration (S):   Do Not Recover
 Image Output Frequency (Hz):

Rest Duration:

Test Parameter	Description
<b>Control Axis</b>	Loading is normally applied in the vertical Z-Compress axis. MicroSquisher systems that have been equipped with a second displacement control axis can also apply horizontal shear loads by selecting the X-Shear axis.
<b>Control Mode</b>	Compression tests are typically performed under displacement control. However, you can select the following control modes to achieve specific testing objectives: <ul style="list-style-type: none"> <li>• For creep testing, use force control with a long hold duration.</li> <li>• For relaxation testing, use displacement control with a long hold duration.</li> </ul>
<b>Control Function</b>	Ramp functions are most commonly used although step functions are also used.
<b>Compress Magnitude</b> (Load Magnitude when in Force Control mode)	Compression magnitude is specified as an absolute position or force relative to the initial state of the microbeam. Positive magnitudes are compressive (the beam moves down). In systems equipped to perform horizontal shear testing, positive magnitudes are to the right.  If you are using Displacement control mode, you can specify the displacement in either $\mu\text{m}$ or as a percentage. For example, the displacement on a $1000\mu\text{m}$ Initial Size specimen can be expressed as either $100\mu\text{m}$ or 10%.  If you are using Force control mode, you can only specify the force magnitude in $\mu\text{N}$ .  Watch the test results carefully to determine which magnitude setting best achieves your test goals.
<b>Tolerance</b>	For step functions only.  A step compression is considered achieved when the current microbeam position (or force) is at the specified magnitude +/- the tolerance. A tolerance that is too small may result in unacceptably long compression durations and/or instability.  Recommended values are at least 10 times the force resolution of the beam. (see chapter: Force Calculation Formulas for more details).
<b>Correct Overshoot</b>	For step functions only.  An overshoot is defined as a compression magnitude that is larger than the specified magnitude plus the tolerance. When checked the control software will move the microbeam up and/or down until the desired step magnitude is achieved (i.e. recover from overshoot). When unchecked, the control software will consider overshoot as the successful completion of the step function.  Not correcting overshoot is important for relaxation testing.
<b>Loading Duration</b>	For evenly spaced images, it is recommended to choose a number that is an integer multiple of the Image Output Frequency.
<b>Hold Duration</b>	Hold Duration is typically set to 0, however it is useful for creep or relaxation testing.
<b>Recovery Duration</b>	Recovery Duration is often set to the same value as the Stretch Duration.
<b>Do Not Recover</b>	Select this to skip the recovery phase.
<b>Rest Duration</b>	Rest duration is typically set to 0, however a non-zero value may be used to mimic <i>in vivo</i> conditions or for specialized testing.
<b>Repetitions</b>	Apply enough repetitions until the force deformation curves from one repetition to the next start to overlie each other.
<b>Data Output Frequency</b>	Typically set to the same frequency as the image output frequency.
<b>Image Output Frequency</b>	Typically set to 1Hz for cycles > 30 seconds and 5 Hz for cycles < 30 seconds.

**SquisherJoy Tip: Multi-Stage Compression Testing**

Recover phases can be skipped by checking the “Do Not Recover” check box. For example, a set that performed 10% compression with the “Do Not Recover” box checked would result in the specimen still being compressed by 10% at the start of the next cycle.

Multi-Stage compression can be achieved by performing multiple sets that have a “Do Not Recover” duration.

For example, to compress a specimen by 300um in 3 stages of 100um, create 3 separate sets (i.e. three separate lines in the test parameter specification table). The first set should have a 100um compression magnitude, the second a 200um compression magnitude, and the third a 300um compression magnitude. (Note the compression magnitude is relative to the start of the test rather than the position at the start of the set).

**SquisherJoy Tip: Testing Tissues that Relax Quickly**

For specimens that relax very quickly, it may be necessary to achieve the desired move in 2 or 3 stages so as not to overshoot and rebound. What can happen in these cases is that the microbeam will achieve its desired displacement, completing the “Compress” phase. At the start of the hold phase, the tendency of the specimen is to relax, causing a drop in force. This dropping force causes the microbeam to want to unload its elastic energy and hence further deform the specimen. The software will react to this by raising the stage (microbeam base) to reduce the applied force and maintain the desired displacement. The difficulty occurs when the relaxation causes these events to happen so quickly that the 5Hz operation of the equipment cannot keep up and maintain the desired displacement. The only remedy is to move the beam part way to its final destination in 1 or more preliminary moves so that the overshoot that takes place never results in the microbeam moving more than its desired displacement.

**SquisherJoy Tip: Applying Preloads**

Preloading can be implemented by creating a set with a force control step function load at the beginning of a testing sequence.

**MicroSquisher Tip: Tension Testing**

The MicroSquisher can be used to perform tension testing by fixing the specimen to the lower and upper compression plates. To achieve this, the compression magnitude should be entered as a negative number.

### Step 3: Specify the Microbeam Properties (Occasional)

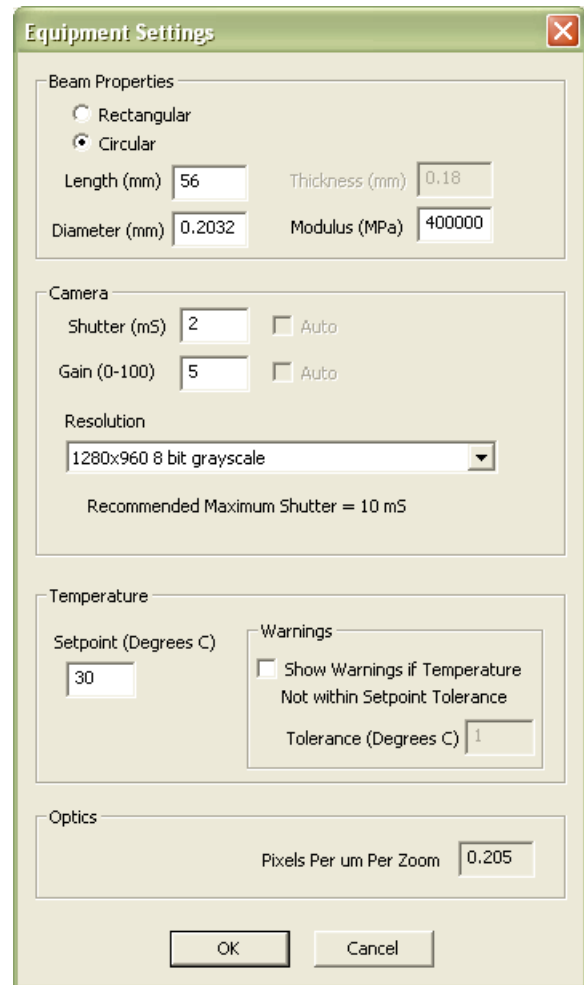
Microbeam properties include dimensions and modulus and must be correctly specified to obtain correct force outputs. You can check and set Beam properties by selecting Equipment from the Settings menu or by pressing the Equipment Settings button in the Live Output section of the main display window. The following dialog will appear.

Note that the “Beam Length” parameter refers to the distance from the fixed end of the microbeam wire (the near side of the clamp) to the point of force application at the free end under the compression platen.

The microbeam length described above **MUST** be accurately measured. Calipers or a finely marked ruler can be used.

Standard microbeam diameters and moduli are shown in the table below. Potentially, other microbeams of other materials could be used. Microbeams may need to be cut before mounting to between 30-60 mm in length. Scissors or side-cutters may be used for this purpose.

**For instructions on preparing and mounting a microbeam, see section 9 of the manual - Mounting Microbeams.**



Diameter (µm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (µN)	0.012	0.038	0.191	0.604	3.056	9.658	34.521
Recommended Max Force (µN)	4	11	57	181	917	2,897	10,536

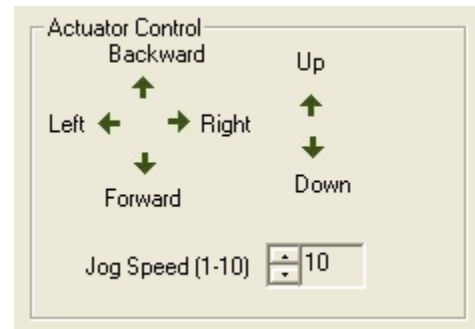
#### **SquisherJoy Tip: Update Templates After Changing Beam Properties**

*It is important to update the beam properties after replacing a microbeam. **The beam properties are stored in the test template along with the test protocol and other equipment settings.** In order for these updated beam properties to be automatically used the next time you start a new test using the same template, you should save these settings to a new template using “Save As Template” from the File menu.*



## Step 4: Load the Specimen

Loading the specimen under the microbeam takes a certain amount of practice. It is important to position the specimen as close as possible to the front glass so that it can be imaged correctly. The end of the microbeam can be used as a micromanipulator for this purpose. The microbeam can be moved using the manual Actuator Controls which allow the user to move each actuator independently at a variety of jog speeds.



### **MicroSquisher Tip: Viewing the Specimen in the Live Video Window**

*The optics and camera are mounted on three axis manual stages which can be used to focus and position the optics and camera to obtain optimal imaging.*

*The optics have a manual zoom control which can be used to change the magnification of the image and increase the field of view. It is often helpful to work at low zoom levels for loading a specimen. The zoom level can then be increased once the specimen is properly loaded.*

*Camera Shutter and Gain can also be adjusted (on the Equipment Settings dialog) if the live image appears too bright or too dark. In addition, the camera iris can be adjusted. See Section 6 for more details.*

### **MicroSquisher Tip: Microbeam Position**

*For most testing it is desirable to move the microbeam forward until it touches the glass and then move it backward a very small amount. It is usually very obvious when the microbeam makes contact with the glass because it will jump suddenly in the live image.*

## Step 5: Set the Optics Zoom Level

A zoom specification pull-down box is located above the Test Parameter Specification table. The “Zoom” pull-down box **MUST** be set correctly (at the same value as the zoom indicator on the lens) so that the software can convert image tracking data from pixel values to  $\mu\text{m}$  values.

Test Sequence				Zoom	Temperature Set Point (C)	Total Time			
Edit Set	Delete	Insert Above	Insert Below	2.0	30	00:01:20			
Set Name	Z-Axis	Compress(S)	Hold(S)	Recover(S)	Rest(S)	Reps	Data(Hz)	Image(Hz)	Parameters
Data1	Ramp 200.0 ( $\mu\text{m}$ )	20	0	20	0	1	5	1	
Data2	Ramp 400.0 ( $\mu\text{m}$ )	20	0	20	0	1	5	1	

### **SquisherJoy Tip: Setting a Default Software Zoom Level**

*The initial software zoom level is stored in the test templates. This value will be saved to the current selected value when saving a template using “Save As Template” from the File menu.*

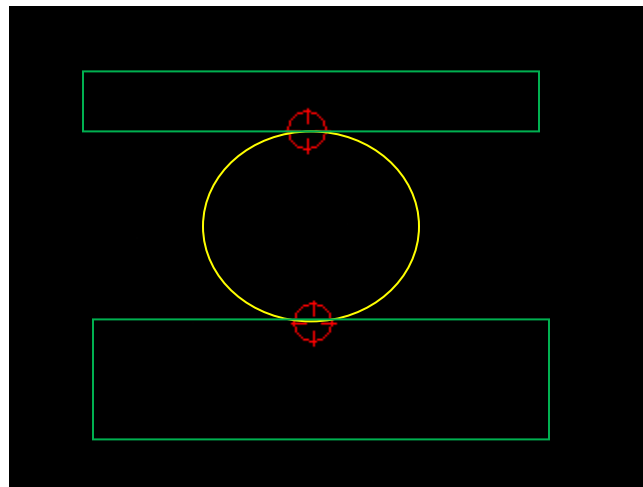
## Step 6: Adjust the Initial Size Crosshairs

There are two red crosshairs overlaid on the live image. If the zoom level is set correctly, the “Initial Size” field on the left will display the distance between these crosshairs. The crosshairs can be moved on the screen by clicking on them and dragging them (they will turn yellow when they are clickable and can be dragged).

Usually the crosshairs are moved to be positioned at the top and bottom of the test specimen.

Note that the absolute distance is measured between the crosshairs, not the linear distance in one particular axis. Thus, the crosshairs must be aligned horizontally if a correct vertical distance is to be measured.

Initial Size is one of the outputs in the .csv data output file.



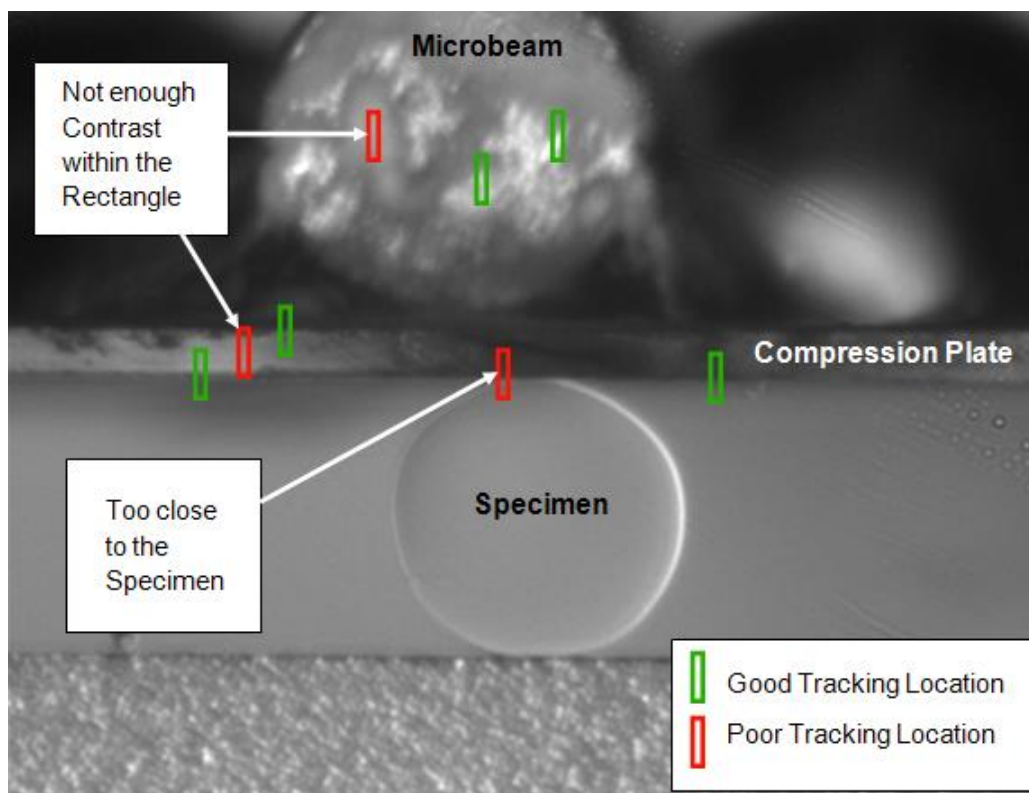
## Step 7: Select a Tracking Point

Select an appropriate tracking point on the microbeam or compression plate by **right-clicking** on the live video window. A green rectangle or square will appear showing the region that will be used to track this location. The selected point should have some contrast (light/dark) within the green rectangle and should not change in appearance due to lighting glare (i.e. it should be a real feature, not an effect created by the lighting). Since force calculations are based on the tracked point locations, accurate tracking is essential for accurate force outputs.


Good choices for the tracking point location, as shown in the following figure, include locations on the microbeam wire end or on the compression plate attached to the microbeam. Features in focus tend to track better than features that are out of focus or blurry.

Good choices on the microbeam include regions of high contrast within the rectangle. A poor location (shown in red) on the microbeam does not have enough contrast within the rectangle for the tracking algorithm to uniquely determine its location.

Good choices on the compression plate will span across a sharp boundary with, for example, the bottom half of the green tracking rectangle covering the compression plate and the top half of the tracking rectangle not on the compression plate. Poor choices on the compression plate (shown in red) include locations that are overlapping with or close to the specimen (which may change in appearance during compression) or do not span across one of the plate edges.




## **Step 8: Execute the Test**

Select Execute from the File menu, or click  on the toolbar.

### ***MicroSquisher Tip: Check Settings***

*Before executing a test, be sure that the microbeam properties and optics zoom level are set correctly. Accurate results are highly dependent on these settings. Also check the position of the initial size crosshairs and tracking point.*

## **Step 9: Terminate the Test Prematurely (optional)**

You can stop a test at any time by clicking  on the toolbar, or by selecting Stop from the File menu.

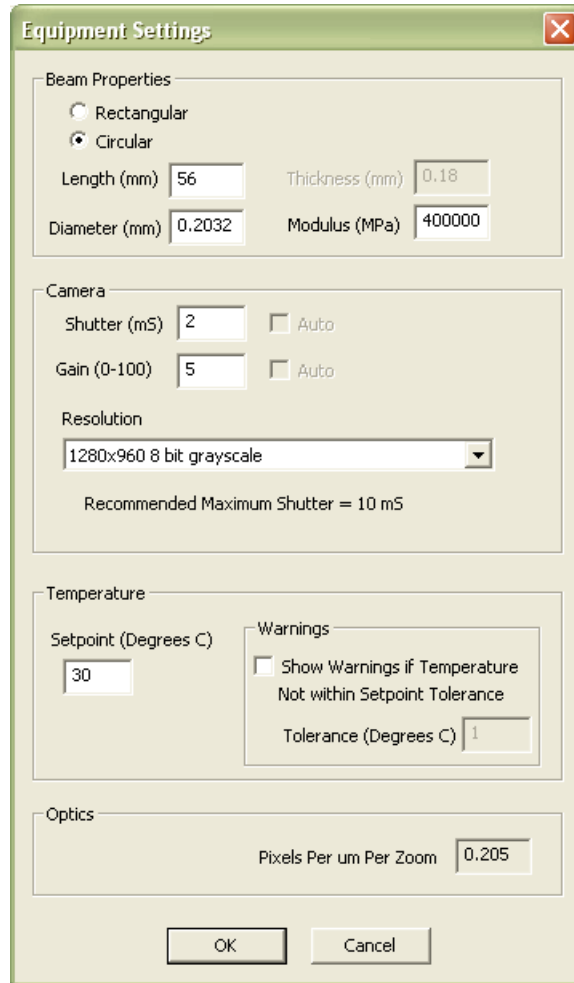
# 6. Additional Settings

## Camera Shutter and Gain

Camera settings can be adjusted by selecting Equipment from the Settings menu or by pressing the Equipment Settings button in the Live Output section of the main display window. The following dialog will appear.

Increasing the camera shutter duration or the camera gain makes the image brighter. Decreasing either of these parameters makes the image darker. If the gain is too large, the image may appear grainy. If the shutter is too long, the image may appear blurry. These settings are stored with the test template file.

Note that camera brightness can also be adjusted using the iris ring on the camera lens. See Section 4 – System Overview: Imaging System for details.

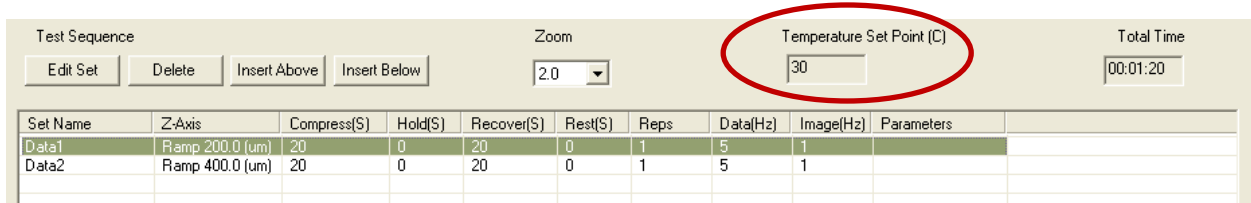


## Temperature

To activate the fluid chamber heater, not only must the ON/OFF switch be turned on, but the "ENABLE" button must be pressed so that the HEATER ON indicator is illuminated. At this point, the heater will warm the fluid to the last used temperature set point. The temperature set point can be changed by selecting Equipment from the Settings menu.

Typically, the heater will take 10 to 30 minutes to warm a full fluid chamber from room temperature to 37°C.

When Show Temperature Warnings is enabled and the Current Temperature is not at the Temperature Set Point plus or minus the specified tolerance -- a red indicator button will be displayed next to the Current Temperature live output and a warning dialog will appear when you execute the test.



The screenshot shows a software interface for a test sequence. At the top, there are control buttons: "Edit Set", "Delete", "Insert Above", and "Insert Below". To the right of these buttons is a "Zoom" dropdown menu set to "2.0". Further right is a "Temperature Set Point (C)" input field containing the value "30", which is circled in red. To the far right is a "Total Time" display showing "00:01:20". Below these controls is a table with the following data:

Set Name	Z-Axis	Compress(S)	Hold(S)	Recover(S)	Rest(S)	Reps	Data(Hz)	Image(Hz)	Parameters
Data1	Ramp 200.0 (um)	20	0	20	0	1	5	1	
Data2	Ramp 400.0 (um)	20	0	20	0	1	5	1	

## Configuring the Real-Time Graphs

The three graphs on the right side of the screen provide real-time user feedback during a test. These graphs are intended to be used for qualitative feedback, not for detailed analysis.

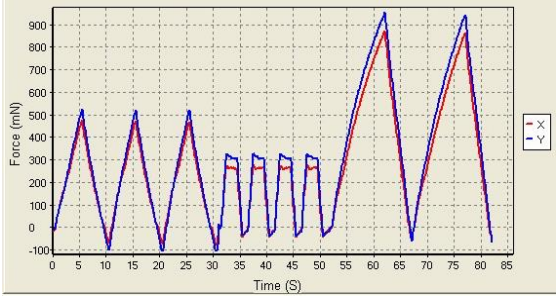
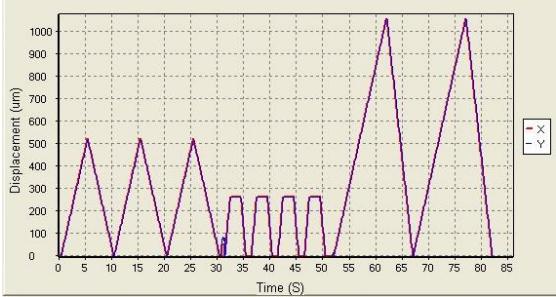
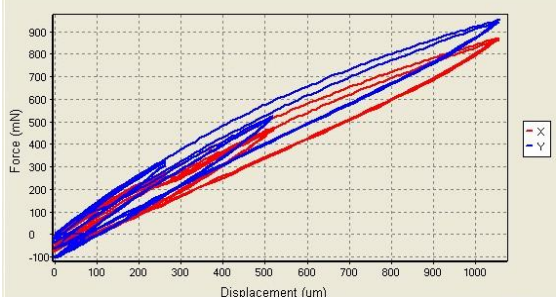
The graph settings can be specified by selecting Graphs from the Settings menu. Auto scaling allows the axis min and max to start at the specified values but expand if the data values are larger or smaller than the initial limits.

The screenshot shows a 'Graph Settings' dialog box with three sections for Graph 1, Graph 2, and Graph 3. Each section has an XAxis and YAxis configuration. The XAxis configuration includes a dropdown menu for the variable name, a dropdown for 'Auto' scaling, and input fields for 'Min' and 'Max' values. The YAxis configuration includes a dropdown menu for the variable name, a dropdown for 'Auto' scaling, and input fields for 'Min' and 'Max' values. There is a checkbox labeled 'Clear Graph Between Sets' at the bottom of each section. At the bottom of the dialog are 'OK' and 'Cancel' buttons.

Graph	XAxis Variable	XAxis Min	XAxis Max	YAxis Variable	YAxis Min	YAxis Max	Clear Graph Between Sets
Graph 1	Time (S)	0	40	Force (mN)	0	500	<input type="checkbox"/>
Graph 2	Time (S)	0	40	Displacement (um)	0	400	<input type="checkbox"/>
Graph 3	Displacement (um)	0	400	Force (mN)	0	500	<input checked="" type="checkbox"/>

It is typical to display Force versus Time, Displacement versus Time and Force versus Displacement as shown below. However, any of the following variables can be plotted in any of the three graphs:

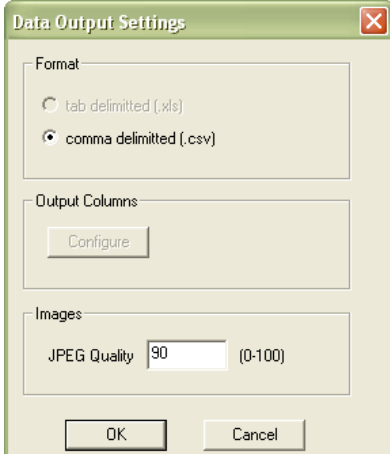
Force ( $\mu$ N)	Displacement ( $\mu$ m)	Time (S)
Force (mN)	Displacement (mm)	Temperature

Graph	Data
<p><b>Force versus Time</b></p> <p>This graph shows how the measured forces are changing with time. Peak loads per cycle, and force relaxation are all easily seen in this type of graph. Force is proportional to nominal (engineering) stress.</p>	 <p>The graph shows Force (mN) on the y-axis (ranging from -100 to 900) and Time (S) on the x-axis (ranging from 0 to 85). It displays several cycles of loading and relaxation. The peaks of the cycles increase in magnitude over time, with the final two cycles reaching approximately 900 mN. The relaxation phase shows a gradual decrease in force between loading events.</p>
<p><b>Displacement versus Time</b></p> <p>This graph shows how the microbeam is moving with time. The phases of the test sequence are readily apparent in this type of graph. Displacement is proportional to nominal (engineering) strain.</p>	 <p>The graph shows Displacement (um) on the y-axis (ranging from 0 to 1000) and Time (S) on the x-axis (ranging from 0 to 85). It displays several cycles of loading and relaxation. The displacement increases linearly during the loading phase and remains constant during the relaxation phase. The peak displacement of the cycles increases over time, with the final two cycles reaching approximately 1000 um.</p>
<p><b>Force versus Displacement</b></p> <p>This graph displays a qualitative representation of the material behavior. Viscoelastic effects (like hysteresis) and material response to different loading phases are apparent in this graph. This graph is proportional to a nominal (engineering) stress-strain graph.</p>	 <p>The graph shows Force (mN) on the y-axis (ranging from -100 to 900) and Displacement (um) on the x-axis (ranging from 0 to 1000). It displays several cycles of loading and relaxation. The loading curves are nearly linear but show a slight upward curvature. The unloading curves are lower than the loading curves, indicating hysteresis. The area between the loading and unloading curves represents energy dissipation.</p>

## Data Output Settings

Select Data Output from the Settings Menu and the following dialog will appear.

Users can specify the \*.jpg image file quality that will be used for saving collected images.



The screenshot shows the "Data Output Settings" dialog box with the following settings:

- Format:**
  - tab delimited (.xls)
  - comma delimited (.csv)
- Output Columns:**
  - Configure
- Images:**
  - JPEG Quality: 90 (0-100)

Buttons: OK, Cancel



## Advanced Settings

Select Advanced from the Settings menu and the following dialog will appear.

Hold Phase Control allows the user to set some limits on actuator movement, in order to improve system stability.

The first parameter limits the maximum movement of the vertical stage during each control loop iteration.

The second parameter (“Slow Ratio”) is similar to a safety factor. For example, if a move of  $10\mu\text{m}$  is desired in a given time step, the actuator will actually attempt to move  $10\mu\text{m} \times$  the slow ratio. See the tip below for more information.

Image Tracking Settings allow users to control the size of the search region and the size of the tracking template. These parameters should normally not be changed.

For systems equipped to measure the X-Shear axis, a square template and search region is recommended.

Advanced Settings

Hold Phase Control

Limit Moves To a Maximum of 50 Steps Per 200 mS Interval

Limiting moves may cause target values to be reached more slowly  
But can be set to ensure that each move completes within each 200 mS Interval  
Allowing for full 5Hz data output with no skipped points

0.75 Slow Ratio

Corrections for overshoot or undershoot are multiplied by the Slow Ratio  
If this ratio is small target values may be achieved slowly  
If this ratio is large oscillations about the target value may occur

Image Tracking Settings

Search Region Minimum (+/- pixels) 3 x 30

Template Region Size (+/- pixels) 5 x 21

Increasing these settings will increase real time computations during data collection  
And may result in 5Hz cycle time not being achieved

OK Cancel

### **SquisherJoy Tip: Slow Ratio**

*For the hold phase of linear elastic materials, both the beam tip and beam base will remain close to stationary during a hold phase, so a slow ratio of 0.3-0.5 is appropriate to improve stability. For highly viscoelastic materials, the beam tip will tend to keep moving downward at the end of the compression phase, resulting in increasing compression and decreasing applied force (due to decreasing beam deflection). To counteract this, the software will have to move the beam base upward for a displacement-controlled test (to maintain the current displacement) or downward for a force-controlled test (to maintain the current force). In these situations, a higher slow ratio may be needed. A ratio of 0.75-1.0 is fairly stable. Higher ratios will be even more responsive, but also potentially more unstable. Trial and error may be required to obtain the optimal results for your material.*

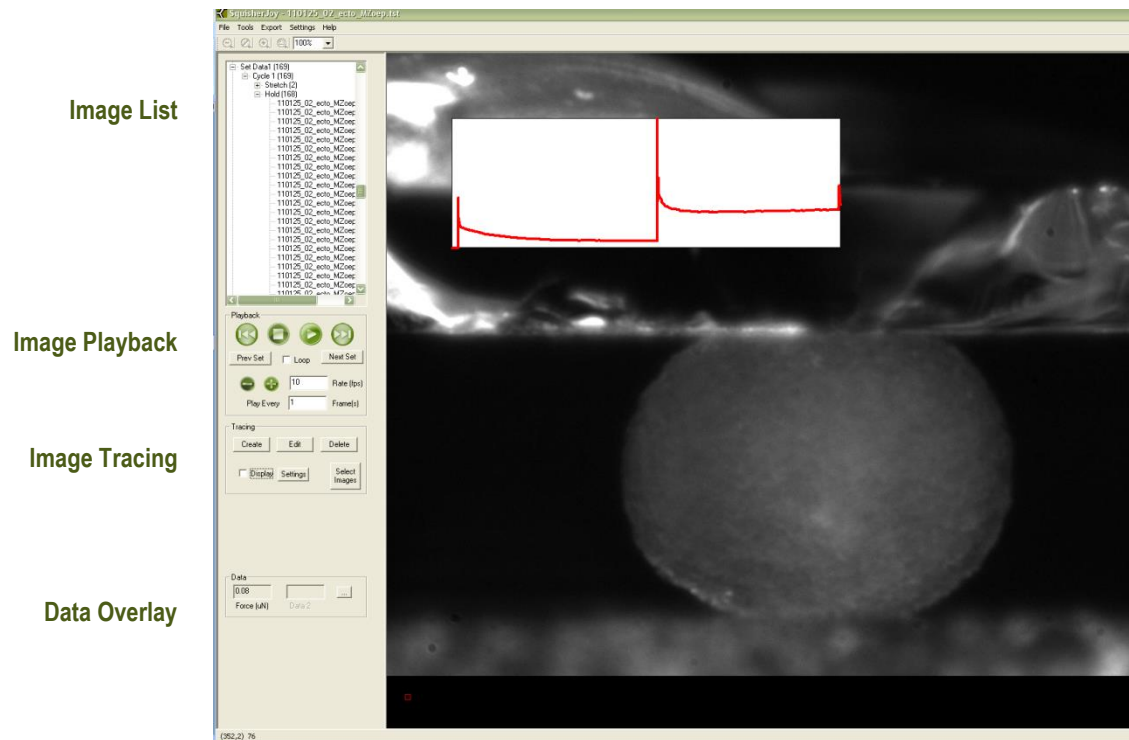
# 7. Reviewing Test Results

## Overview

The MicroSquisher software has an integrated image analysis module which can be accessed by selecting Analyze and Review Images from the File menu, then selecting the appropriate test file. The test file is a text file with a .tst extension that contains information about the test parameters so that the software can display the images and data in a useful fashion.

The “Analyze and Review Images” module allows for post processing of the test images. It is used for 3 main purposes:

1. Review test for quality assurance purposes
2. Generate movies and other material for presentations
3. Trace specimen profiles to calculate geometry values



## Selecting Images

The Images are shown in a tree structure on the top left panel. The tree structure organizes the images by:

**Set** (line on the test parameter window)

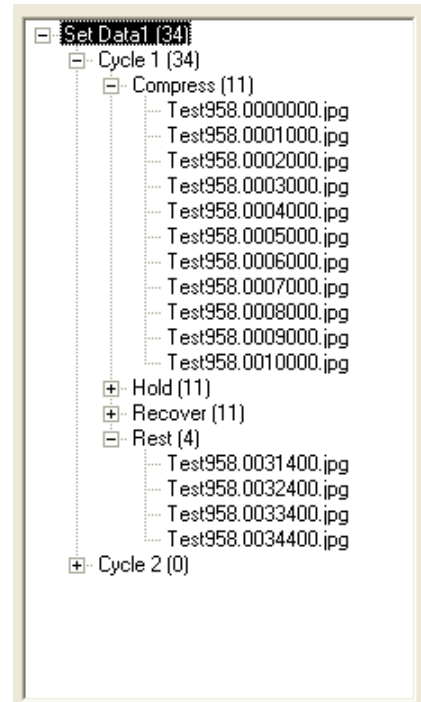
**Cycle** (iteration of a given Set)

**Phase** (Compress/Hold/Recovery/Rest of each Cycle)

**Image** (the one or more individual images from each Phase)

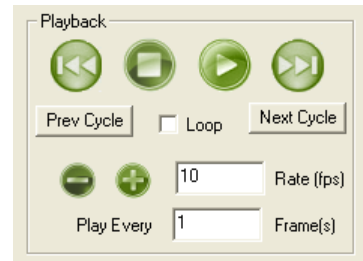
You can select which images to include in a playback set in one of several ways:

- Hold down the shift key and click on individual images
- Select entire sets, cycles, or phases (will playback all contained images)
- Select images which have data associated with a particular tracking set



## Image Playback Options


The controls on the left of the screen are broken into 2 sections: Playback and Tracking. The playback buttons allow the user to review the images taken during a test.

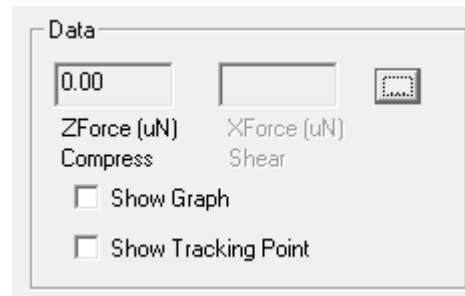
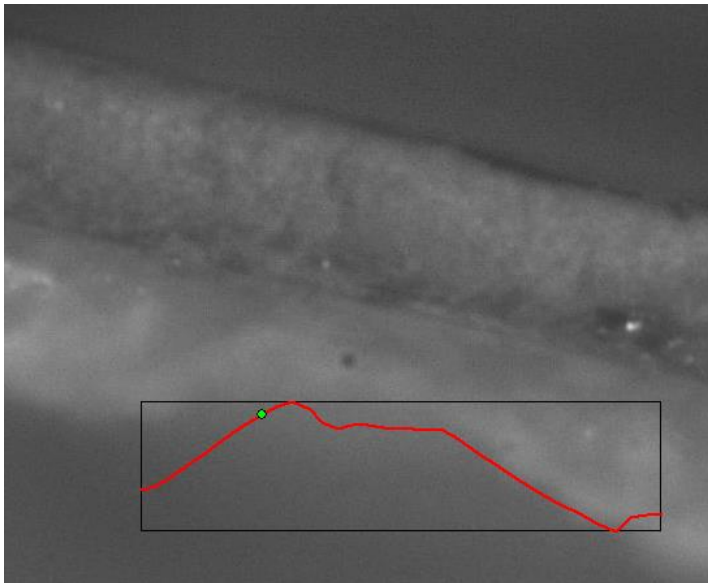


Next/Prev	Display next or previous image (as dictated by "Play Every" parameter).
Play/Stop	Start and stop the playback.
Prev/Next Cycle	Jumps to a corresponding image and phase in the next or previous cycle. Useful when reviewing sets that have multiple cycles.
Loop	Sequences can be played continuously in a loop or only once.
Playback Rate	+/- changes the speed of the playback. A playback rate can also be manually entered in the display box.
Play Every	Allows the users to skip some frames to expedite playback.

## Data Overlay

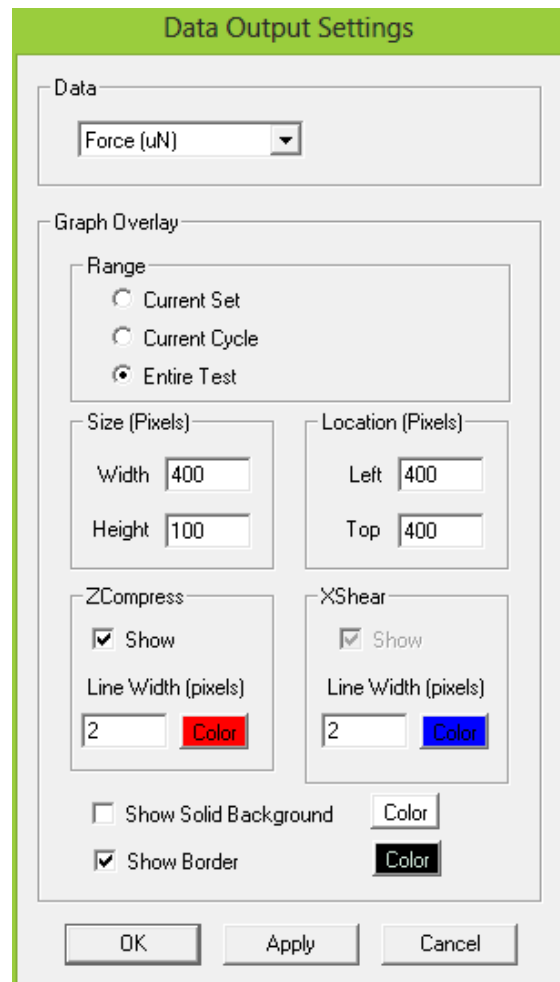
Data associated with each image is displayed below the tracking controls on the left side of the main window. Clicking the Show Graph check box will display the collected force data associated with this test as a graph overlaid on the images. Clicking on the Show Tracking Point check box will display the tracked point used during the collection of this test. Since force calculations are based on the tracked point locations, a test where the tracked point suddenly jumps or loses its correct location indicates invalid force outputs.

Clicking the details icon  displays the dialog shown below. The user can specify the graph's size, location and appearance. The data points associated with the current image are marked on the graph with green circles. The overlaid data graph will be drawn on top of any image tracking displays (when they overlap). This can be useful for correlating force events with image events and for generating presentation material.



The Data dialog box contains the following elements:

- A text input field with the value "0.00".
- A text input field for "ZForce (uN)".
- A text input field for "XForce (uN)".
- A "Compress" label with a corresponding checkbox.
- An "XShear" label with a corresponding checkbox.
- A "Show Graph" checkbox.
- A "Show Tracking Point" checkbox.



The Data Output Settings dialog box contains the following elements:

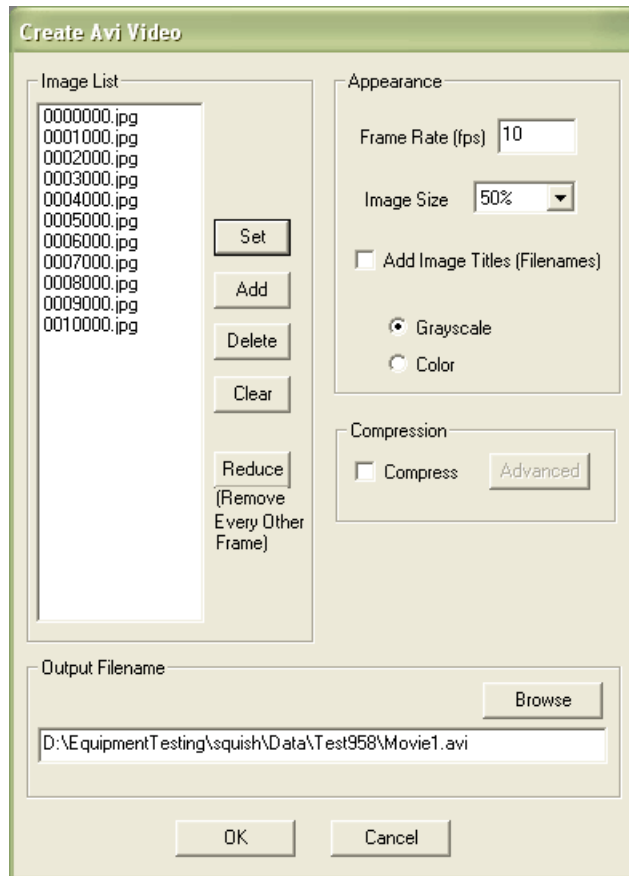
- A "Data" section with a dropdown menu set to "Force (uN)".
- A "Graph Overlay" section with a "Range" group containing three radio buttons: "Current Set", "Current Cycle", and "Entire Test" (selected).
- A "Size (Pixels)" section with "Width" (400) and "Height" (100) input fields.
- A "Location (Pixels)" section with "Left" (400) and "Top" (400) input fields.
- A "ZCompress" section with a "Show" checkbox (checked) and a "Line Width (pixels)" input field (2) with a "Color" button.
- An "XShear" section with a "Show" checkbox (checked) and a "Line Width (pixels)" input field (2) with a "Color" button.
- A "Show Solid Background" checkbox (unchecked) with a "Color" button.
- A "Show Border" checkbox (checked) with a "Color" button.
- Buttons for "OK", "Apply", and "Cancel" at the bottom.

## Exporting Images and Movies

The current Image view, including all displayed tracing data (see section on Image Tracing) and overlaid data can be exported as a metafile (wmf) by selecting Current Image from the Export menu. Alternately, the current image view can be copied to the clipboard by selecting Ctrl-C from the keyboard.

To export a series of image views, including all displayed tracing data and overlaid data as an .avi movie, select Movie from the Export menu. The dialog shown on the right will be displayed. The image list is automatically set to the currently selected images in the test image list tree. This image list can be changed by selecting new images in the test image list tree and pressing the set button. Since there can be a large number of images in a single set or phase, clicking the “Reduce” button can be useful to remove every other frame in the Image List. This button can be used multiple times to pare down the image list so that the movie file will not be too large.

Options are available for setting the frame rate or image size (resolution), adding filenames, and choosing color or grayscale output. Compression options are also available.

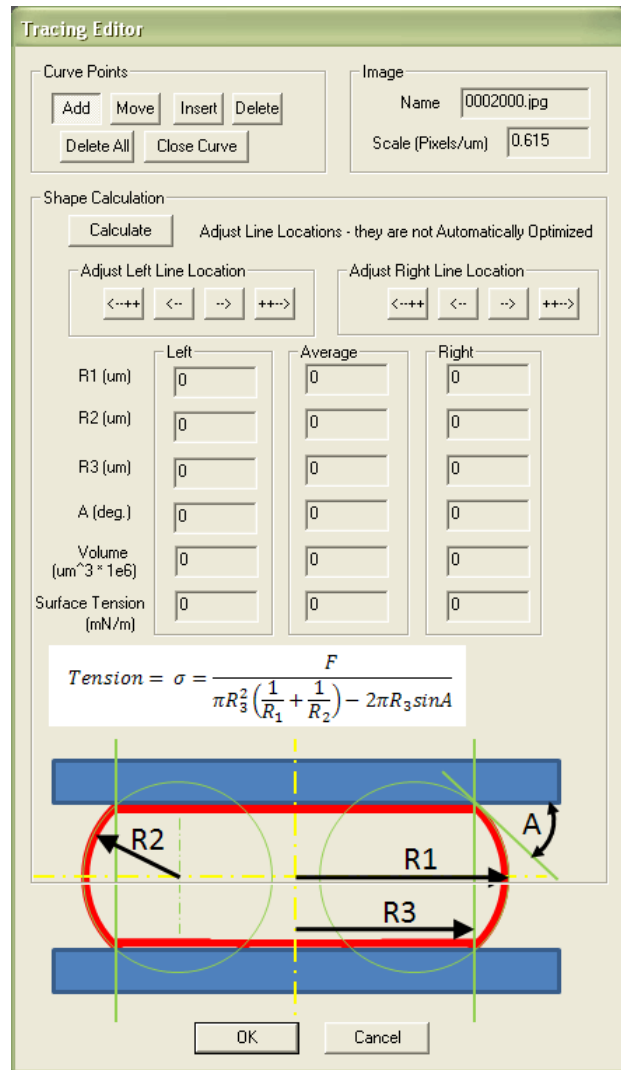
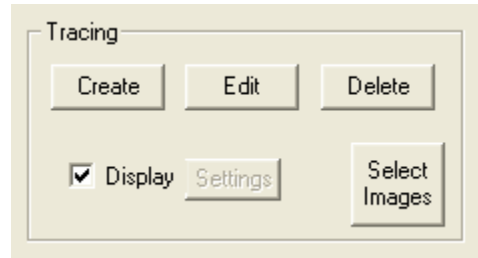


## Image Tracing

Image Tracing is a function that can be used to determine aggregate geometries and surface tensions during cell aggregate compression testing.

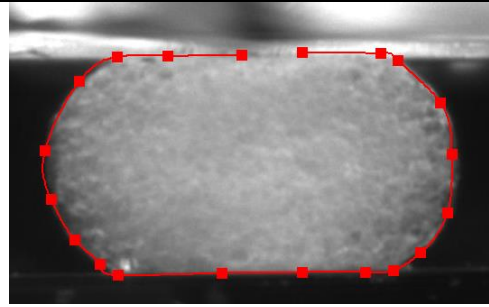
The tracing portion of the screen allows the user to create traces, which are associated with a particular image. If the “Display” checkbox is checked, then any traces will show up on the screen when the images are being viewed. The “Select Images” button will automatically select all of images that have associated traces. This is useful if your data set is large and the traced images are spread out.

Clicking “Create” (or if an existing trace exists, clicking “Edit”) brings up the following dialog. This dialog floats so that it can be positioned to allow the user to visualize the image and the dialog simultaneously.

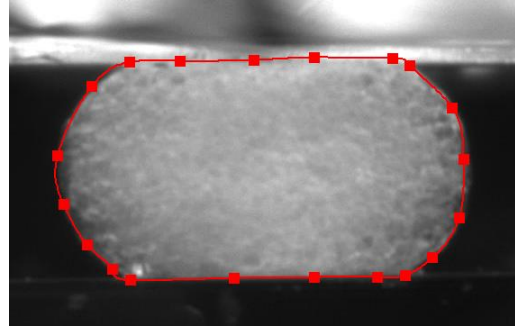


The general procedure for creating a specimen tracing is as follows:

1. While the "Add" button is depressed, select a number of points around the perimeter of the image.

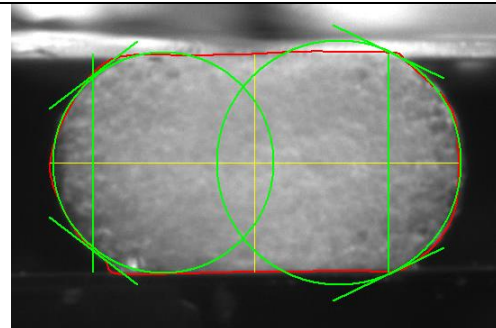


2. Click the "Close Curve" button to attach the first and last point.

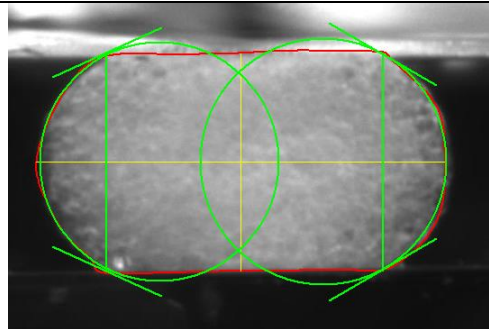


3. Use the "Move" and/or "Insert" buttons to modify the points as needed. There is an image zoom feature available on the top toolbar if needed.

4. Press "Calculate" to generate the curve fit geometries.



5. Use the line location adjustment arrows to position the vertical lines such that they intersect the circles at the point where the aggregate surface intersects the compression plates.

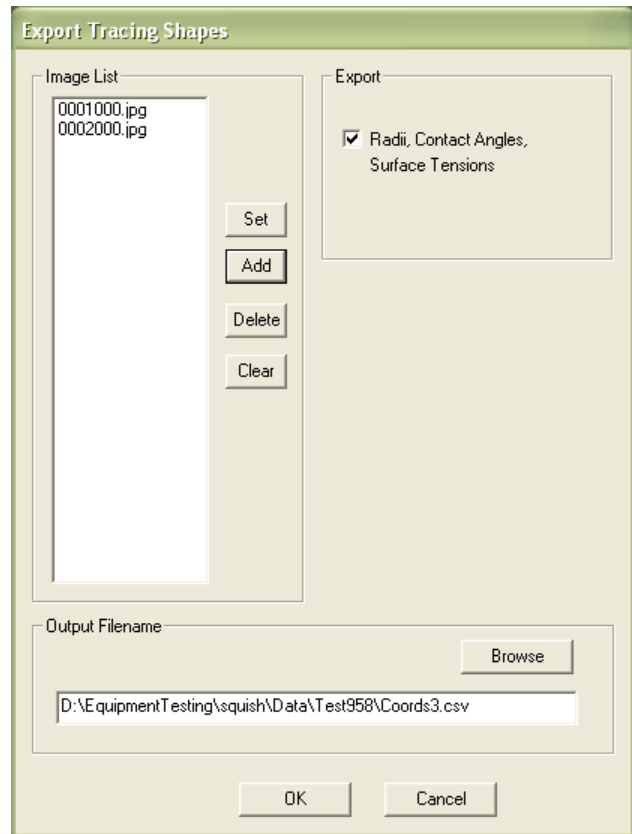


6. Click "OK"

## Exporting Traced Data

Geometries and Surface Tensions calculated using the image tracing module can be exported to a .csv text file by selecting Tracing Results from the Export menu.

The default list contains the images that were selected when the “Tracing Results” menu item was selected. Items can be added or deleted using the dialog buttons.





## 8. System Calibration and Advanced Tools

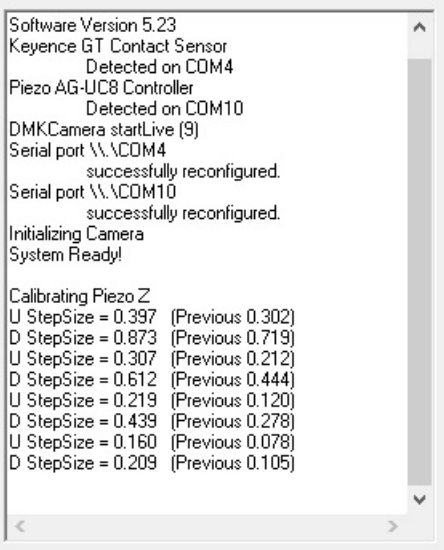
### *Piezo Calibration*

Piezo Calibration can be selected from the Advanced submenu of the Tools menu.

While the vertical piezo stage relies on the displacement sensor for determining the amount of travel, a calibration routine is necessary so that the software can estimate the correlation between movement pulses and displacement. This calibration routine will attempt to move the piezo actuator a certain amount and will measure the actual movement achieved. It is recommended that the piezo calibration be performed when the equipment is being set up for the first time in your facility. It is also required to be performed whenever the SquisherJoy software is used on a new computer as the calibration factor is stored on the computer.

Before calibration, make sure the vertical piezo stage is in the mid-range of its travel, preferably at the height from which a test will be started.

The calibration results are reported in the log window on the left side of the main screen.



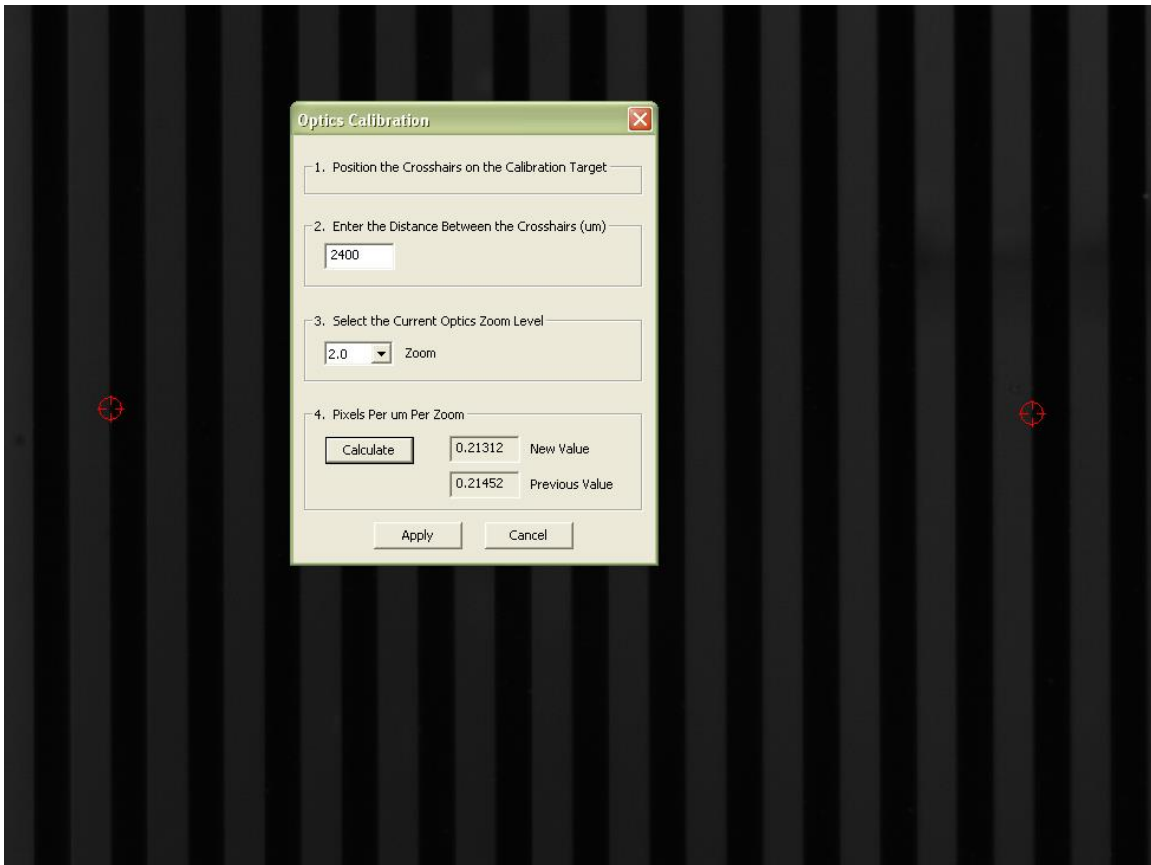
```
Software Version 5.23
Keyence GT Contact Sensor
    Detected on COM4
Piezo AG-UC8 Controller
    Detected on COM10
DMKCamera startLive (9)
Serial port \\.\COM4
    successfully reconfigured.
Serial port \\.\COM10
    successfully reconfigured.
Initializing Camera
System Ready!

Calibrating Piezo Z
U StepSize = 0.397 (Previous 0.302)
D StepSize = 0.873 (Previous 0.719)
U StepSize = 0.307 (Previous 0.212)
D StepSize = 0.612 (Previous 0.444)
U StepSize = 0.219 (Previous 0.120)
D StepSize = 0.439 (Previous 0.278)
U StepSize = 0.160 (Previous 0.078)
D StepSize = 0.209 (Previous 0.105)
```

## Optics Calibration

Optics calibration must be completed when the system is first set up and every time the camera and lens system is disassembled or changed. It must also be performed every time a new computer is used to run the MicroSquisher. The procedure is as follows:

1. Launch the SquisherJoy software and launch a new test in order to see the live video window.
2. Bring up the Optics Calibration Dialog by selecting Optics Calibration from the Advanced submenu of the Tools menu.
3. Position a calibrated target with features of known size on the front of the fluid chamber. A glass target with 5 lines/mm (1mm = 1000  $\mu\text{m}$ ) is provided with your equipment for this purpose. Note that this means 5 **pairs** of lines/mm (light/dark alternating). This target can be positioned with removable tape. Focus the Image using the three axis manual stages on which the optics and camera are mounted.
4. Drag the crosshairs on the live video window such that they are positioned a known distance apart. Be sure that the crosshairs are **aligned with each other** as the computer measures the **absolute distance** between them, not just the horizontal or vertical distance.
5. Enter the distance between the crosshairs in the dialog field.
6. Select the optics zoom level.
7. Click the "Calibrate" button in the dialog box.
8. Selecting the "Apply" button will cause the software to use the current calibration value. It is useful to compare the current calculated value with the previous value as the value should not change dramatically. A typical calibration value is around **0.24 pixels/ $\mu\text{m}$ /zoom**.



## ***Tuning the Temperature Controller***

The MicroSquisher is equipped with a Thorlabs TC 200 temperature controller which uses a built-in PID controller to regulate the fluid bath temperature. This controller is capable of heating the fluid up to about 39°C (given a room temperature of about 23°C) and maintaining the setpoint temperature within +/-0.2°C.

**NOTE: The temperature controller works best if the temperature probe tip is completely submerged and not touching the side of the fluid chamber.**

Under normal operating conditions, the temperature is set in the SquisherJoy software and there is no reason for the user to have to use the button interface on the front of the control module other than to turn the unit on and off and enable the heater. For the heater power to be on, not only must the ON/OFF switch be turned on, but the “ENABLE” button must be pressed so that the HEATER ON indicator is illuminated.

**Prior to being shipped to the user, the controller has been tuned at the CellScale facility. If at some point the unit is not keeping the fluid at the set point temperature, it may be necessary to reset the controller. This procedure is simple and requires about 2 hours.**

1. Turn the controller on and ensure that the bath is full of fluid to the normal level.
2. Ensure that the temperature probe tip is completely submerged in the fluid and is not touching the chamber walls
3. Press the “MODE” button until the PID/TUNE screen is visible. Check that **P = 250, I = 10, and D = 0.**
4. Press the “ENTER” button
5. Using the “UP” and “DOWN” keys, adjust the temperature as desired.
6. If the “HEATER ON” light is not illuminated, press the “ENABLE” button to turn on power to the heater.
7. Press the “MODE” button until the PID/TUNE screen is visible.
8. Press the “SELECT” button until the cursor is on the “DETUNE” function (if the screen displays “TUNE”, then disregard this step, press “MODE” until the main screen is visible and skip to step 10).
9. Press the “ENTER” button
10. Wait until the fluid temperature is stable (approx. 1.5 hours). It may not be at the right temperature at this point, but it is important that the temperature is no longer changing.
11. Press the “MODE” button until the PID/TUNE screen is visible.
12. Press the “SELECT” button until the cursor is on the “TUNE” function
13. Press the “ENTER” button
14. The temperature should now rise to the correct value and the controller should function properly on subsequent restarts.

## ***Snap Image***

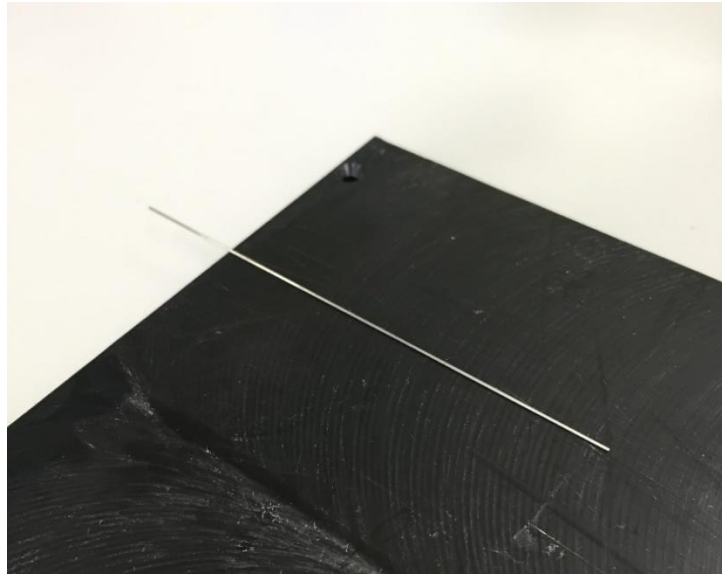
Single Images can be captured and saved from the live video stream by selecting Snap Image from the Tools menu.

This feature allows the user to capture a single image during the setup phase. This can be useful for documenting specimen loading activity and neutral (non-contact) beam position.

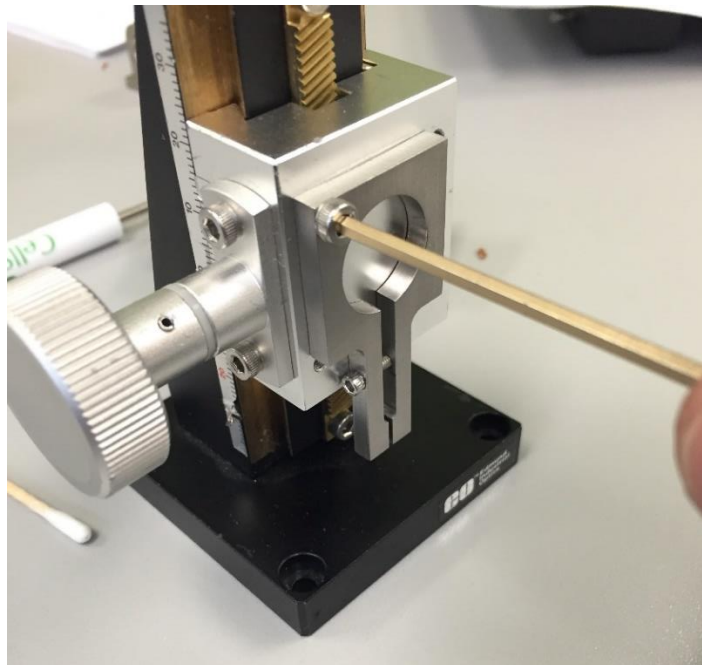
## 9. Mounting a MicroBeam

The exact details of how to best adhere the upper compression plate and mount the microbeam to the grips may vary depending on your specific application, but a general protocol is outlined here.

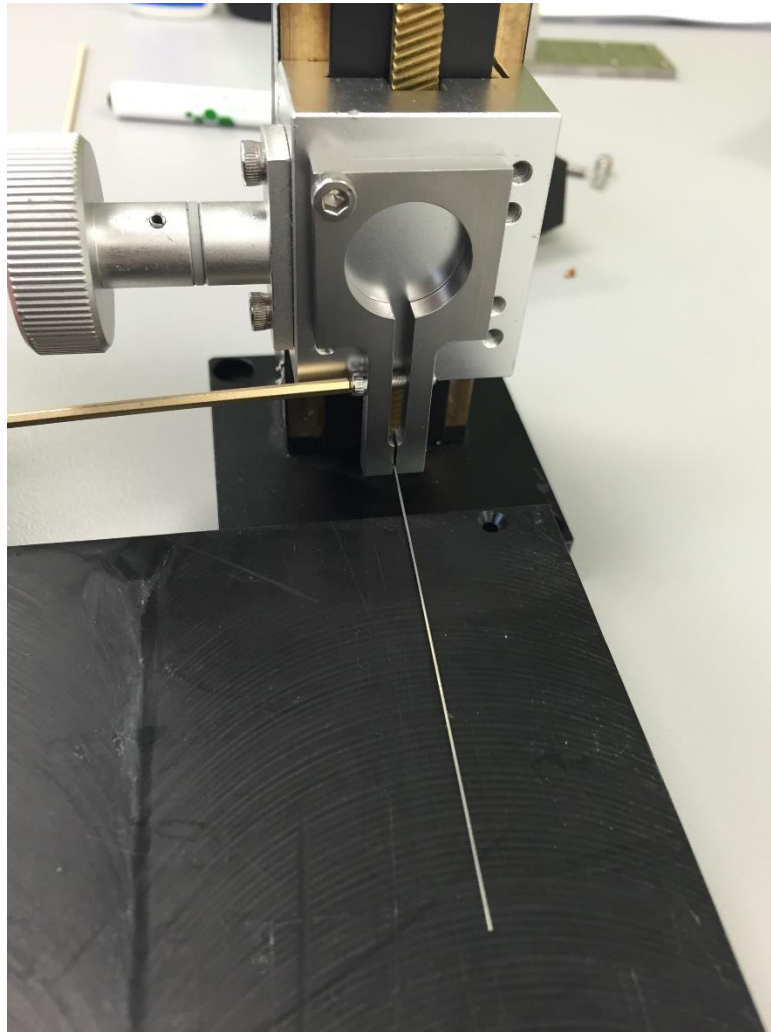
1. Place the microbeam on an elevated level surface



2. Mount the grip to the included vertical stage



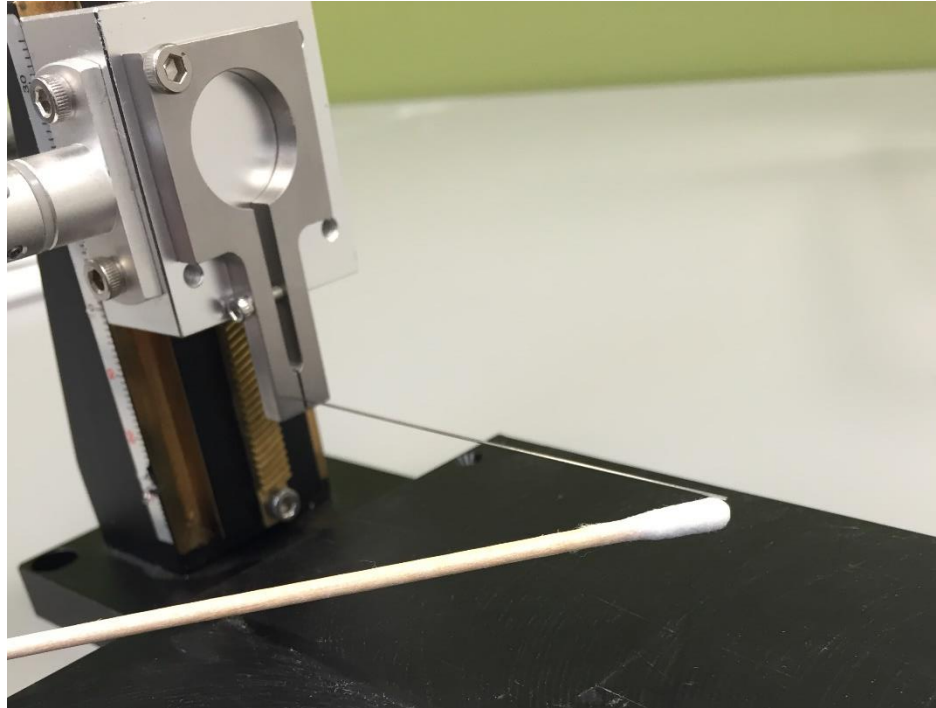
3. Carefully position the stage to grip the microbeam near the tips of the grip and close the grip using the included hex key. This procedure should ensure that the microbeam is mounted horizontally.



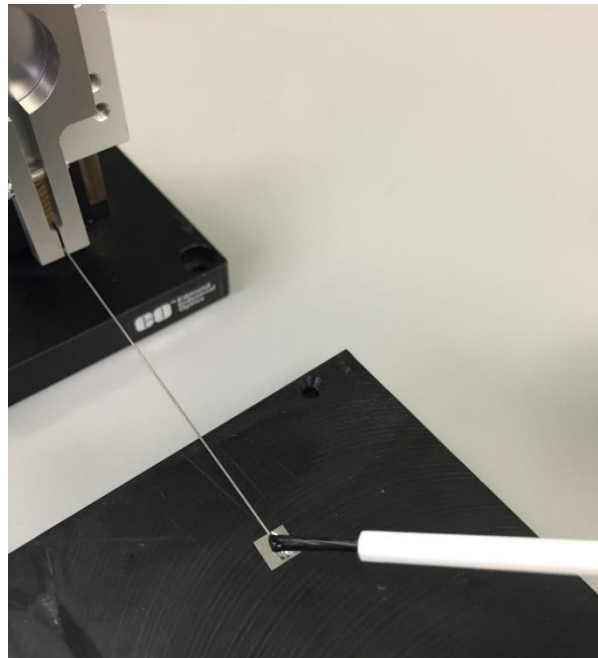
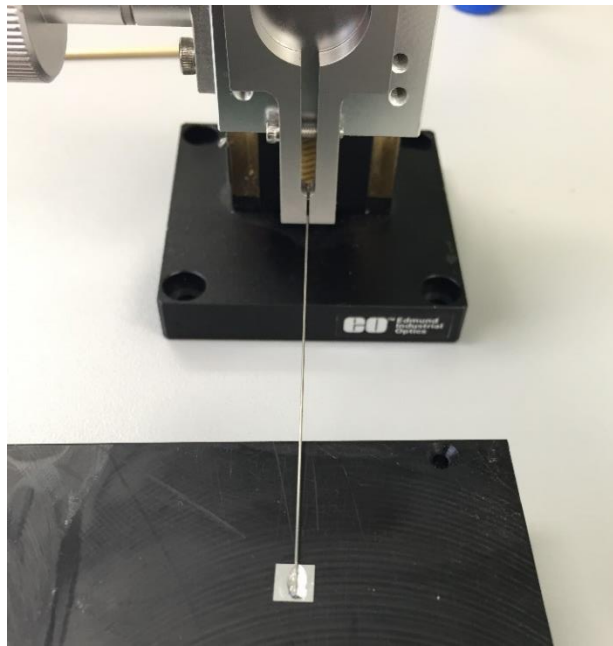
***MicroSquisher Tip: Microbeam Position***

*Very thin and/or delicate microbeams will tend to sag under their own weight (e.g. 100 $\mu$ m diameter and smaller metal microbeams). To ensure that the upper compression plate is level after mounting, it may be desirable to initially place the microbeam on an inclined surface.*

4. Place the compression platen on the elevated level surface.
5. Position the microbeam above the compression platen and then carefully lift the microbeam up to allow application of the adhesive. This may be easier to do under a dissection microscope if one is available.
6. Put a drop of adhesive on a cotton swab and apply to the end of the microbeam. Cyanoacrylate is included with the system for this purpose.



7. Slowly lower the microbeam onto the center of the compression platen. The end of the microbeam should be flush with the end of the platen. Cure the adhesive with a small amount of adhesive accelerator. This can be applied with the included brush.



***MicroSquisher Tip: Cyanoacrylate Adhesive***

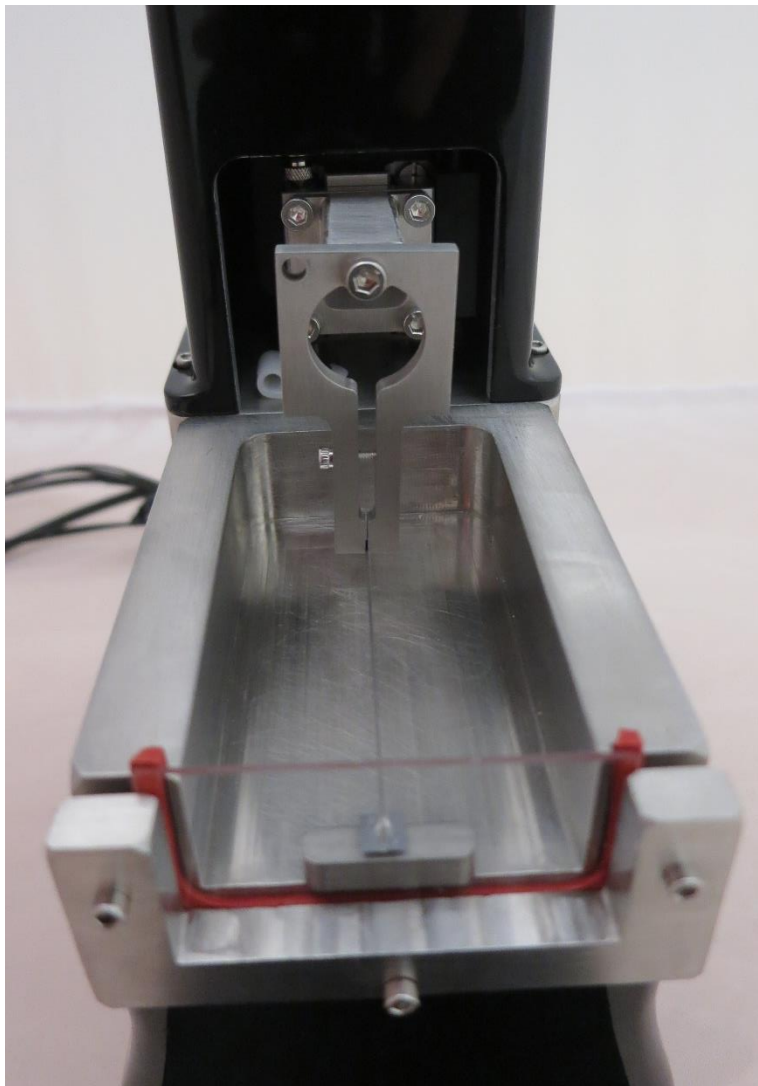
*Cyanoacrylate adhesive is provided complimentary with the MicroSquisher system to bond the microbeam and compression platen. This adhesive will best retain its properties if it is **refrigerated** when not being used.*

The microbeam clamp is attached to the vertical piezo stage with a single M3 fastener. This single connection allows for the angle of the upper compression platen to be adjusted under guidance from the live image. It is important that the upper compression platen and the lower compression anvil are parallel for proper compression testing.

The microbeam length must be measured and the dimensions and modulus correctly specified before a test. See Section 3 - Step 5 for more details.

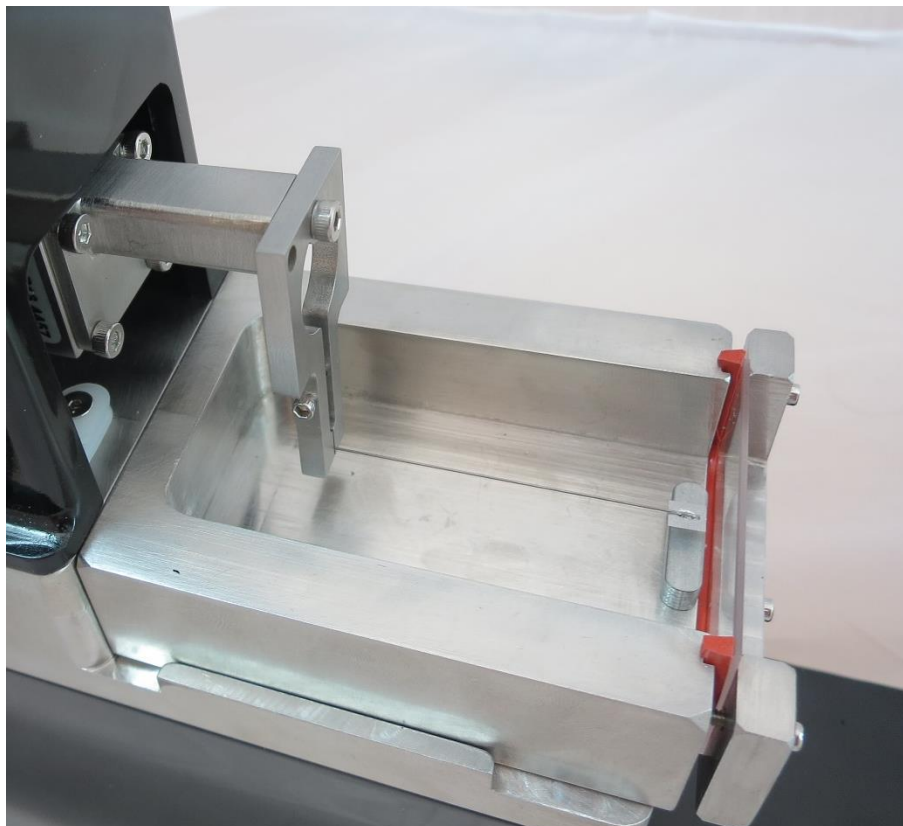
***MicroSquisher Warning: Adhesive and Accelerator***

*Cyanoacrylate adhesive and accelerator are provided with the MicroSquisher system to bond the microbeam and compression platen. These are eye, skin, and respiratory irritants. Cyanoacrylate will instantly bond skin. The use of gloves is recommended.*

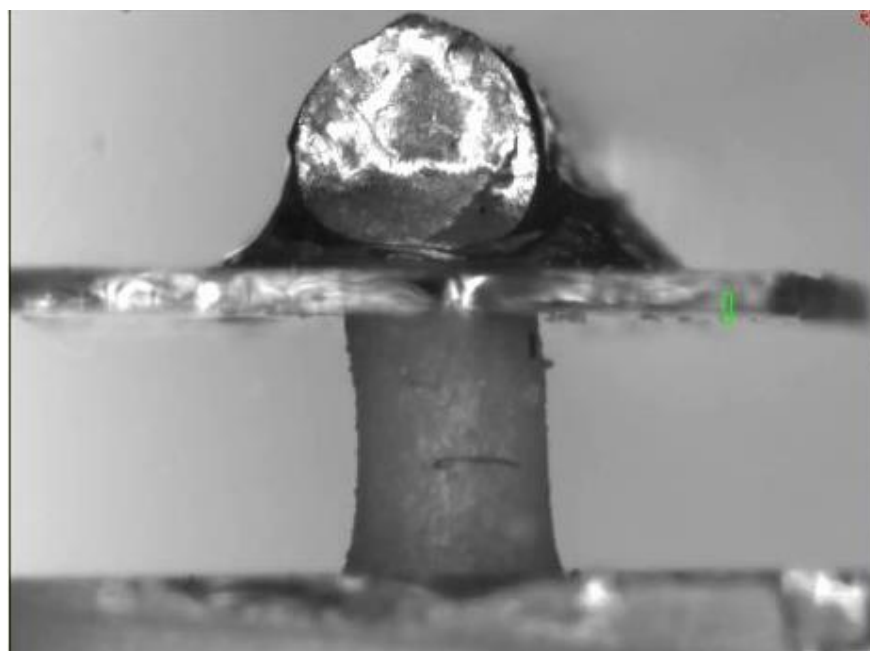


*Clamp with microbeam and platen attached to the vertical piezo stage, with testing anvil and sample*





*Clamp with MicroBeam and platen attached to the vertical piezo stage, with testing anvil and sample*



*Live software camera view MicroBeam and platen, with testing anvil and sample*

# 10. Force Calculation Formulas

The force applied to the specimen is calculated using the deflection of the microbeam according to the formula:

$$Deflection = \frac{Force \times Beam Length^3}{3 \times Beam Modulus \times Beam Area Moment of Inertia}$$

where

$$Beam Area Moment of Inertia (round) = \frac{\pi \times radius^4}{4}$$

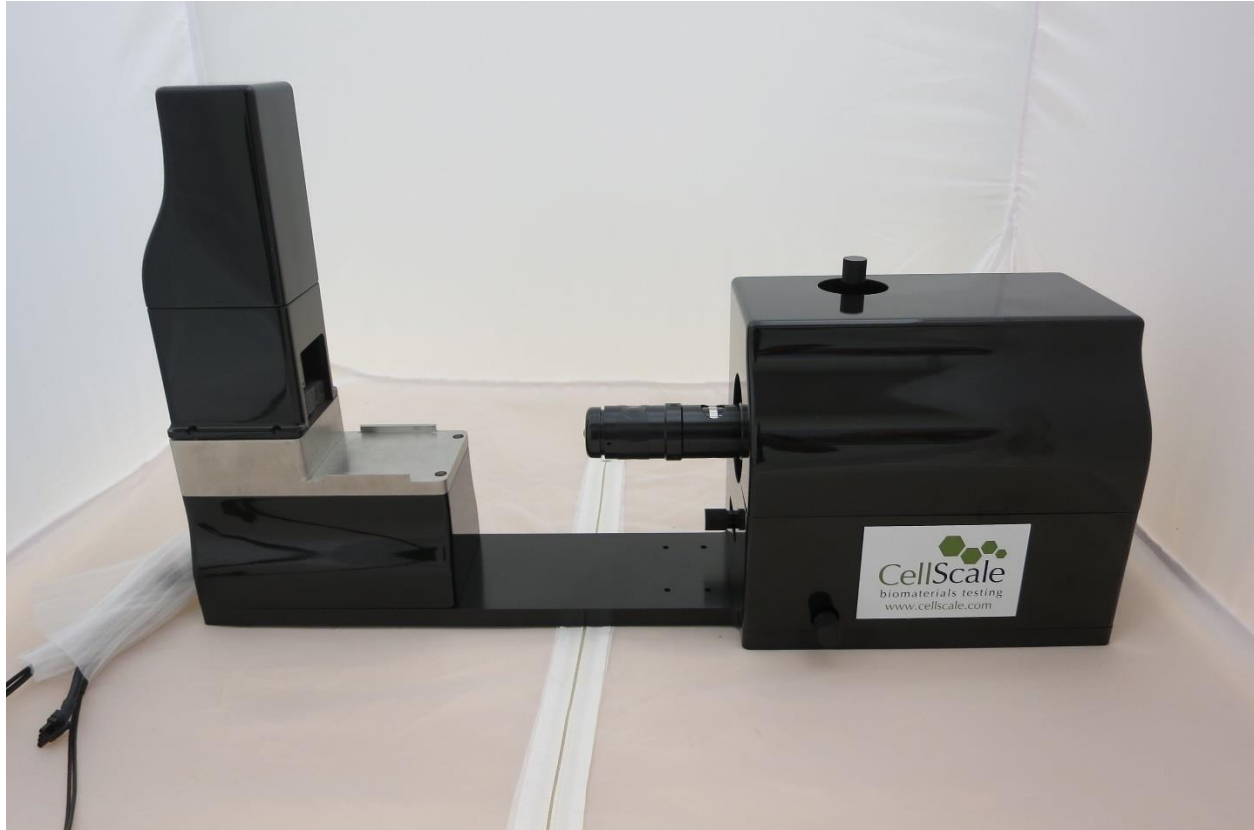
$$Beam Area Moment of Inertia (rectangular) = \frac{Width \times Height^3}{12}$$

Typically, the microbeams are made from tungsten wire. CellScale has this wire available in 7 different diameters. With a beam length of 55mm, the expected force resolutions and recommended maximum forces are given below.

Diameter (µm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (µN)	0.012	0.038	0.191	0.604	3.056	9.658	34.521
Recommended Max Force (µN)	4	11	57	181	917	2,897	10,536

# 11. Appendix A: System Setup

## *Unpack Main Unit, Controllers, and Accessories*



Remove the main unit from its packing material. A foam “clamshell” encases the main unit inside a cardboard box for shipping. This material must be carefully removed. Take care not to lift the main unit by the camera lens or positioning knobs.

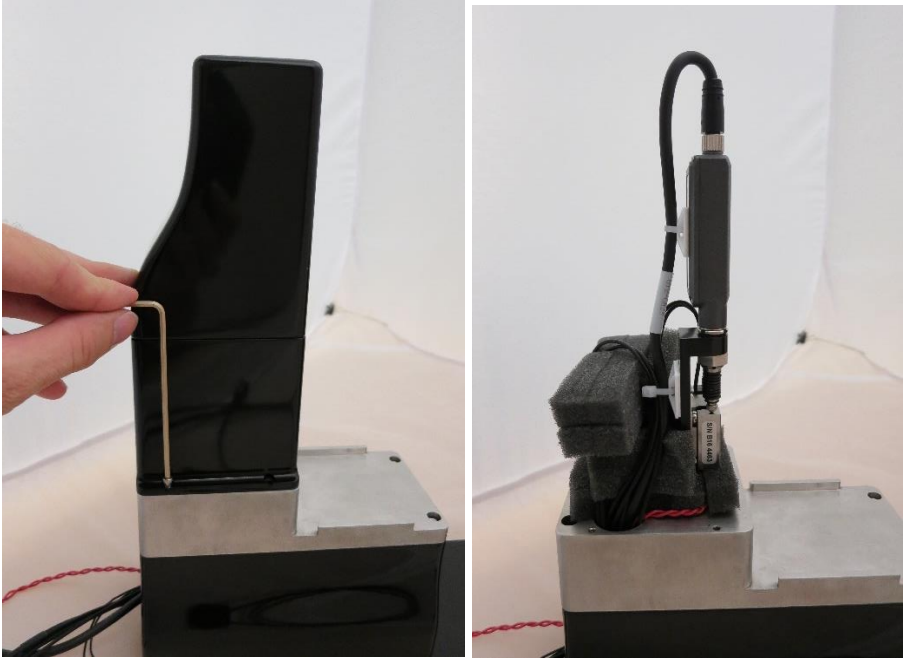
The temperature controller, piezo stage controller, control box, and all system cables and accessories are packed in a separate container.

## ***Remove Packing Material from the Main Unit Cables***

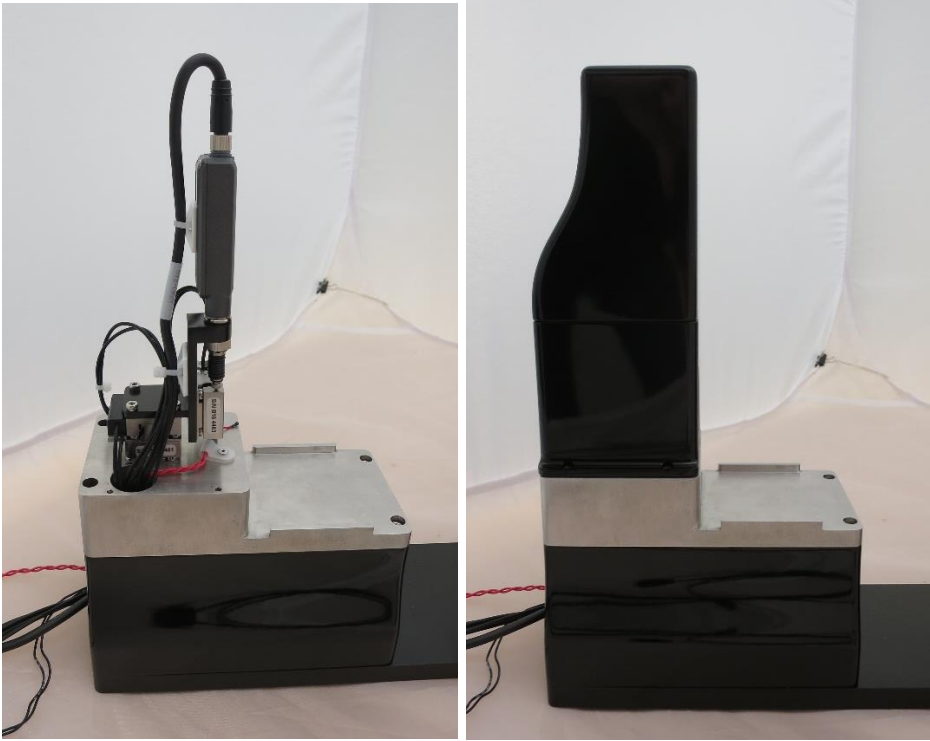


The cables exiting the main unit are encased in packing material for protection during shipping. This material must be removed from the length of the cables and from the point where the cables exit the main unit.

**Remove Plastic Cap and Foam from the Piezo Stack**



The piezo actuator stack and displacement sensor are protected by a plastic cap. Foam surrounds these components inside the cap during shipping. Remove the cap using a hex key and remove all pieces of foam inside. Replace the cap and fasteners.



### ***Attach Spacer to Vertical Piezo Stage***



A spacer is attached to the vertical piezo stage. This allows the microbeam clamp to reach the test chamber. 4 x M3x6 fasteners are used to attach this component using the supplied 2.5mm hex key.

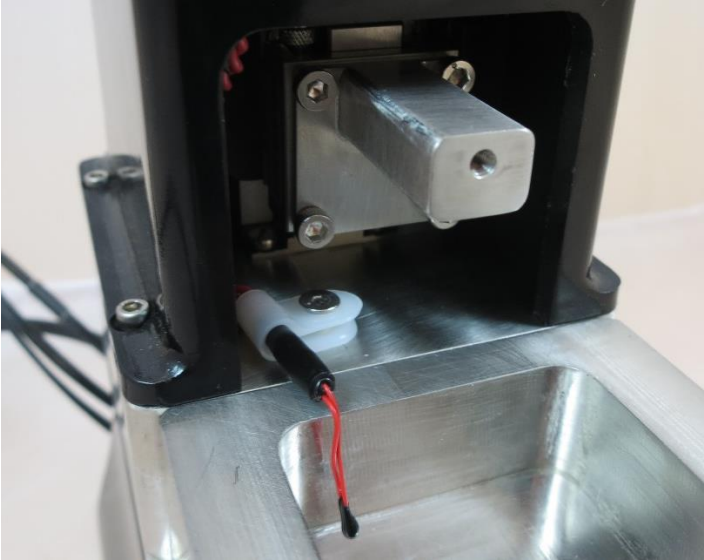
***Set Test Chamber on the Chamber Holder***



Set the fluid bath (test chamber) in the chamber holder on the main unit. This chamber is pre-assembled with a glass slide and gasket. Replacements are provided for these components.

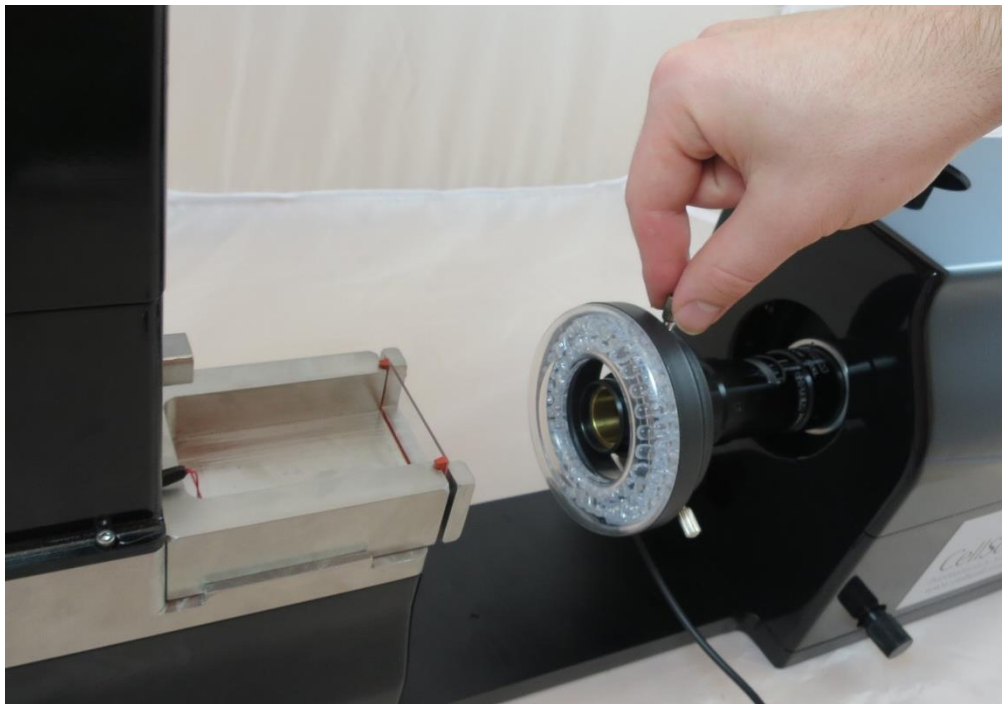
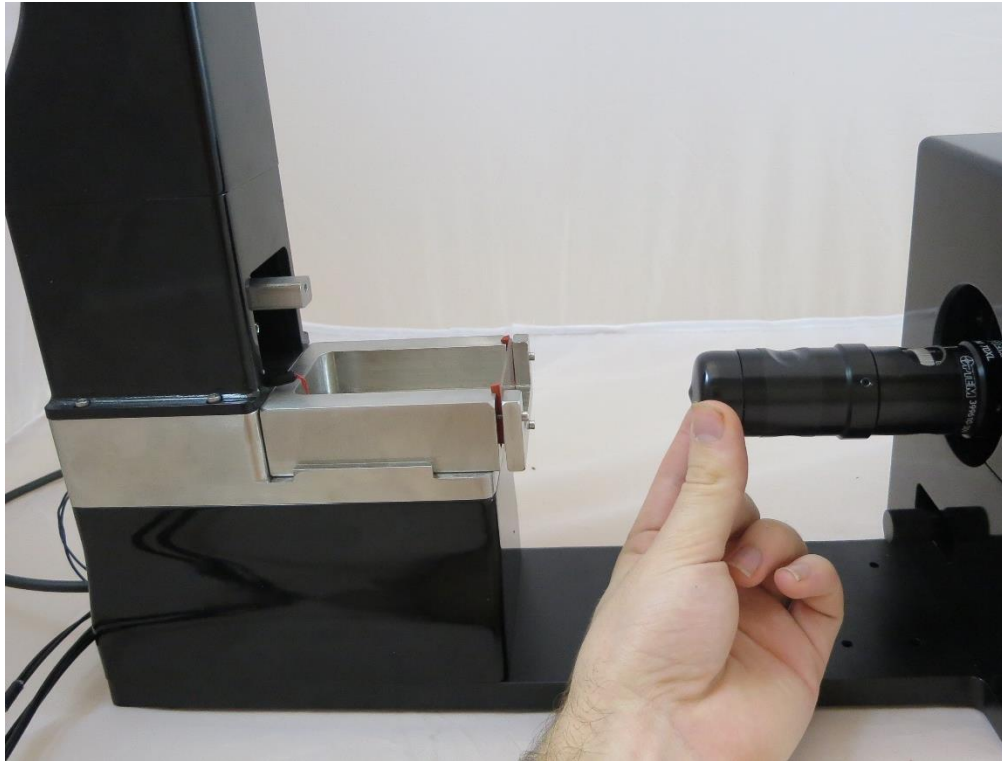
***Place Temperature Sensor in the Test Chamber***

Bend the temperature sensor into the test chamber. This chamber can be filled with fluid for which temperature control can be specified. The temperature sensor functions best when it is fully immersed in fluid and is not touching the side of the chamber.





## ***Remove Lens Cap and Mount the Ring Light on the Lens Assembly***



Remove the lens cap from the lens assembly. Attach the ring light to the end of the lens assembly using the 3 set screws spaced around the ring light.



## ***Connect Cables***

A number of power and communication cables need to be connected between the main unit, control box, temperature controller, piezo controller, and computer:

- 20V power supply to power input jack on the control box
- LED ring light to control box power extension
- Displacement sensor cable to cable extension from control box
- USB Cable to back of camera (do not yet connect to PC)
- 3 piezo stages to piezo controller
  - Channel 1 Axis 1 (Left/Right)
  - Channel 2 Axis 1 (Forward/Back)
  - Channel 3 Axis 1 (Up/Down)
- Mini USB cable to piezo controller (do not connect to PC yet)
- Temperature control harness to temperature controller (main output)
- Heater element to temperature control harness
- Temperature sensor to temperature control harness
- Power cable to temperature controller
- USB cable to temperature controller (do not connect to PC yet)

NOTE: At the completion of this step, there should be 4 USB connections ready to connect to the PC USB ports

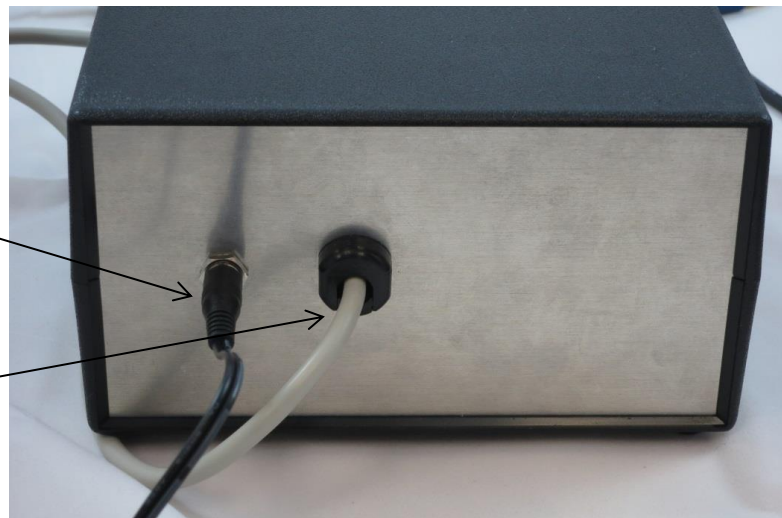
- Black USB cable from camera
- Black USB cable from temperature controller
- White USB from piezo controller
- Blue USB plug with Serial/USB converter connected to beige serial cable from control box



The temperature controller USB can be connected via a hub with keyboards, a mouse etc. All other devices require **their own** USB port.

20V Power Supply Input

Position Sensor Control Box Output  
(with Serial/USB converter)

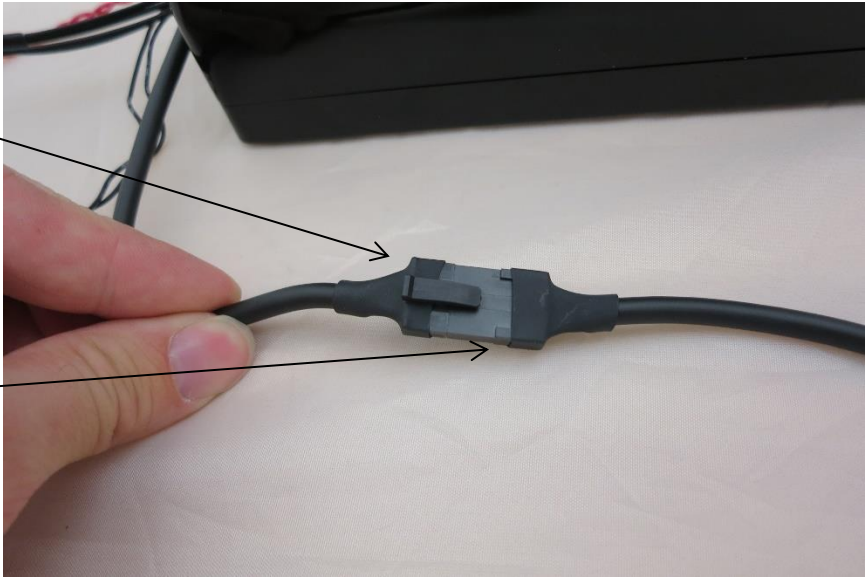




Position Sensor Cable Extension

LED Ring Light Power Extension

Power Switch



Displacement Sensor Cable

Displacement Sensor Cable Extension (from control box)

Temp Sensor/Heater Connector

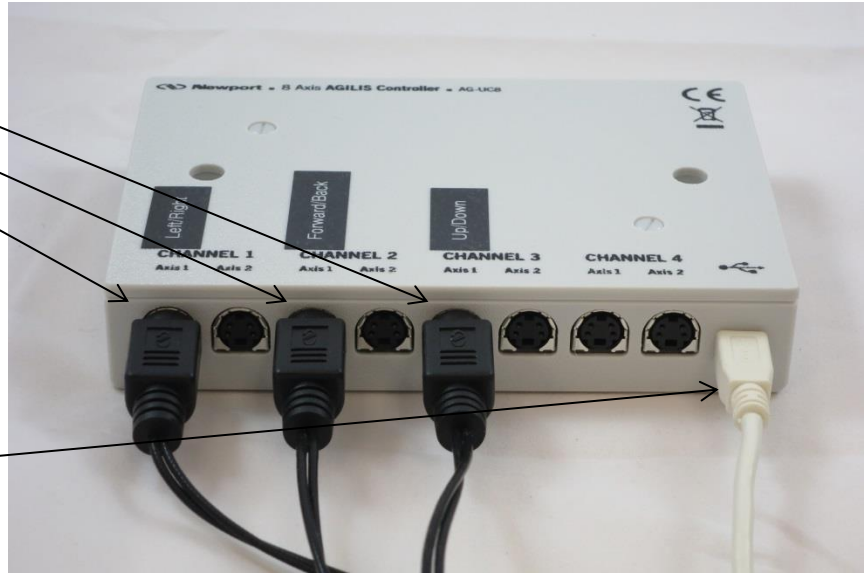
Temperature Controller USB

Temp Controller Power Input



Piezo Stage Connectors

Piezo Controller USB

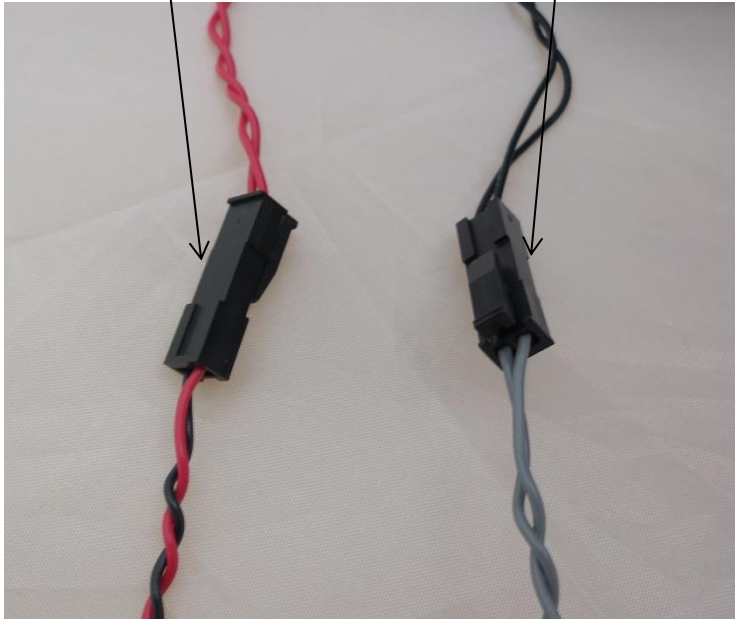


The piezo stage connectors are labelled according to the port they should be plugged into



Temp Sensor  
Input/Output

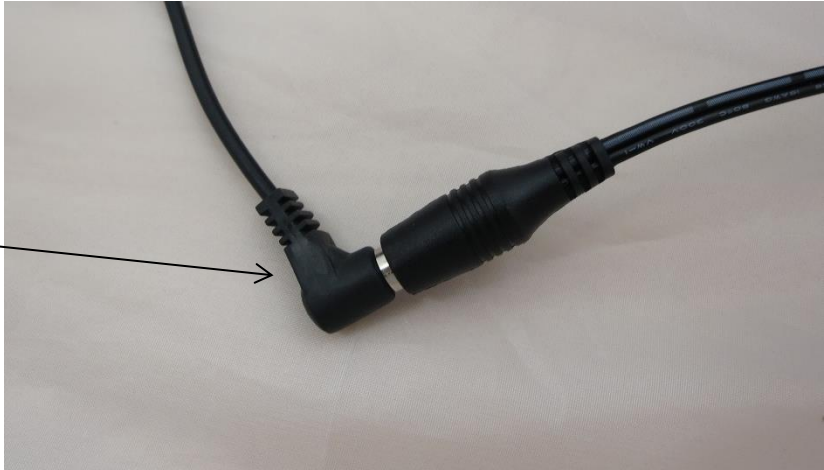
Heater Input/Output





Camera USB

LED Ringlight Power Extension



### **System Calibration Check**

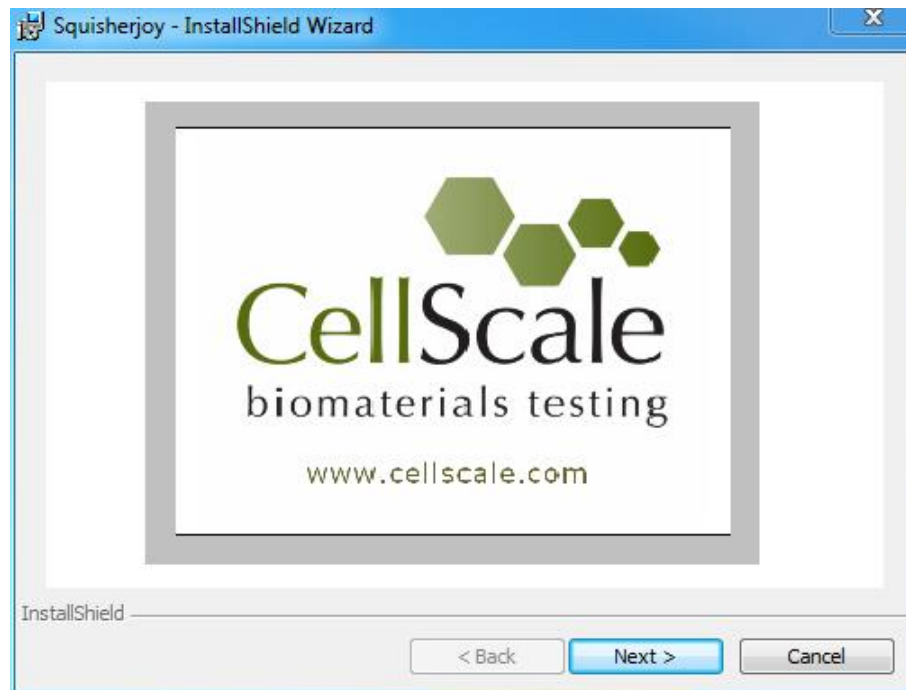
After the MicroSquisher is fully assembled and set up, the SquisherJoy software must be installed (see Appendix B). The system can then be switched on and the computer connectivity may be tested by opening an initial test. However, before any tests are run, it is recommended to check the piezo and optics calibrations. Instructions for these operations can be found in Section 8: System Calibration and Advanced Tools.

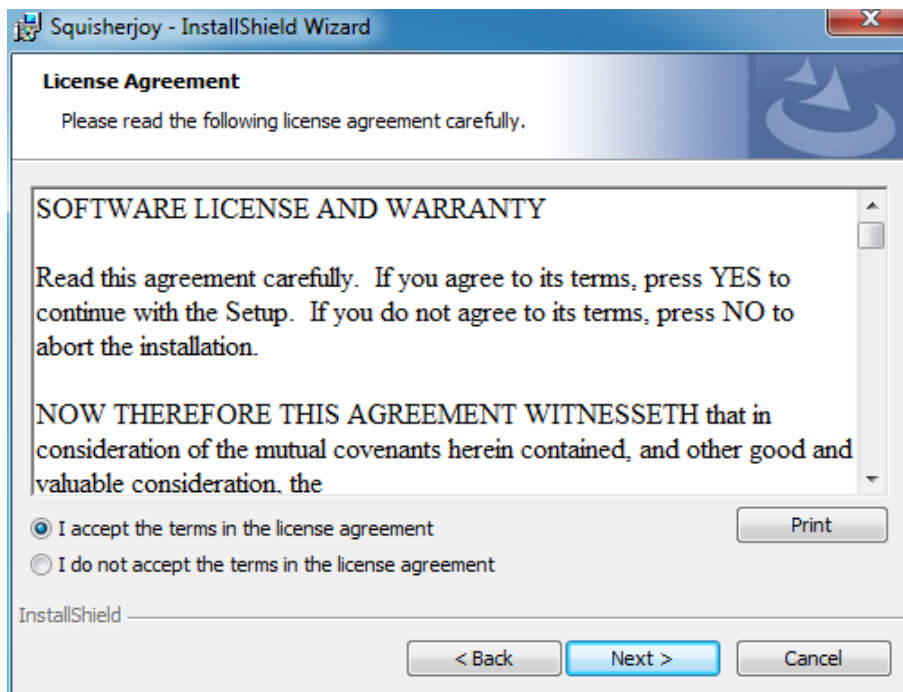
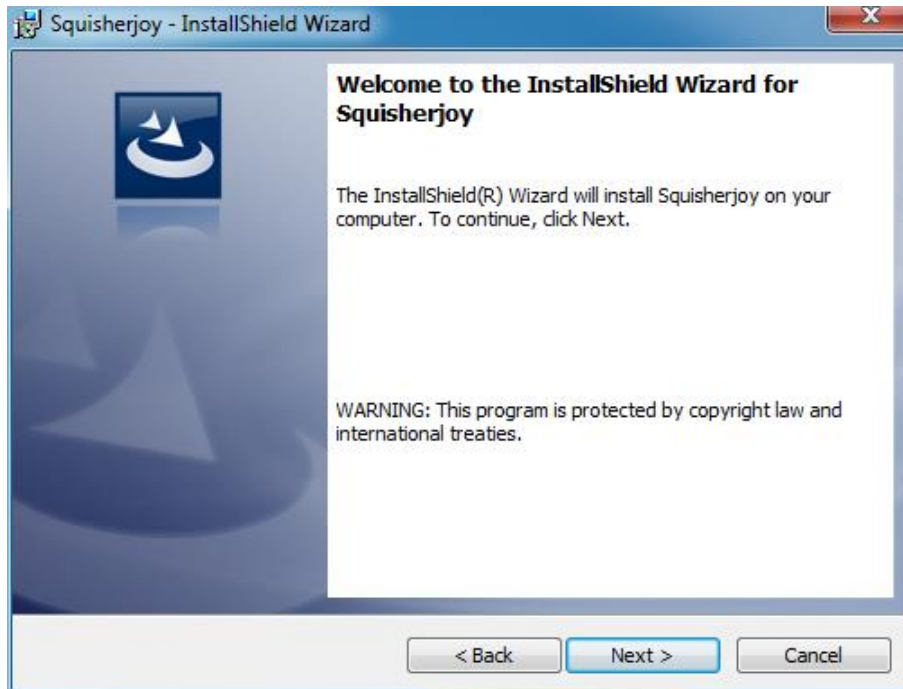


## 12. Appendix B: Computer Setup

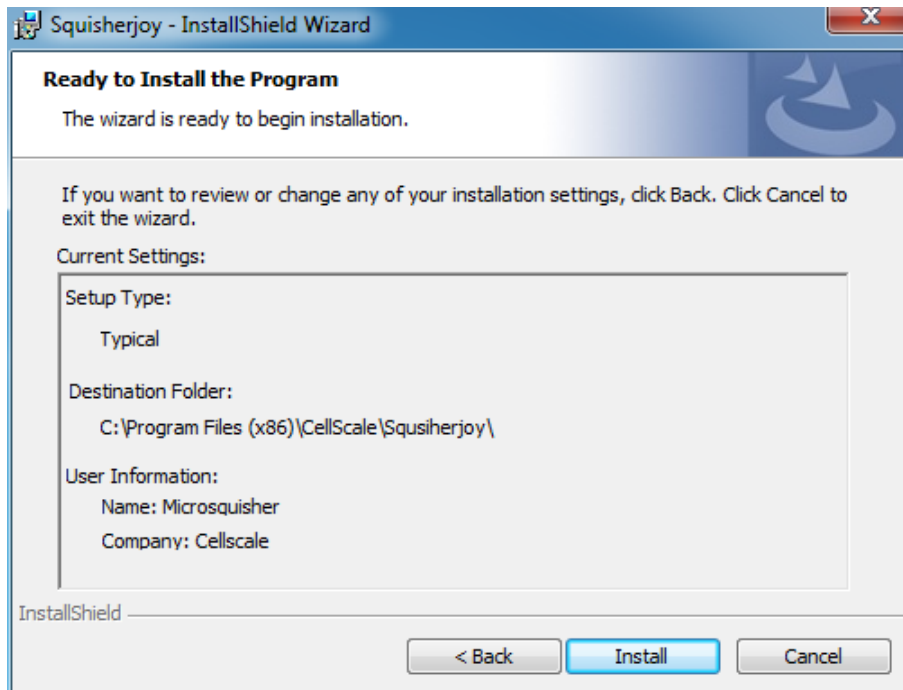
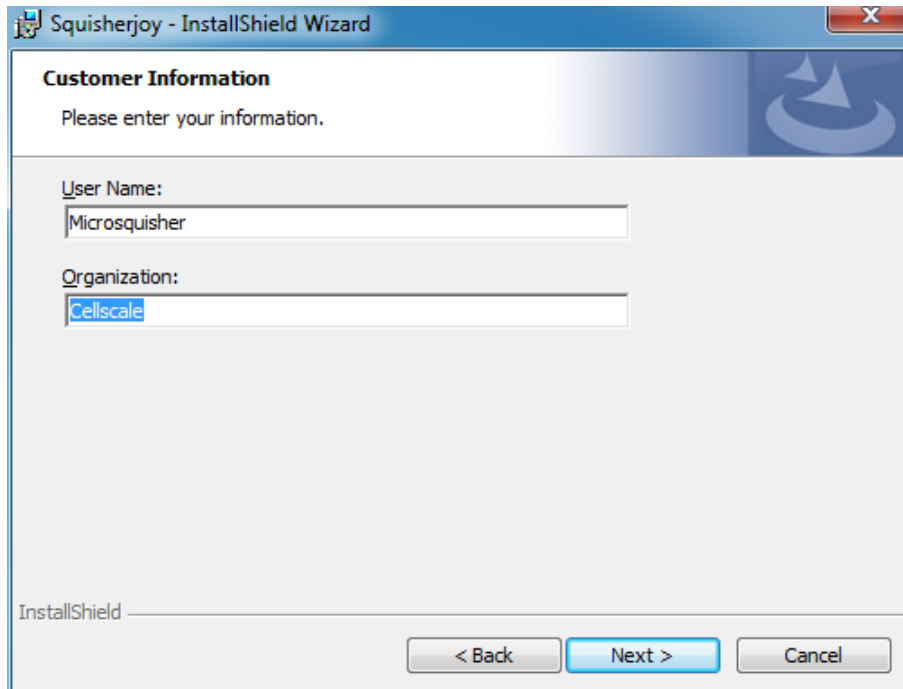
1. Download the software installation package from the provided link and save to a known location
2. Right click on the .zip installation file, right click, and select “Extract All...” to unzip the installation package
3. Go to the uncompressed folder and select “setup.exe”.
4. Follow the prompts as shown on the next few pages.
5. Once the installer is finished, plug the USB cables into a USB port for the following components:
  - Camera (black USB cable)
  - Position Sensor (beige serial cable with USB converter and plug)
    - A 2<sup>nd</sup> position sensor may be used for specially equipped 2-axis systems
  - Temperature Controller (black USB cable)
  - Piezo Actuator Controller (white mini-USB cable)

The MicroSquisher requires at least 4 USB ports for operation. For computers with insufficient ports, the heater controller can be hubbed with other peripherals like a mouse or keyboard. The piezo controller, camera, and position sensor all require their own USB port and **cannot** be hubbed.



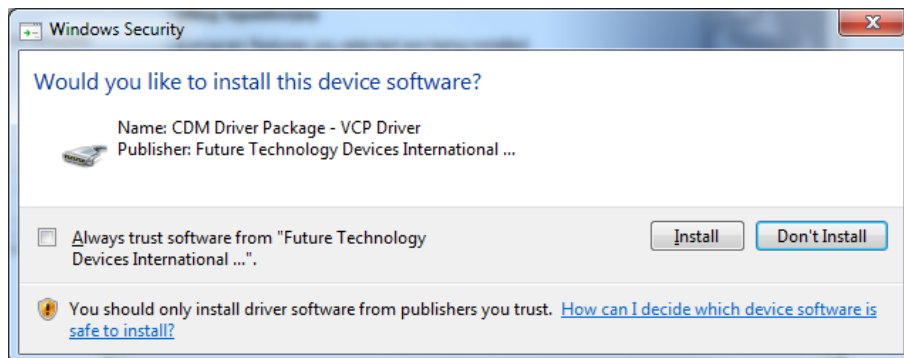


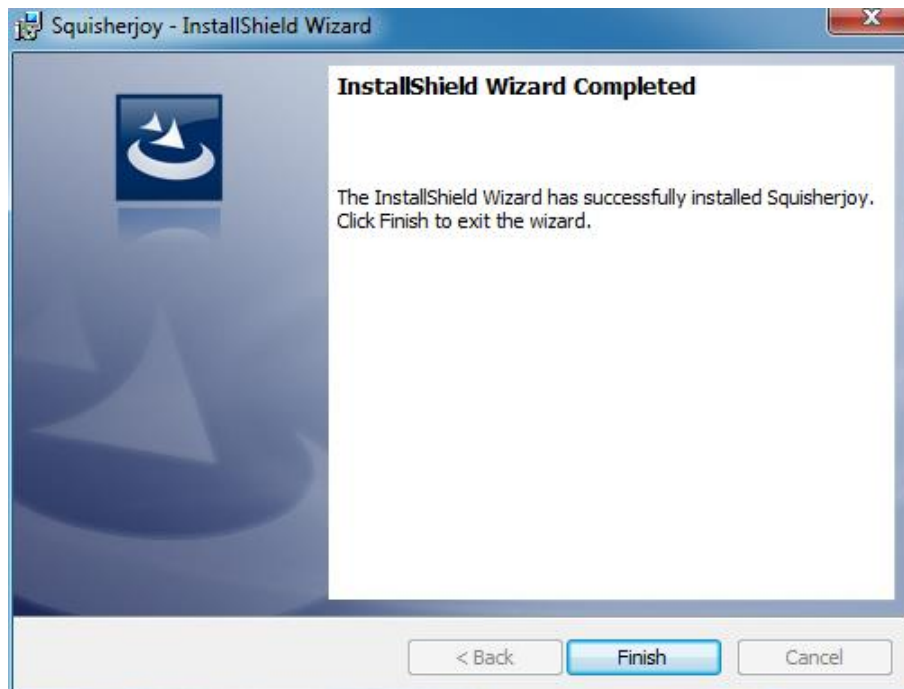
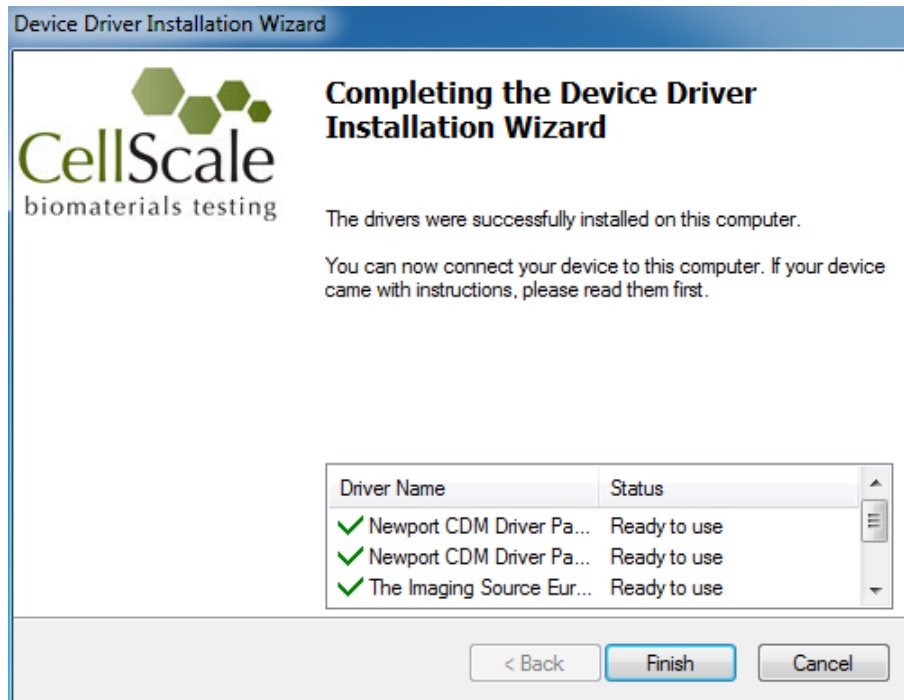


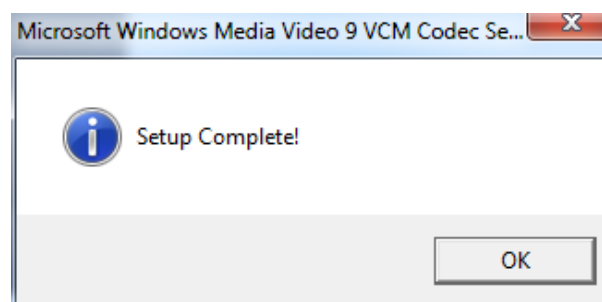
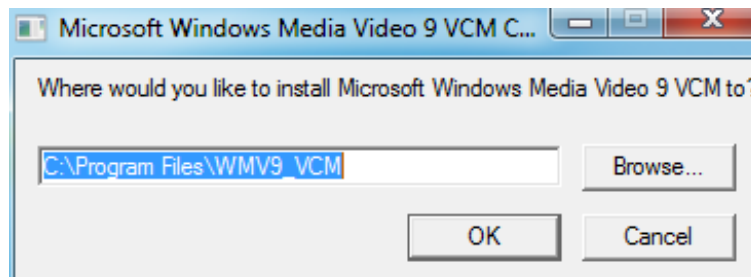
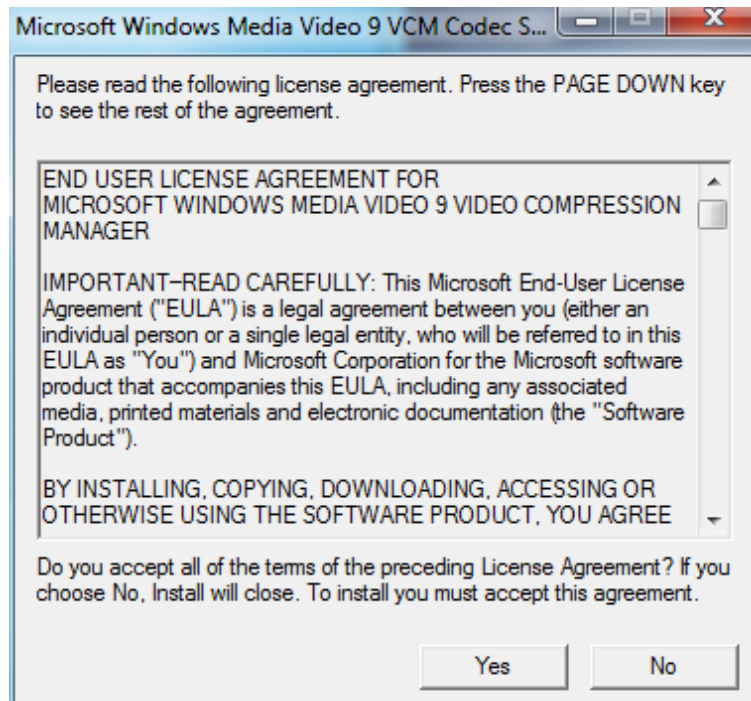
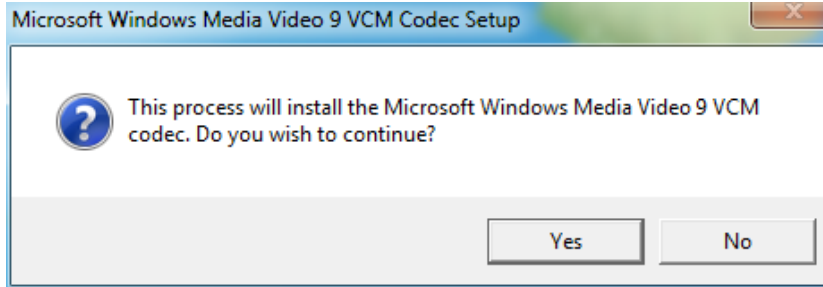




Depending on security settings, the following message may appear.







## 13. Appendix C: Shear Testing



Some MicroSquisher systems are equipped with an additional displacement sensor to measure horizontal displacement of the microbeam during a test so that tests can be executed in this axis. This allows a type of shear testing to be performed on samples.

On systems equipped for shear testing, a special version of the software is provided directly to the user (the link on the CellScale website contains the single-axis version of the software). In the "Set Parameter Editor", this allows X-Shear to be selected as the Control Axis. Compression and Shear movements can be executed in the same test sequence by selected a different Control Axis for different test sets. For shear testing, positive magnitudes are to the right.

Set Parameter Editor

Name: SHEAR (Example: "Squish1", "TestData1")

Axis

Control Axis: X-Shear

Control Mode: Displacement

Control Function: Ramp

Shear Magnitude: 100 um

Tolerance (um): 5

Correct Overshoot

Cycle

Loading Duration (S): 30  Do Not Load

Hold Duration (S): 0

Recovery Duration (S): 10  Do Not Recover

Rest Duration: 0

Repetitions: 1

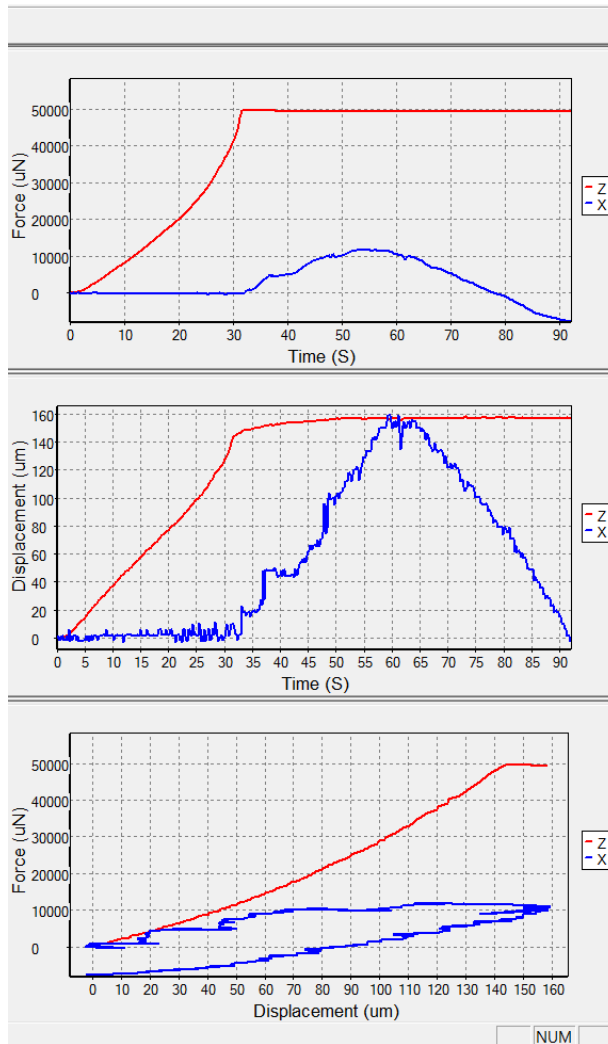
Data Output Frequency (Hz): 1

Image Output Frequency (Hz): 1

OK Cancel

Additional data columns for the X-Shear axis are output in the data .csv file for these specially equipped systems. In addition, the live graphs during a test will contain additional data output for this axis.

- □ ×



Also, the image tracking settings will typically differ from that of a single axis MicroSquisher system. The tracking template and search region settings can be found in Advanced in the Settings menu. This will be preset as a square template under the demo template provided so that image tracking can be performed equally well in both axes. If single axis testing is being performed, these settings can be altered so that the template and search region is optimized for the axis being used.

Advanced Settings

Hold Phase Control

Limit Moves To a Maximum of 50 Steps Per 200 mS

Limiting moves may cause target values to be reached more slowly  
But can be set to ensure that each move completes within each 200 mS  
Allowing for full 5Hz data output with no skipped points

0.75 Slow Ratio

Corrections for overshoot or undershoot are multiplied by the Slow Ratio  
If this ratio is small target values may be achieved slowly  
If this ratio is large oscillations about the target value may occur

Image Tracking Settings

Search Region Minimum (+/- pixels) 3 x 8

Template Region Size (+/- pixels) 12 x 12

Increasing these settings will increase real time computations during data  
And may result in 5Hz cycle time not being achieved.

OK Cancel