



# **BioTester 5000**

## **Biaxial Test System**

***User Manual  
version 8.7***



## *Mechanical measurement and analysis of biomedical materials*

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

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# 1. General Information

The BioTester 5000 is a precision test instrument specifically designed for the measurement and analysis of small biological specimens. The system includes a compact biaxial test station and an integrated software interface to run and analyze test results. The system is intended for indoor use only.

## ***Environmental and Electrical Specifications***

<b>Electrical Input</b>	100-240VAC, 50-60Hz
<b>Current Rating</b>	2.71 Amp
<b>Environmental Conditions</b>	Maximum Operating Temperature 25°C 0% - 95% Relative Humidity
<b>Installation Category</b>	Category II
<b>Pollution Degree</b>	Degree 2
<b>Maximum Altitude</b>	2000m
<b>Data Connections</b>	1 – USB 3.0 for camera/PC communication 1 – USB for controller/PC communication

## ***System Assembly***

The BioTester 5000 unit requires some assembly. See Appendix A and C.

## ***Power Connections***

Connect power supply into properly grounded 100-240VAC power source to ensure safe operation. Ensure that the power cord is always easily accessible. The use of an Uninterruptible Power Supply (UPS) is recommended to protect against data loss.

The mains supply voltage fluctuations should not exceed 10% of the nominal supply voltage.

## ***Safety Warnings***

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

### ***System Alert***

*The plastic covers must remain on this unit at all times to prevent injury and/or damage.*

*This equipment must not be disassembled by the user or modified in any way.*

## ***Approvals and Certification***

This product conforms to EN61010-1 and EN61326-1.

## Manual Operating Controls

There are a few manual controls on the unit. The camera housing contains two adjustment knobs that are used for fine adjustment of the horizontal camera position, and the camera lens has focus, zoom, and iris rings which can be set by the user.

### **BioTester 5000 Tip: Setting Camera Controls**

*The camera housing knobs are used to ensure that the specimen is centered in the field of view. The iris is usually left 100% open; the brightness of the images is better adjusted by changing the camera gain and exposure in the software settings.*

There are two switches on the front of the unit, one for powering the controller and the LED lighting, and the second for powering the heating element.

The front of the unit also contains a pull rod which is used to raise and lower the specimen stage and fluid chamber. This rod is spring-loaded so that the chamber can be held at any height. To raise or lower the stage, lift the rod and then pull or push.

## Software

LabJoy software is included and is required to run the BioTester 5000. 2 x USB ports are required for data connections, with at least one of those ports being USB 3.0. Windows 10 or higher is required to run LabJoy.

## General Maintenance

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

### **BioTester 5000 Tip: Removing the fluid chamber**

*The fluid chamber can be removed by resetting (fully retracting) the actuators followed by carefully lifting and twisting the chamber. This is best accomplished when the chamber is emptied (The large syringe supplied works well to drain the fluid bath).*

## 2. Testing Terminology

The BioTester 5000 is designed to apply biaxial forces to soft tissue specimens (deforms by more than 1% *in vivo*) with an in-plane dimension of 3 - 15 mm. This includes biological material such as eye tissue, heart valves, pericardium, joint capsules, large blood vessels, scaffolds, and soft polymers.

### ***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.

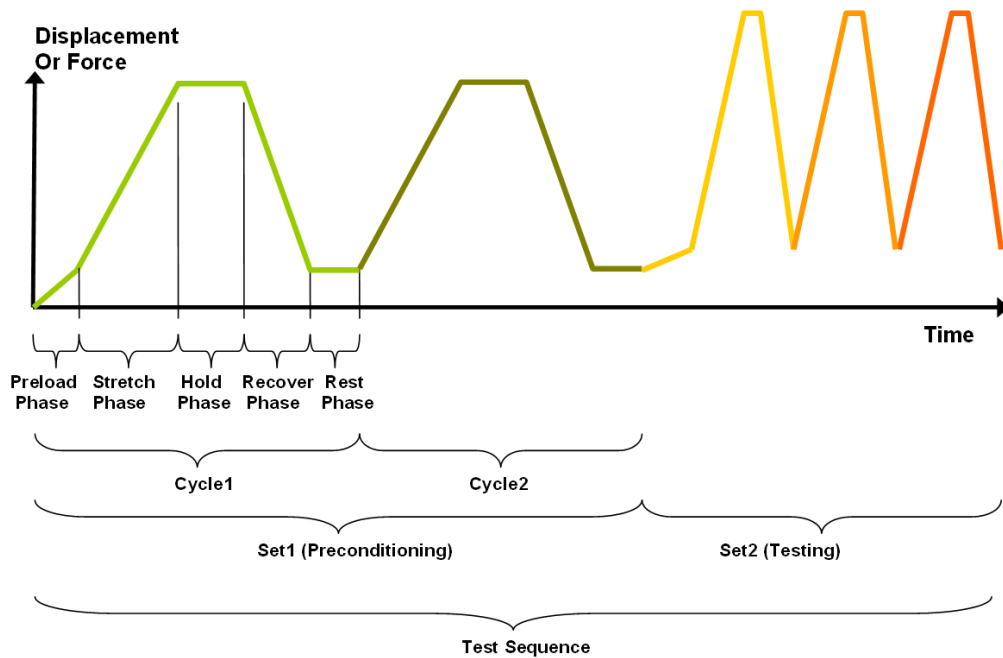
***Reproducing Physiological Conditions During Testing*** - By applying various loads and load rates, natural expansion and contraction of a specimen can be reproduced (for example, the pulse pressure in an organ). In as much as the physiological conditions can be recreated, the specimen can be tested 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*: the first set applies preconditioning to the specimen; the second set executes the actual test on the specimen.
- Within the first set (preconditioning), two identical *test cycles* are implemented to bring the sample to a satisfactory in vivo state.
- Finally, the second set (testing) 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 five phases within a cycle. Each phase serves a specific purpose:

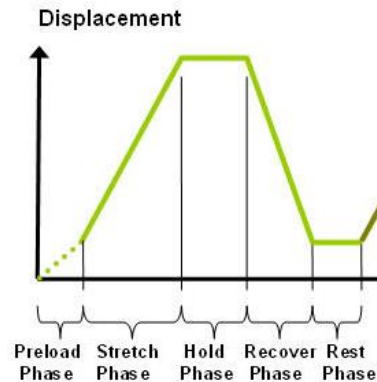
**Preloading**- Preloads are applied to bring a test to a well-defined starting point. Because the dimensions of a specimen may change as a result of a loading cycle (stretching of fibers, viscoelastic effects, plastic deformation, or localized material failure at the attachment points), the preload adjustment compensates for any of these changes in specimen geometry.

**Stretching** - During the stretch 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 BioTester 5000 stretches the specimen until the predefined displacement is achieved. The force required to achieve the displacement is an output of the test. Several control functions are available for displacement control.

Under **force control**, the force applied to the specimen is predefined. The BioTester 5000 stretches the specimen until the predefined force is achieved. The displacement required to achieve the force is an output of the test. Force control can be applied as a ramp or sinusoid function.

In addition, strain control from image feedback is available for the BioTester 5000. This control mode utilizes displacements based on a region within a specimen rather than global actuator displacements. For more information on this control mode and how to set up a protocol with this mode, see the **Strain Control from Image Tracking** section below.

## ***Control Functions***

The BioTester 5000 makes it possible to test specimens under several control functions:

Under Displacement or Force control:

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

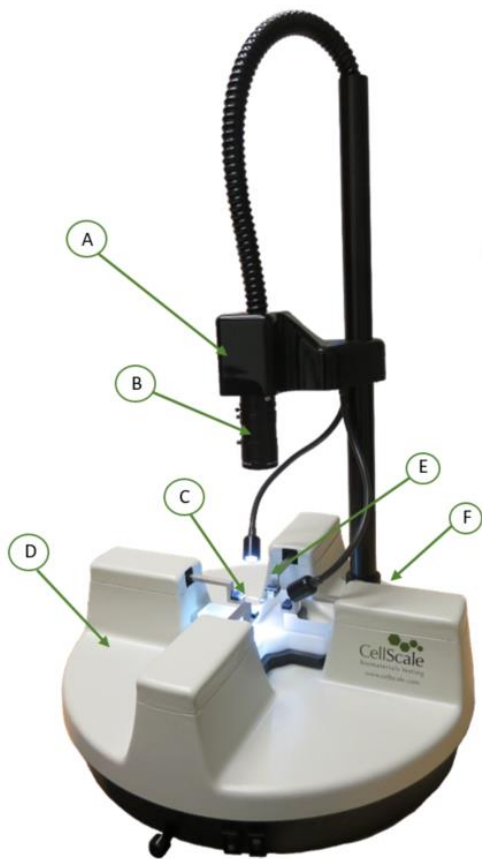
The **sine function** applies the displacement or force according to a sinusoid with the desired displacement magnitude and duration.

The control mode, load function and load magnitude can be set independently for each of the two loading directions.

### 3. System Overview

#### System Components

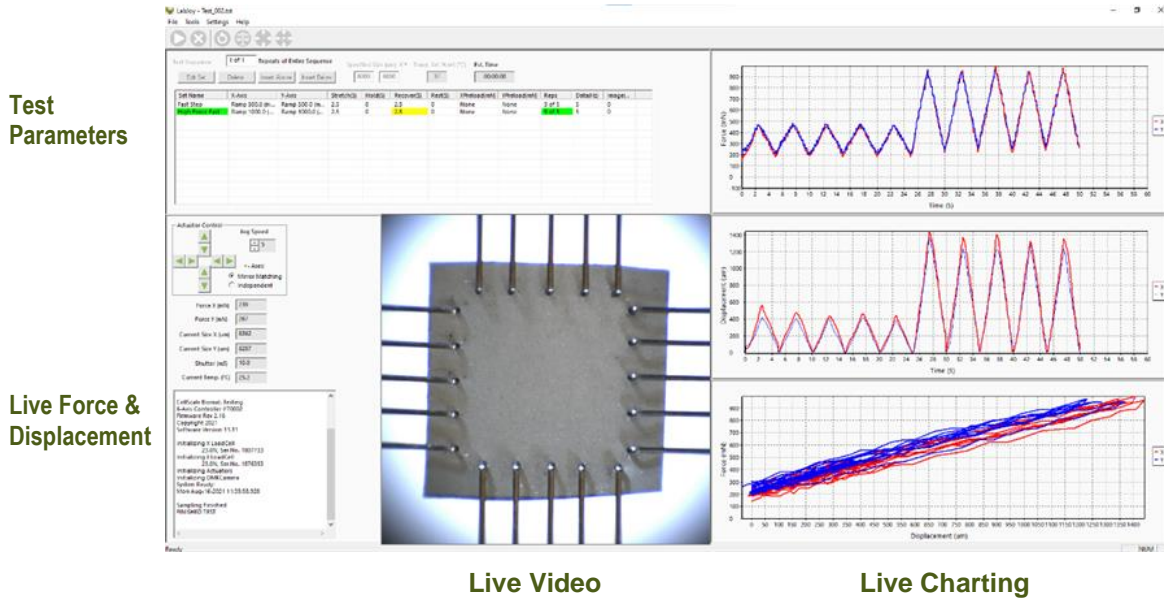
The BioTester 5000 brings together several high-precision components, making it the state-of-the-art instrument for testing biomaterials.



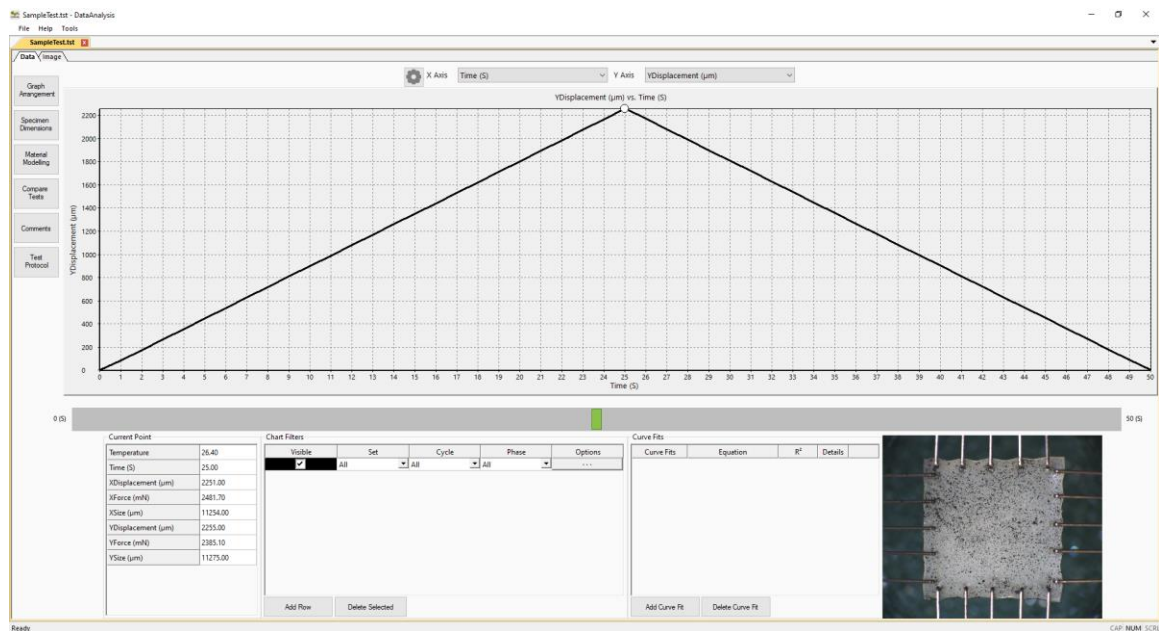
A	High-resolution CCD camera provides synchronized video tracking and analysis.
B	Illumination and lens provided to capture excellent image quality
C	Our patented BioRake attachment method provides quick and easy mounting of samples.
D	Integrated machine controls and data collection are compact and designed for testing flexibility.
E	High resolution control delivers precision measurement on small samples.
F	A simple USB interface for easy connection to the host computer.

## Software Overview

The software included with the BioTester 5000 is called LabJoy. 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:



The Data Analysis software is an additional program used to review and analyze data and images collected by the BioTester 5000. For more details, see the included Data Analysis user manual in the Documentation folder (*Cellscale>Labjoy>Documentation*).



## Output Files and Data Structures

For each test, the BioTester 5000 creates and saves three file types. The following table describes the three 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 Sample1.000010.3.jpg, which would correspond to an image captured at 10.3 seconds 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 dialogue portion of the main screen. It is useful as a reference to troubleshoot problems with your system, should they occur.

Additional “Analysis” and “Tracking” folders will be created if the data file and/or images are reviewed and processed in the Data Analysis software module. These folders will store tracking information and other preference settings. For more information, see the Data Analysis user manual in the Documentation folder.

When working with the data, you should be aware of a few details regarding the data output:

1. The output specimen sizes are based on the spacing between the BioRake tines and do not account for the specimen material outside of the test region.
2. The software cannot calculate stresses since the thickness of the specimen is not known. To calculate stress, you will have to manually measure the thickness of the material before or after the test is performed.
3. Strains can be calculated using the output displacement values (which are based on the BioRake tine motions). The specimen may actually be subjected to less strain than the calculated values due to attachment site effects and tissue tearing. The image tracking module is useful for determining the actual strain values and variations within the specimen.

## 4. Setting Up & Starting a Test

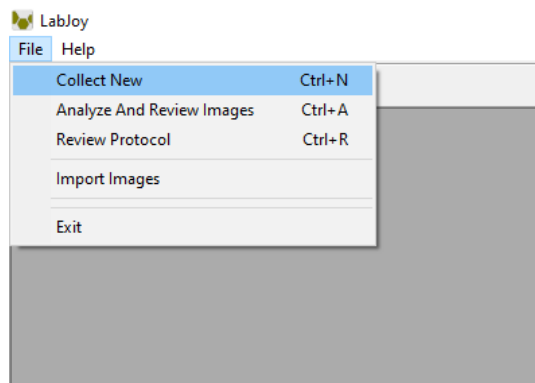
### Overview

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

- Step 1: Start a New Test
- Step 2: Reset the Actuators
- Step 3: Move the Actuators to a Specified Size
- Step 4: Zero the Load Cells (occasional)
- Step 5: Modify the Test Parameters (optional)
- Step 6: Mount the Specimen
- Step 7: Execute the Test
- Step 8: Terminate the Test Prematurely (optional)
- Step 9: End of Test Controls (optional)

### Step 1: Start a New Test

Launch the LabJoy software, and then select *Collect New* from the File menu. In the Create Test from Template dialogue, perform the following steps:



1. Select a template that matches the type of test you wish to perform. See the *BioTester 5000 Tip* on how to select and use a template. You can modify the template parameters in Step 4, below.
2. Name your test. The dialogue 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 'Create Test From Template' dialog box. It contains a 'Select' dropdown menu with 'demo' selected. Below it are three text input fields: 'Test Name' (containing 'Test\_001'), 'Data Directory' (containing 'C:\Cellscale\Labjoy\Data'), and 'Template Directory' (containing 'C:\Cellscale\Labjoy\Templates'). Each of the last two fields has a 'Browse' button next to it. At the bottom are 'OK' and 'Cancel' buttons.

**BioTester 5000 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.*

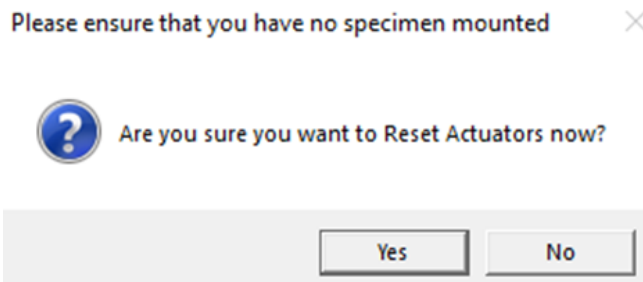


## Step 2: Reset the Actuators

We suggest performing an actuator reset at the start of a new test. Also, if you have stopped the previous test in mid cycle, then you should reset the actuators. By resetting the actuators, you are moving the actuators to the precise home position ensuring that the displacement measurements taken by the BioTester 5000 are accurate.

To reset the actuators, select *Reset Actuator* from the tools menu, or press  on the toolbar.

If the below warning dialogue appears, select *Yes*. This warning is to remind the user not to reset the actuator with a specimen mounted.




### System Alert

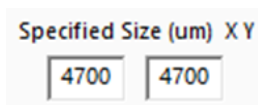
The actuators **SHOULD NOT** be reset if the specimen is already mounted. Doing so will damage your specimen as well as possibly damage the BioRakes or Load Cells.

## Step 3: Move the Actuators to a Specified Size

After the actuators have been reset, they will remain in their fully retracted positions and must be moved to specified (reference or starting) positions.


To move the actuators to the specified position, select *Move Actuators to Specified Size* from the

Tools menu, or press  on the toolbar. The desired size must be entered in the boxes labelled *Specified Size (um) X Y*.



Step 4: Zero the Load Cells (occasionally)

While it is not necessary to zero the load cells with every test sequence, we suggest zeroing the load cells at the start of a new test session. With repeated use, the zero point of the load cells can drift. By zeroing the load cells, you are ensuring that the force measurements are accurate.

To zero the load cells, select *Zero Load Cells* from the Tools menu or press  on the toolbar.

System Alert

*You should not zero the load cells if the specimen is already loaded, or the load cell zeroing process will not work properly and an offset will be introduced into your force data.*

Step 5: Modify Testing Parameters (optional)

You can select and modify parameter sets by clicking on their row in the Test Sequence table and then pressing the *Edit Set* button (or by double clicking on their row). When you do so, the Set Parameter Editor dialogue will appear.

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

The following table describes each of the parameters.

Set Parameter Editor

NamePrecon1(Example: 'Precon1', 'HoldA')Same As X

X Axis

Control ModeDisplacementControl FunctionRampStretch Magnitude10%PreloadNot AppliedApplied on 1st Repetition OnlyReApply On Every RepetitionPreload Magnitude (mN)100

Y Axis

Control ModeDisplacementControl FunctionRampStretch Magnitude10%PreloadNot AppliedApplied on 1st Repetition OnlyReApply On Every RepetitionPreload Magnitude (mN)100

Cycle

Stretch Duration (S)20Do Not StretchRepetitions3Hold Duration (S)0Data Output Frequency (Hz)1Recovery Duration (S)20Do Not RecoverImage Output Frequency (Hz)1Rest Duration0Edit Custom Load Function

OKCancel

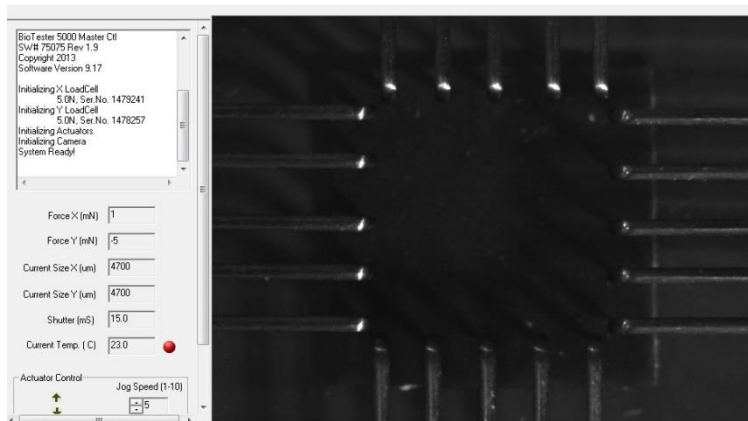
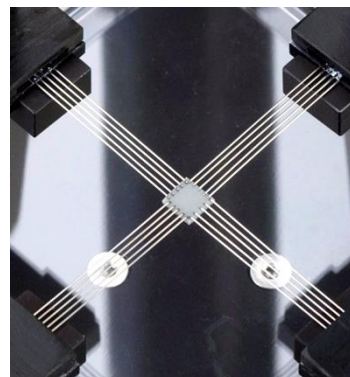
Test Parameter	Description
<b>Control Mode</b>	<p>Biaxial tests are typically performed under displacement control; however, you can select the following control modes to achieve specific testing objectives:</p> <ul style="list-style-type: none"> <li>To obtain uniaxial properties for a biaxially mounted specimen, specify displacement control in one axis and Force control with zero Load Magnitude in the other axis.</li> <li>For creep testing, use a preload with a long hold duration.</li> <li>For relaxation testing, use displacement control with a long hold duration.</li> </ul>
<b>Control Function</b>	<p>True Strain (Displacement Control only), Sinusoidal, or Ramp is typically selected for tests performed in displacement or force control mode. True Strain tests can only be run in displacement control mode.</p>
<b>Stretch Magnitude</b> (Load Magnitude when in Force Control mode)	<p>Selecting a stretch magnitude is entirely dependent on the material you are testing. A sound approach is to begin with a small magnitude and iteratively move up to larger magnitudes.</p> <p>If you are using Displacement control mode, you can specify the displacement in either <math>\mu\text{m}</math> or as a percentage. For example, the displacement on a <math>5000\mu\text{m} \times 5000\mu\text{m}</math> specimen can be expressed as either <math>500\mu\text{m}</math> or 10%.</p> <p>If you are using Force control mode, you can only specify the force in mN.</p> <p>Watch the test results carefully to determine which magnitude setting best achieves your test goals.</p>
<b>Preload</b>	<p>Preload is typically reapplied on every repetition during a preconditioning set as well as on the first repetition of a testing set. Specimen size is adjusted after a preload adjustment. Strain calculations are based on the specimen size after the last preload adjustment. If you are working with a material for which preload values have been suggested, you can set the value accordingly. Otherwise, zero is a good initial choice.</p>
<b>Preload Magnitude</b>	<p>As with stretch magnitude, the preload magnitude settings depend on the material you are testing. While Preload can be set at zero, typically you would set the preload magnitude somewhere between zero and 10% of the peak load you expect to achieve.</p>
<b>Stretch Duration</b>	<p>For evenly spaced images, it is recommended to choose a number that is an integer multiple of the Image Output Frequency.</p>
<b>Hold Duration</b>	<p>Hold Duration is typically set to 0, however it is useful for creep or relaxation testing.</p>
<b>Recovery Duration</b>	<p>Recovery Duration is typically set to the same value as the Stretch Duration.</p>
<b>Rest Duration</b>	<p>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.</p>
<b>Repetitions</b>	<p>Apply enough repetitions until the force deformation curves from one repetition to the next start to overlie each other.</p>
<b>Data Output Frequency</b>	<p>Typically set to the same frequency as the image output frequency.</p>
<b>Image Output Frequency</b>	<p>Typically set to 1Hz for cycles &gt; 5 seconds and 10 Hz for cycles &lt; 5 seconds. To capture images higher than 15Hz, the image resolution can be lowered to accommodate that.</p>

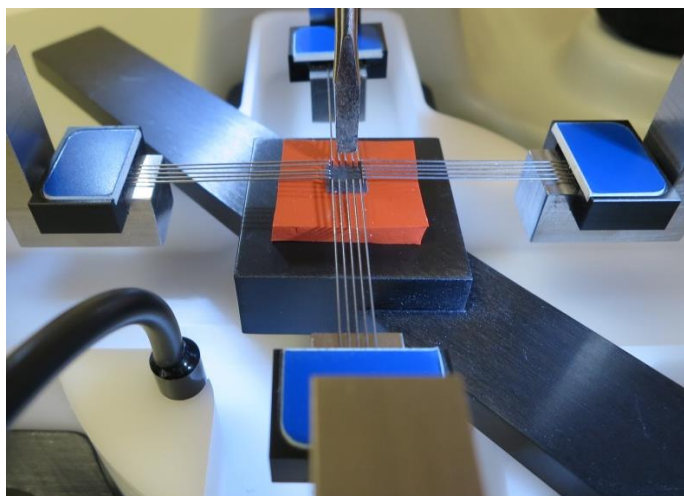
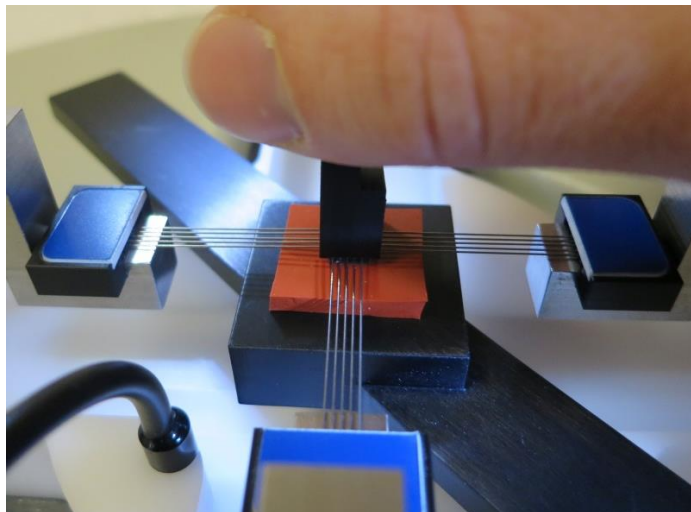
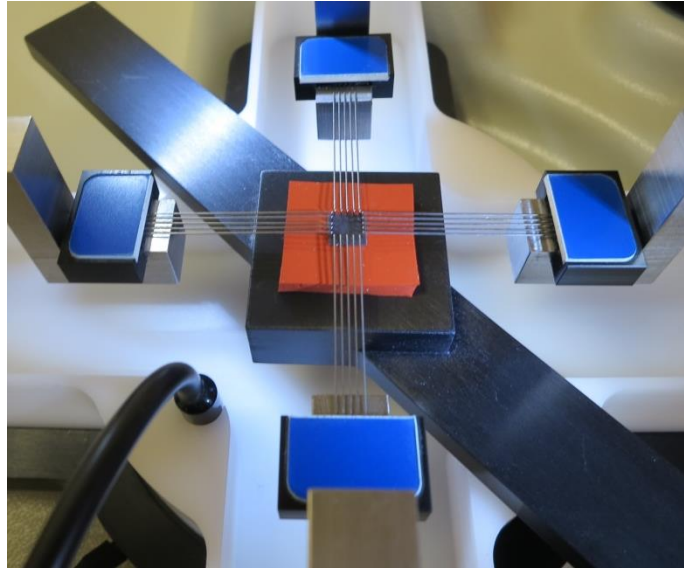
## Step 6: Mount the Specimen

Proper specimen mounting is critical to ensuring accurate and repeatable results. It is best to have the tines spaced evenly across the edge of the specimen and as closely to the edges as possible.

To mount the specimen, follow these steps:

1. Place the specimen in the mounting press on top of a backing material such as the supplied soft silicon or a piece of foam.
2. Place the mounting bridge across the fluid chamber.
3. Partially raise the mounting bridge.
4. Centre the specimen between the rakes using a small implement, such as forceps, and the live video window as feedback. Adjustment of the actuators may be necessary to ensure the rake tines are positioned correctly for non-ideally sized specimens.
5. Raise the stage until the rake tines are pressing gently on the specimen.
6. Push all 20 tines into the specimen, either all at once using the press block or one at a time using a small implement such as a precision screwdriver.
7. Partially lower the stage and remove the backing material.
8. Fully lower the stage and remove the mounting bridge.
9. Fully raise the stage to submerge the specimen, if desired. Typically, you will want to match a specimen's physiological state, which for most biological materials is in a saline solution heated to the organism's body temperature.
10. To minimize the effect of surface debris and surface ripples, a cut-out has been made on the top of the fluid chamber that is sized to hold a standard microscope slide.






**BioTester 5000 Tip: Ideal Specimen Size**

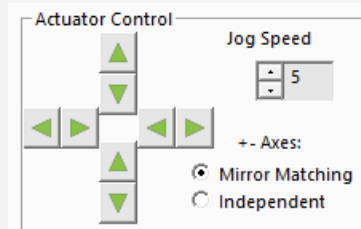
The BioTester 5000 is designed for small specimen sizes. For Standard BioRakes (5 tines spaced at 1 mm) the ideal specimen size is square with side lengths of 5.5 - 6 mm. If a specimen is not in the ideal range, it may still be mounted, but the placement of the rake tips will need to be adjusted. Also, BioRakes of different size are available and may be used.

Manual adjustment of rake positions during specimen loading may be necessary to ensure that the rakes puncture the sample correctly.


These manual adjustments result in a change in specimen size, which in turn results in a change in actuator displacement profiles. The specified specimen size is displayed at the top of the screen while the current specimen size is displayed on the left of the screen just below the force output.

At any point before the test has started, specimen size can be changed using one of the following methods:


- Press  on the tool bar, or from the Tools menu select Move Actuators to Specified Size
- Use the Actuator Control jog button arrows.



## Step 7: Execute the Test

Select *Execute* from the File menu or press the  button on the toolbar.

## Step 8: 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. This is preferred method as opposed to powering down the equipment, as it allows the software to store the current actuator position.

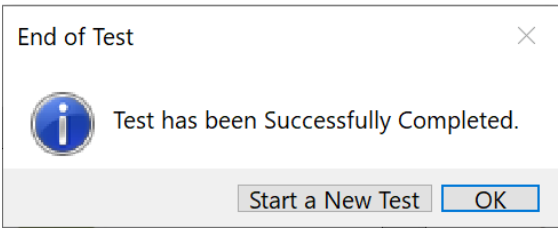
At the end of the completed test (both normally and prematurely terminated) the actuators will maintain their current position.

**BioTester 5000 Tip: Specimen Thickness**

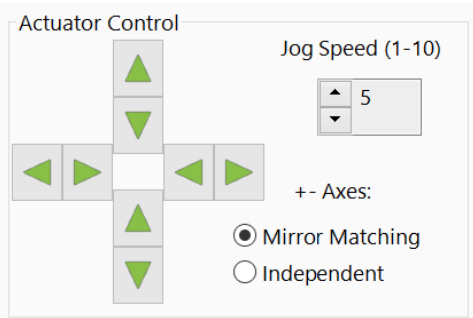
Measuring the specimen thickness before and/or after a test is performed will allow stress calculations to be made from your output data. You can input this data in the software under the Specimen Menu

**Step 9: End of Test Controls (optional)**

Once the test has been completed, you can choose to start a new test or use the system controls by selecting the “OK” button.



System controls include the top ribbon as well as Actuator Control. These controls can be useful if you need to release a specimen from the text fixtures at the end of a test.



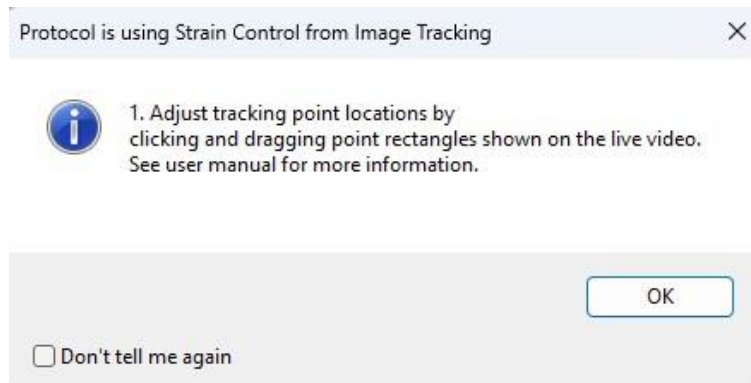
## 5. Strain Control from Image Tracking

Strain control from image tracking is an alternate test control mode that can specify and control the test displacements based on a region within a specimen rather than the global actuator displacements. When deforming a specimen, strain values often vary near the attachment sites compared to the middle region of a specimen. This is especially evident when using BioRakes which tend to deform more around the rake line puncture points compared to other parts of the specimen. Clamped specimens usually have lower strains around the clamps because the clamps prevent lateral deformations.

In biaxial testing, strains in the center of a specimen can be significantly less than the actuator strains and local strains in the X and Y directions are often different from each other.

Controlling the applied strain in a region of a specimen is achieved by tracking image features in real time during test execution.

When a template with Strain Control from Image Tracking protocols is opened, the pop-up window shown below will appear to indicate so.





## Specify Testing Parameters

To use strain control:

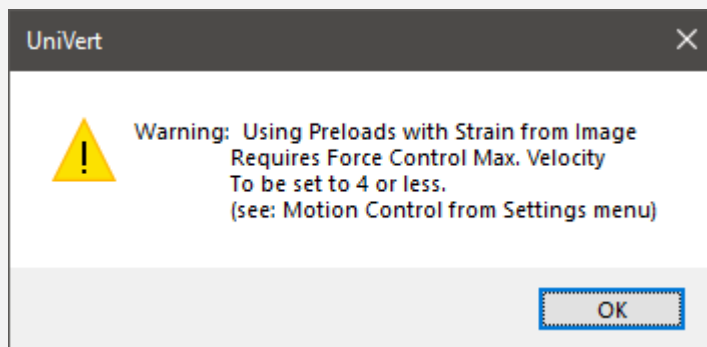
1. Double click or select Edit on the desired protocol line to open Set Parameter Editor
2. Select *Strain From Image* as the Control Mode. This mode will only be available if a scientific camera set up is connected.
3. Select Sinusoid or Ramp control function.
4. Magnitude is in % and is calculated as the change in length divided by the initial length at the start of the stretch phase. The initial length is updated after preloads.

The 'Set Parameter Editor' dialog box is shown with the 'Name' field set to 'Precon1'. The 'X Axis' and 'Y Axis' sections are both configured with 'Strain From Image' as the Control Mode (highlighted with a red circle), 'Sinusoid' as the Control Function, and a Magnitude of 20%. The Preload section for both axes has 'Not Applied' selected. The Cycle section shows Stretch Duration (S) as 10, Hold Duration (S) as 0, Recovery Duration (S) as 10, and Rest Duration as 0. Repetitions are set to 1, Data Output Frequency (Hz) to 10, and Image Output Frequency (Hz) to 1. The 'Do Not Stretch' and 'Do Not Recover' checkboxes are unchecked. The 'OK' and 'Cancel' buttons are at the bottom.

### System Alert: Preload with Strain from Images

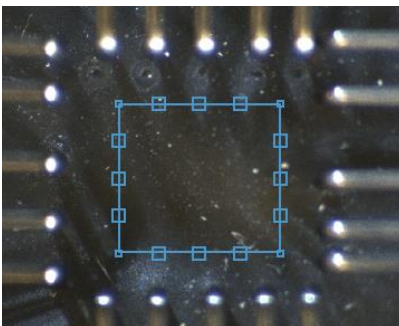
*When using preloads with the strain from images control mode, the force control velocity parameter must be set to a value of 4 or lower. Higher velocity settings may result in failure to execute the preload due to the image tracking algorithm speeds.*

*If the force control velocity setting is greater than 4, it will automatically be reduced to 4 upon executing the test to ensure successful completion of the preloads and test protocol.*



## Selecting the Tracking Point Locations

A test protocol that is using strain control with image tracking will display a rectangle in the live video feed as follows:



The small squares are the points that will be tracked. The location of the tracking points can be adjusted by clicking and dragging the corners of the box or the center tracking points on each side.

The average distance between all pairs of points is used for strain calculations. Tracking is performed using a digital image cross-correlation technique with updates at 20 Hz. For strain control using image tracking to be successful, there must be sufficient texture patterns or fiducial markers present in the images. Texture patterns can be added to specimens with marking pens or a light dusting of graphite or other contrasting powders on the specimen.

The colour of the tracking box and points can be adjusted in the Strain Control from Image Tracking Settings (see below).

## Configuring the Live Charting Graphs

The strains can be displayed on the live charting graphs when using the strain control from image tracking feature. The graphs can display measured strain or nominal strain.

- Measured Strain is the calculated strain from the image tracking.
- Nominal Strain is calculated from the motion of the actuators.

The graph settings can be specified by selecting *Graphs* from the Settings menu.

Graph Settings

Graph 1

XAxis

Time (S) Auto Min 0 Max 48

YAxis

Force (N) Auto Min 0 Max 1

☐ Clear Graph Between Sets

Graph 2

XAxis

Time (S) Auto Min 0 Max 48

YAxis

Measured Strain (%) Auto Min 0 Max 0.1

☐ Clear Graph Between Sets

Graph 3

XAxis

Time (S) Auto Min 0 Max 48

YAxis

Nominal Strain (%) Auto Min 0 Max 1

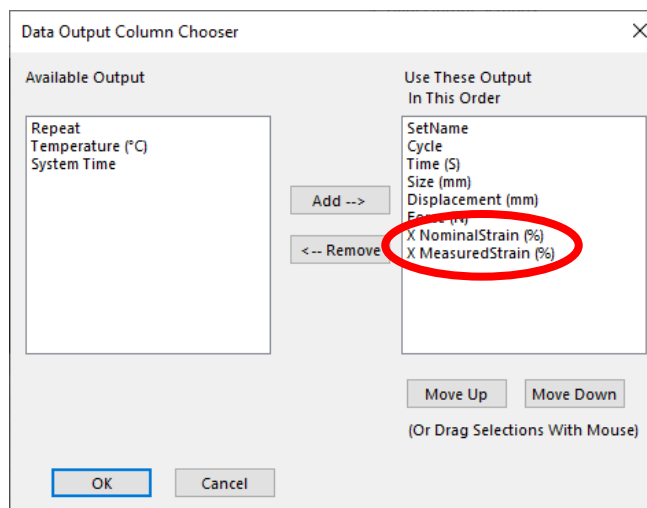
☐ Clear Graph Between Sets

☐ Clear Graph Between Cycles

OK Cancel

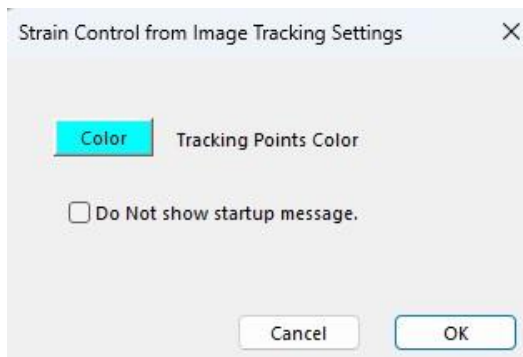
## Configuring Output Data Files

Measured and Nominal strains are available as test output columns for the csv data file. From the Settings menu, select *Data Output*. Then click the *Configure* button to select the desired output columns.



## Strain Control from Image Tracking Settings

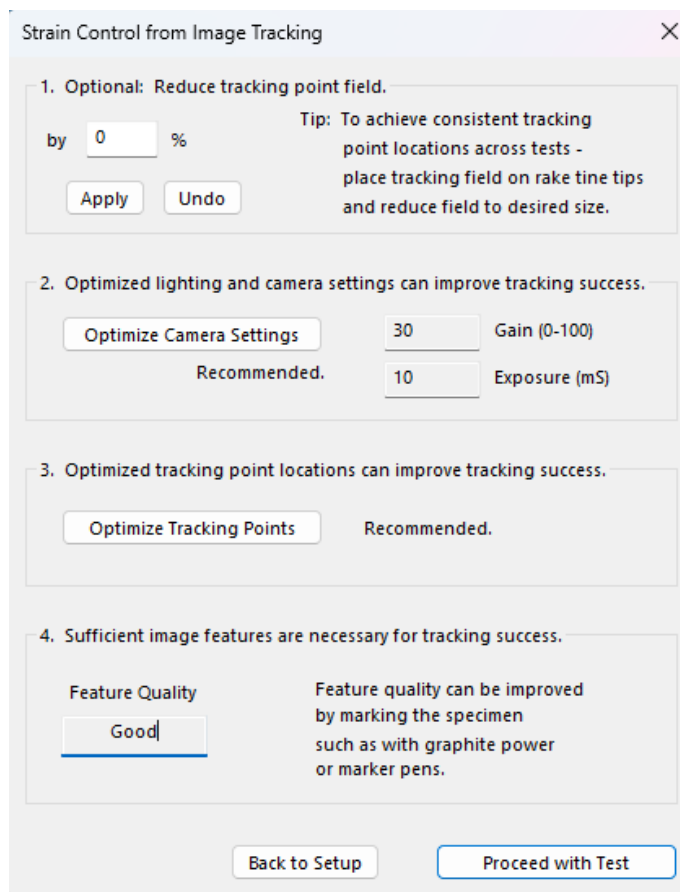
To access the settings for Strain Control from Image Tracking, select *Settings* in the top ribbon and *Strain from Image*. The colour of the tracking points can be modified as well as disabling/enabling the pop-up window at the beginning of each test.



## Executing the Test

Upon executing a test, the dialog box shown below will appear. It gives options to automatically adjust camera settings and to automatically adjust tracking point locations. Optimized lighting and camera settings and adjusted tracking points locations may improve the success of tracking.

Click proceed with Test to execute the test. If the software is unable to continuously track a point, the test will automatically stop to prevent any damage to the system. If this occurs, more textured patterns or unique features may be required to generate better tracking points for the software to follow.



The dialog box, titled "Strain Control from Image Tracking", contains four numbered sections for optimizing tracking performance. Section 1 allows reducing the tracking point field by a percentage, with a tip to place the field on rake tine tips. Section 2 offers to optimize camera settings, showing current values for Gain (30) and Exposure (10 ms) against recommended values. Section 3 offers to optimize tracking points, with a recommended status. Section 4 shows the current feature quality as "Good" and provides a tip on improving it with markers. At the bottom are "Back to Setup" and "Proceed with Test" buttons.

**Strain Control from Image Tracking**

1. Optional: Reduce tracking point field.

by  %

Tip: To achieve consistent tracking point locations across tests - place tracking field on rake tine tips and reduce field to desired size.

Apply Undo

2. Optimized lighting and camera settings can improve tracking success.

Optimize Camera Settings

Recommended.

Gain (0-100)

Exposure (mS)

3. Optimized tracking point locations can improve tracking success.

Optimize Tracking Points

Recommended.

4. Sufficient image features are necessary for tracking success.

Feature Quality

Good

Feature quality can be improved by marking the specimen such as with graphite power or marker pens.

Back to Setup Proceed with Test

## Advanced Controls

Strain control from image tracking is controlled with a PID control algorithm. The PID control parameters can be adjusted by selecting *Motion Control* from the *Settings* menu in the top ribbon.

Check the *Edit Advanced Settings* check box then select *Strain from Image* in the control mode drop down menu.

Increase the Kp value if the system is not responsive enough or cannot achieve the specified strains.

Decrease the Kp value if the system is producing unstable or oscillating results.

The hardware defaults should work under most conditions and can be restored with the *Reset to Hardware Defaults* button.

Motion Control Settings

X Axis

Max. Velocity (1-10) (Force Control): 8

Acceleration (1-10): 10

Y Axis

Max. Velocity (1-10) (Force Control): 8

Acceleration (1-10): 10

Note: To Save Velocity and Acceleration settings to a Template  
Select Save As Template from the File Menu.

☒ Edit Advanced Settings

Reset to Hardware Defaults

Control Mode: Strain From Image

Algorithm: ☐ Adaptive ☒ PID

Kp: 200

Ki: 0

Kd: 0

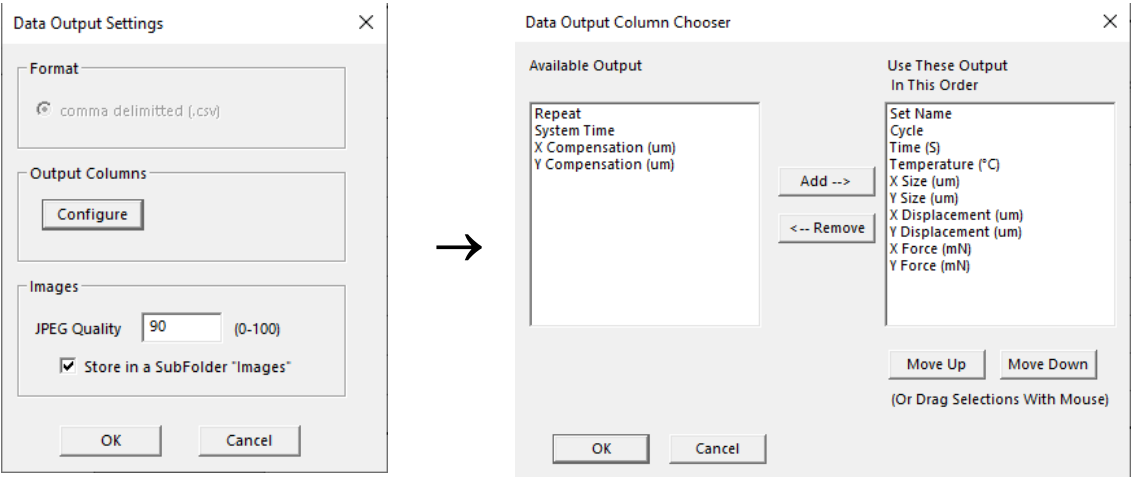
Note: Improperly tuned PID settings may result in unstable behavior.

Apply Done

## 6. Additional Settings

### Configuring Output Data Files

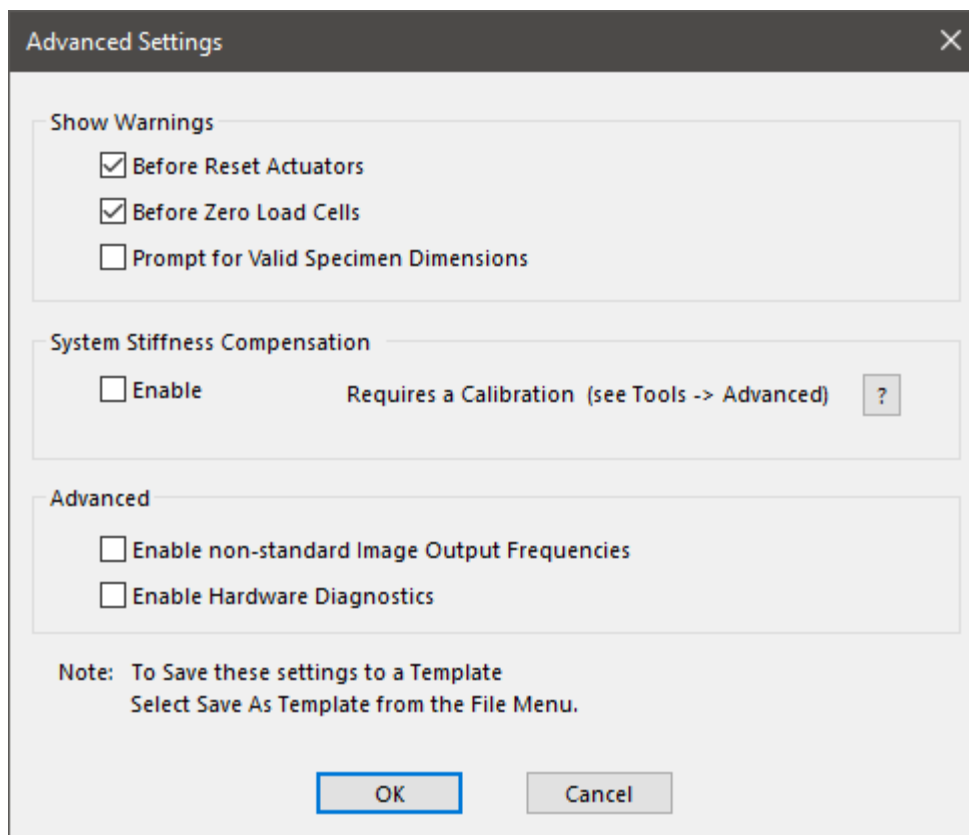
From the Settings menu, select *Data Output* to display the dialogue shown on the left. Click the *Configure* button to select which of the following columns to output.



Output Column	Description
Set Name	User defined name of each set
Cycle	Cycle and Phase information
Time (S)	Time in seconds
X/Y Size (µm)	Distance between opposite pairs of BioRakes
X/Y Force (mN)	Load Cell reading
X/Y Displacement	Reports the change in size relative to the initial size
Set Name	Name specified for each set
Temperature (°C)	Current temperature

## Advanced Settings

Advanced settings are available by selecting *Advanced* from the Settings menu. All of these settings are template specific unless otherwise noted.



### Show Warnings

By selecting the check boxes, a warning dialogue will appear before either action button (i.e. Reset Actuators or Zero Load Cells).

Resetting the actuators with a sample attached may damage the specimen, BioRakes, and/or load cells. To avoid resetting the actuators with a sample attached, a warning dialogue will appear after “Reset Actuators” is selected, prompting the user to proceed.

### System Stiffness Compensation

System Stiffness Compensation can be used to compensate for the small displacements that occur in the system during testing due to flexion and load cell compression/tension. These displacements arise from any strains in the load cell and any bending or deformation of the system during loading. Under most loading conditions of the BioTester5000, these displacements are negligible and system stiffness compensation is not required. It is recommended to leave the System Stiffness Compensation as Enable>Auto.

## Advanced

Checking the “Enable non-standard Image Output Frequencies” will enable Image Output Frequencies options between 1 and 20 in the Parameter Editor. It is important to note, the scientific camera will not be running at those frame rates natively. The camera will operate at its highest frame rate (45 fps) and the nearest frame to the selected frequency interval will be selected. This could result in an output image frame that does not exactly match the desired frequency output. This setting can be saved to a template.

Checking the Enable Hardware Diagnostics will enable an LED light on the main controller circuit board to flash continuously. By default, the LED is not enabled as it can cause discernable noise on load cell signal communications within the circuit board. This checkbox should only be used for troubleshooting purposes only. **This setting will not be saved to a template and must be checked for each test if required.**



## Motion Control Settings

Motion Control Settings affect the rate and stability at which forces are achieved in force-controlled procedures. These settings apply to preloads as well as Force Control Modes. Changes to these settings are saved to the test template.

### Max. Velocity

Maximum velocity setting can be set to an integer value between 1 and 10 (see chart below for corresponding velocity values). The velocity integer matches actuator jog speed on the BioTester software home screen. Typically for stiffer or smaller specimens (i.e. requires a short displacement to achieve desired force), use a lower velocity setting as this will allow for smoother force-controlled protocols. It may take a few trials to optimize your test protocols and settings for your experiment.

Velocity Jog Integer	um/s
1	25
2	50
3	100
4	200
5	400
6	800
7	1575
8	3125
9	3250
10	12500

## Acceleration

Acceleration setting can be set as an integer value between 1 and 10 (see chart below for corresponding acceleration values). Changes made to the acceleration will affect tests under both force and displacement control. Reducing this value may help smooth force-controlled tests under low loads or displacement-controlled tests at high speeds. Tests run with lower accelerations will have softer transitions at direction or speed changes but may take longer than specified to achieve specified testing cycles.

Acceleration value	um/s/s
1	3200
2	6400
3	12800
4	25600
5	51200
6	102400
7	204800
8	409600
9	819200
10	1638400

Control Algorithm Settings Editing (Advanced)

This setting offers the option to change from the programmed adaptive control algorithm to a custom algorithm. We recommend using the adaptive algorithm that has already been optimized for the device, as improperly tuned PID settings may result in unstable behavior. We suggest only advanced users with specialized tests modify these PID settings. Contact CellScale for more information regarding these settings.

For adaptive control, an initial gain value (K0) can be modified. This value must be greater than zero, and it is set to 1 by default. The force control may undershoot or overshoot its target when testing very soft or stiff specimens, respectively. The K0 value can be reduced to adjust for overshooting and increased to adjust for undershoot.

Motion Control Settings

Max. Velocity (1-10)  
(Force Control)

8

Acceleration (1-10)

10

X Axis

Y Axis

Note: To Save Velocity and Acceleration settings to a Template  
Select Save As Template from the File Menu.

☒ Edit Advanced Settings

Reset to Hardware Defaults

Control Mode

Force

Force

Algorithm

☒ Adaptive

☐ PID

☒ Adaptive

☐ PID

K0

1

1

Kp

5

5

Ki

0

0

Kd

0

0


Notes: Improperly tuned PID settings may result in unstable behavior.

Apply

Done

BioTester Tip: Testing Force Control Settings

It is important to have appropriate Force Control Settings that match the material you are testing.

To safely test Force Control Settings: press  on the tool bar, or from the Tools menu select Move Actuators to Specified Load. The dialogue shown on the right will appear. The BioTester will attempt to achieve the specified loads (forces) but will stop when either the specified loads are reached, the actuators have displaced by the maximum specified amount, or the timeout time has elapsed.

Move to Specified Load

X Direction

Specified Load (mN)

0

☒ Apply

Maximum Displacement

1000

(um)

Y Direction

Specified Load (mN)

0

☒ Apply

Maximum Displacement

1000

(um)

Timeout (S)

10

Move to Specified Load will stop when  
The specified load is reached  
Or the actuators have displaced (moved)  
by the maximum specified amount  
Or the Timeout time has elapsed

OK

Cancel

## Range Limits

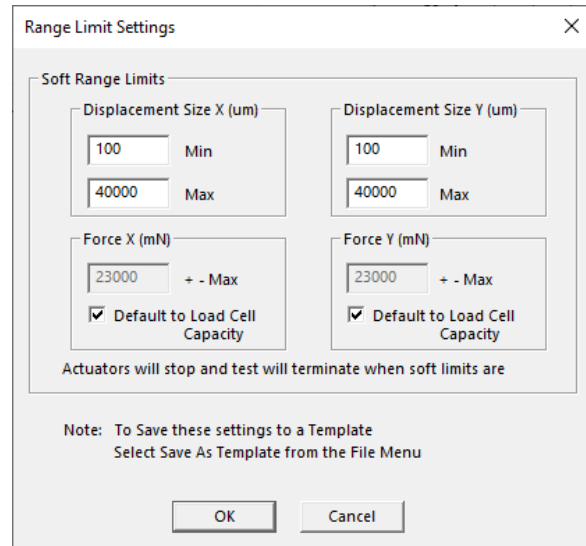
Range limits can be accessed by selecting *Range Limits* from the Settings menu.

### Soft Limits

To avoid rake collisions or sample destruction, it is useful to assign reasonable travel limits to the actuators. This is especially important during force control or preloading, where if specified loads cannot be reached, the actuators will keep moving until they reach the soft limits.

**Min and Max Displacement**, in  $\mu\text{m}$ , set the minimum and maximum positions to which the rakes are allowed to move.

**Max Force**, in mN, sets the maximum value of force at which a test will be automatically stopped. The default value is set to the capacity of the load cells. Setting this value may be useful to prevent sample destruction or overloading of the load cells.



The 'Range Limit Settings' dialog box contains the following elements:

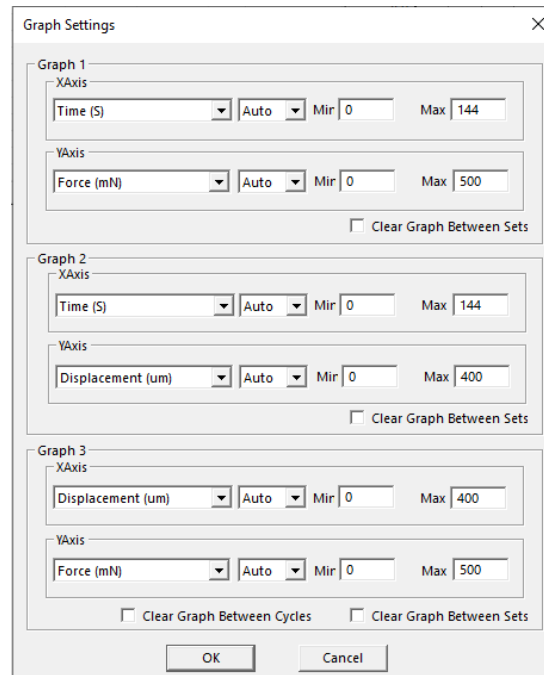
- Soft Range Limits** section:
  - Displacement Size X (um)**: Min 100, Max 40000.
  - Displacement Size Y (um)**: Min 100, Max 40000.
  - Force X (mN)**: 23000, +/- Max. Includes a checked checkbox for 'Default to Load Cell Capacity'.
  - Force Y (mN)**: 23000, +/- Max. Includes a checked checkbox for 'Default to Load Cell Capacity'.
- Text: 'Actuators will stop and test will terminate when soft limits are'.
- Note**: 'To Save these settings to a Template Select Save As Template from the File Menu'.
- Buttons: 'OK' and 'Cancel'.

## Configuring the Live Charting 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.

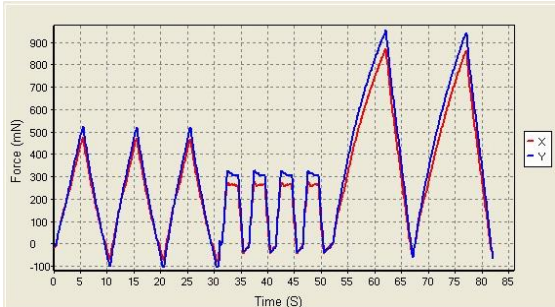
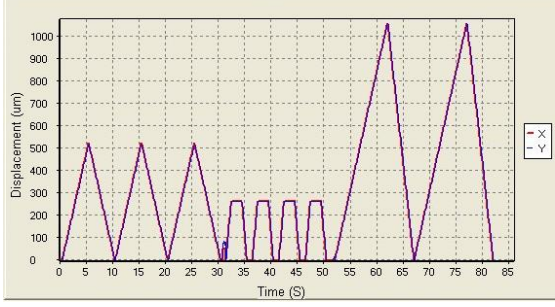
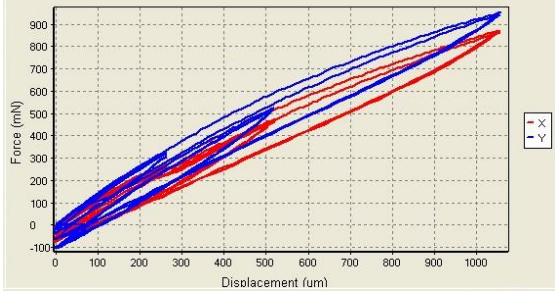
The graphs are updated at a maximum frequency of 10Hz; certain short-duration tests may not show all the detail that is actually present.

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 'Graph Settings' dialog box contains the following elements:

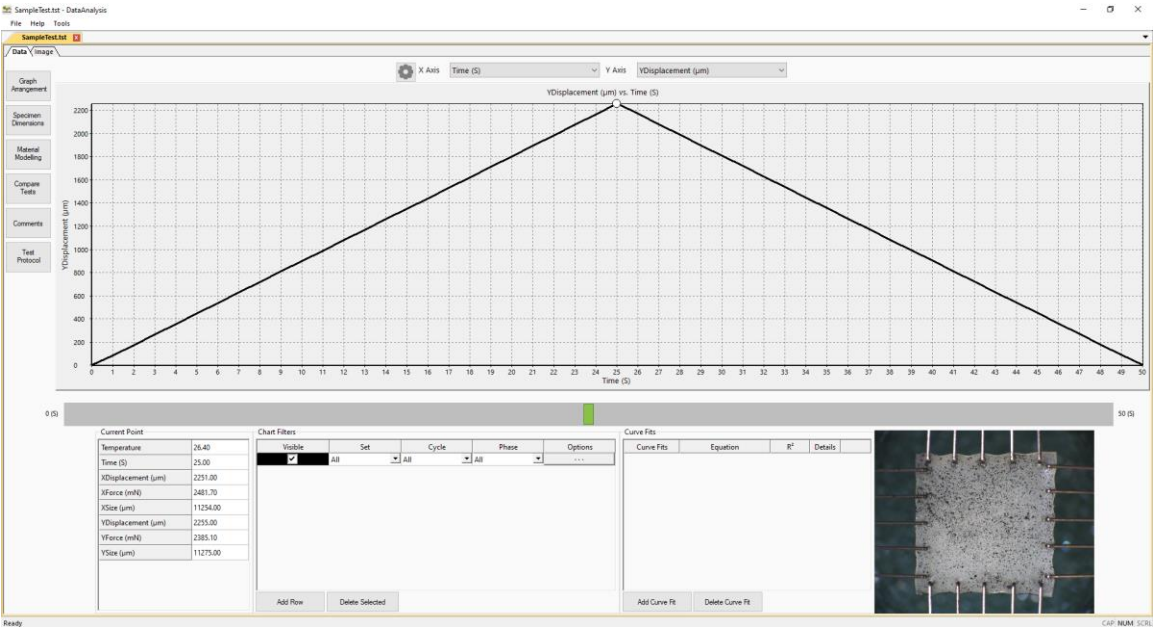
- Graph 1**:
  - XAxis**: Time (S), Auto, Min 0, Max 144.
  - YAxis**: Force (mN), Auto, Min 0, Max 500.
  - Checkbox: 'Clear Graph Between Sets'.
- Graph 2**:
  - XAxis**: Time (S), Auto, Min 0, Max 144.
  - YAxis**: Displacement (um), Auto, Min 0, Max 400.
  - Checkbox: 'Clear Graph Between Sets'.
- Graph 3**:
  - XAxis**: Displacement (um), Auto, Min 0, Max 400.
  - YAxis**: Force (mN), Auto, Min 0, Max 500.
  - Checkboxes: 'Clear Graph Between Cycles' and 'Clear Graph Between Sets'.
- Buttons: 'OK' and 'Cancel'.

Graph	Data
<p><b>Force versus Time</b></p> <p>This graph shows how the measured X and Y forces are changing with time. Peak loads per cycle, differences in load between the X and Y axes, and force relaxation are all easily seen in this type of graph. Force is proportional to nominal (engineering) stress.</p>	
<p><b>Displacement versus Time</b></p> <p>This graph shows how the rakes are moving with time. The phases of the test sequence and size adjustments due to preloading are readily apparent in this type of graph. Displacement is proportional to nominal (engineering) strain.</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>	

## 7. Reviewing Test Results

The BioTester 5000 includes an additional software, Data Analysis, to review and analyze data collected from the device. This includes graphing, curve fitting, material modeling and image processing to determine detailed strain maps.

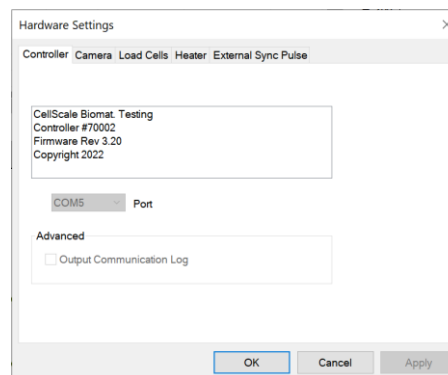
To open the Data Analysis program, select *File>Analyze and Review Images* from the Labjoy software home page. More details about Data Analysis can be found in its user manual located in the Documentation folder (*Cellscale>Labjoy>Documentation*).



## 8. System Hardware Settings

### Controller

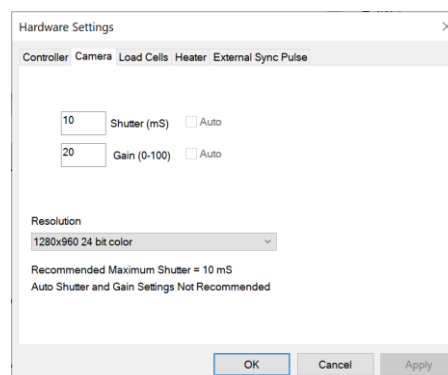
The BioTester 5000 uses an integrated control board rather than a group of PC controlled device drivers. This minimizes communication lag and allows the device to operate asynchronously with the PC. The control board details can be seen by selecting *Hardware* from the Settings menu, which brings up the window shown to the right.



### Camera

The camera uses a 2/3" monochrome CMOS sensor to obtain a resolution of 2048 pixels by 2048 pixels. The camera shutter and gain can be controlled during the setup phase of the test.

The camera captures images at 15 frames per second during every test and writes these images to a buffer. The test controller extracts images from this buffer at the frequency specified in the Set Parameter Editor (50Hz, 25Hz, 15Hz, 5Hz, 1Hz, 0.1Hz, 0.01Hz, or 0.001Hz). These images are written to the output directory. The file name is the ideal capture time. The actual capture time may differ from the ideal capture time by as much as 1/30 of a second since the images are extracted from a buffer.



The test software creates an image capture log file in the output directory should this difference be significant. This log stores the actual capture time and the idealized capture time.

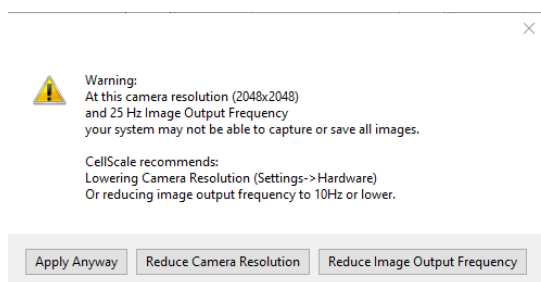
The camera default shutter and gain are specific to each test template and can be set by selecting the *Camera* tab in *Hardware* from the *Settings* menu. The recommended maximum shutter speed is 10mS. Typically, the camera iris is left fully open and the shutter and gain are adjusted to achieve the desired image brightness.

## Changing Camera Image Resolution

The default resolution of the camera is 2048x2048, the current image resolution is written at the bottom of the screen.

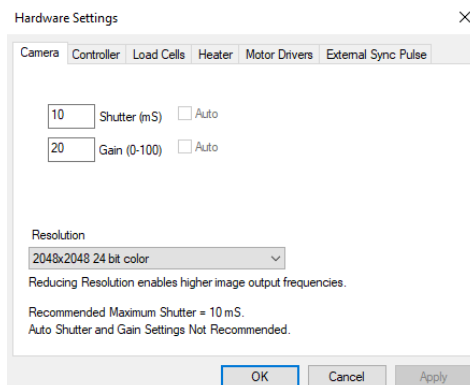


If you would like to capture images at a higher frequency (25Hz or 50Hz) you can reduce this resolution to 1024x1024. When you select the higher frequency, you will get the below prompt where you can reduce the image output frequency, Reduce Camera Resolution or for 25Hz you can leave it the same but note that images may be missed on long tests or if your computer cannot keep up.



Selecting “Reduce Image Output Frequency” will return you to the Set Editor, selecting “Reduce Camera Resolution” will bring you to the hardware settings window for the camera where you can change the resolution.

To change this setting, you can access the camera settings from the Settings-> Hardware from the top menu.





## Load Cells

The load cells are semiconductor strain gage-based with mechanical overload protection (for the lower capacity load cells). They have an accuracy equal to 0.2% of the rated full scale load. The load cells are available in 0.5N, 1.5N, 2.5N, 5N, 10N and 23N sizes. Each load cell has a unique calibration factor which is stored in a chip attached to the load cell connector.

While the load cells should not have significant hysteresis or thermal drift, but temperature changes or prolonged loading can introduce small offsets. It is recommended that the zero load state be redefined periodically. This can be done by selecting *Zero Load*

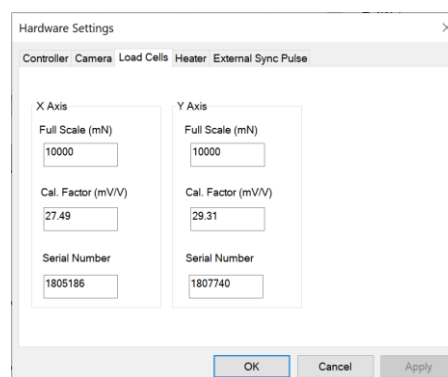
*Cells* from the Tools menu or pressing .

The load cells are sampled at 100Hz, with hardware sample averaging to reduce noise in the data. Further sample averaging can be implemented by selecting *Advanced* from the Settings menu.

Each load cell is calibrated and has a specific calibration factor which is stored on a memory chip in the load cell connector. The full-scale capacity, calibration factor, and serial number can be viewed by selecting *Hardware* from the *Settings* menu. This window only displays the values stored on the load cell's memory chip and cannot be modified.

In addition, it is recommended to run a calibration check on the load cells during the initial set up of the system or when load cells are changed. See the **System Calibration and Advanced Tools** section of this manual for this procedure.

Note: The Diagnostics button is only intended to be used for troubleshooting with CellScale's Technical Support Team. If you suspect your load cell may be damaged, contact [support@cellsacle.com](mailto:support@cellsacle.com).



Hardware Settings	
Controller Camera Load Cells Heater External Sync Pulse	
<b>X Axis</b>	<b>Y Axis</b>
Full Scale (mN)	Full Scale (mN)
10000	10000
Cal. Factor (mV/V)	Cal. Factor (mV/V)
27.49	29.31
Serial Number	Serial Number
1805186	1807740
<input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="Apply"/>	

### System Alert

*Though the load cells have some mechanical overload protection, they are sensitive to **excessive torque, excessive overload, transverse loading, and bending forces**. Such forces may **permanently damage the load cells**.*

*Care should be taken to apply force to the goosenecks only in the linear direction within the rated load of the load cells being used.*

**DO NOT overtighten the load cell stud.**

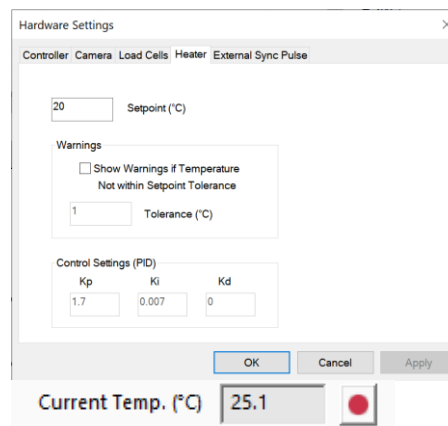
## Heater

To activate the fluid chamber heater, both power switches on the front of the BioTester 5000 need to be in the On position (the computer and software do not need to be on yet). 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 Hardware from the Settings menu.

Typically, the heater will take 20 to 40 minutes to warm a full fluid chamber from room temperature to 37°C and will take up to 90 minutes to stabilize at this temperature.

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 dialogue will appear when you execute the test.

The Control Settings (PID) section displays the programmed settings for the temperature control of the heated bath. These have already been optimized for the BioTester heated bath. This section only displays the setting values (i.e. cannot be edited) to ensure the correct values have been programmed.



### System Alert

*In the event of a dislodged or faulty temperature sensor, or if there is a lack of fluid in the fluid chamber, the system is designed to reach a maximum temperature of 80°C.*



**Caution:** Avoid touching the specimen stage when the heater is on, it can become very hot!

### BioTester 5000 Tip: Preheat Fluid

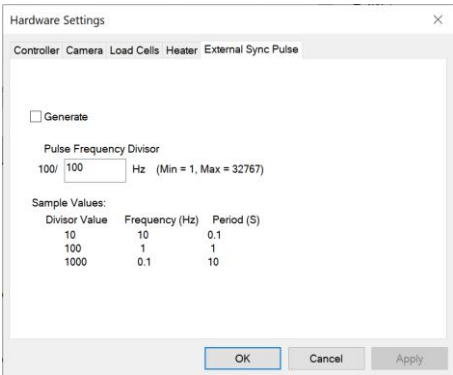
*You can add preheated fluid to the fluid chamber to reduce warm up time.*

**External Sync Pulse**

A 5-volt TTL external sync pulse signal can be generated on BioTester 5000 models containing a BNC connector jack on the back panel.

The sync pulse will be pulsed on the very first sample pulse, which occurs when the load cells are measured and the motor positions of all 4 axes are read. Thereafter a sync pulse will occur at a user definable frequency as fast as 100 Hz (1 pulse every 10 mS) and as slow as 0.00305 Hz (1 pulse every 327 s). The sync pulse will stop when the sampling has been stopped.

The sync pulse is active high for 10 mS except when operating at maximum speed (100 Hz) where the active high pulse duration is about 550 nS.



## 9. System Calibration and Advanced Tools

### Load Cell Calibration Check

If the load cells are changed, moved, or impacted, it is good practice to perform a load cell calibration check.

The calibration check procedure is as follows:

1. Launch the calibration utility by selecting Tools, Advanced, then *Load Cell Calibration*

**Load Cell Calibration**

**X Axis**

1. Enter Spring Calibration Value

X Load Cell (N)

Spring K (N/mm)

Calibration Preload (mN)

2. Move Actuators to Calibration Position

a. Remove Rakes

b. Insert Spring Calibration Brackets

c.

d. Attach Calibration Spring (Jog Actuators if necessary)

e. Apply Calibration Preload

3. Calibrate

	Calculated	Factory
<input type="button" value="Run"/>	<input type="text" value="0"/>	<input type="text" value="23.8104"/>

**Y Axis**

1. Enter Spring Calibration Value

Y Load Cell (N)

Spring K (N/mm)

Calibration Preload (mN)

2. Move Actuators to Calibration Position

a. Remove Rakes

b. Insert Spring Calibration Brackets

c.

d. Attach Calibration Spring (Jog Actuators if necessary)

e. Apply Calibration Preload

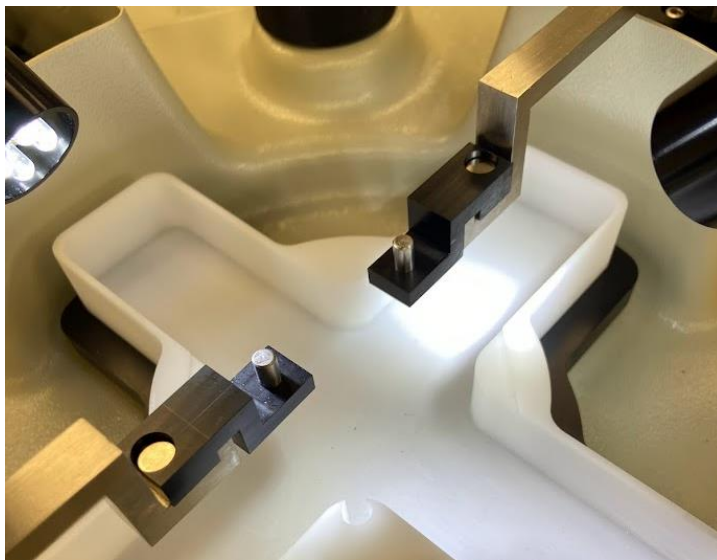
3. Calibrate

	Calculated	Factory
<input type="button" value="Run"/>	<input type="text" value="0"/>	<input type="text" value="58.1612"/>

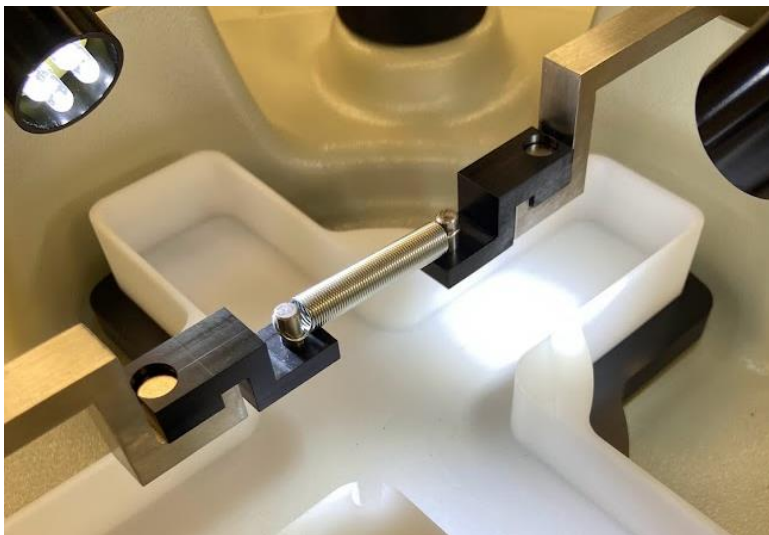
Note: Soft Range Limits for Force are template specific  
 You may wish to adjust Force Soft Limits when changing load cells  
 Soft Range Limits can be set by selecting Range Limits from the Settings menu

2. Input the K value (spring constant) for the calibration spring provided for the installed load cell. This value is shown on the spring case label (provided upon system delivery). If you have multiple load cells, ensure you are using the correct spring for the capacity of the installed load cell.
3. The load cell capacity should be automatically read and displayed, and the appropriate calibration preload calculated.

4. Lower the fluid chamber and install the calibration spring brackets on the goosenecks.

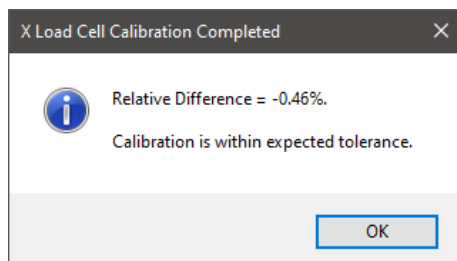


5. Zero the load cell using the *Zero Load Cells* button.
6. Using the *Jog+ and Jog-* buttons, drive the actuators until the posts are correctly spaced so that the calibration spring can be installed without applying any significant load. Set the calibration spring on the posts. The spring should be set level and in line with the goosenecks.

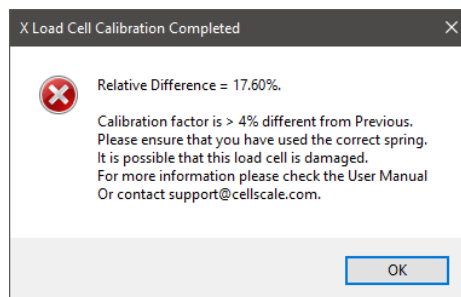


7. Once the spring is installed, click the *Move to Preload* button. The actuators will automatically move to the calibration preload for that load cell. For most setups, the spring should be driven to 30% of the load cell rating i.e. for a 2.5N load cell, a preload of 750mN is specified. However, for the 0.5N load cell only, a preload of 60% of the load cell rating is required (300mN).
8. Visually verify that the spring is loaded in a stable, horizontal, and in-line position.

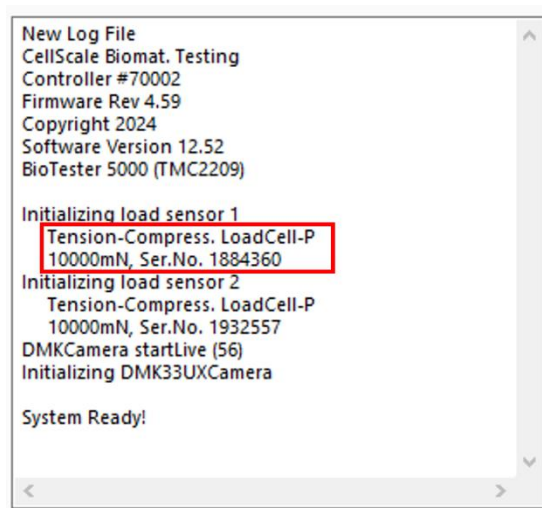
9. Click the *Run* button, wait for calibration to complete.
10. Once the calibration procedure is complete, a dialog window will appear displaying the relative % difference. This value reflected the difference between the factory calibrated factor and the calculated factor from the calibration procedure.



- a. If the relative difference is greater than 4%, this could indicate damage to the load cell. Click OK and repeat the calibration procedure. Check that the correct calibration spring is loaded, and the correct calibration preload is applied. If you suspect your load cell may be damaged or is reading improper values, contact our Support team ([support@cellscale.com](mailto:support@cellscale.com)).



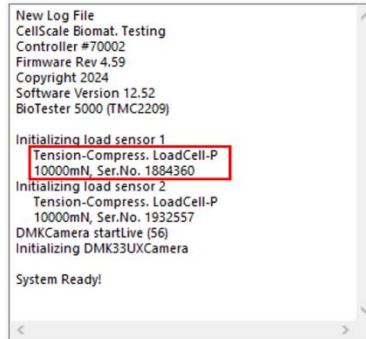
- b. If you have a legacy load cell (labelled Type P in the log window), follow the instructions in the next section.



11. The load cell calibration procedure must be carried out for both load cells (X and Y axis).

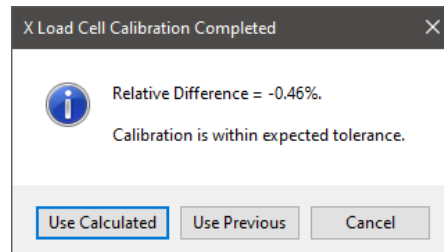
## Legacy Load Cell Calibration (Type P)

Load cells marked as Type P were factory calibrated with a different procedure. These types of load cells **require** calibration if they are changed, moved, or impacted. The load cell information and type is displayed in the log window in the bottom left corner of the Labjoy software.

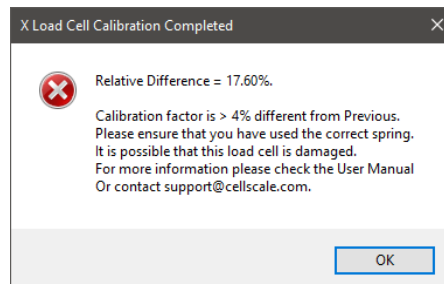


These load cells are calibrated with the same procedure outlined above. Complete Steps 1-9, and follow the below steps:

1. Once the calibration procedure is completed, a dialog window will appear displaying the relative % difference. This value reflected the difference between the previous calibrated factor and the calculated factor from the calibration procedure.
  - a. If the relative difference is less than 4%, click the *Use Calculated* button to apply the calculated calibration factor. This is recommended if the load cells were changed, moved, or impacted. Alternatively, you may choose to select the *Use Previous* button to continue using the previous calibration factor.



- b. If the relative difference is greater than 4%, this could indicate damage to the load cell. Click *Use Previous* or *Cancel* to exit the window and repeat the calibration procedure. Check that the correct calibration spring is loaded, and the correct calibration preload is applied. If you suspect your load cell may be damaged or is reading improper values, contact our Support team ([support@cellscale.com](mailto:support@cellscale.com)).

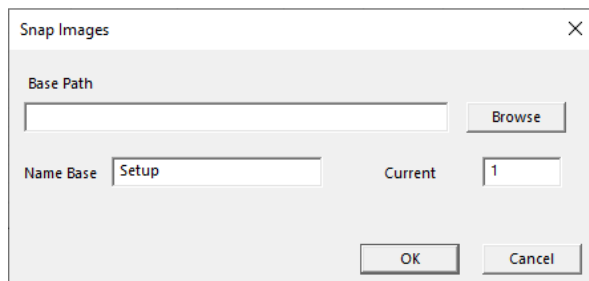


2. The load cell calibration procedure must be carried out for both load cells (X and Y axis).

## Snap Image Feature

Located under >Tools>Snap Image

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.



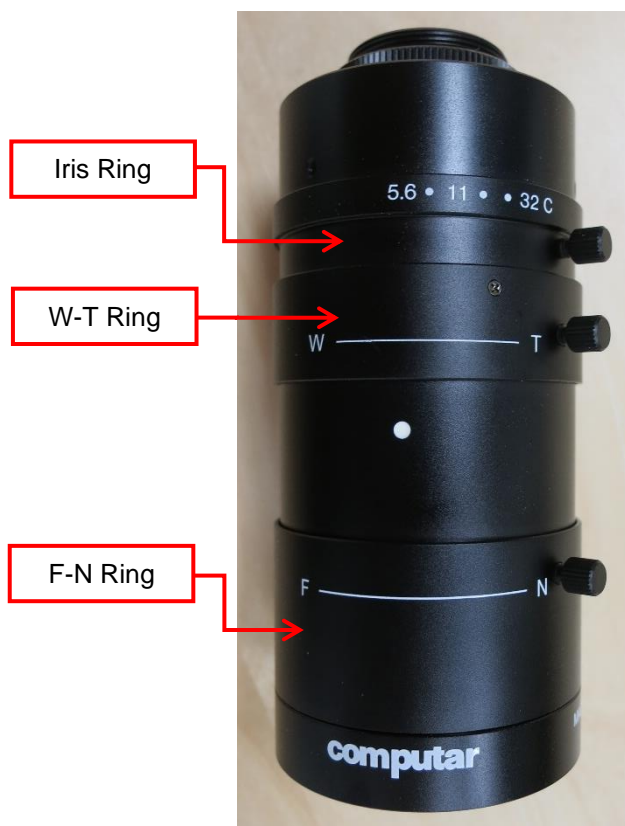
## Adjusting the Camera Position and Image Magnification

The horizontal camera position can be adjusted using the knobs at the back and side of the camera housing. If larger position adjustments are needed, the camera mast can be rotated to allow alignment of the camera image with the testing axes.

The BioTester 5000 comes equipped with a 10X zoom lens as shown below. Other versions of the BioTester 5000 may be equipped with a fixed magnification 75mm focal length lens (see **Appendix E: Fixed Focal Length Lens**).

The image magnification can be changed by rotating the adjustable rings found on the lens. Once the rings are adjusted to the desired settings, they can be locked using thumb screws. Adjusting the three rings has the following effect:

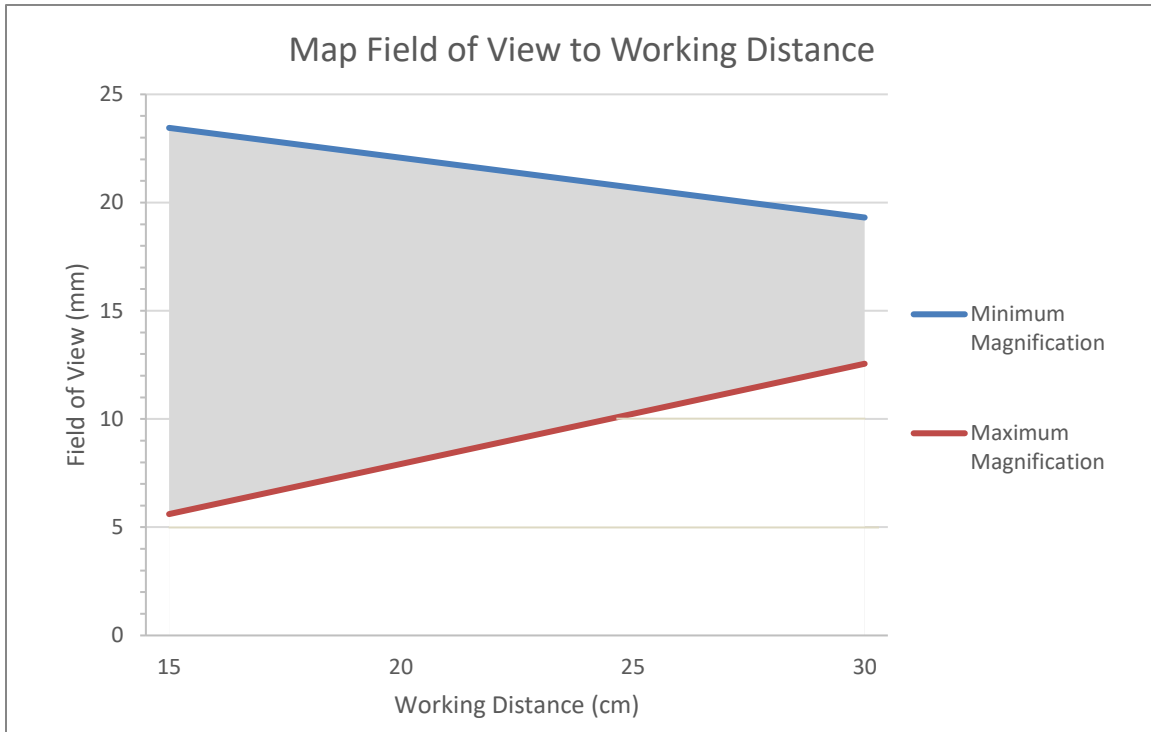
1. The iris ring changes the brightness of the image. It is recommended to leave this ring fully open and control image brightness by adjusting the camera shutter and gain in the Camera tab of the Hardware Settings.
2. The W – T ring changes the lens zoom.
3. The F – N ring changes the lens focus.





Using this lens, it is possible to achieve a wide ranging field of view as shown in the graph below. Possible values for field of view are shown by the shaded gray region of this graph and can range from 5.5mm to 24mm. To adjust the working distance (vertical position of the lens and camera assembly), loosen the fasteners that clamp the camera housing to the mast and raise or lower the camera then retighten the fasteners. The ideal working distance from the specimen to the lens is **15cm**. It is not recommended to have the lens any closer than this.

If the camera position or magnification is changed, it may be useful to capture a single image scale shot. A glass calibration target or a ruler is acceptable for this purpose. The scale shot will be useful later to characterize the size of test specimens or test specimen features.



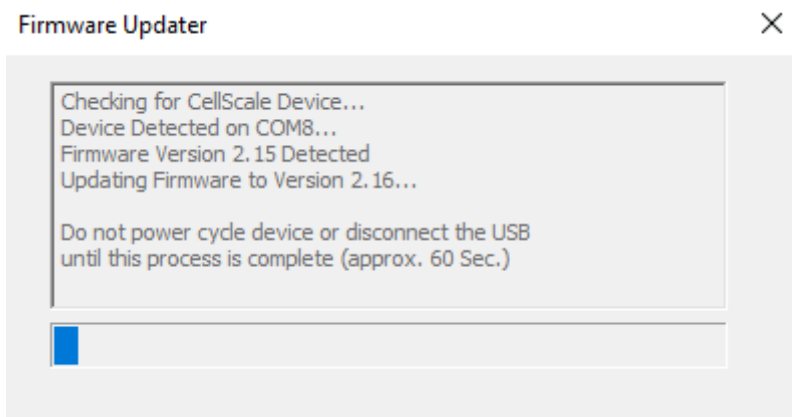
## Update Firmware

Periodic firmware updates may be issued by CellScale for the BioTester. Updates are available at <https://www.cellscale.com/downloads/>.

The device firmware will automatically update as part of the software installer if your device is connected via USB and powered on.

You may update the firmware for your device at a later time if it is not available when updating your software:

1. Connect your device via USB to your computer and power it on
2. Run the *UpdateFirmware.exe* located under  
CellScale>LabJoy>Software>Utilities>Update Firmware



## 10. Troubleshooting

### Image Appears Out of Focus

With the live image on the screen, adjust the lens focus until the image appears to be in focus.

Whenever you adjust the focus, the image magnification may change slightly. If you want to know the absolute scale of your Images you can snap an image of a calibrated target (such as a precision graticule or ruler) by selecting Snap Image from the Tools menu. You can estimate the image scale by comparing the number of pixels between rake tips in the images with their known  $\mu\text{m}$  spacing (size) in the data output files.

### ERROR - Sample Buffer Overflow Continuing Test...

Or

### System Lagging, Camera Frame Lost missed capture

These errors can be caused by lack of data throughput to the location where the test data is being saved. This has been noted by users who are saving their data to a network drive or a USB-connected external hard drive. Another cause for these errors can be a lack of computer resources, especially at high image capture frequencies (5 or 15Hz). It may be necessary to upgrade the computer that is controlling the device.

### Communication Errors: ReadFile failed with error 6.

When communication errors occur the live message window will display a ReadFile failed message. Communication errors can occur if:

- The BioTester 5000 is not powered up.
- USB cables between the computer and BioTester 5000 are disconnected.
- The supply power surges or is interrupted.

When a communication error occurs, close LabJoy, check connections, power cycle the BioTester 5000, and restart LabJoy.

#### ***BioTester 5000 Tip: Preventing Power Disruptions***

*We recommend plugging the BioTester 5000 (and host computer) into an uninterruptable power supply (UPS) or battery back-up.*

# 11. Appendix A: Initial System Setup

The BioTester 5000 has been packed in a special container to protect it during shipping. In addition, system accessories have been packed in several cartons contained in a separate box. The system components listed below may be unpacked and laid out before system setup. A space 60cm x 60cm with 100cm overhead clearance is required for setup.

## Tools

- Small flat head screwdriver
- SAE hex wrenches: 1/16", 3/32", 7/64", 9/64", 5/32", 5/16"
- Metric hex wrenches: 2mm, 2.5mm, 3mm
- SAE Combination Wrench 5/16"

## Fasteners & Parts

1	BioTester 5000 main unit	1	Press Block
16	M4 x 10mm Socket Head Cap Screws	1	Sheet of Rubber Backing Material
6	6-32 Hex Nuts (4 + 2 extra)	4	Actuator Caps
20	BioRakes (5 sets of 4 each)	1	3"x1" Microscope Slide
2	Load Cells	1	Pull knob
1	Calibration Spring	1	Temperature sensor
2	Calibration Spring Brackets	1	Set extra fasteners
1	Camera Lens (10X Zoom)		
1	Specimen Mounting Bridge		
2	Load Cell Goosenecks		
2	No-Load Cell Goosenecks		
2	Load Cell Brackets		
1	Standard Specimen Cutting Jig		
4	Razor Blades for Cutting Jig (2 + 2 extra)		
1	Fluid Bath		
1	Power Cord and Supply		
1	6' (1.8m) USB Cable		
1	Set of Shims (Thin and Thick)		

## ***Unpack System***

### **Remove the BioTester 5000 from the shipping container**

Carefully remove the protective shipping material from the container such that the BioTester 5000 can be lifted out. Remove the camera mast first then the BioTester 5000 main unit. In addition, protective foam will have to be removed from the main unit and camera mast after lifting out the BioTester 5000.



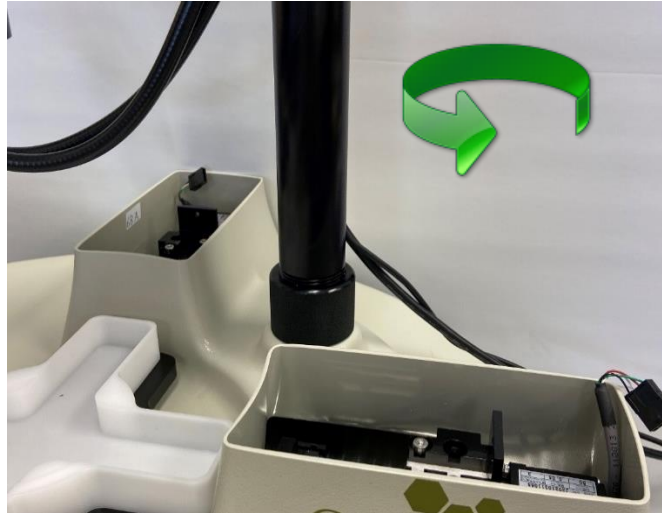


### ***Attach Camera Mast***

Thread the camera post into the receiver. The **knurled collar must be rotated** to fasten the camera post. It will take many turns but eventually the mast will lock into place. Try to position the camera approximately as shown, although the final position can be adjusted later in the procedure. Be sure to position the cables from the camera mast post towards the back where the power entry panel is located.







Once the camera mast is in place, the cables for the lights and camera USB will be routed out the back of the mounting pole. The camera USB can be plugged into the PC and the connector for the lights can be plugged into the back panel of the BioTester 5000.





### ***Place Fluid Chamber on Riser Stage***

Put the fluid chamber in place on the riser stage and align the temperature probe mounting hole with the temperature probe cable.



## Install Camera Lens

Remove the protective caps on the lens and camera body and then screw the lens onto the camera. The correct camera setup can be fine-tuned at a later step. If using a fixed focal length lens (typically 75mm), see Appendix E for installation.



Lens (10X Zoom)

Camera (inside housing)

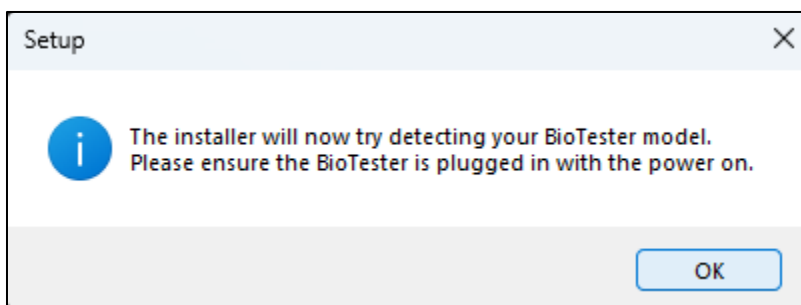
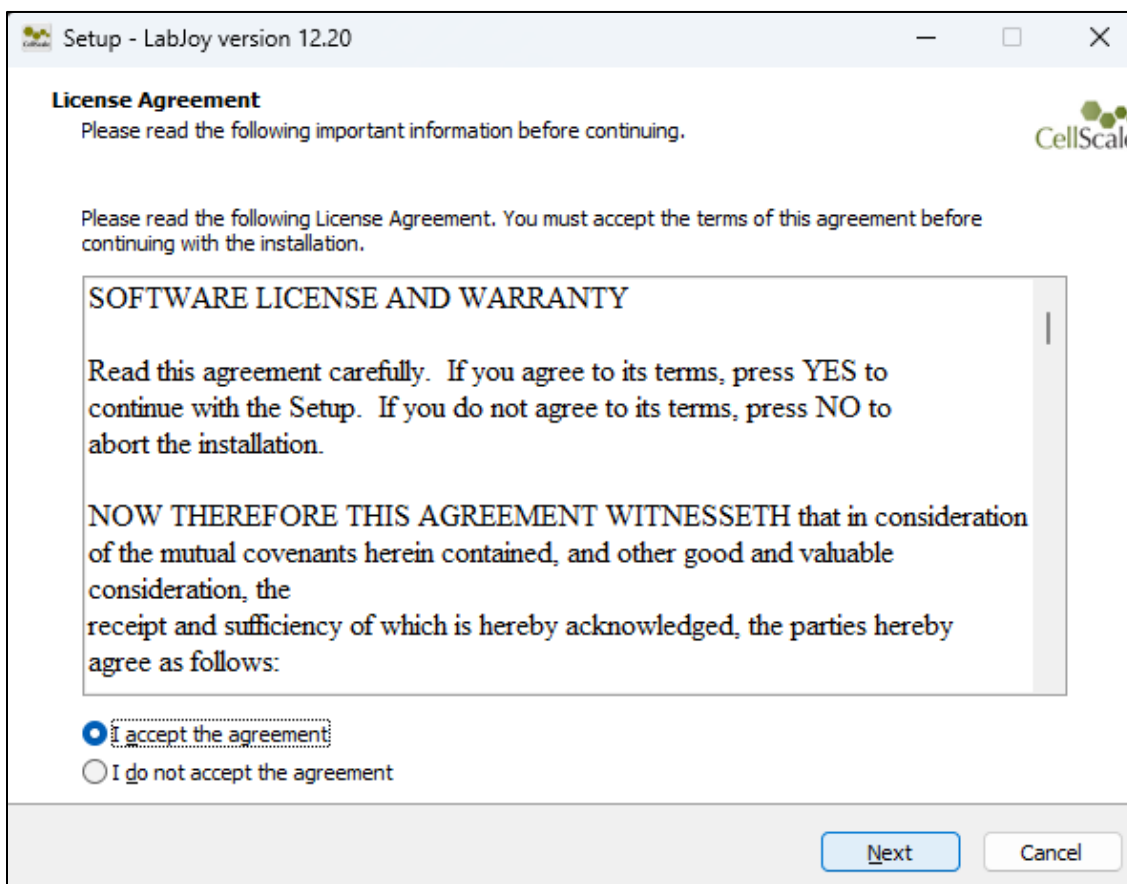


## 12. Appendix B: Software Installation

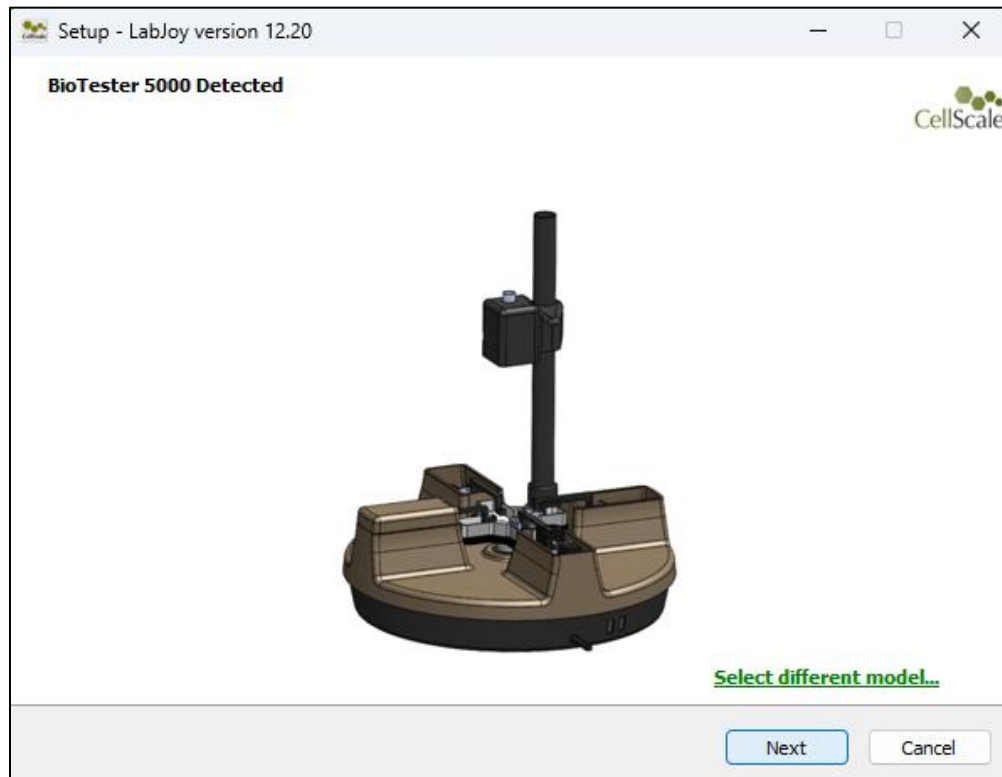
A Windows based PC (Windows 10 or higher) with at least 2 USB ports (1x USB 3.0 minimum) is required to operate the BioTester 5000. A label on the cover of the manual contains a web address to download the LabJoy installation package. These files contain the software installer for LabJoy, which is the user interface and control software for the BioTester 5000.

To install the software:

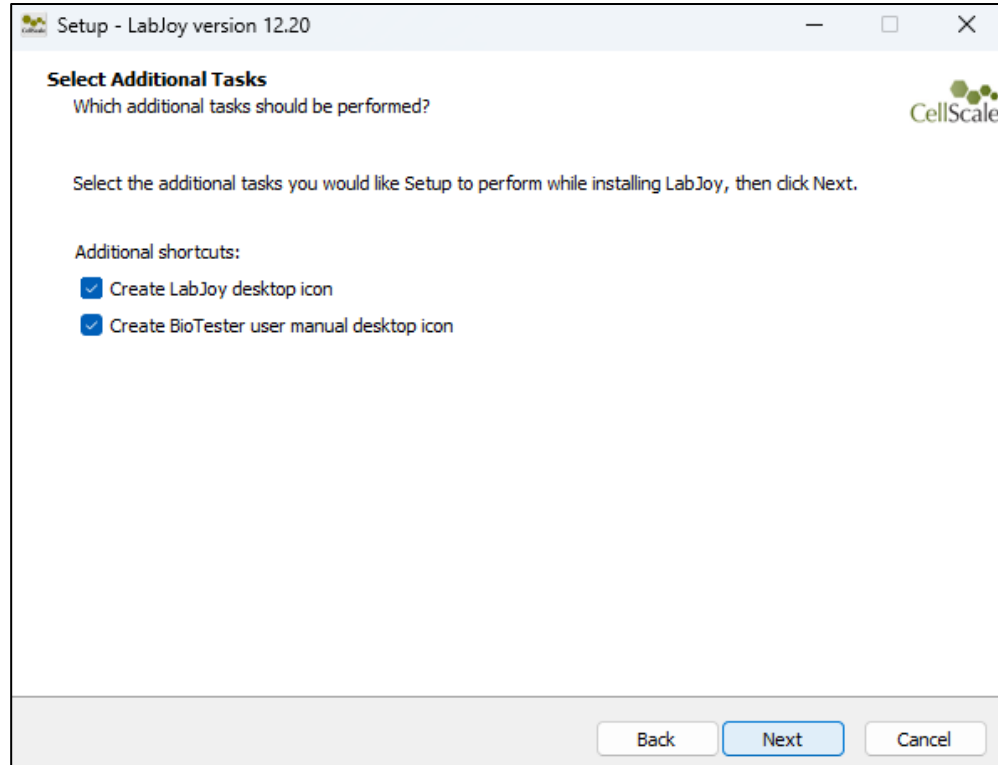
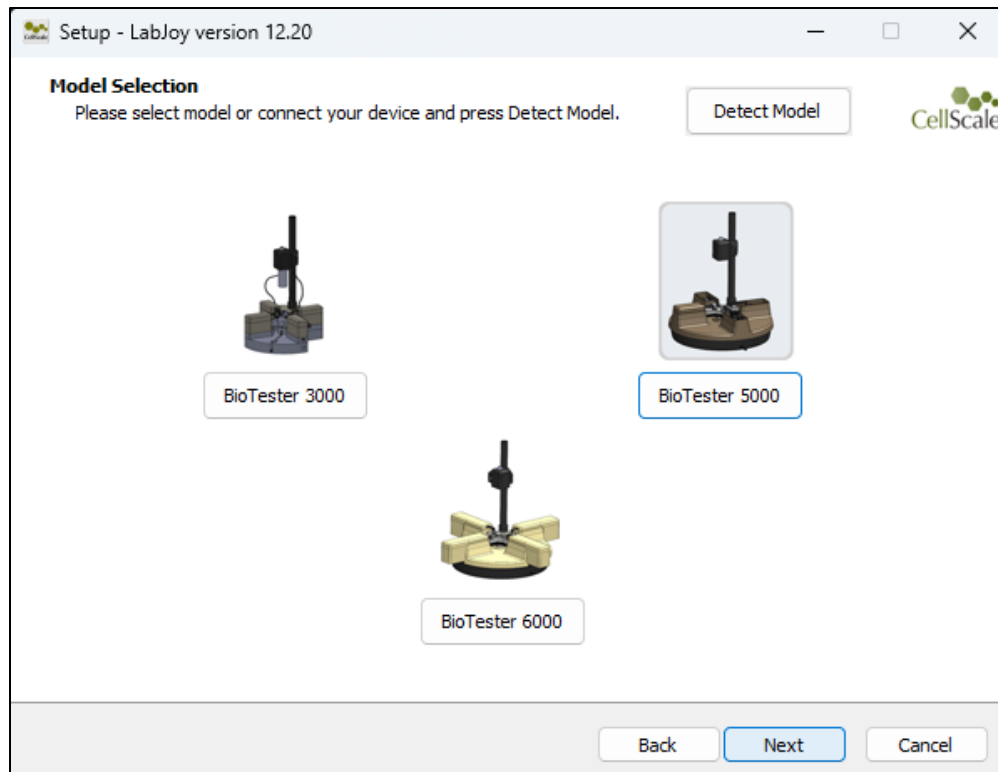
1. Go to the provided web address and download the compressed installation package.
2. Transfer this file to the computer which will be running the BioTester device.
3. After this is complete, go to the uncompressed installation folder and select *LabJoy Installer.exe*. Details of typical dialogue boxes are shown below.

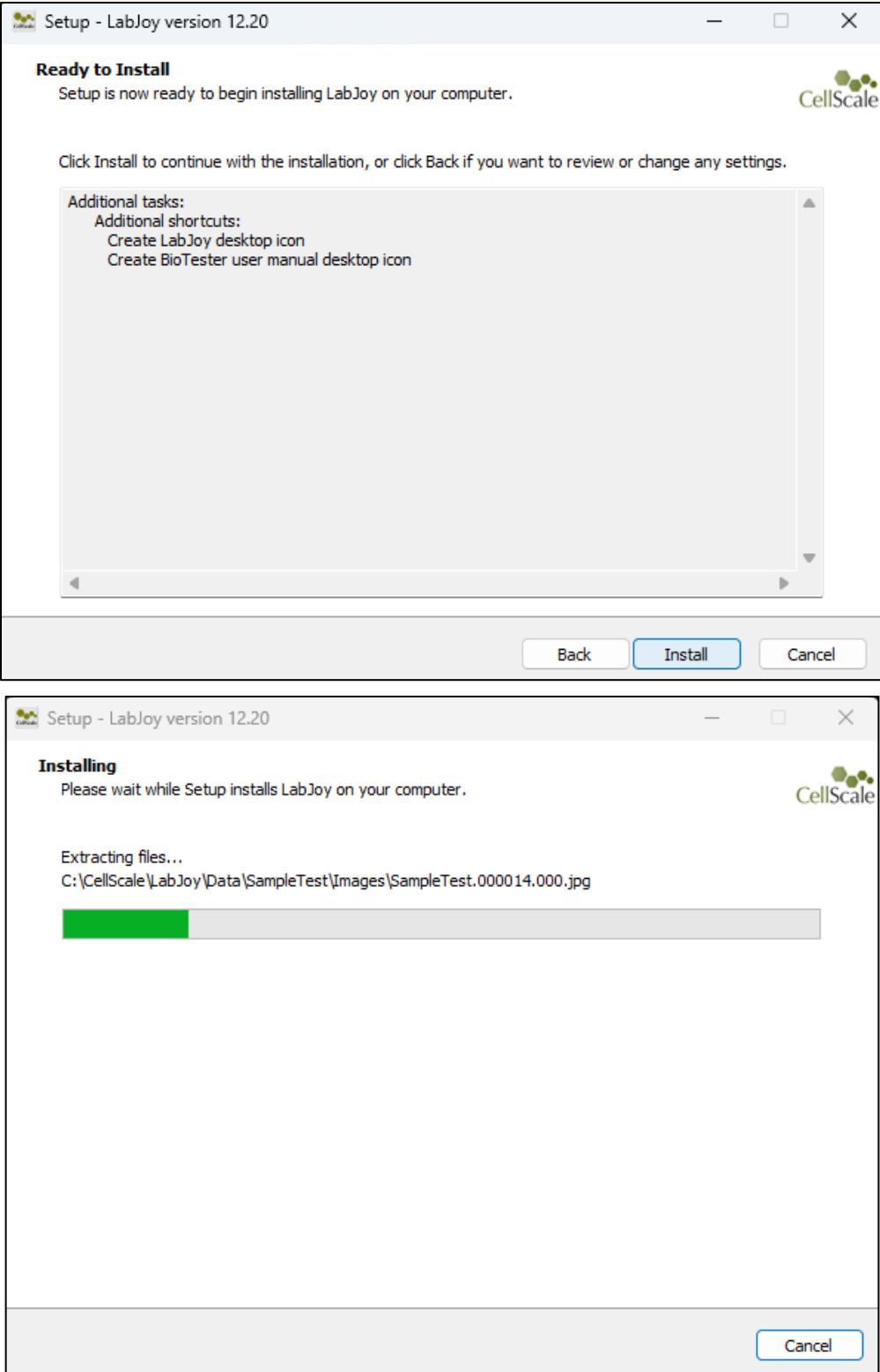


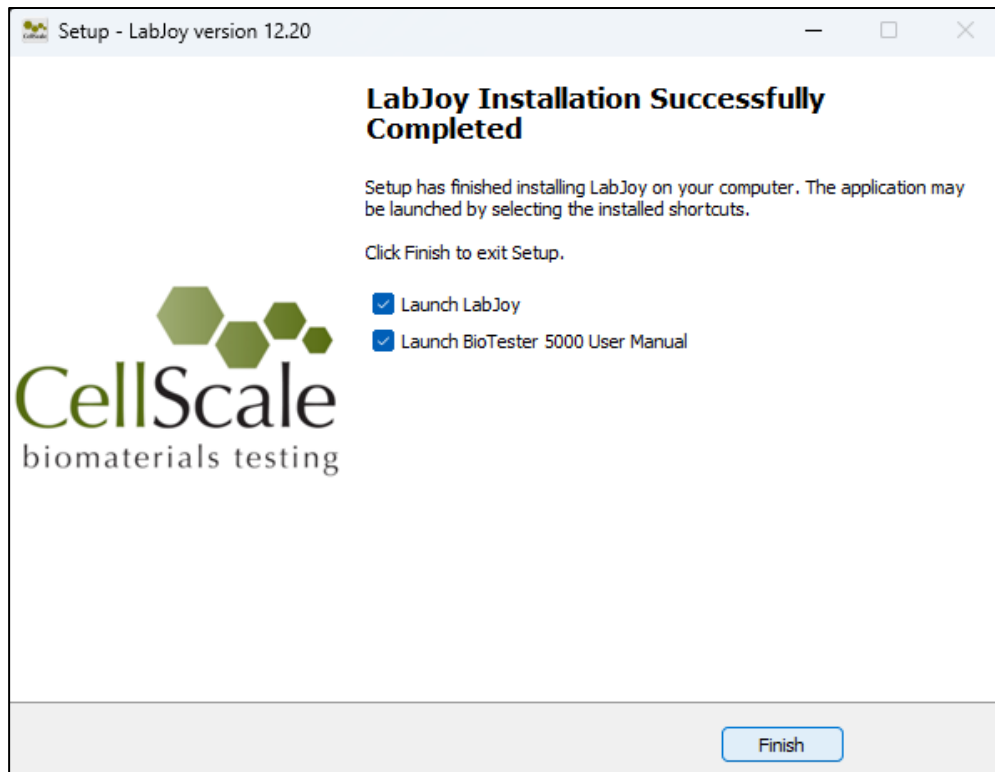
If your BioTester is detected this screen will popup:



If BioTester is not detected, then you will be taken to a model selection screen where you can select your BioTester model. To install the correct user manual and templates please select the appropriate model.







## 13. Appendix C: Install Load Cells / Perform Camera and Gooseneck Alignment

Connect the camera USB cable to a PC USB port. Connect the included USB cable to the main unit and a PC USB port. Plug in the power supply to the BioTester 5000 and then proceed with the following steps. **Do not turn the BioTester 5000 on yet.**

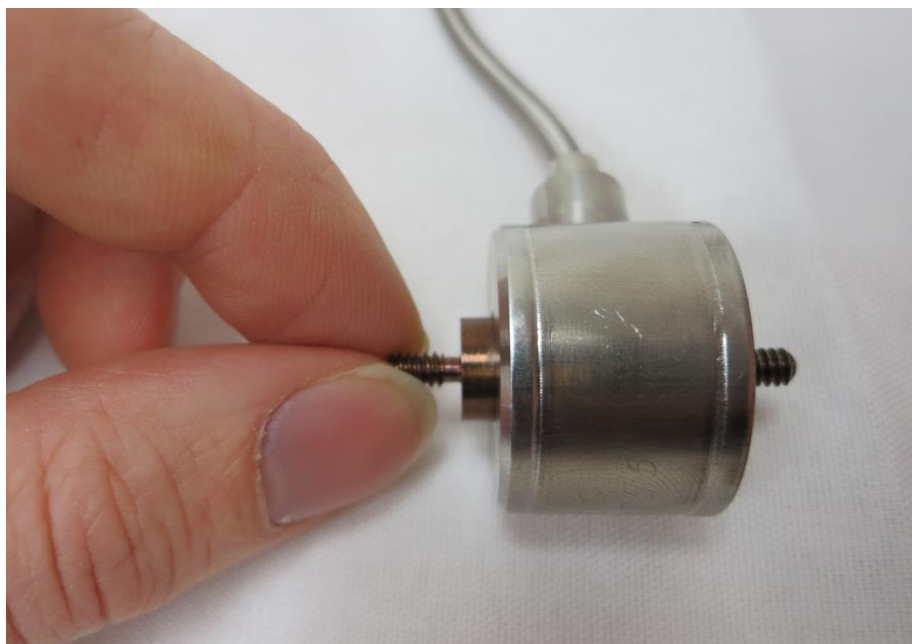
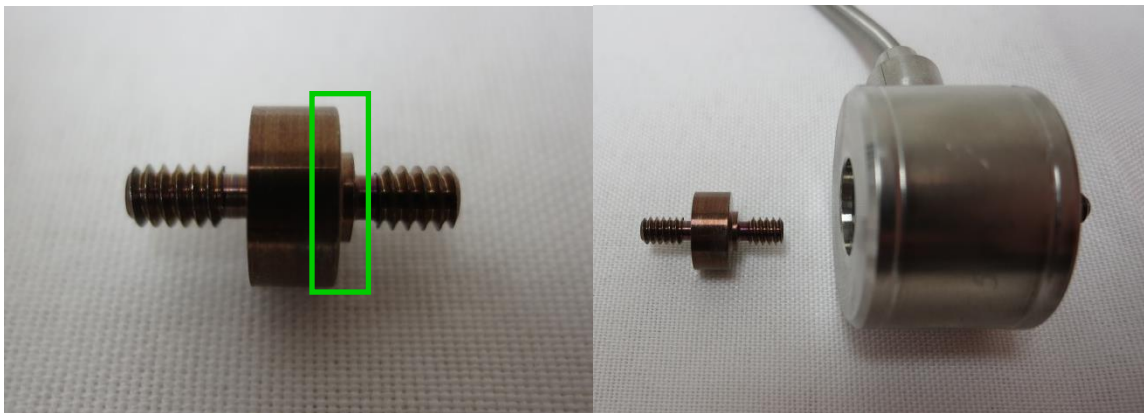
### ***Attach the Load Cells to the Goosenecks***

Ensure the correct orientation of the load cell components as shown in the below pictures. Tighten the nuts gently to avoid damaging the load cells (just a little bit tighter than finger tight). **Keep in mind that the load cells are sensitive to excessive torque, excessive overload, transverse loading, and bending forces.**

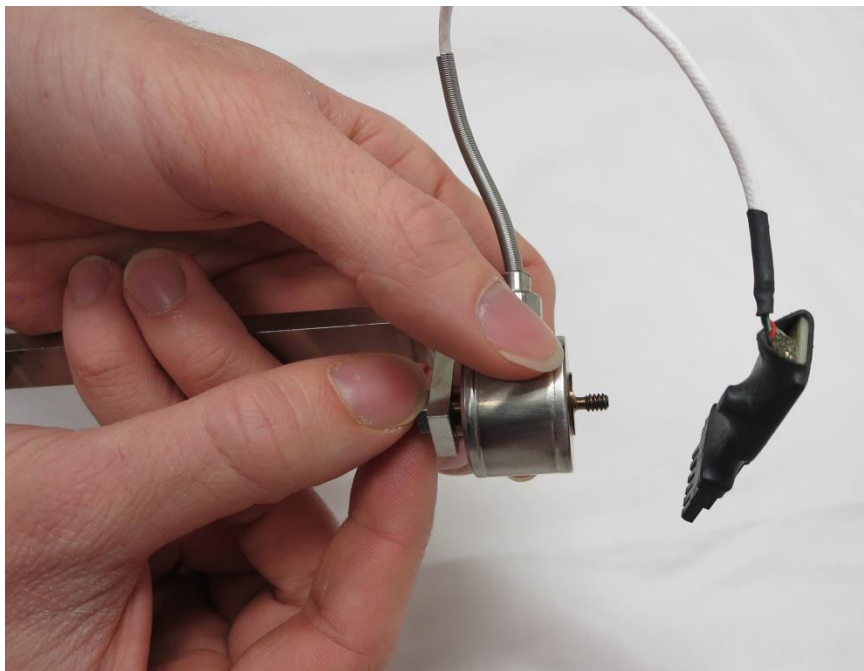




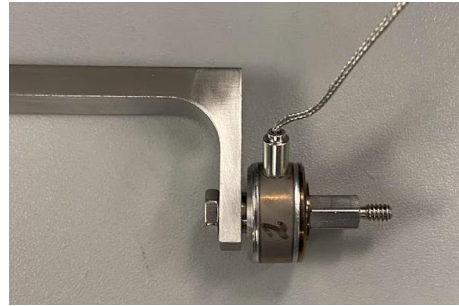
Attach the load cell stud to the load cell using your fingers. The load cell stud has one side with a shoulder below the threads which should be attached to the load cell. The other end is attached to the gooseneck. **If the orientation of the stud is reversed, the load cell may not function properly.**



Attach the other end of the load cell stud to the gooseneck. Finger tighten a 6-32 nut onto the threaded stud. The load cell cable should be at about a 45° angle to the gooseneck as shown below.



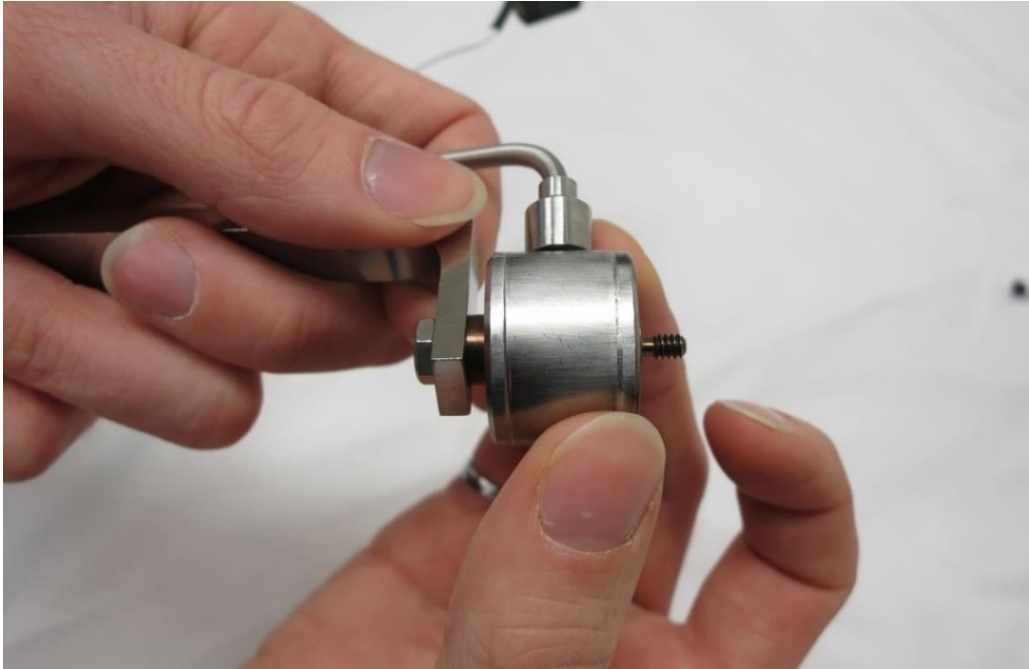
The load cell and gooseneck assembly should look like this when assembled. There are two styles of load cells available (both are shown below). For 10N and 23N load cells, ensure the included spacer is attached before mounting it to the actuator bracket. This is important for proper spacing between the goosenecks.



The nut may be gently tightened against the gooseneck using the included wrench, **while grasping the gooseneck only** to avoid transmitting torque through the body of the load cell.

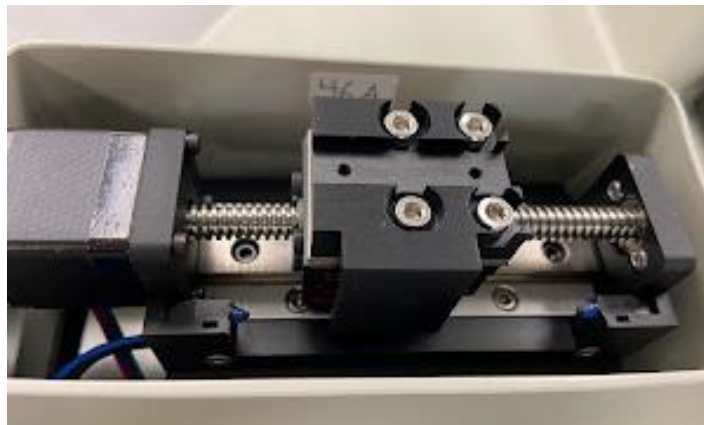
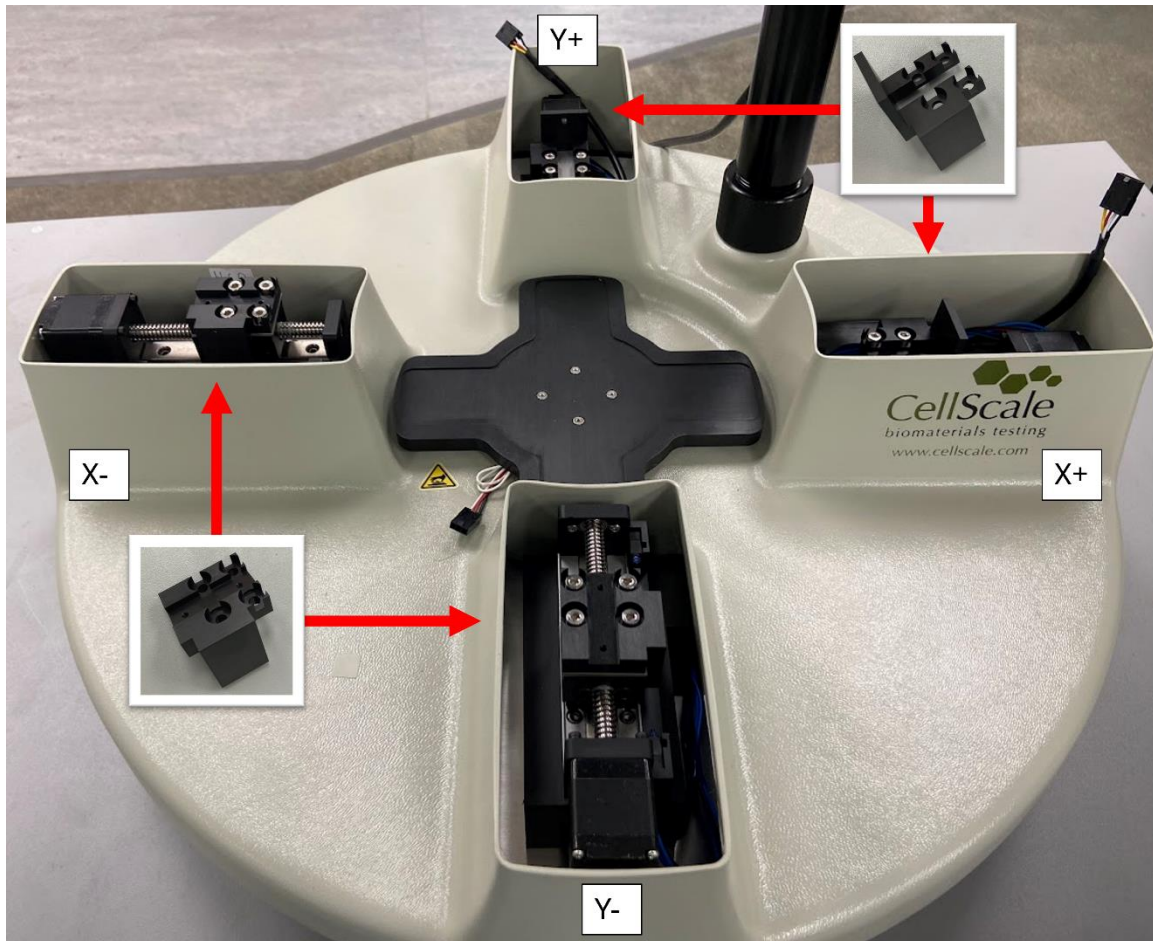


Check that all components are snugly in place by gently turning the base of the load cell against the gooseneck. All components should only be **finger tight** while being snug enough so that the components don't freely rotate while using the BioTester 5000.



## Attach the Load Cell Brackets to the Actuators

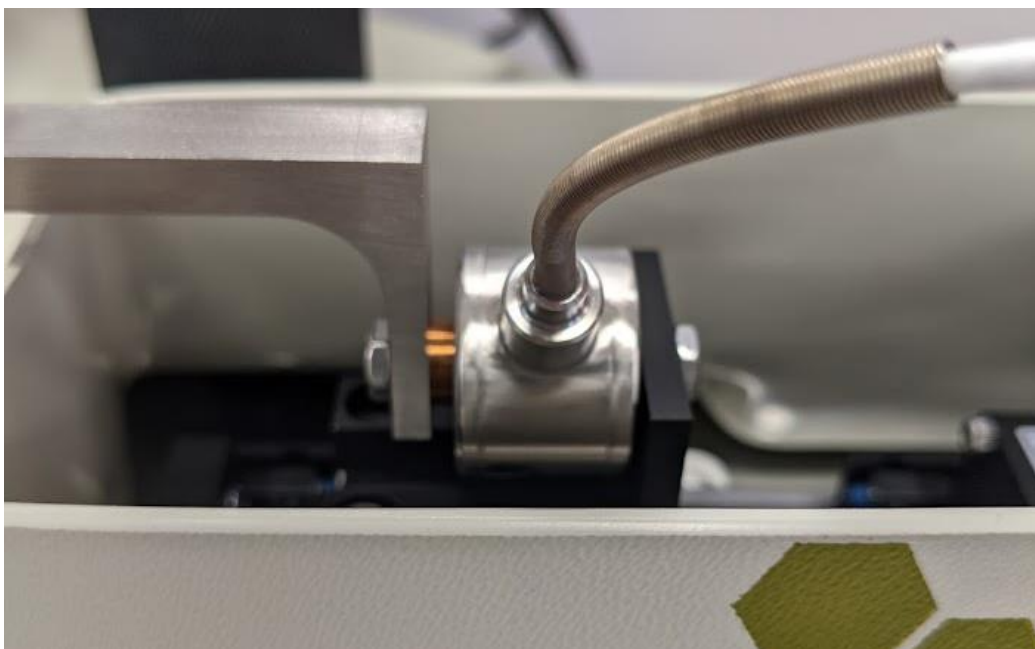
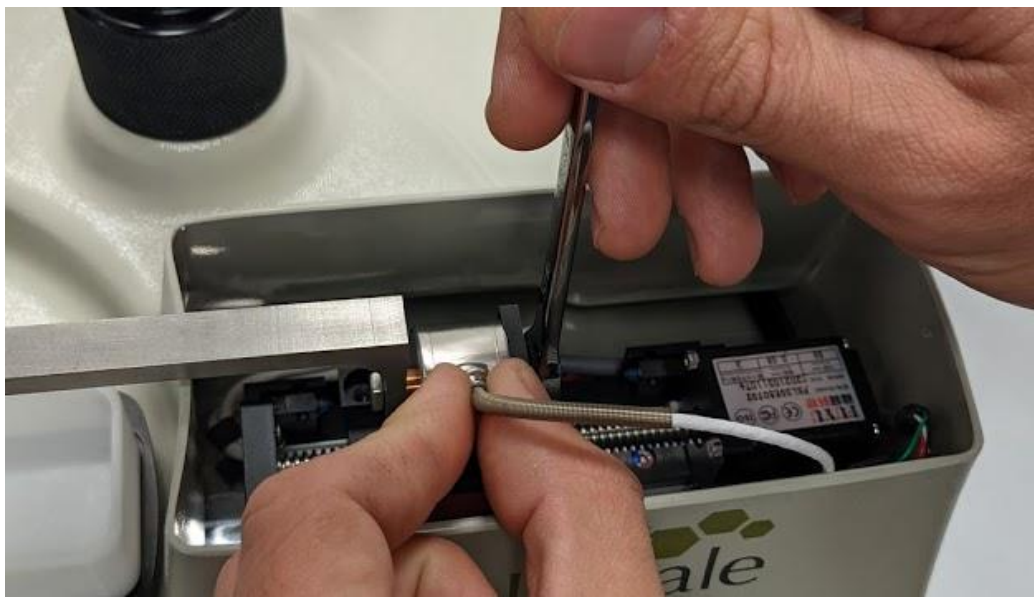
Before attaching the load cell/gooseneck assembly, ensure the load cell brackets are installed on the appropriate actuators. Use M4 x 10mm (x4) fasteners provided for each bracket as shown below. The load cell brackets are mounted on the X+ and Y+ actuators (on either side of the camera post). The remaining brackets are mount on the other actuators. Ensure the brackets are oriented correctly (shown below).

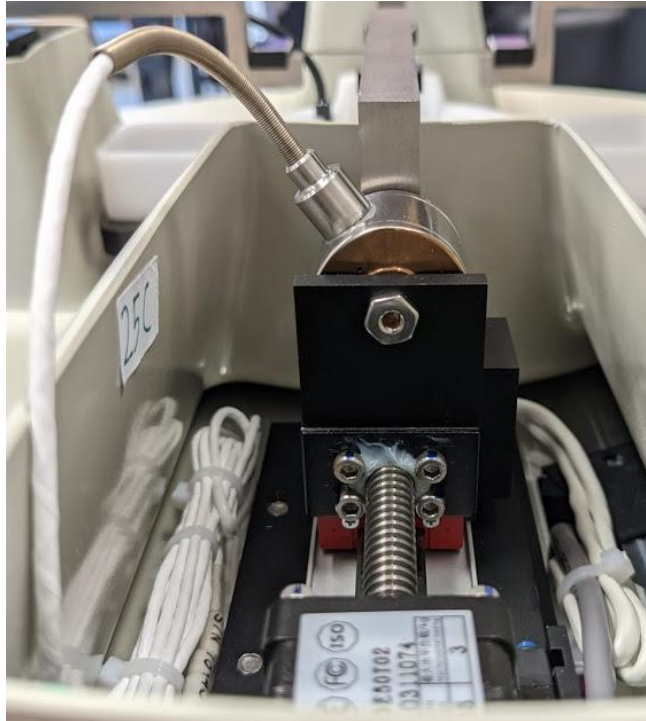




### ***Attach the Goosenecks to the Actuators***

Attach each load cell/gooseneck assembly to the load cell bracket on the appropriate linear actuator using a 6-32 nut. The load cells are attached to the actuators on either side of the camera post. See the following images. The nut on the back of the load cell is not as sensitive as the front side nut and may be snugly tightened using the wrench, while grasping the body of the load cell. Make sure that the gooseneck is rotated such that it is level when the nut is fastened.





Attach the 2 remaining goosenecks to the appropriate linear actuators using 4-40 fasteners (2 each).







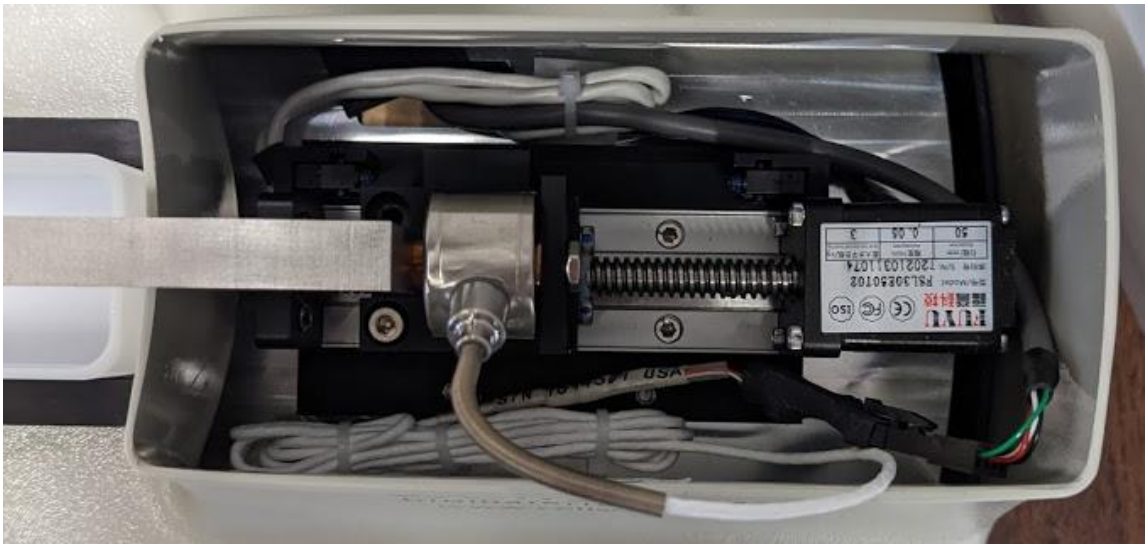
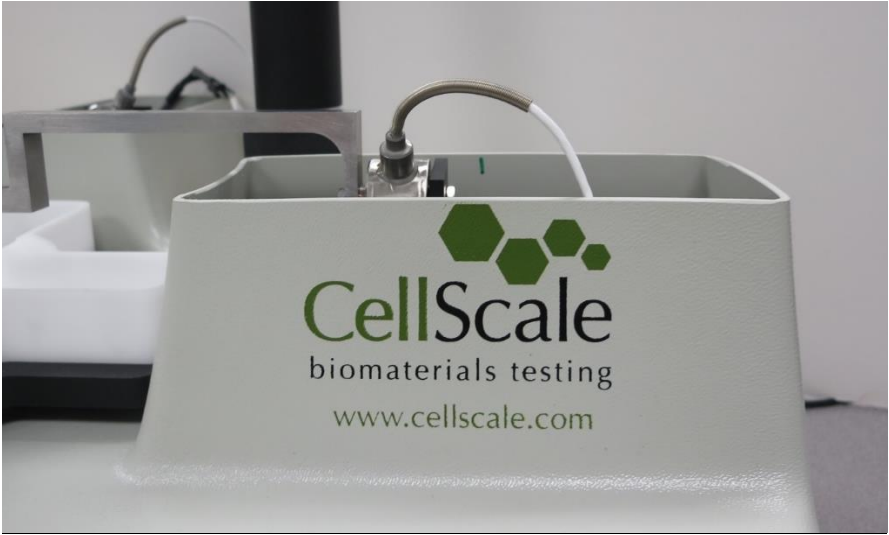
## ***Stow Cables and Plug in the Load Cells***

Before connecting the load cells, stow the cables inside the plastic cover beside the actuators, taking care to handle the connectors by the edges. A circuit board assembly may be attached to the connector and must be handled carefully. Also, the load cells may have a coiled length of cable which must be carefully stowed. The initial length of cable should bend towards the **outside edge** of the BioTester 5000 as shown.

Position the cables inside the cover so that they do not interfere with the actuator carriage travel, particularly during actuator reset where the carriages are fully retracted. Plug in the load cells and stow the connector inside the cover as well.

Also use care when unplugging the load cells. This may be done by firmly pressing on the latch mechanism before pulling it apart. See below photos for reference.





### ***Attach the Temperature Probe***

Attach the temperature probe and place it in the sensor pocket in the fluid chamber.



### ***Attach the Knob to the Pull Rod***

A small knob screws into the pull rod at the front of the BioTester 5000. This pull rod is used to raise and lower the stage.



### ***Turn on the BioTester 5000***

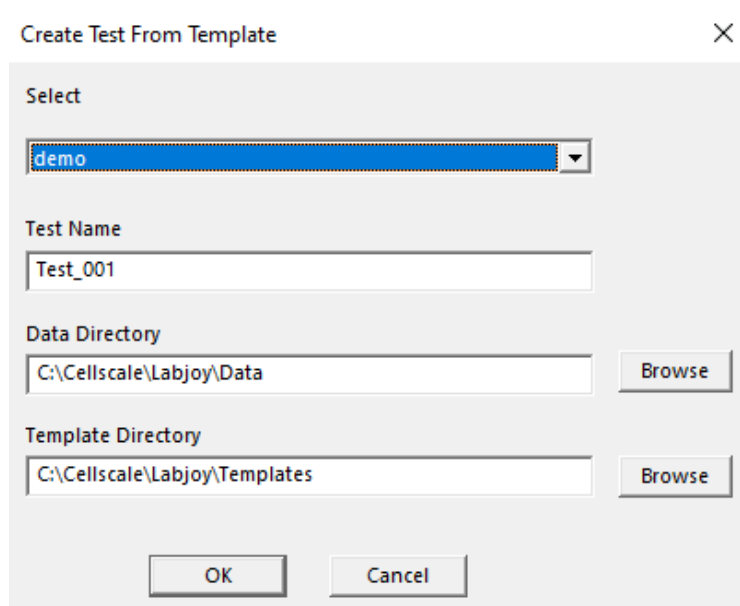
Plug the provided power supply into the BioTester 5000 and a standard electrical outlet. The BioTester 5000 has 2 switches. The “Main” switch turns on the BioTester 5000 main unit and lights. The “Heater” switch turns on the heater below the stage and may be used to warm fluid inside the fluid chamber to the temperature specified in the software. For the heater to be used, the main switch must also be activated. However, the heater is not required to use the system. For the set up procedure, turn on the “Main” switch only and check that the LED lights turn on.

## ***Launch the LabJoy Software***

Launch the LabJoy software. The default screen should appear as:



Selecting File>Collect New should bring up this window:

A screenshot of a dialog box titled "Create Test From Template". The dialog box has a close button (X) in the top right corner. It contains several fields and buttons:

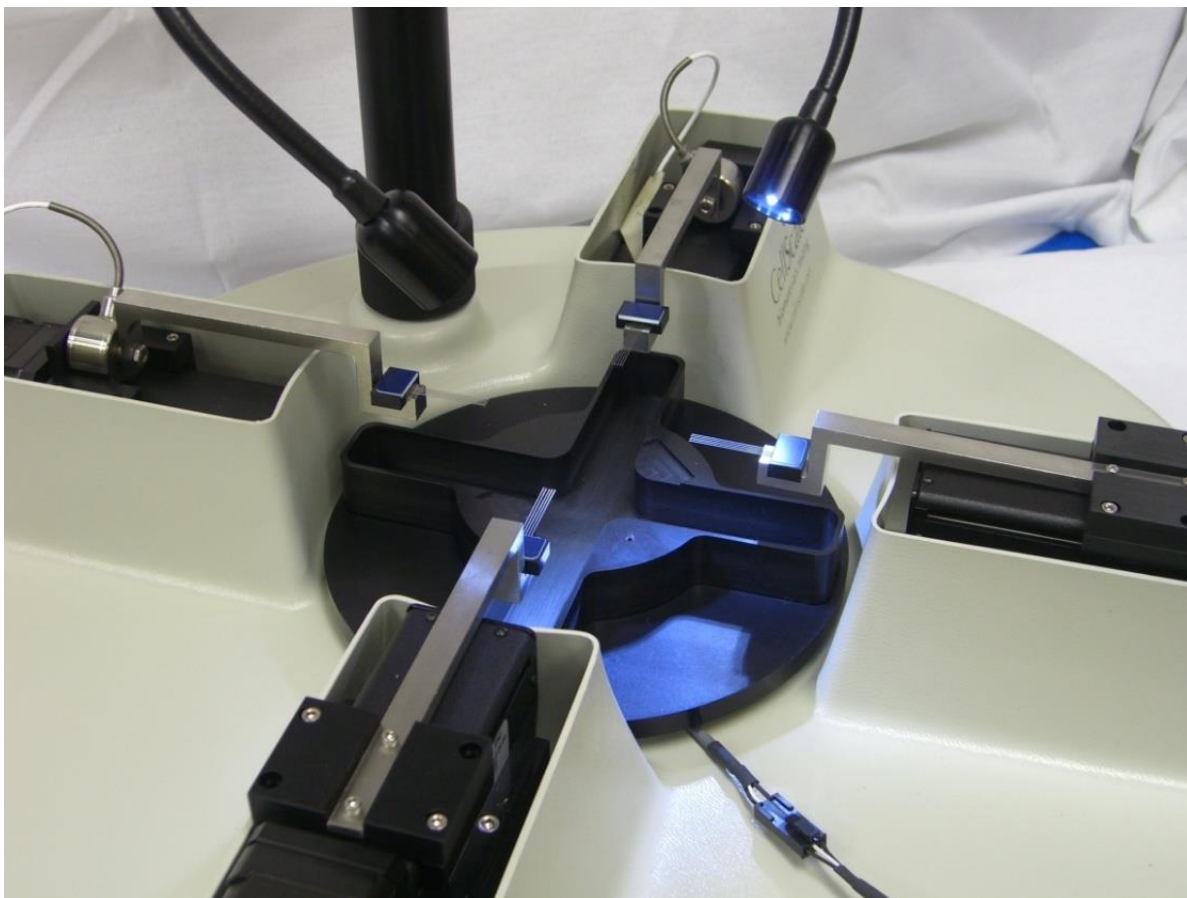
- A "Select" dropdown menu with "demo" selected.
- A "Test Name" text field containing "Test\_001".
- A "Data Directory" text field containing "C:\Cellscale\Labjoy\Data" and a "Browse" button to its right.
- A "Template Directory" text field containing "C:\Cellscale\Labjoy\Templates" and a "Browse" button to its right.
- "OK" and "Cancel" buttons at the bottom.


The details of the software are outlined in this user's manual. For assembly purposes, it is only required that we initiate the default test. Select OK.

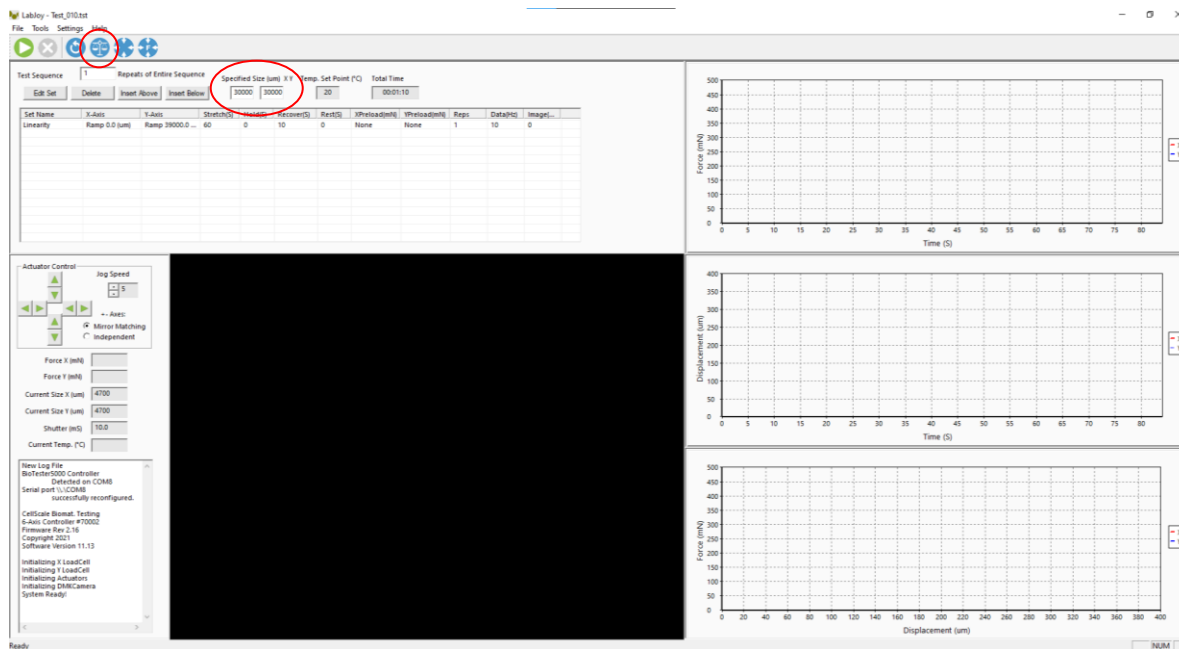
If prompted to Reset Actuators, select "Yes".

If prompted to Calibrate Load Cells, select "No".

Next place a standard size BioRake (1.0mm spacing, blue caps) in each of the 4 goosenecks so that the BioTester 5000 looks like this:



On the LabJoy control screen, ensure that the specimen size is set to 5000 $\mu$ m in X and Y and then click the *Move to Size* button .



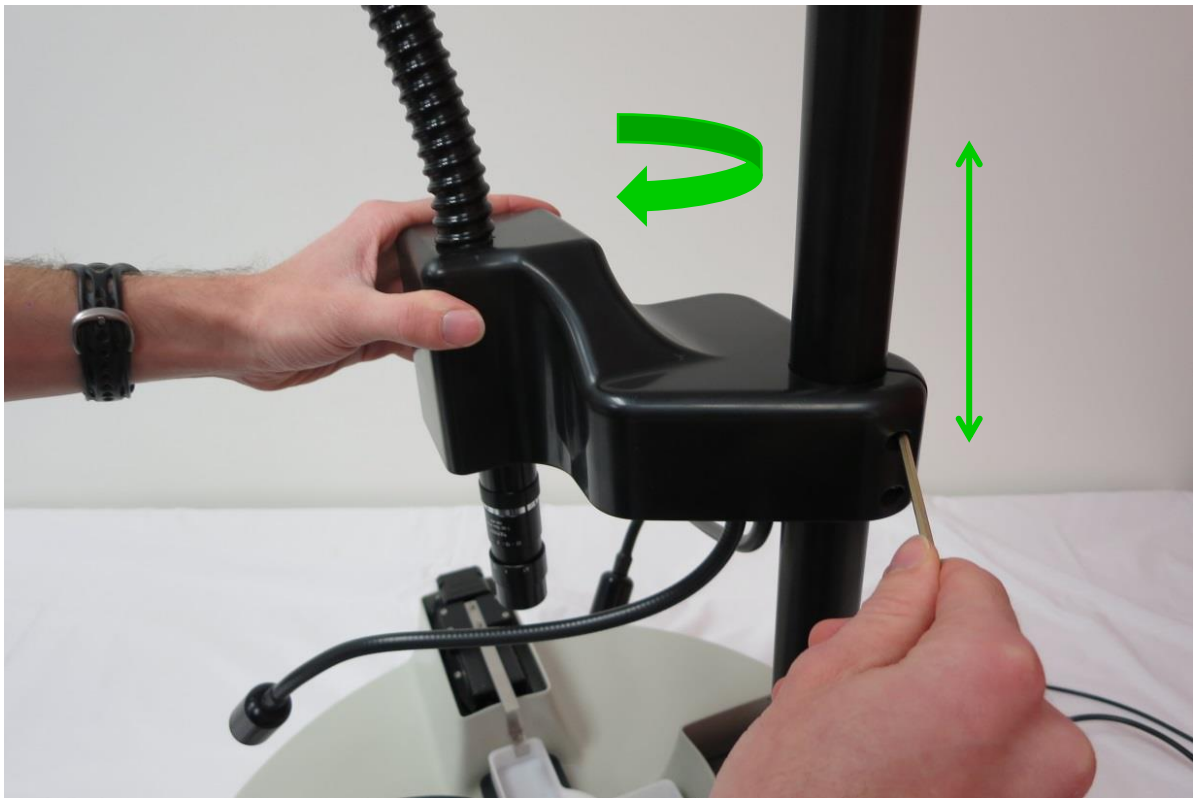


## ***Perform Camera Alignment***

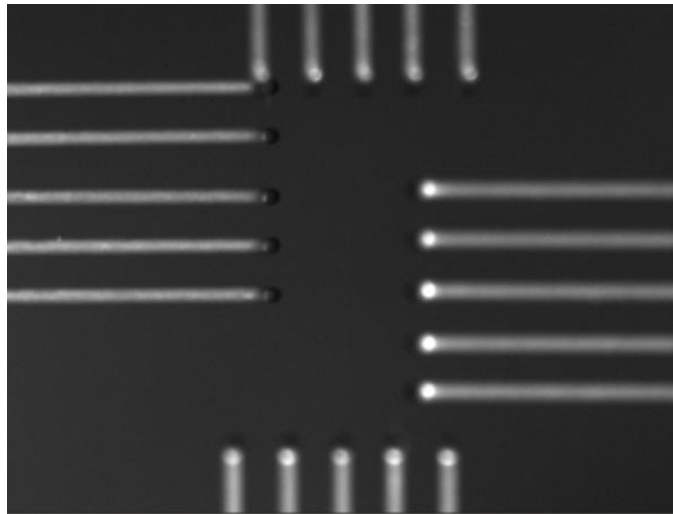
The camera position has both coarse and fine position adjustments and coarse and fine focus adjustments. To start, loosen the fasteners which tighten to camera cover to the mast. Position the camera cover vertically such that the working distance from the lens surface to the BioRakes is **15cm**. Next, adjust the zoom and focus rings on the lens until the BioRake tines are brought into focus. The camera assembly can then be rotated until the camera is positioned approximately over the center of the 4 BioRakes.

To achieve different ranges for field of view, the camera cover can be moved up and down to change the working distance but a 15cm working distance will accommodate most applications.

Be careful to move the camera housing gently in small increments, supporting it with your hand at all times, so as not to crash it into the goosenecks or BioRakes.



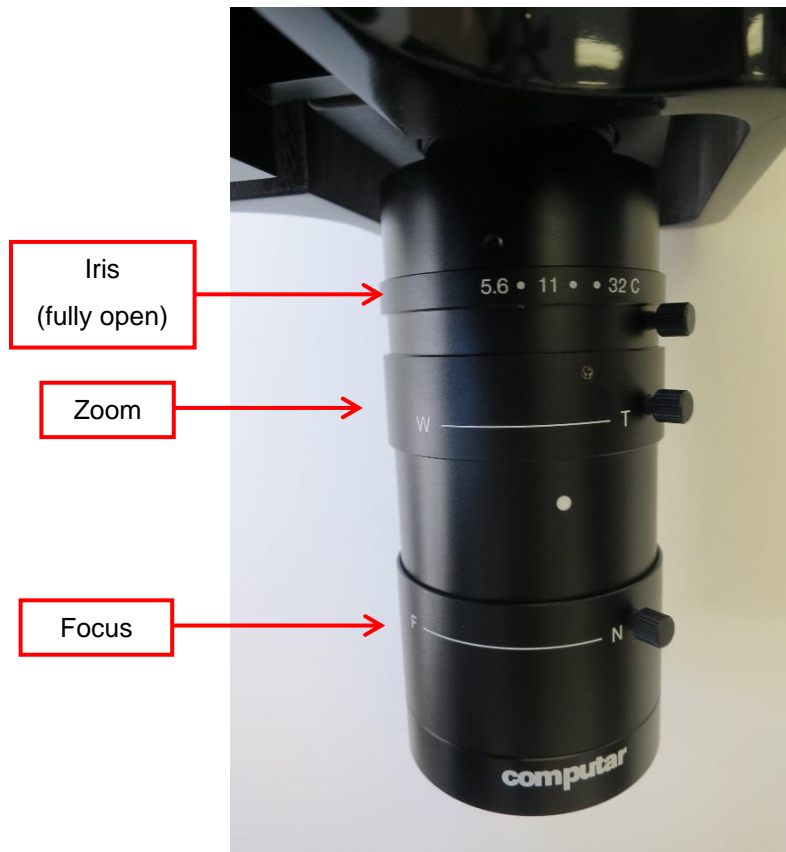
Attempt to bring all of the BioRakes into the image, as shown below:



Next, fine tune the camera position using the knobs at the back and side of the camera housing and fine tune the image focus using the lowest ring on the lens.



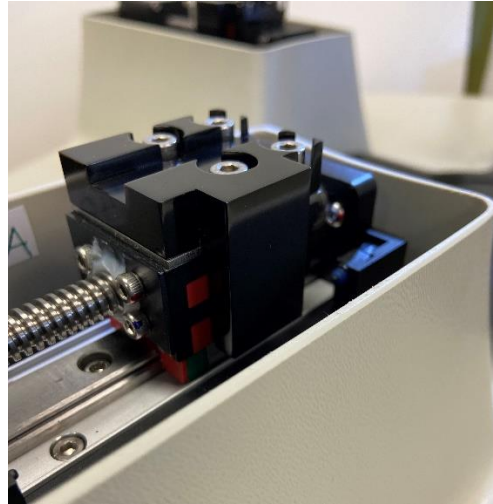




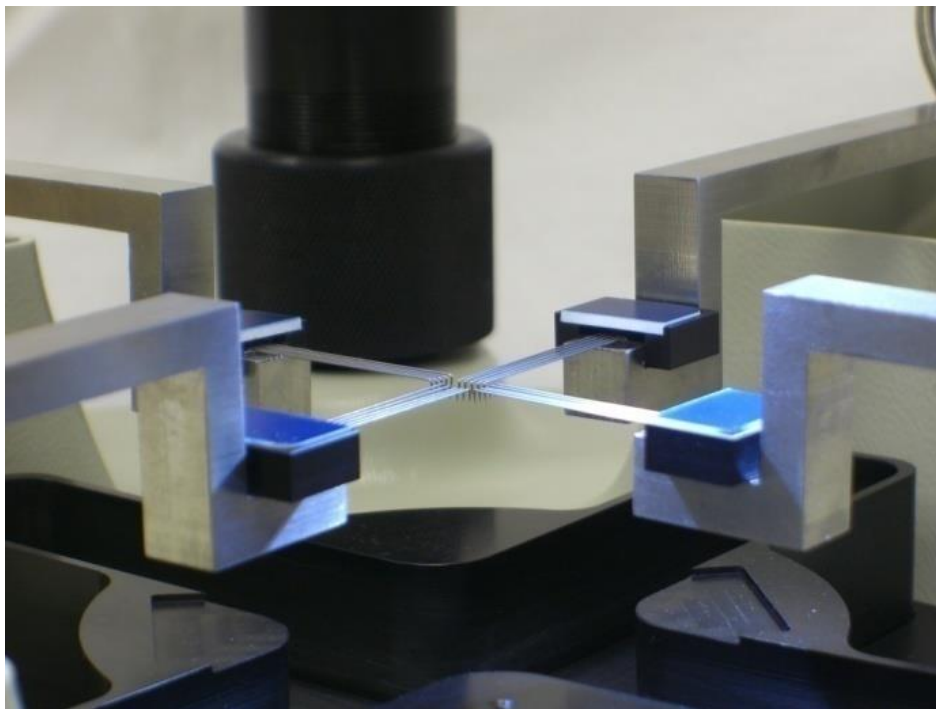
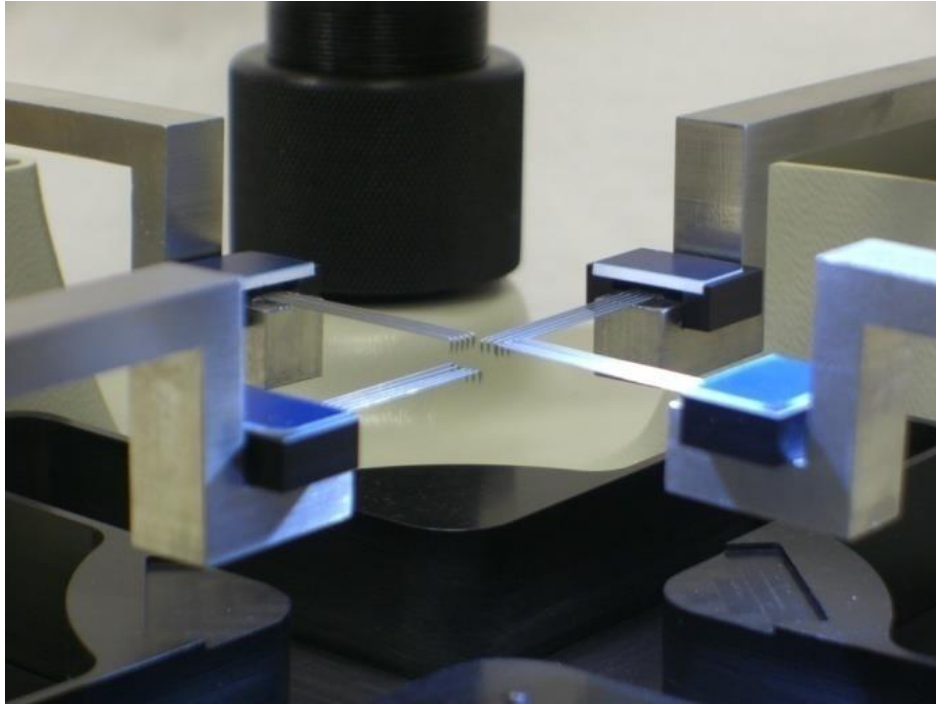
Adjustments to the zoom can be made by adjusting the middle ring on the lens. See the section on “Adjusting the Camera Position and Image Magnification” in section 8 of this manual for more detail.

## ***Perform Vertical Gooseneck Alignment***

Because the goosenecks attached to the load cells are connected by only 1 threaded stud, the vertical and transverse alignment of the end of the gooseneck can be somewhat different each time the goosenecks are removed and reattached. To accommodate the vertical variation, shims between the gooseneck brackets and the actuators may need to be added or removed. Thick and thin shims are provided (2 thin shims = 1 thick shim). At this stage, do not be concerned with the transverse or axial positioning of the goosenecks as these will be aligned in subsequent steps.

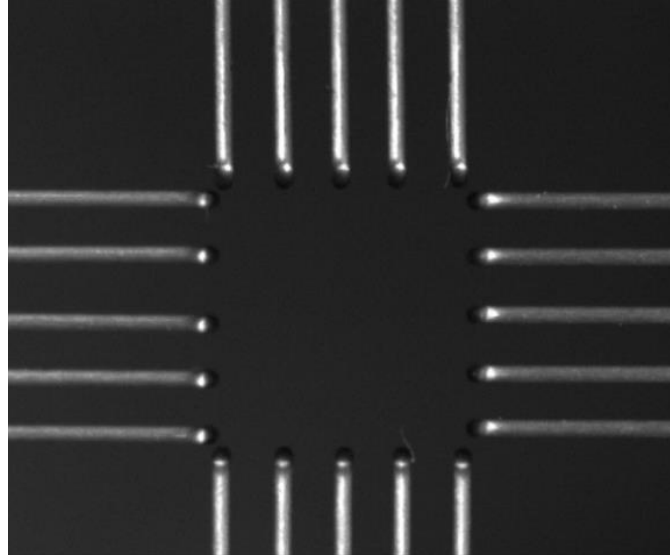


By looking at the BioRakes from the side, it is possible to observe their vertical alignment. Please add or remove shims to vertically align the BioRakes. Here are photos of poor vertical alignment and good vertical alignment:

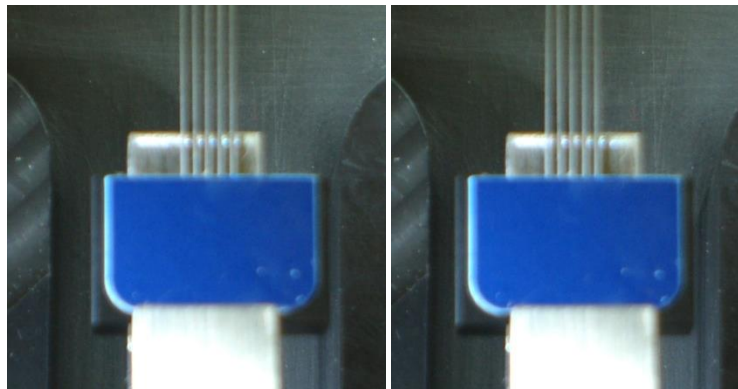


## ***Perform Transverse and Axial Gooseneck Alignment***

This step requires 2 different adjustment mechanisms. The goal is to make the BioRakes look like this:

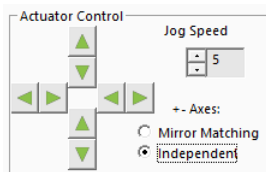


First, make sure the BioRakes are positioned in the transverse center of the gooseneck. The magnetic mounts that hold the BioRakes to the gooseneck fix them in the axial direction, but allow a small amount of adjustment in the transverse direction. This is useful for alignment during regular testing, but for the purposes of gooseneck alignment they should be in the neutral position. The first image below shows a transversely offset BioRake and the second shows a transversely centered BioRake.



To transversely adjust the goosenecks, loosen the fasteners that attach the goosenecks to the actuators, slightly rotate the gooseneck, and retighten the fasteners. The on-screen image should be helpful as a guide. The goal is to make the rake tines of opposing BioRakes collinear.

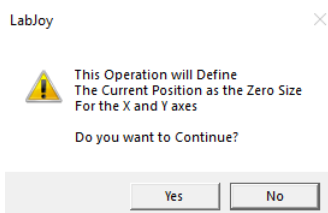
To axially adjust the goosenecks, use the actuator jog icons in the lower left of the screen. Make sure the “**independent**” radio button is selected and **not** the “**mirror matching**” button. The jog speed should be about 7. The goal is to make a square as shown in the figure above.




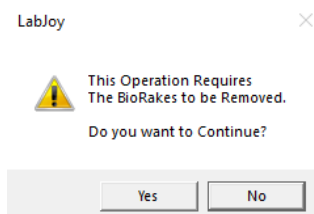
Adjust all 4 goosenecks until you are satisfied with their transverse and axial alignment and the rake tips form a square as shown above. It may also be necessary to make small adjustments to the camera position. The camera alignment should be parallel to the rake alignment and the BioRake tips should be in the middle of the image.

It is now necessary to perform a center position calibration. First, it will be necessary to temporarily define the current position as the center position. This calibration can be performed in the following steps:

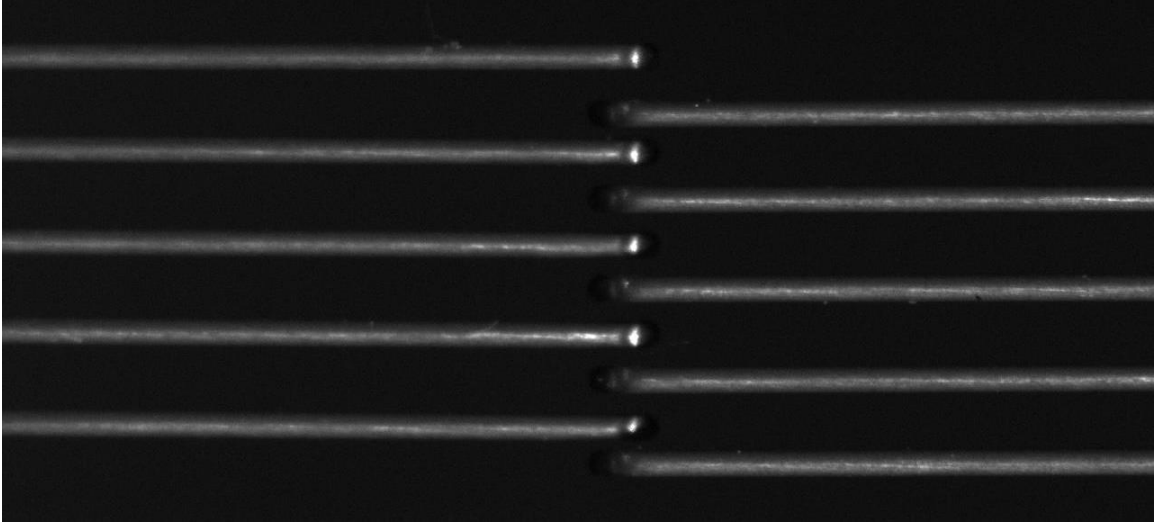
1. Lower the fluid chamber and remove the BioRakes.
2. From the Tools menu select *Advanced* and then select *Centre Position Calibration*. This should bring up this dialogue box:



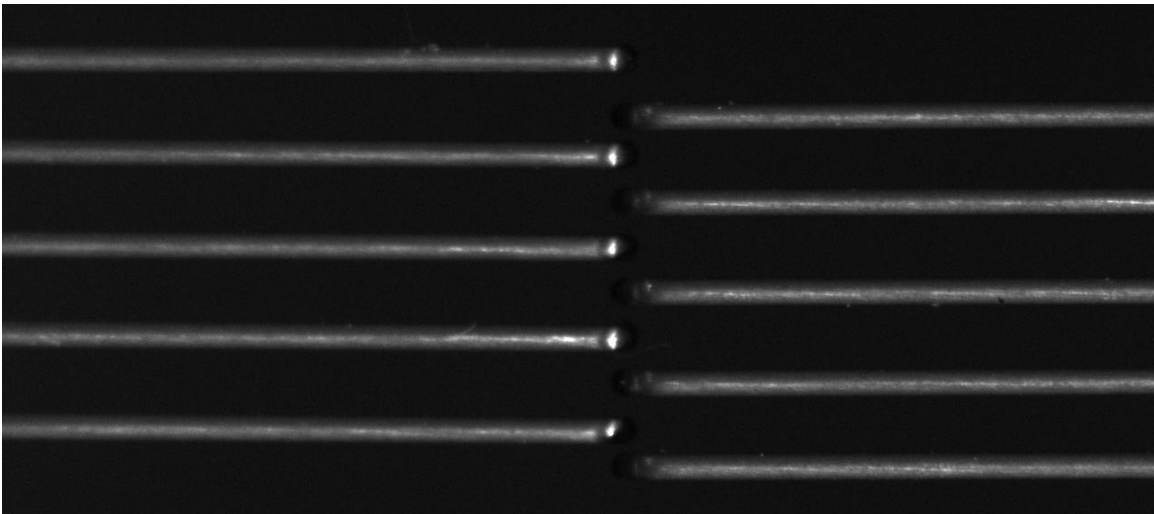
3. Press Yes.
4. Select the *Reset Actuators* button  (or use *Tools>Reset Actuators*).
5. From the Tools menu select *Advanced* then select *Move to Centre*. This should bring up this dialogue box:



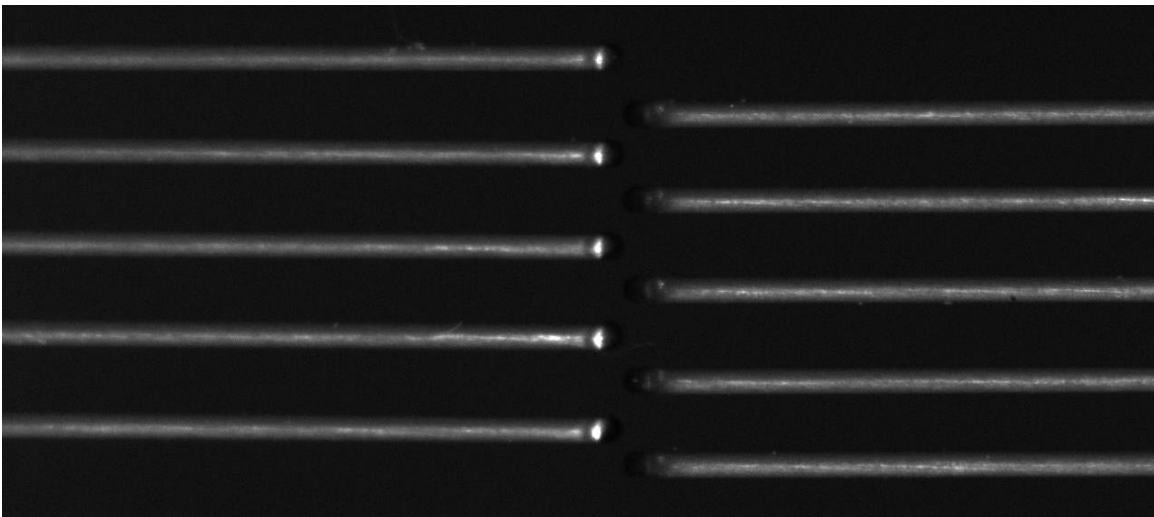
6. Press Yes. Once the actuators have stopped moving, put 2 opposing BioRakes on the X axis.
7. Using the actuator jog control arrows at the lower left of the screen, move the actuators until all 10 BioRake tips are collinear. Make sure the “**mirror matching**” button is selected for this step and **not** the “**independent**” button! Note that it is the tips of the BioRakes that should be collinear and not the outside edge. Because the tips are underneath the BioRakes and cannot be seen on screen, it is necessary to estimate their position. Please use the image below as a reference:



Example 1: BioRakes have too much overlap.

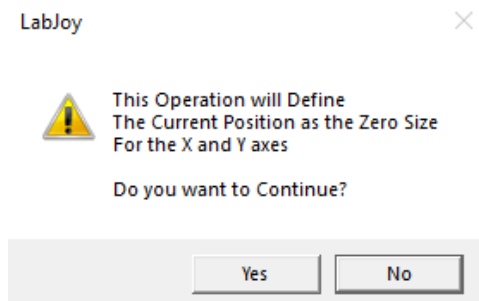


Example 2: BioRakes are correctly positioned.



Example 3: BioRakes do not have any overlap so tips are not collinear.

8. Remove the BioRakes and place them on the Y axis.
9. Using the actuator control arrows, drive the Y actuators until all 10 BioRake tips are collinear as in step 4 (**IN MIRROR MATCHING MODE ONLY!**).
10. From the Tools menu select *Advanced* and select *Centre Position Calibration*. This is the final Centre Position Calibration after temporarily assigning this position in step 2. This should bring up the following dialogue box:



11. Press Yes.
12. Select the reset actuators button  (or use *Tools>Reset Actuators*).

To check the center position calibration, use the Tools>Advanced>Move to Centre command and place 2 opposing BioRakes. Compare the position of the rakes to the image in step 7 above.

After a successful center position calibration, the actuators can be successfully moved to a given sample size before beginning a test (for example, 4700um x 4700 um) and the tips should form a square.



## ***Install Actuator Covers***

Each actuator cover has a number written on the inside. These numbers match up with numbers written on the inside of the main cover to ensure the best possible fit between the actuator covers and the main cover.

The best way to install the covers is to place the back 2 pegs into the main cover and then lower the front 2 pegs while squeezing them together. Be careful not to let the cover slip and accidentally apply a transverse load to the gooseneck.



## ***Perform Load Cell Calibration***

The final step is to perform a load cell calibration. This procedure is detailed in **Section 8** under **Load Cell Calibration**.



## 14. Appendix D: Mechanical Grip Usage

In addition to BioRakes, the BioTester 5000 can optionally be equipped with mechanical grips for sample attachment.

Two types of grips are available: Screw Close (Stainless Steel) and Spring Close (Plastic).

### ***Screw Close Grips (Stainless Steel)***

The screw close grips include an 8-32 screw to close the grip faces, and a spring to keep the grip faces open as the screw is loosened.

These grips can provide a much higher clamping force and are made of stainless steel. It is recommended these be used with higher force load cells (e.g. 5N, 10, or 23N) to avoid overloading or inertial force effects.

The grips and specimen can be installed with the following steps:

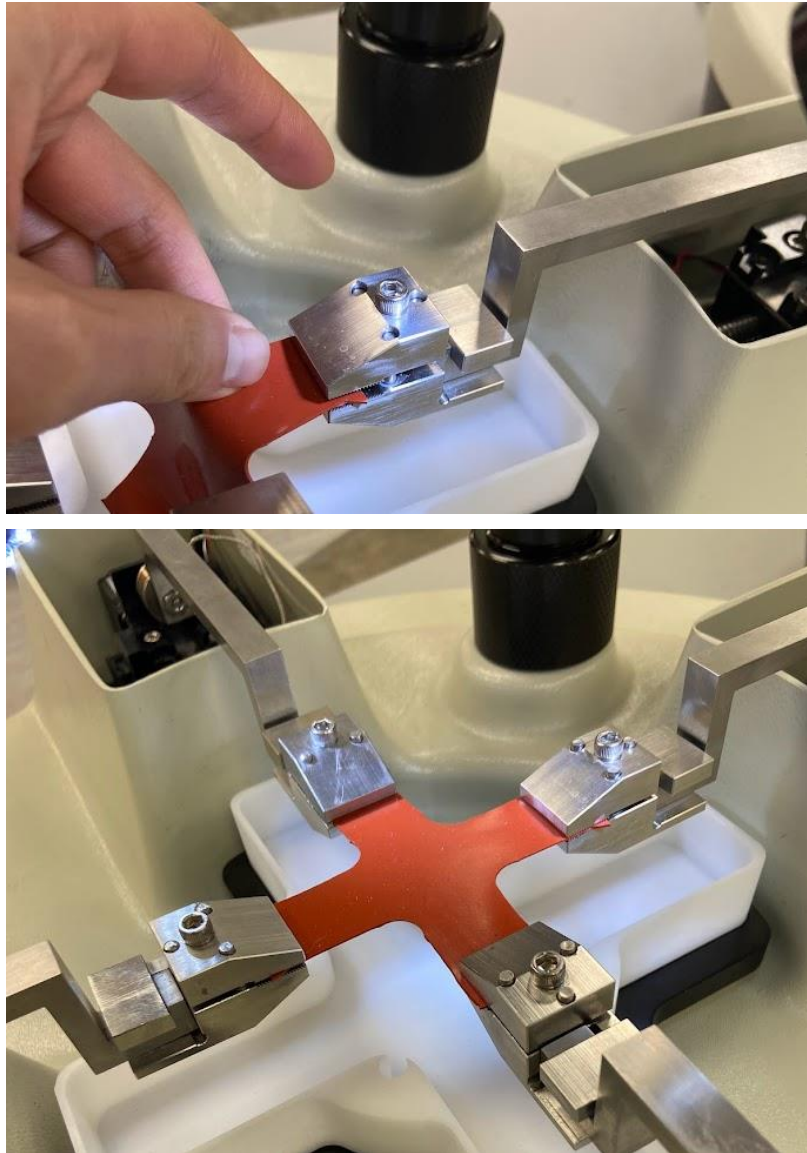
1. Slide each grip onto a gooseneck. For uniaxial testing, only install 2 grips along a single axis.



2. Loosen the 8-32 fastener with a 9/64" hex key.



3. Jog the actuators to fit the size of the specimen.
4. Place the specimen on the grip face and tighten the fastener. **Be careful to not apply excessive downward force on the screw!**



## Spring Close Grips (Plastic)

The spring close grips can be equipped with three different springs of varying stiffness to exert different gripping forces. These springs are provided with the grips and may be switched at any time using the provided hook tool.

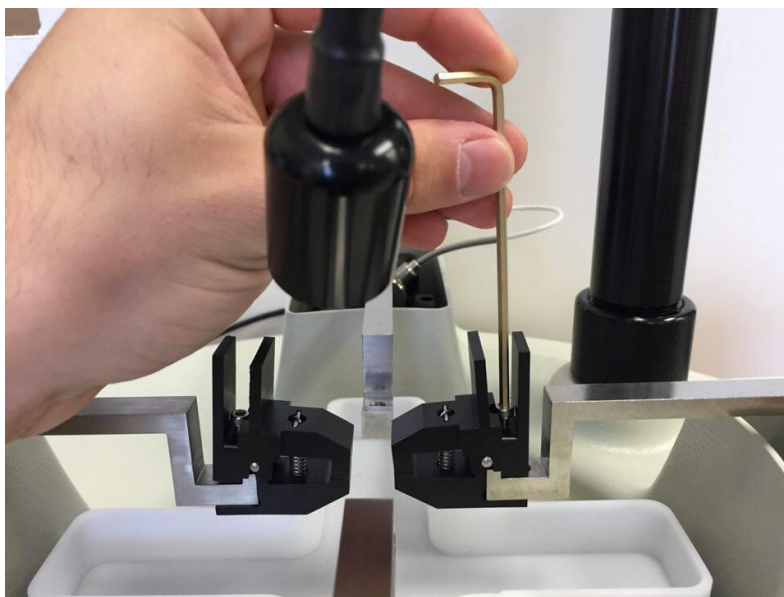
The light, medium and heavy springs will exert **1.5N**, **6N**, and **15N** of gripping force respectively.

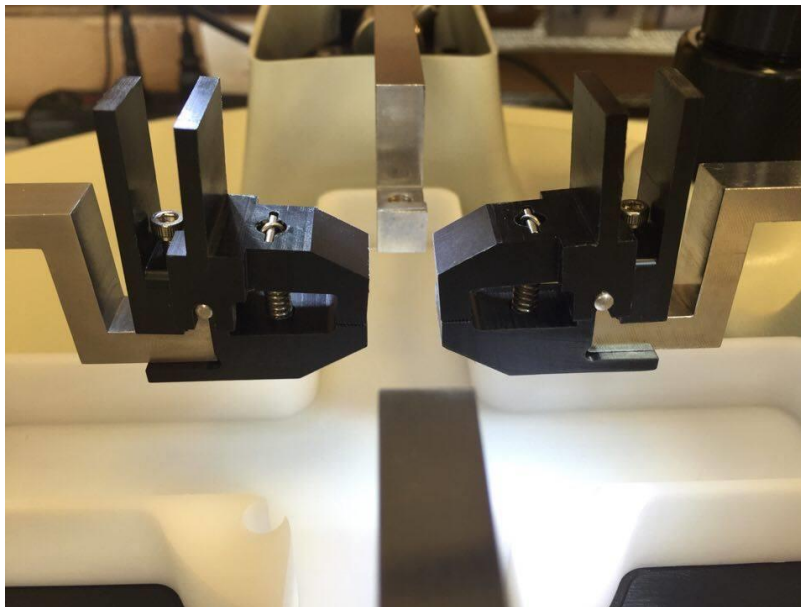
### System Alert

*Select the appropriate grip spring for the load cell in use. Using a grip with the stiffest spring in combination with a low capacity load cell (0.5-2.5N) may produce high forces when mounting a sample. Load cells can be damaged by large off-axis or bending forces.*

The grips can be attached using the following steps:

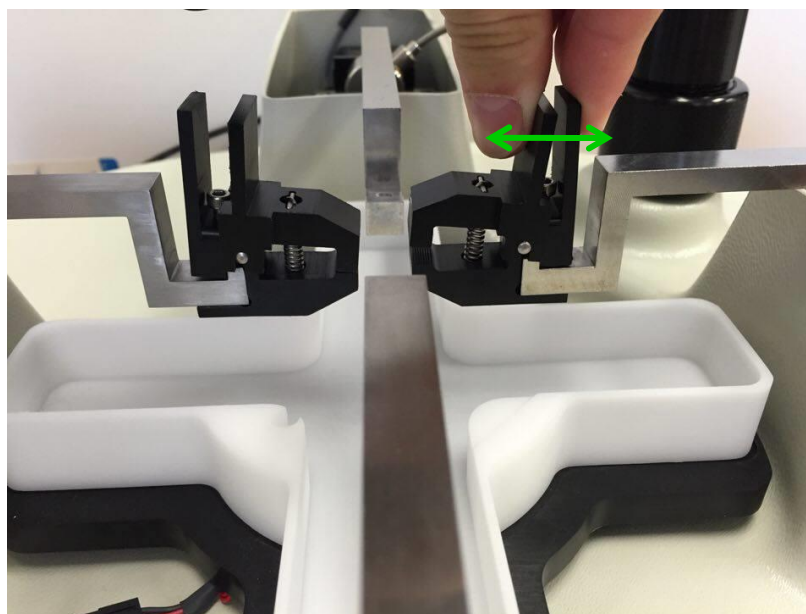
1. Slide the grips onto the goosenecks as pictured below.
2. Tighten the locking fastener to hold the grips in place on the gooseneck. Do not over-tighten the locking fastener as this may push the tip of the grip out of alignment.





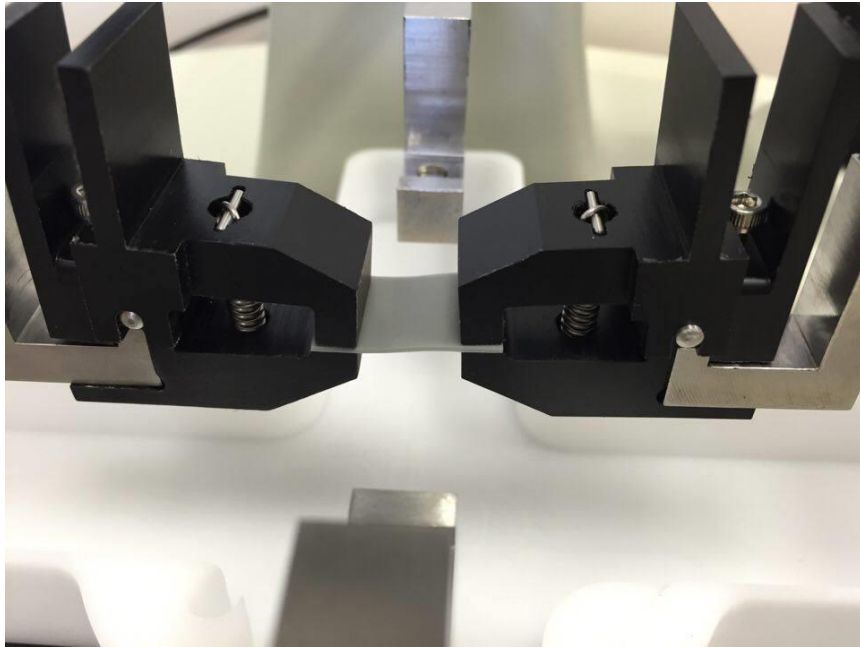
### Sample Mounting

Squeeze the grip levers to open the jaws of the grip. Slide the end of a test specimen between the jaws and gently release the levers. Specimens may be mounted and tested using 2 grips (uniaxial) or 4 grips (biaxial).

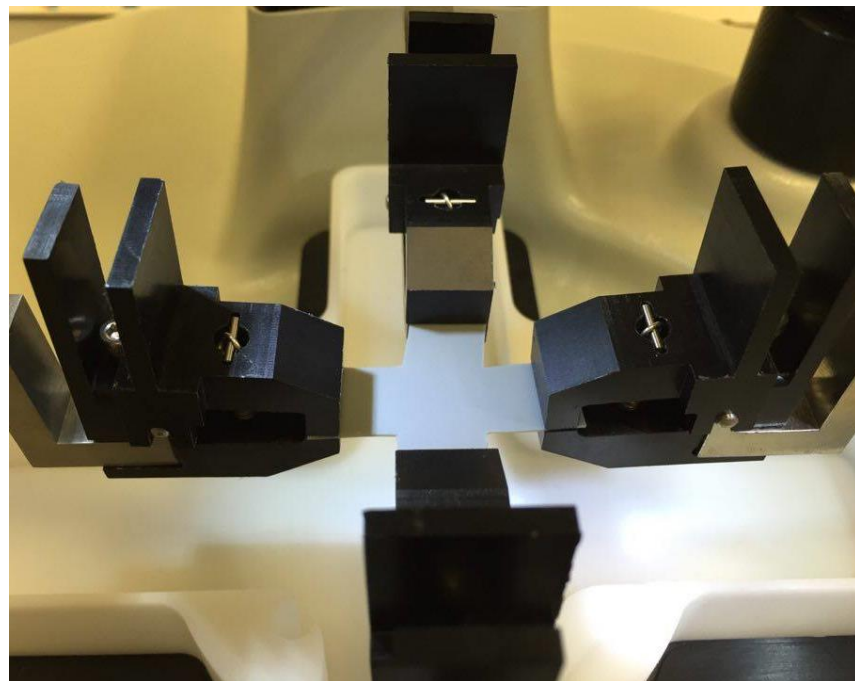


#### **System Alert**

*When squeezing the grip levers to open the jaws, exert force as much as possible in a linear direction through the body of the load cell to avoid off-axis or bending forces.*



*Figure 1 - Uniaxial Test Setup*

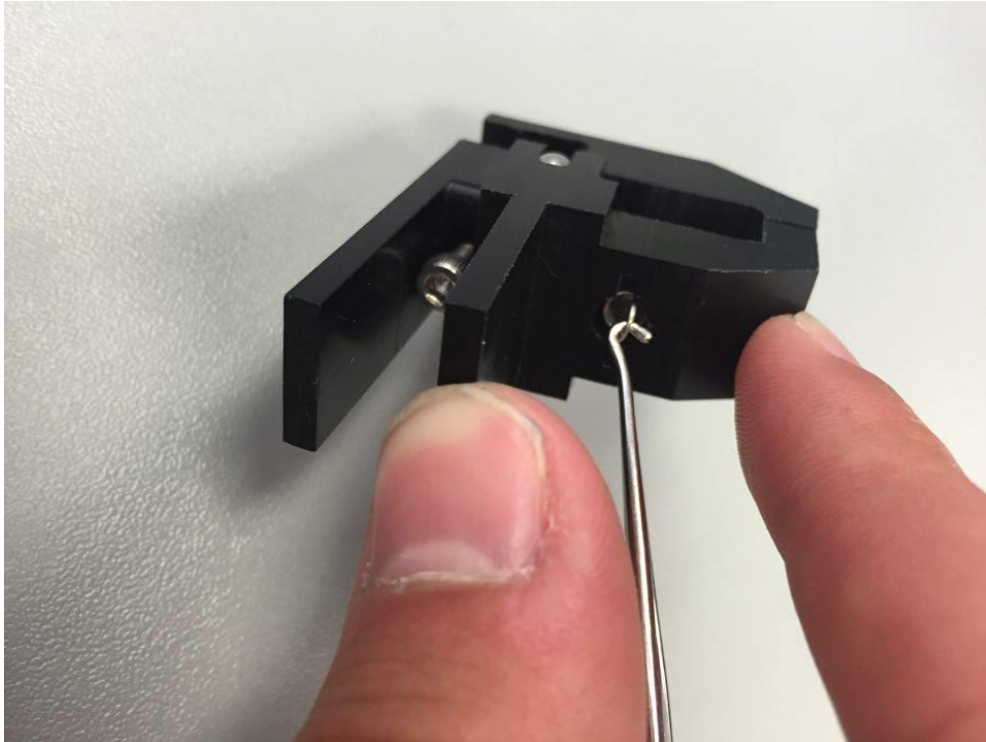


*Figure 2 - Biaxial Test Setup*



## Changing Grip Springs

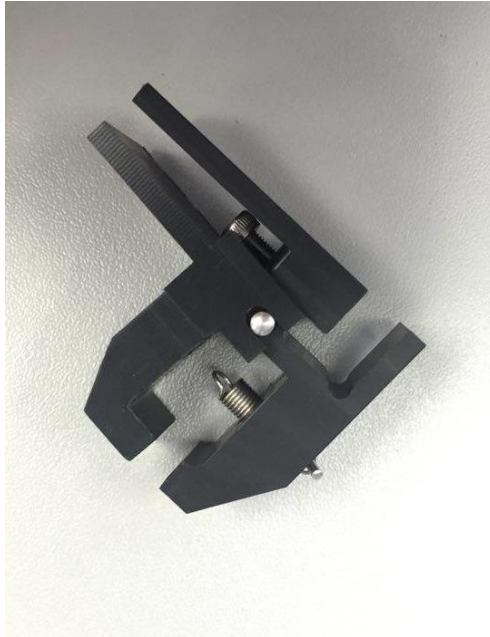
1. Using the hook tool provided, pull on the spring and remove the metal dowel holding the grip spring in place. After this step, the spring may be slowly released.



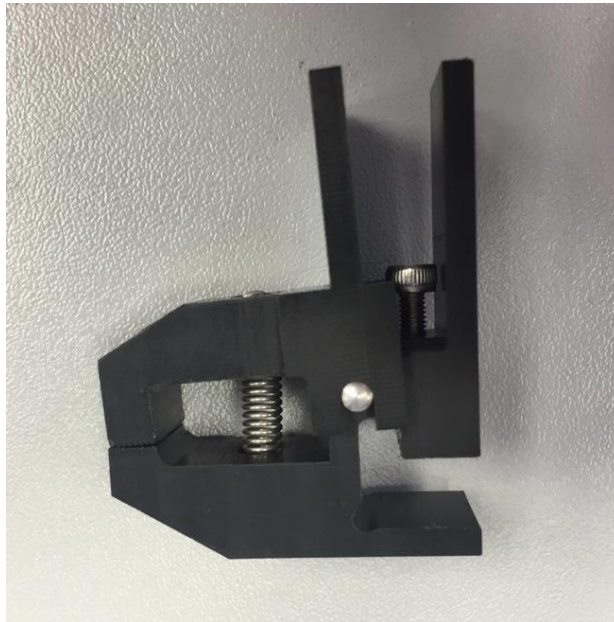
### **System Alert**

*A fully installed spring experiences a high amount of tension. When the springs are disengaged, be sure not to let the hook tool slip or the spring may be released at high velocity. **The use of safety glasses is recommended.***

2. Replace the old spring with a new spring inside the mounting hole.



3. Secure one side of the spring onto the grip with the metal dowel
4. Use the hook tool to pull the other side of the spring into place and secure it with another metal dowel (Note: This step may be easier with two people).

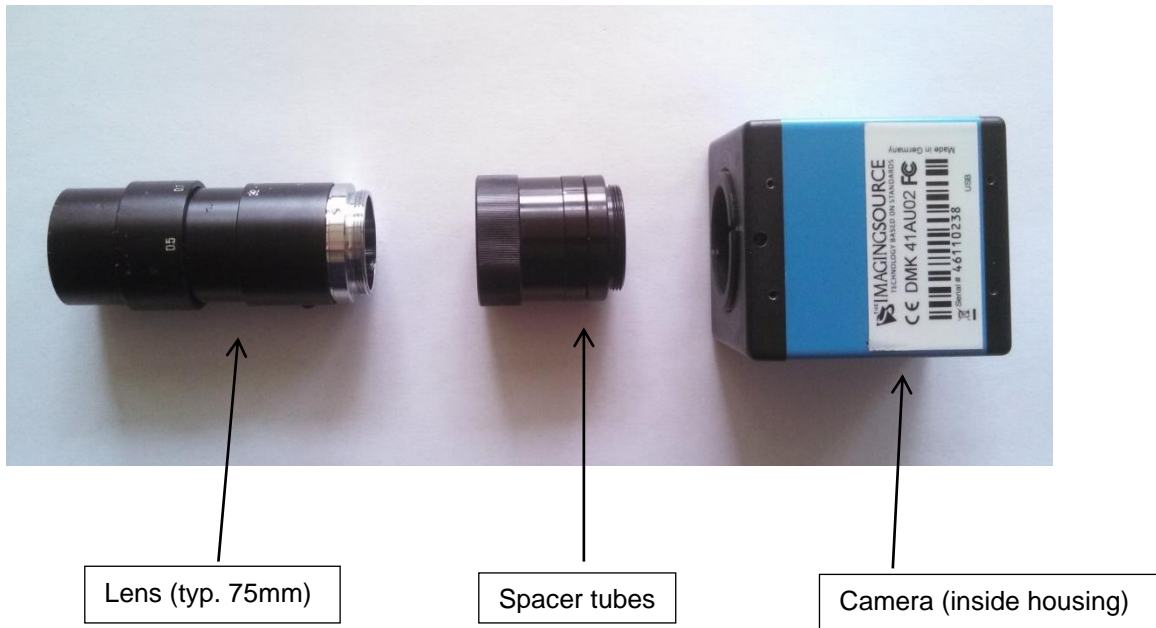


## 15. Appendix E: Fixed Focal Length Lens

In addition to the 10X zoom lens described in Section 8, the BioTester 5000 system can also use fixed focal length lenses. Other BioTester 5000 models may have a 75mm focal length lens and a set of lens spacers. Other focal lengths are available and are compatible with the BioTester 5000 system.

### ***Install Camera Lens***

Remove the protective caps on the lens and camera body and then screw the lens into the appropriate series of spacer tubes. The correct camera setup can be fine-tuned at a later step. The spacer tube is then screwed into the camera body. Spacer tubes are placed between the lens and the camera body to change the image scale. One 40mm spacer (largest one) is a good starting point for setting up the camera. The spacers also affect the working distance of the lens and therefore the camera housing may need to be moved up or down on the camera mast to accommodate changes in the number of spacer tubes.







## ***Adjusting Camera Position and Image Magnification***

The camera position can be adjusted using the knobs at the back and side of the camera housing. If larger position adjustments are needed, the bolts that attach the camera to the mast can be loosened to allow rotation of the camera housing.

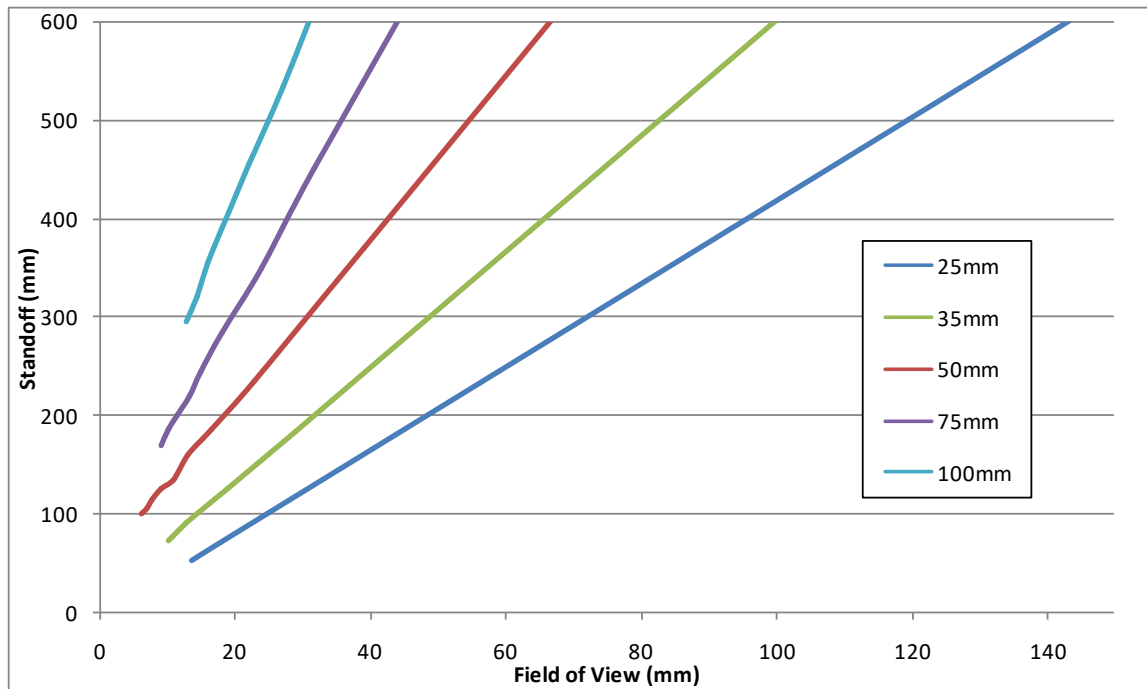
Using the 75mm lens and lens spacers, it is possible to achieve a field of view ranging from 9mm to 30mm. The details of the appropriate number of spacers and the required standoff are shown in the table below. The image magnification can be changed by adding or removing spacer tubes located between the camera body and the lens. Increasing the total spacer tube length will result in higher magnification while reducing the tube length will result in lower magnification. Changing the tube length will also change the working distance of the lens. To adjust the vertical position of the lens and camera assembly, loosen the bolts that clamp the camera housing to the mast and raise or lower the camera housing until the image is in focus. It is helpful to make sure the lens focus ring is in the middle of its rotation so that fine tuning the image focus can be done once the camera housing is re-clamped.

**INCREASING THE TOTAL LENGTH OF THE SPACER TUBES WILL INCREASE MAGNIFICATION (DECREASE FIELD OF VIEW) AND DECREASE WORKING DISTANCE (STAND-OFF).**

If the camera position or magnification is changed, it is important to capture a single image scale shot. A glass calibration target or a ruler is acceptable for this purpose. The scale shot will be useful later to characterize the size of test specimens or test specimen features.

As the table and graph also show, it is also possible to swap in a lens with a different focal length to achieve a different range of magnifications. This data was collected using the family of fixed focal length lenses available from Edmund Optics (the part numbers are shown in the table). The camera has a C-mount connection, making it possible to mount a wide variety of optical components to best suit any application. Currently, only the standard camera is supported.

	25mm (NT56-529)			35mm (NT56-530)			50mm (NT56-531)			75mm (NT56-532)			100mm (NT56-675)		
Spacer (mm)	Stand off (mm)	pix/c m	FOV (mm)	Stand off (mm)	pix/c m	FOV (mm)	Stand off (mm)	pix/c m	FOV (mm)	Stand off (mm)	pix/c m	FOV (mm)	Stand off (mm)	pix/c m	FOV (mm)
0	685	78.6	162.8	1070	71	179.3	930	121	105.9						
5	100	520	24.6	215	373	34.3	370	329	38.9	820	206	62.2	1540	147	86.8
10	53	935	13.7	125	682	18.8	235	560	22.9	450	404	31.7	950	258	49.6
15				90	993	12.9	185	779	16.4	350	534	24.0	675	366	34.9
20				73	1273	10.1	160	978	13.1	285	711	18.0	535	473	27.0
25							135	1186	10.8	245	859	14.9	455	578	22.1
30							125	1410	9.1	220	959	13.3	400	687	18.6
35							115	1630	7.9	200	1111	11.5	355	797	16.1
40							105	1842	6.9	185	1260	10.2	320	897	14.3
45							100	2068	6.2	170	1392	9.2	295	1003	12.8



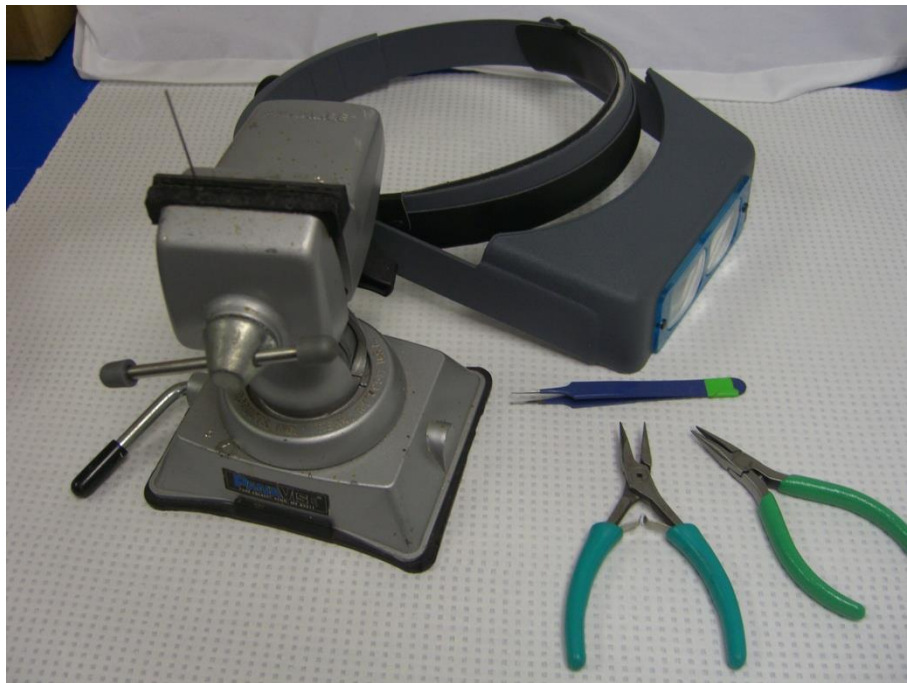
NOTE: The physical camera standoff will not go below 100mm or exceed 500mm, the preceding table and chart provides guidelines only

## 16. Appendix F: Protocol for Tying and Cutting Sutures for Biaxial Testing

In addition to BioRakes and grips, the BioTester 5000 can optionally be equipped with bracket and pulley system to attach samples using sutures.

There are many ways to attach specimens to the suture brackets using a variety of suture designs. This procedure outlines one way to use double-armed sutures to accomplish specimen attachment.

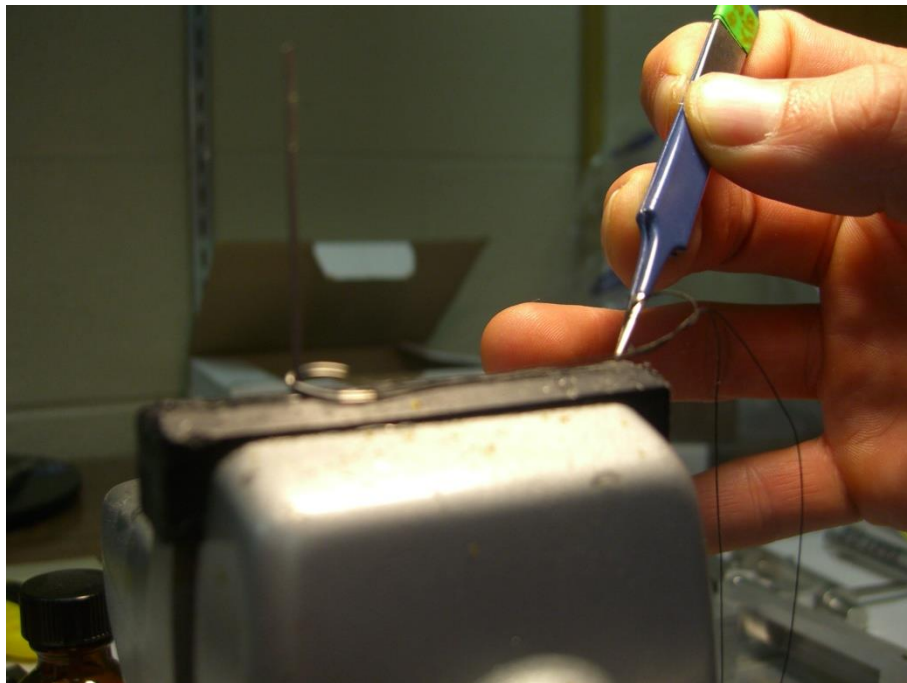
1. Assemble equipment: small vice, magnifying goggles, 2 needle nose pliers, small forceps.



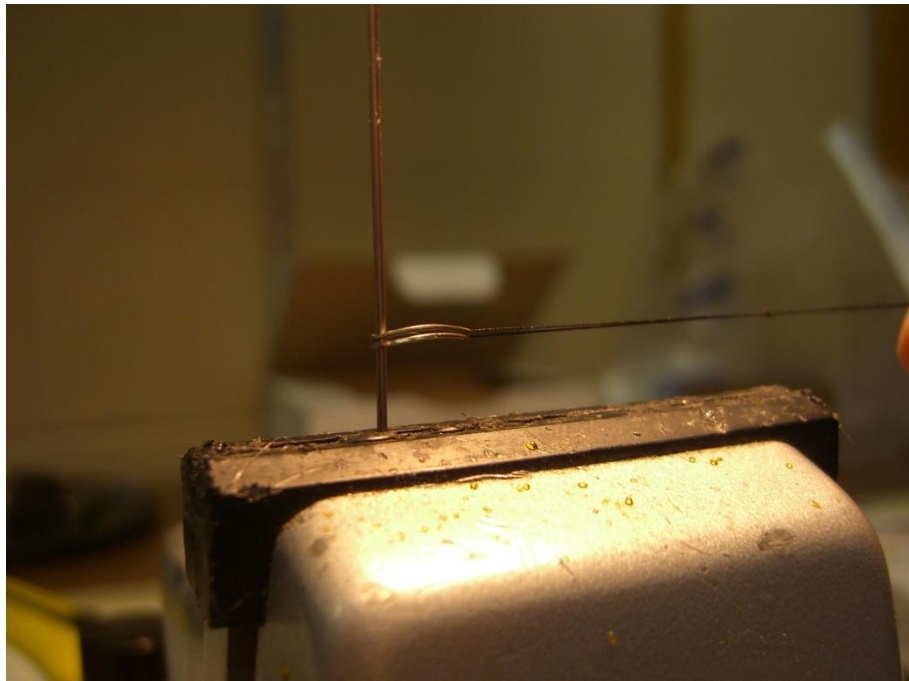
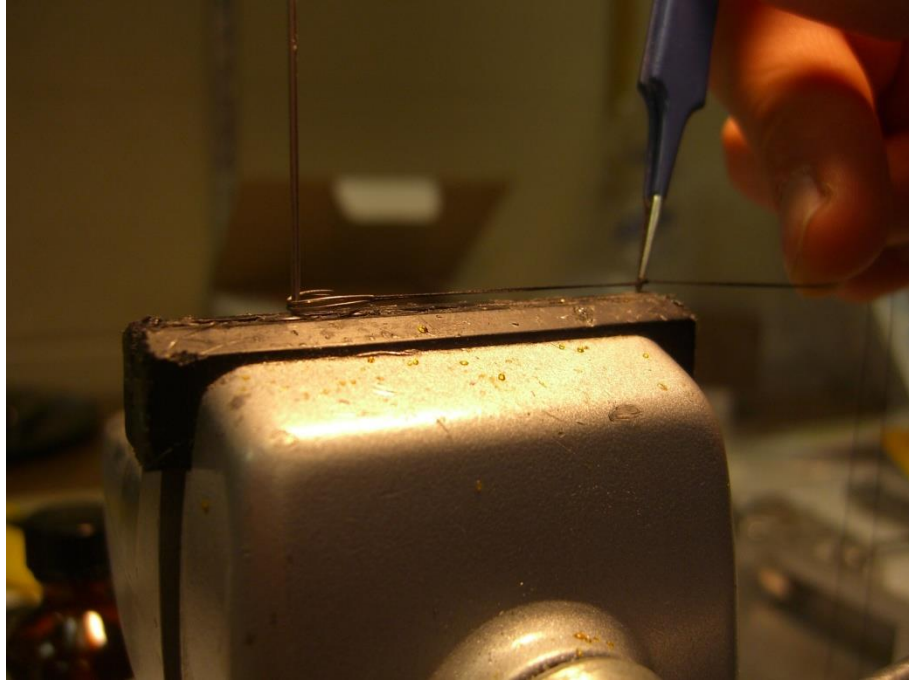
2. Bend suture hooks



3. Tie off sutures. First hook each bent hook around a secured post held in the vice. Then tie a standard knot in the suture and use forceps to guide the location of the knot so that it tightens at the correct location.







4. Use pliers to tighten knot



5. Cut off excess.





The resulting mounted specimen should ideally look something like this:

