



BioTester 3000

Biaxial Test System

***User Manual
version 8.1***



Mechanical measurement and analysis of biomedical materials

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

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

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

Explore our web site www.cellscale.com or contact us to learn more about our measurement systems.

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Table of Contents

1. General Information.....	1
Environmental and Electrical Specifications	1
System Assembly.....	1
Power Connections	1
Safety Warnings.....	1
Approvals and Certification	1
Manual Operating Controls	2
Software	2
General Maintenance.....	2
2. Testing Terminology	3
Multiphase Test Cycles	3
Phases, Cycles, and Test Sequences	4
Test Phases: The Smallest Unit of Testing.....	5
Control Modes.....	5
Control Functions	6
3. System Overview	7
System Components.....	7
Software Overview	8
Output Files and Data Structures.....	9
4. Setting Up & Starting a Test.....	10
Overview	10
Step 1: Start a New Test.....	10
Step 2: Reset the Actuators	11
Step 3: Move the Actuators to a Specified Size.....	12
Step 4: Zero the Load Cells (occasionally)	12
Step 5: Modify Testing Parameters (optional)	13
Step 6: Mount the Specimen.....	15
Step 7: Execute the Test.....	17
Step 8: Terminate the Test Prematurely (optional)	17
Step 9: End of Test Controls (optional)	18
5. Additional Settings.....	19
Configuring Output Data Files.....	19
Advanced Settings	20
Motion Control Settings.....	21
Range Limits	23

Configuring the Live Charting Graphs	24
6. System Hardware Settings	26
Controller	26
Load Cells	26
External Sync Pulse	27
Heater (optional)	28
Camera (optional)	29
7. System Calibration and Advanced Tools	30
Load Cell Calibration	30
Snap Image Feature	32
8. Troubleshooting	33
9. Appendix A: Initial System Setup	34
Tools	34
Fasteners & Parts	34
Unpack System	35
Remove the BioTester 3000 from the shipping container	35
Attach Camera Mast (optional)	36
Place Fluid Chamber on Riser Stage (optional)	37
Install Camera Lens (optional)	39
10. Appendix B: Software Installation	40
11. Appendix C: Install Load Cells / Perform Camera and Gooseneck Alignment	46
Attach the Load Cells to the Goosenecks	46
Attach the Load Cell Brackets to the Actuators	50
Attach the Goosenecks to the Actuators	51
Stow Cables and Plug in the Load Cells	54
Turn on the BioTester 3000	56
Launch the LabJoy Software	56
Perform Camera Alignment (optional)	59
Perform Vertical Gooseneck Alignment	62
Perform Transverse and Axial Gooseneck Alignment	64
Install Actuator Covers	68
Perform Load Cell Calibration	68
12. Appendix D: Reviewing Test Results	69
Overview	69
Selecting Images	70
Image Playback Options	70
Image Tracking: Overview	71

Image Tracking: The Points Display Option	73
Image Tracking: The Displacement Option	74
Image Tracking: The Strains Option	75
Data Overlay	76
Exporting Tracked Data	77
Exporting Images and Movies.....	78
Importing and Analyzing Non- BioTester 3000 Images	78
13. Appendix E: Mechanical Grip Usage	79
Installing Grips	79
Sample Mounting	80
Changing Grip Springs.....	82
14. Appendix F: Specimen Mounting with Temperature Controlled Fluid Bath	84
Adjusting the Camera Position and Image Magnification	86
15. Appendix G: Protocol for Tying and Cutting Sutures for Biaxial Testing	88

1. General Information

The BioTester 3000 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

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

System Assembly

The BioTester 3000 unit requires some assembly depending on what options you have chosen for you system, please see Appendix A and C for further instructions on assembly.

Power Connections

Connect power supply into properly grounded 100-240VAC power source to ensure safe operation. Ensure that the power cord is easily accessible at all times. 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 EN61010-1:2001 and EN61326-1.

Manual Operating Controls

There are a few manual controls included with the optional camera and heated fluid bath if they were included. The camera housing contains two adjustment knobs that are used for fine adjustment of the camera position. The camera lens has 3 rings which control the focus, zoom, and iris which can be set by the user.

BioTester 3000 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 is one switch on the front of the unit for powering the system on and off.

If your system has the upgraded temperature-controlled fluid bath there will be an additional switch to control the heater. As well the front of the unit will also contain 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 3000. 1 x USB port is required for data connections and 1 more USB port is required if the system has the optional camera. Windows 7 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 3000 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 supplied large syringe works well to empty the dish).

2. Testing Terminology

The BioTester 3000 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 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:

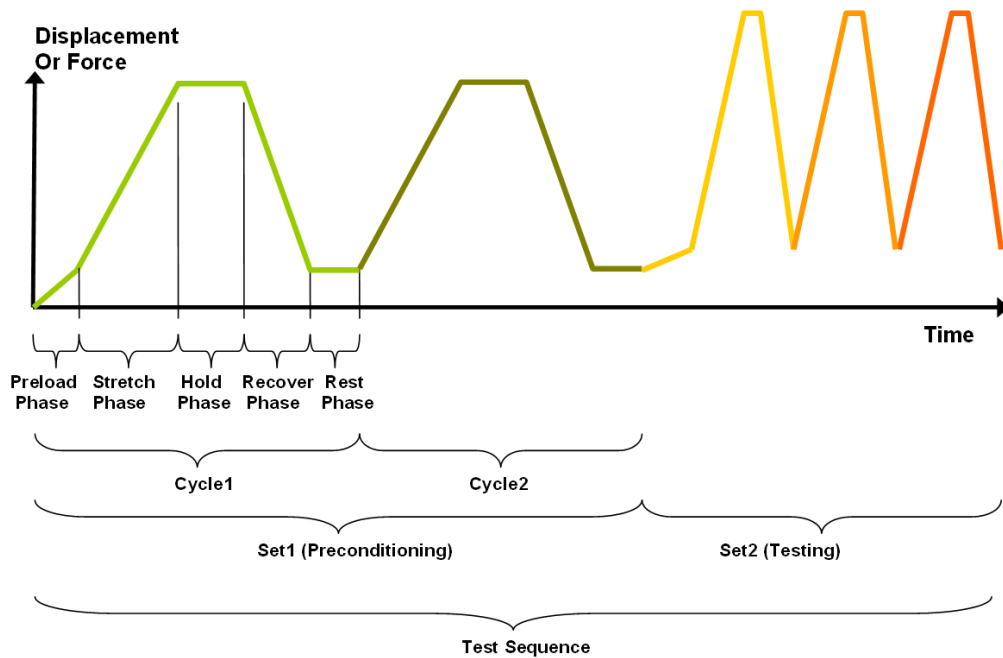
Preconditioning - The goal of preconditioning is to restore a specimen to its physiological or *in vivo* state. During the process of specimen storage and preparation, a specimen may swell, dry out, have its material fibers realign, or its molecules reorganize. It may take multiple preconditioning cycles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

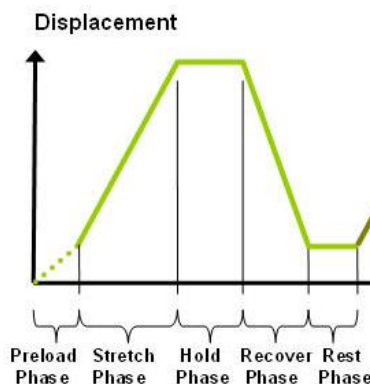
Preloading- Preloads are applied to bring a test to a well-defined starting point. Because the dimensions of a specimen may change as a result of a loading cycle (stretching of fibers, viscoelastic effects, plastic deformation, or localized material failure at the attachment points), the preload adjustment 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 3000 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 3000 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 only be applied as a step function.

BioTester 3000 Note

The standard system only comes with displacement control capabilities. The software can be upgraded to unlock the force control mode, please speak with your sales representative about this.

Control Functions

The BioTester 3000 makes it possible to test specimens under several control functions:

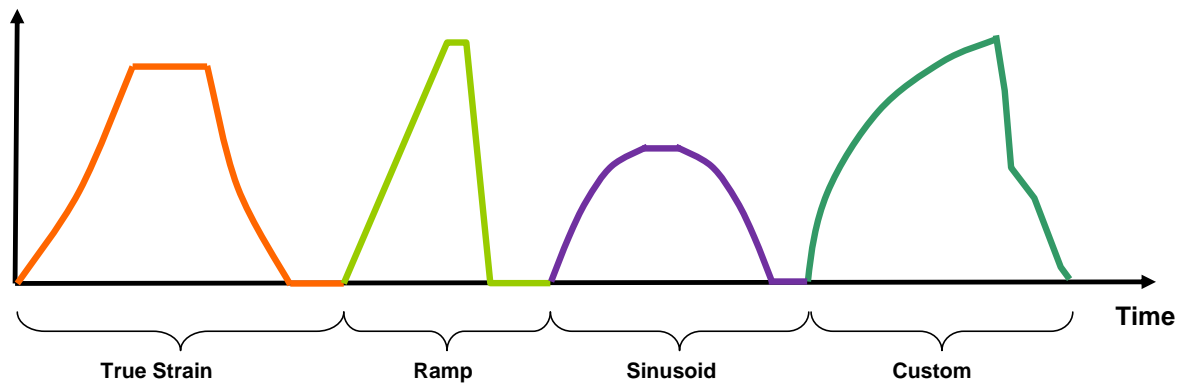
Under displacement control:

The **true strain function** applies the displacement at a constant true strain rate, which accounts for the current specimen length while the specimen is being stretched.

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

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

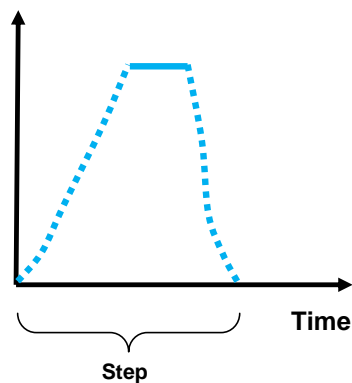
Displacement



Under force control:

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

Force

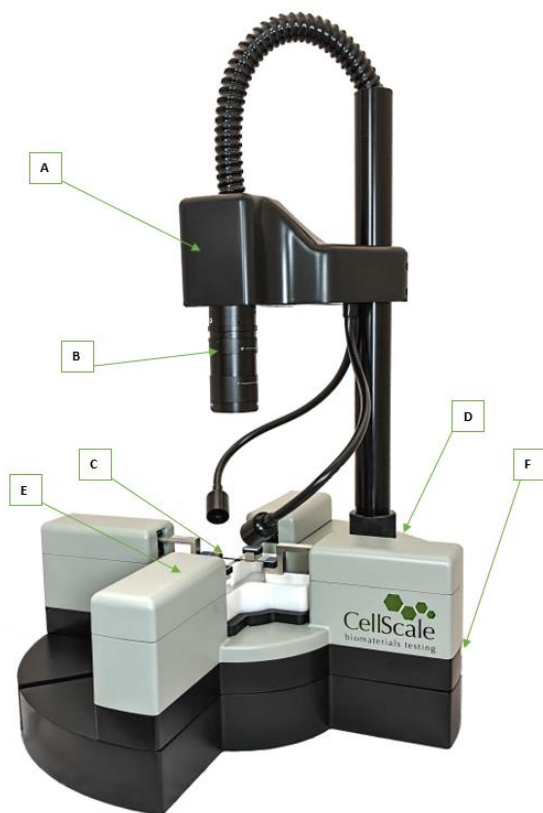


The control mode, load function and load magnitude can be set independently for each of the two loading directions.

3. System Overview

System Components

The BioTester 3000 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. (Optional Upgrade)

B Illumination and lens provided to capture excellent image quality. (Optional Upgrade)

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.

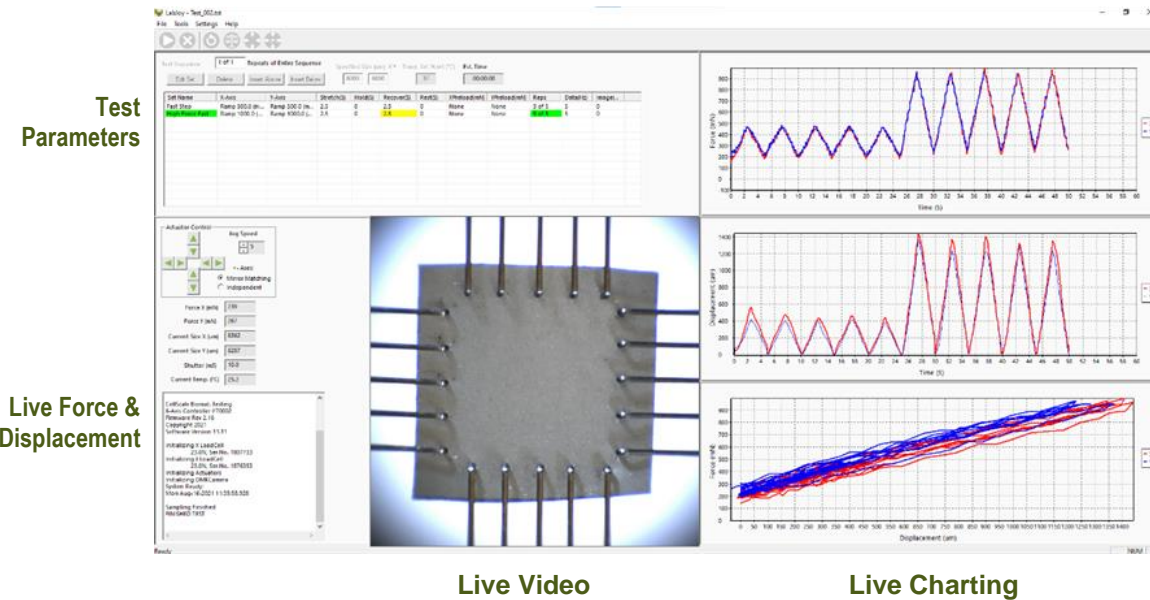
E High resolution control delivers precision measurement on small samples.

F A simple USB interface for easy connection to the host computer.

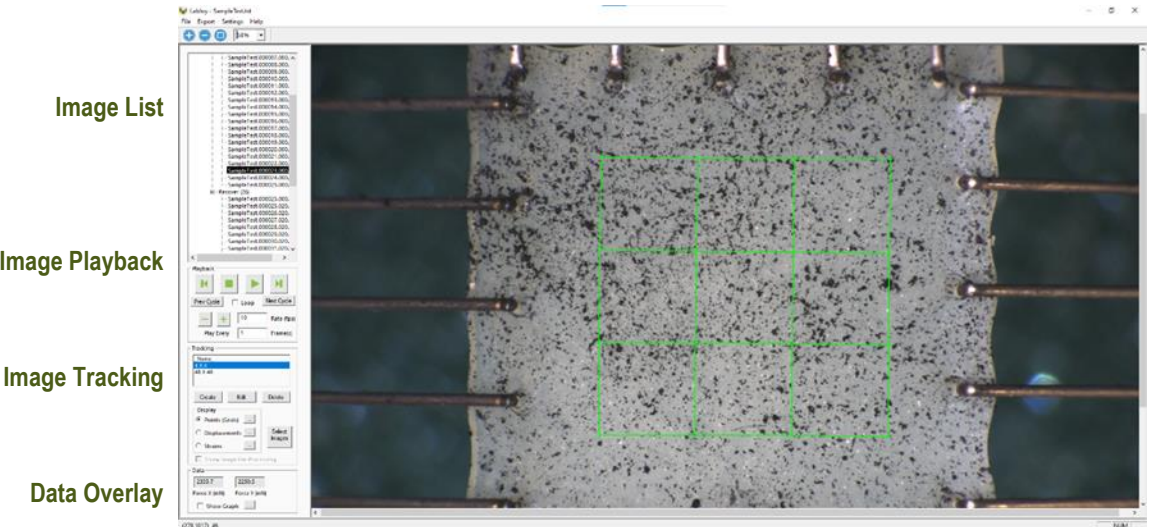
The BioTester 3000

Software Overview

The software included with the BioTester 3000 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 review and analysis module is used to playback accelerated or decelerated test images, perform image analysis and tracking, and output video files for presentation purposes. The screen layout for this module is shown below. The review and analysis module is only compatible with the upgraded camera system:



Output Files and Data Structures

For each test, the BioTester 3000 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 reference to troubleshoot problems with your system, should they occur.

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

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

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

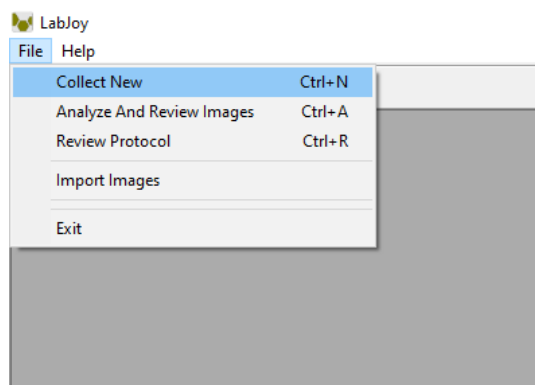
Overview

Setting up and running a new test is a simple process made up of both mandatory and optional steps. The following list presents all of 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 3000 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.

Create Test From Template

Select

demo

Test Name

Test_001

Data Directory

C:\Cellscale\Labjoy\Data

Browse

Template Directory

C:\Cellscale\Labjoy\Templates

Browse

OK Cancel


BioTester 3000 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 3000 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.

Please ensure that you have no specimen mounted ×



Are you sure you want to Reset Actuators now?

Yes


No

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*.

Specified Size (um) X Y

4700

4700

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 dialog will appear.

Note that displacements are specified in either % strain, or μm . Force loads are specified in mN, and durations are specified in seconds.

The following table describes each of the parameters.

Set Parameter Editor

Name

Precon1

(Example: "Precon1", "HoldA")

Same As X

X Axis

Control Mode

Displacement

Control Function

Ramp

Stretch Magnitude

10

%

Preload

☒ Not Applied

☐ Applied on 1st Repetition Only

☐ ReApply On Every Repetition

Preload Magnitude (mN)

100

Y Axis

Control Mode

Displacement

Control Function

Ramp

Stretch Magnitude

10

%

Preload

☒ Not Applied

☐ Applied on 1st Repetition Only

☐ ReApply On Every Repetition

Preload Magnitude (mN)

100

Cycle

Stretch Duration (S)

20

☐ Do Not Stretch

Repetitions

3

Hold Duration (S)

0

Data Output Frequency (Hz)

1

Recovery Duration (S)

20

☐ Do Not Recover

Image Output Frequency (Hz)

1

Rest Duration

0

Edit Custom Load Function

OK

Cancel

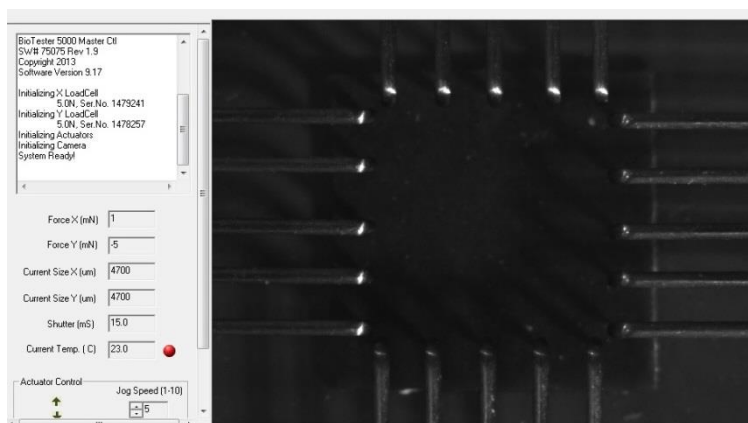
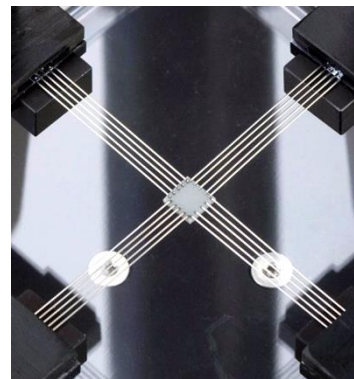
Test Parameter	Description
Control Mode	<p>Biaxial tests are typically performed under displacement control, however, you can select the following control modes to achieve specific testing objectives:</p> <ul style="list-style-type: none"> To obtain uniaxial properties for a biaxially mounted specimen, specify displacement control in one axis and Force control with zero Load Magnitude in the other axis. For creep testing, use force control with a long hold duration. For relaxation testing, use displacement control with a long hold duration.
Control Function	<p>True Strain or Ramp is typically selected for tests performed in displacement control mode. For tests performed in force control mode, you select step controls.</p>
Stretch Magnitude (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 μm or as a percentage. For example, the displacement on a $5000\mu\text{m} \times 5000\mu\text{m}$ specimen can be expressed as either $500\mu\text{m}$ 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>
Preload	<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>
Preload Magnitude	<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>
Stretch Duration	<p>For evenly spaced images, it is recommended to choose a number that is an integer multiple of the Image Output Frequency.</p>
Hold Duration	<p>Hold Duration is typically set to 0, however it is useful for creep or relaxation testing.</p>
Recovery Duration	<p>Recovery Duration is typically set to the same value as the Stretch Duration.</p>
Rest Duration	<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>
Repetitions	<p>Apply enough repetitions until the force deformation curves from one repetition to the next start to overlie each other.</p>
Data Output Frequency	<p>Typically set to the same frequency as the image output frequency.</p>
Image Output Frequency	<p>Typically set to 1Hz for cycles > 5 seconds and 10 Hz for cycles < 5 seconds.</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. If you have the temperature-controlled fluid bath, please refer to Appendix F for specimen mounting instructions.

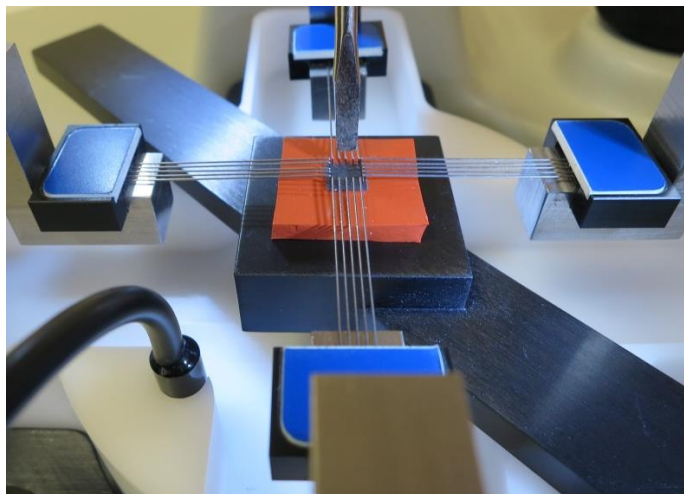
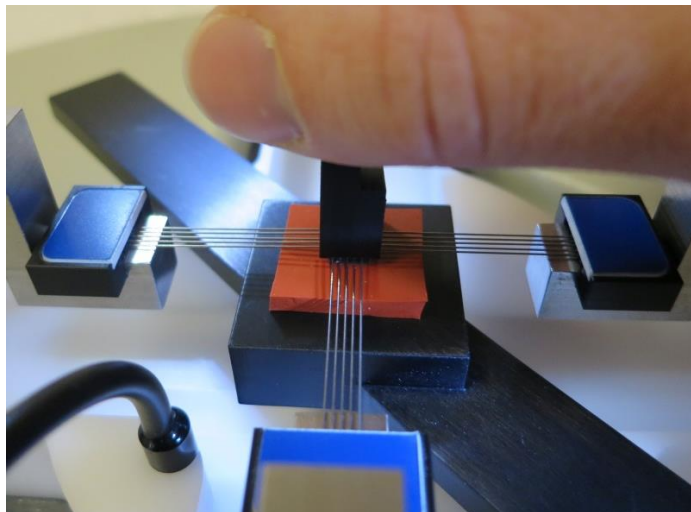
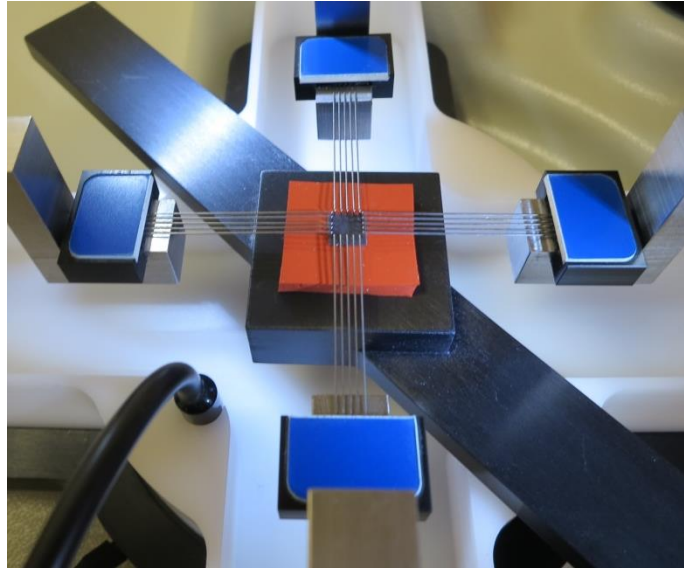
To mount the specimen, follow these steps:

1. Place the specimen in the mounting press on top of a backing material such as soft silicon or foam.
2. Place the mounting bridge under the BioRakes
3. Partially raise the mounting bridge.
4. Centre the specimen between the rakes using a small implement, such as forceps.
5. Raise the bridge until the rake tines are pressing gently on the specimen.
6. Push all the BioRake 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. Slowly lower the mounting bridge and ensure the sample stays mounted to the BioRakes.



System Note

If the temperature controlled fluid bath was included with your system, specimen mounting instructions can be found in Appendix A




BioTester 3000 Tip: Ideal Specimen Size

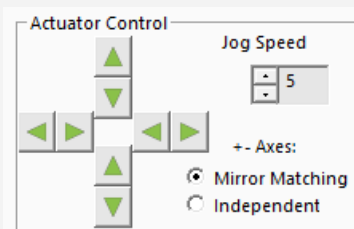
The BioTester 3000 is designed for small specimen sizes. For Standard BioRakes (5 tines spaced at 1 mm) the ideal specimen size is square with side lengths of 5.5 - 6 mm. If a specimen is not in the ideal range, it may still be mounted, but the placement of the rake tips will need to be adjusted. Also, 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 of terminating a test opposed to powering the equipment off, as it allows the software to store the current actuator position.

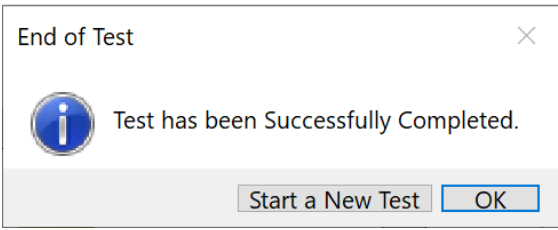
At the end of the completed test (both normally or prematurely terminated) the actuators will maintain their current position.

BioTester 3000 Tip: Specimen Thickness

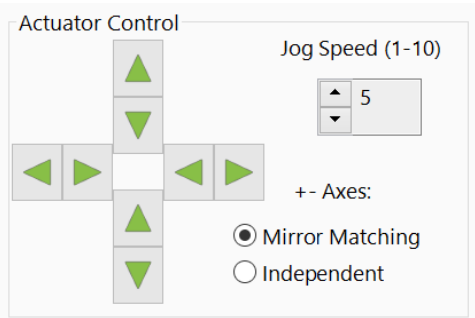
Measuring the specimen thickness before and/or after a test is performed will allow stress calculations to be made from your output data.

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.



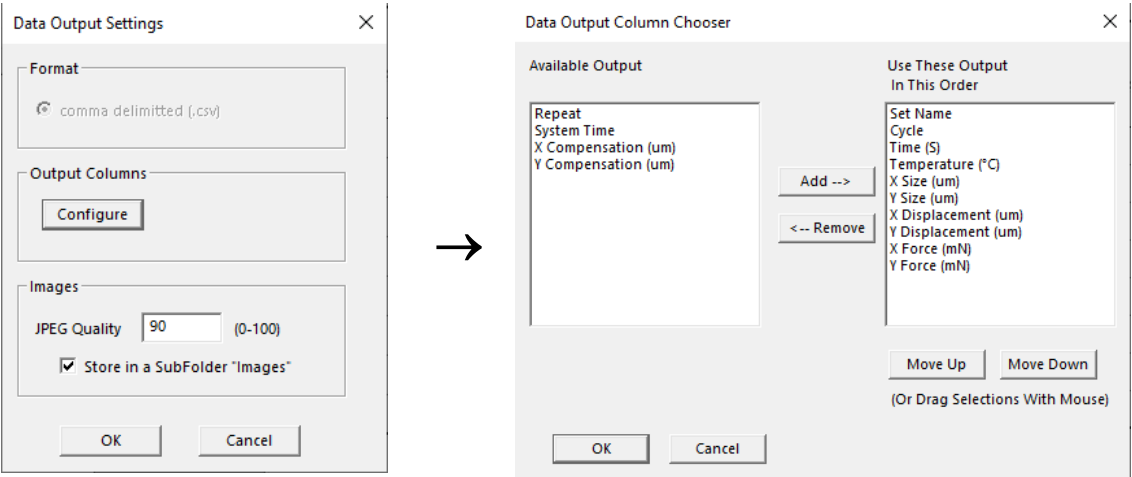
System controls include the top ribbon as well as Actuator Control. These controls can be useful if you need to release a specimen from the text fixtures at the end of a test.



5.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.

Advanced Settings

Show Warnings

☒ Before Reset Actuators

☐ Before Zero Load Cells

System Stiffness Compensation

☒ Enable ☐ Always

☒ Auto

Note: To Save these settings to a Template
Select Save As Template from the File Menu.

OK Cancel

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.

Motion Control Settings

Motion Control Settings

X Axis Y Axis

Max. Velocity (1-10) (Force Control) 6 6

Acceleration (1-10) 10 10

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

☐ Enable Control Algorithm Settings Editing (Advanced) Reset to Hardware Defaults

Control Mode Force Force

Algorithm

☒ Adaptive ☐ PID

Kp 5 5

Ki 0 0

Kd 0 0

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

Apply Done

Velocity (1-10 integer values) matches actuator jog speed and sets the maximum speed at which the actuators can travel in a force-controlled mode. Typically for stiffer materials, this value should be between 6 and 8 and for soft materials, this value should be between 8 and 10.


Acceleration (1-10 integer values). Typically, this should be set to the default value of 10 (maximum acceleration). 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 testing cycles.

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 BioTester3000, 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.

BioTester 3000 Tip: Testing Force Control Settings

It is important to have appropriate Force Control Settings that match the materials 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

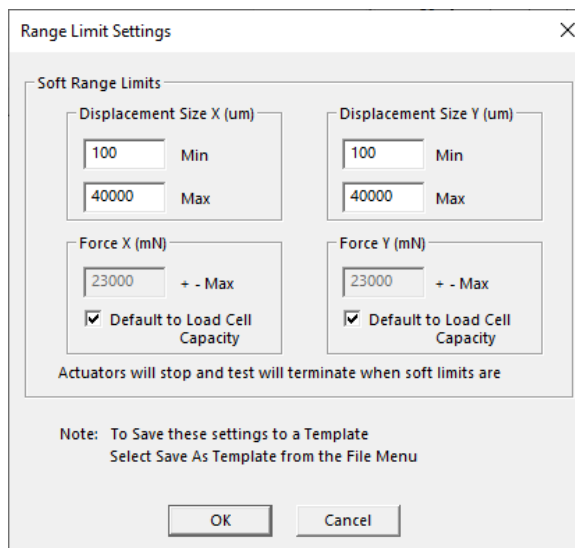
Range limits can be accessed by selecting *Range Limits* from the Settings menu.

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 μm , 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 image shows a 'Range Limit Settings' dialog box with a close button (X) in the top right corner. The dialog is titled 'Range Limit Settings'. Inside, there is a section titled 'Soft Range Limits' which contains four input fields arranged in a 2x2 grid. The top row is for 'Displacement Size' in μm , with 'X' on the left and 'Y' on the right. Each has a 'Min' field set to '100' and a 'Max' field set to '40000'. The bottom row is for 'Force' in mN, with 'X' on the left and 'Y' on the right. Each has a field set to '23000' and a '+ - Max' label. Below each force field is a checked checkbox labeled 'Default to Load Cell Capacity'. Below the displacement and force fields, there is a note: 'Actuators will stop and test will terminate when soft limits are'. At the bottom of the dialog, there is a 'Note: To Save these settings to a Template Select Save As Template from the File Menu' and two buttons: 'OK' and 'Cancel'.

Soft Range Limits	
Displacement Size X (μm)	Displacement Size Y (μm)
100 Min	100 Min
40000 Max	40000 Max
Force X (mN)	Force Y (mN)
23000 + - Max	23000 + - Max
<input checked="" type="checkbox"/> Default to Load Cell Capacity	<input checked="" type="checkbox"/> Default to Load Cell Capacity

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

OK 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 is used to configure three live charting graphs. It contains three sections, one for each graph, with settings for the X-axis and Y-axis. Each section includes a dropdown for the axis type, a dropdown for the unit, a 'Mir' checkbox, and a 'Max' value field. There are also checkboxes for 'Clear Graph Between Sets' and 'Clear Graph Between Cycles'.

Graph	XAxis	YAxis	Clear Graph Between Sets	Clear Graph Between Cycles
Graph 1	Time (S)	Force (mN)	<input type="checkbox"/>	<input type="checkbox"/>
Graph 2	Time (S)	Displacement (um)	<input type="checkbox"/>	<input type="checkbox"/>
Graph 3	Displacement (um)	Force (mN)	<input type="checkbox"/>	<input type="checkbox"/>

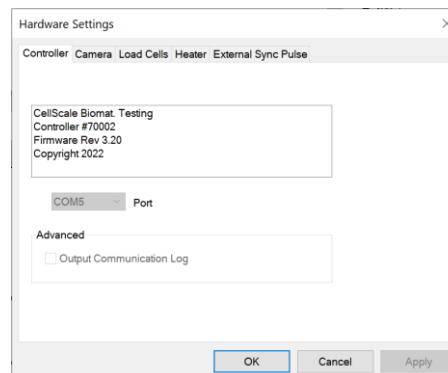
Buttons: OK, Cancel

Graph	Data
<p>Force versus Time</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>Displacement versus Time</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>Force versus Displacement</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>	

6. System Hardware Settings

Controller

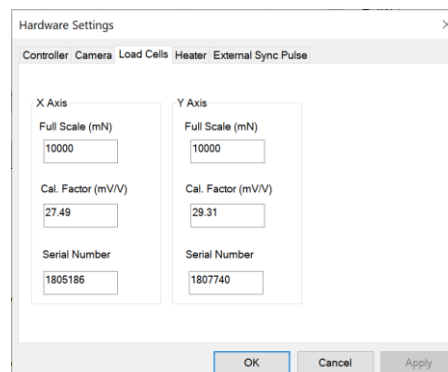
The BioTester 3000 uses an integrated control board rather than a group of PC controlled device drivers. This minimizes communication lag and allows the device to operate asynchronously with the PC. The control board details can be seen by selecting *Hardware* from the Settings menu, which brings up the window shown to the right.




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, 1N, 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, 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 chip in the load cell connector. The range and calibration factor can be viewed by selecting *Hardware* from the Settings menu, but are not user controlled. In addition, the load cells need to be calibrated when the system is initially set up and whenever load cells are changed or re-mounted. See Section 8 of this manual for this procedure.

System Alert

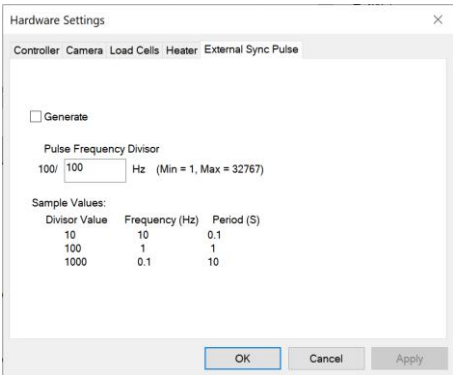
*Though they have some mechanical overload protection, the load cells are sensitive to **excessive torque, excessive overload, transverse loading, and bending forces.** Such forces may **permanently damage the load cells.** Care should be taken to apply force to the goosenecks only in the linear direction within the rated load of the load cells being used. Also, the load cell stud should not be overtightened.*

External Sync Pulse

A 5-volt TTL external sync pulse signal can be generated on BioTester 3000 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.

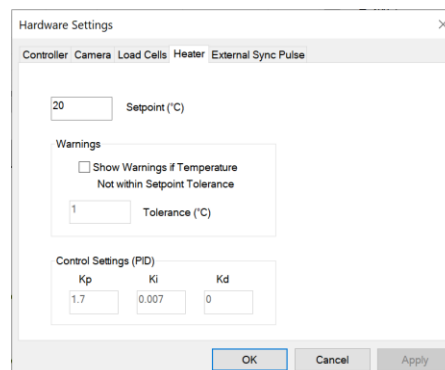
The sync pulse is active high for 10 mS except when operating at maximum speed (100 Hz) where the active high pulse duration is about 550 nS.



Heater (optional)

To activate the fluid chamber heater, both power switches on the front of the BioTester 3000 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 3000 Tip: Preheat Fluid

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

Note:

These warnings are only applicable if your system has the optional fluid bath

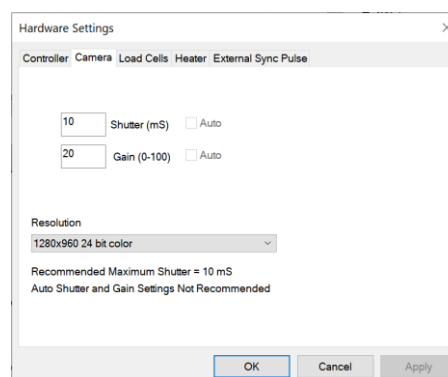
Camera (optional)

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 (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.

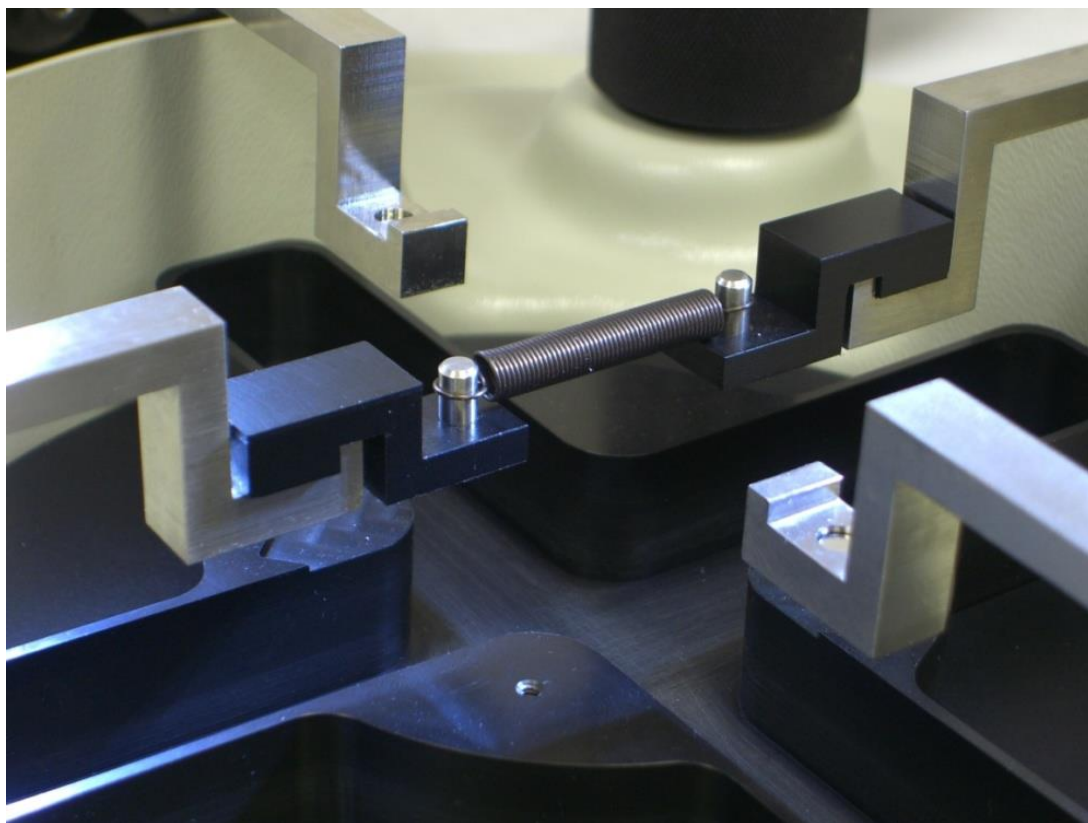
The test software creates an image capture log file in the output directory should this difference be significant. This log stores the actual capture time and the idealized capture time.

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.



7. System Calibration and Advanced Tools

Load Cell Calibration



If the load cells are changed, moved, or impacted, it is good practice to perform the load cell calibration routine. This calibration can be performed in the following steps:

1. Launch the calibration utility by selecting Tools, Advanced, then *Load Cell Calibration*
2. Input the K value (spring constant) for the calibration spring provided for the load cell being used. This value is shown on the spring case label (provided upon system delivery)
3. The load cell capacity should be automatically read and displayed, and the appropriate calibration preload calculated
4. Lower the fluid chamber (if present) 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 (see above figure). The spring should be set level and in line with the goosenecks.

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. Jog Actuators until
Calibration Preload is Achieved

3. Calibrate

	Calculated	Previous
<input type="button" value="Run"/>	<input type="text" value="0"/>	<input type="text" value="27.4879"/>

4. Apply Calibration Factor

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. Jog Actuators until
Calibration Preload is Achieved

3. Calibrate

	Calculated	Previous
<input type="button" value="Run"/>	<input type="text" value="0"/>	<input type="text" value="29.3143"/>

4. Apply Calibration Factor

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

- Using the *Jog+* and *Jog-* buttons, move the actuators until the spring is carrying a tension that is out of the non-linear “dead zone” and reaches the appropriate preload. The current force reading for each axis is displayed in the lower left of the LabJoy main screen.

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). If it is suspected that the load cell is more than 15% out of calibration, contact the manufacturer for another recommendation for calibration pre-load.

BioTester 5000 Master Ctl
 SW# 75075 Rev 1.9
 Copyright 2013
 Software Version 10.45

Initializing X LoadCell
 1.5N, Ser.No. 1539133
 Initializing Y LoadCell
 1.5N, Ser.No. 1539214
 Initializing Actuators
 Initializing Camera
 System Ready!

Force X (mN)

Force Y (mN)

Current Size X (um)

Current Size Y (um)

Shutter (mS)

Current Temp. (°C) ●

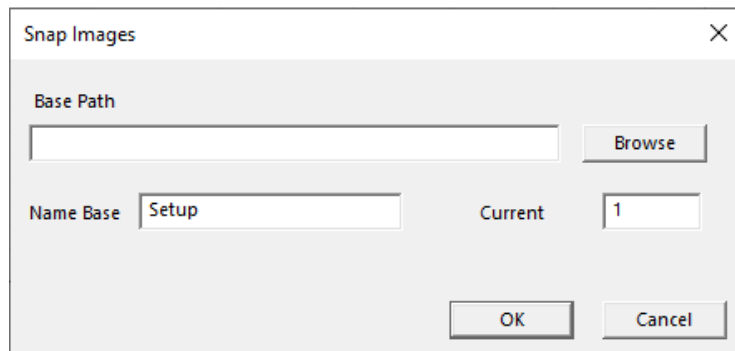
8. Visually verify that the spring is loaded in a stable, horizontal, and in-line position.
9. Click the *Run* button, wait for calibration to complete.
10. A new calculated calibration factor will be displayed in the *Calculated* field. The *Previous* field shows the previous calibration factor (i.e. the factor currently programmed to the load cell).
11. Click the *Use Calculated* button to apply the calculated calibration factor. Alternatively, you may choose to select the *Use Previous* button to continue using the previous calibration factor.
12. The load cell calibration procedure must be carried out for both the X and Y axis.

Snap Image Feature

This feature is available to use if the optional camera is installed.

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.



8. Troubleshooting

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 3000 is not powered up.
- USB cables between the computer and BioTester 3000 are disconnected.
- The supply power surges or is interrupted.

When a communication error occurs, close LabJoy, check connections, power cycle the BioTester 3000, and restart LabJoy.

BioTester 3000 Tip: Preventing Power Disruptions

We recommend plugging the BioTester 3000 (and host computer) into an uninterruptable power supply (UPS) or battery back-up.

Zero Displacement Causes Software Crash

Under certain conditions involving preloads and cyclic loading, the software may crash if a test phase has zero displacement in one axis. If this happens, the easiest solution is to replace the zero displacement with a small non-zero displacement value (i.e. 0.1% or 10µm).

Troubleshooting With Optional Camera System

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 µm 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.

9. Appendix A: Initial System Setup

The BioTester 3000 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 3000 main unit	1	Press Block
16	M4x8mm Socket Head Cap Screws	1	Sheet of Rubber Backing Material
6	6-32 Hex Nuts (4 + 2 extra)	4	Actuator Caps
12	5mm Magnets	1	Set extra fasteners
4	BioRakes (1 sets of 4 each)	1	Camera Lens (10X Zoom) (optional)
2	Load Cells	1	Fluid Bath (optional)
1	Calibration Spring	1	3"x1" Microscope Slide (optional)
2	Calibration Spring Brackets	1	Temperature sensor (optional)
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	Power Cord and Supply		
1	6' (1.8m) USB Cable		
1	Set of Shims (Thin and Thick)		

Unpack System

Remove the BioTester 3000 from the shipping container

Carefully remove the protective shipping material from the container such that the BioTester 3000 can be lifted out.



Attach Camera Mast (optional)

If the camera was added 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.



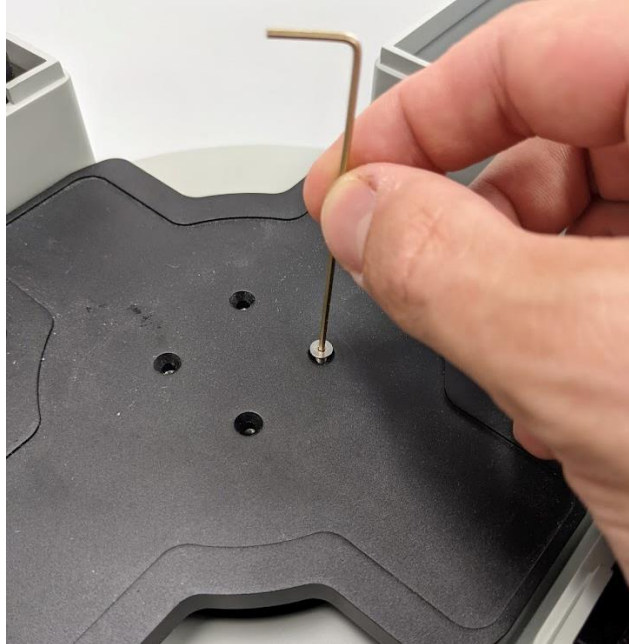
Once the camera mast is in place, plug the lighting cable into its corresponding place on the back panel and run the USB line to the computer.



Place Fluid Chamber on Riser Stage (optional)

If the heater attachment was included remove it from the box and set the Main unit on top of the heater base. The heater plate can be installed using the provided fasteners and the heater and temp probe cables can be attached. Next the heater and temperature probe cables can be plugged into the back panel of the main unit.





Install Camera Lens (optional)

Remove the protective caps on the lens and camera body and then screw the lens and spacer tube onto the camera. The 5mm spacer tubes come attached to the camera and lens from the production facility. 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)

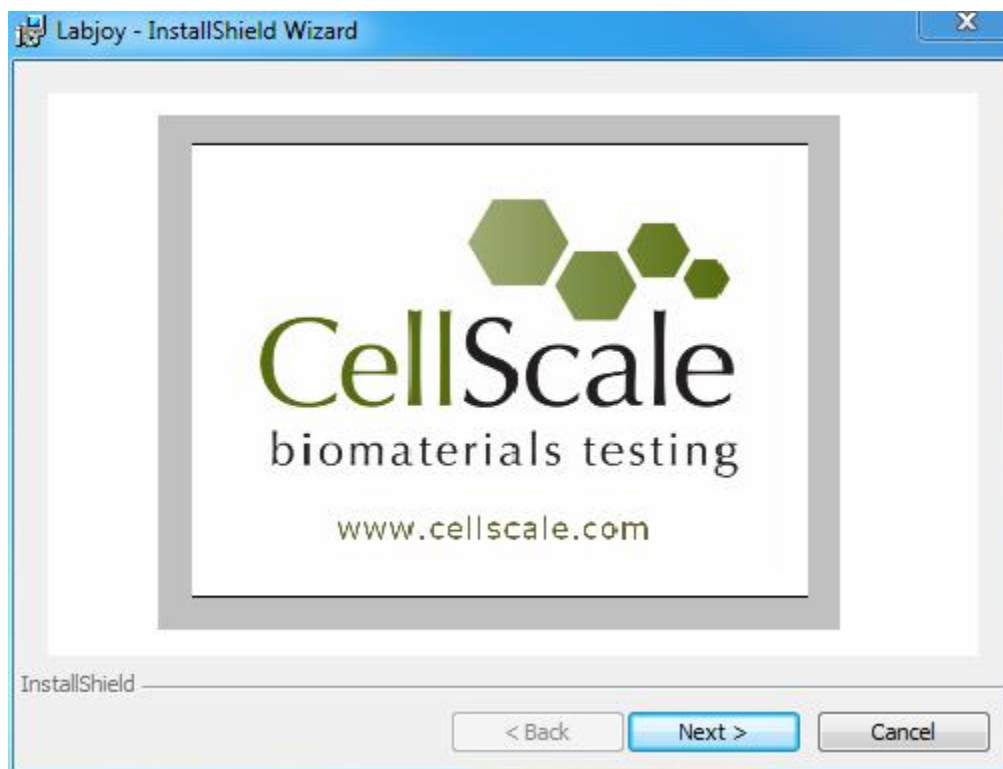
Camera (inside housing)

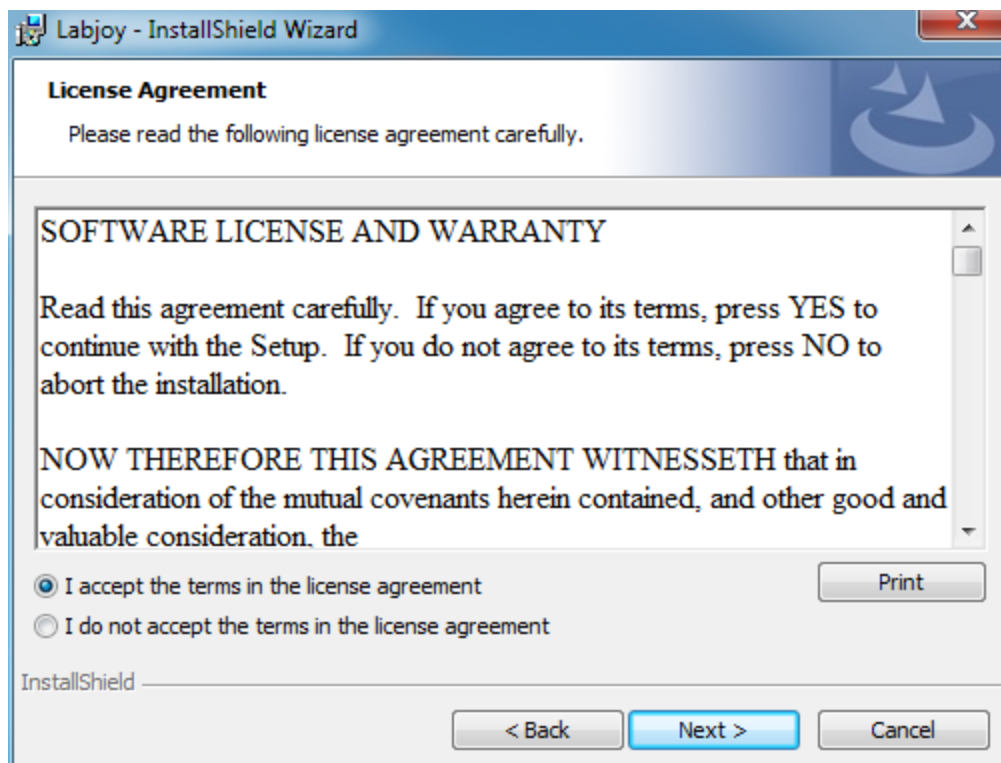
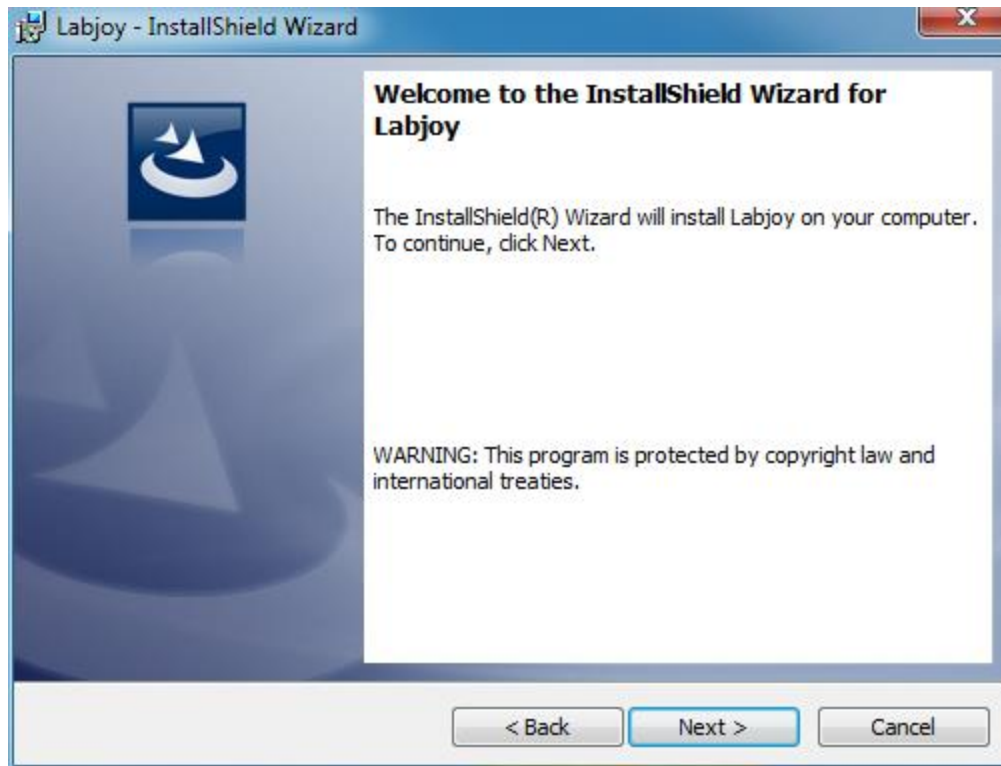


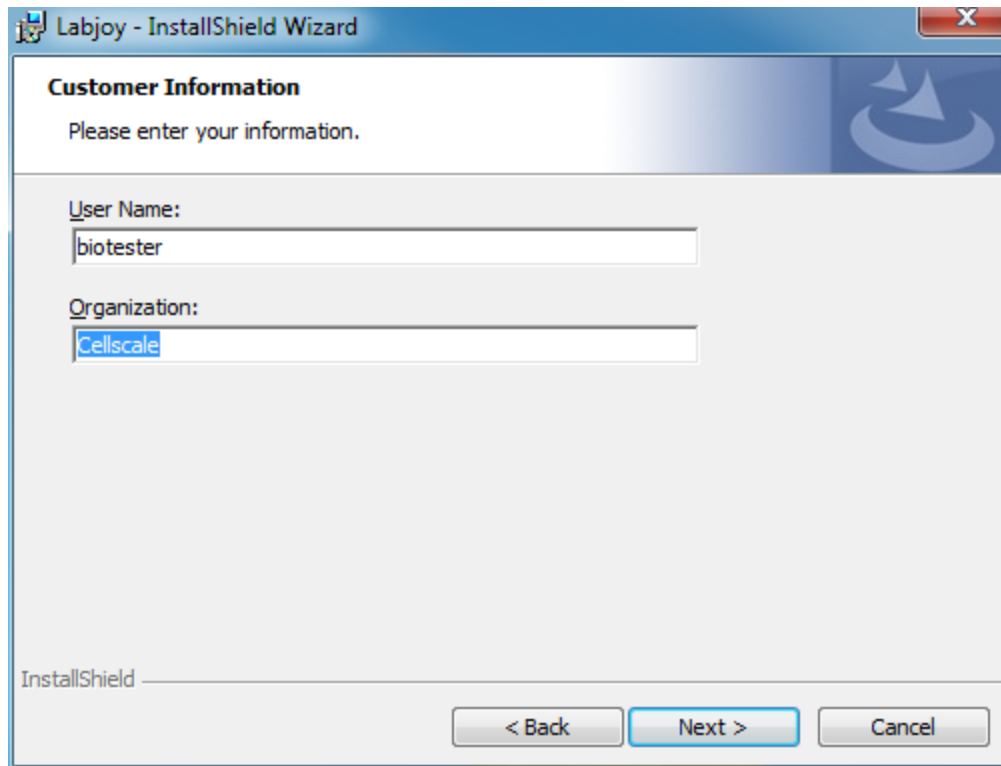
10. Appendix B: Software Installation

A Windows based PC (Windows 7 or above) with at least 2 USB ports is required to operate the BioTester 3000. 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 3000.

To install the software, go to the provided web address and download the compressed installation package. Transfer this file to the computer which will be running LabJoy. Right click on the file and select “Extract All...” to unzip. After this is complete, go to the unzipped installation folder and select “setup.exe”. Details of typical dialogue boxes are shown below.







Labjoy - InstallShield Wizard

Customer Information

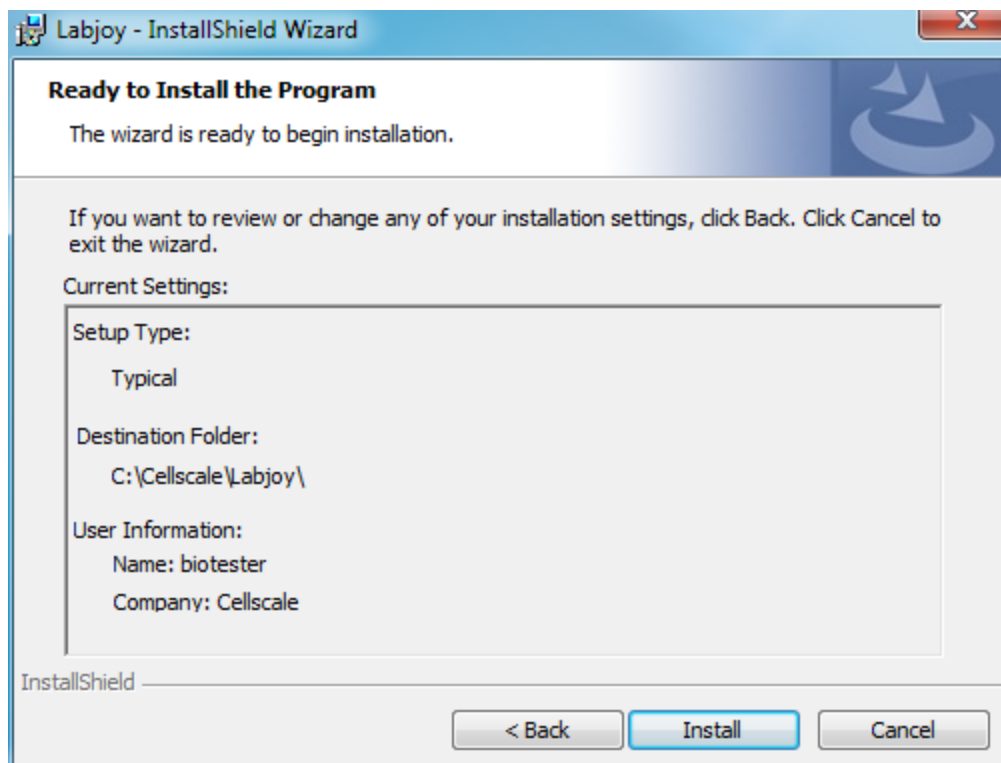
Please enter your information.

User Name:

Organization:

InstallShield

< Back Next > Cancel



Labjoy - InstallShield Wizard

Ready to Install the Program

The wizard is ready to begin installation.

If you want to review or change any of your installation settings, click Back. Click Cancel to exit the wizard.

Current Settings:

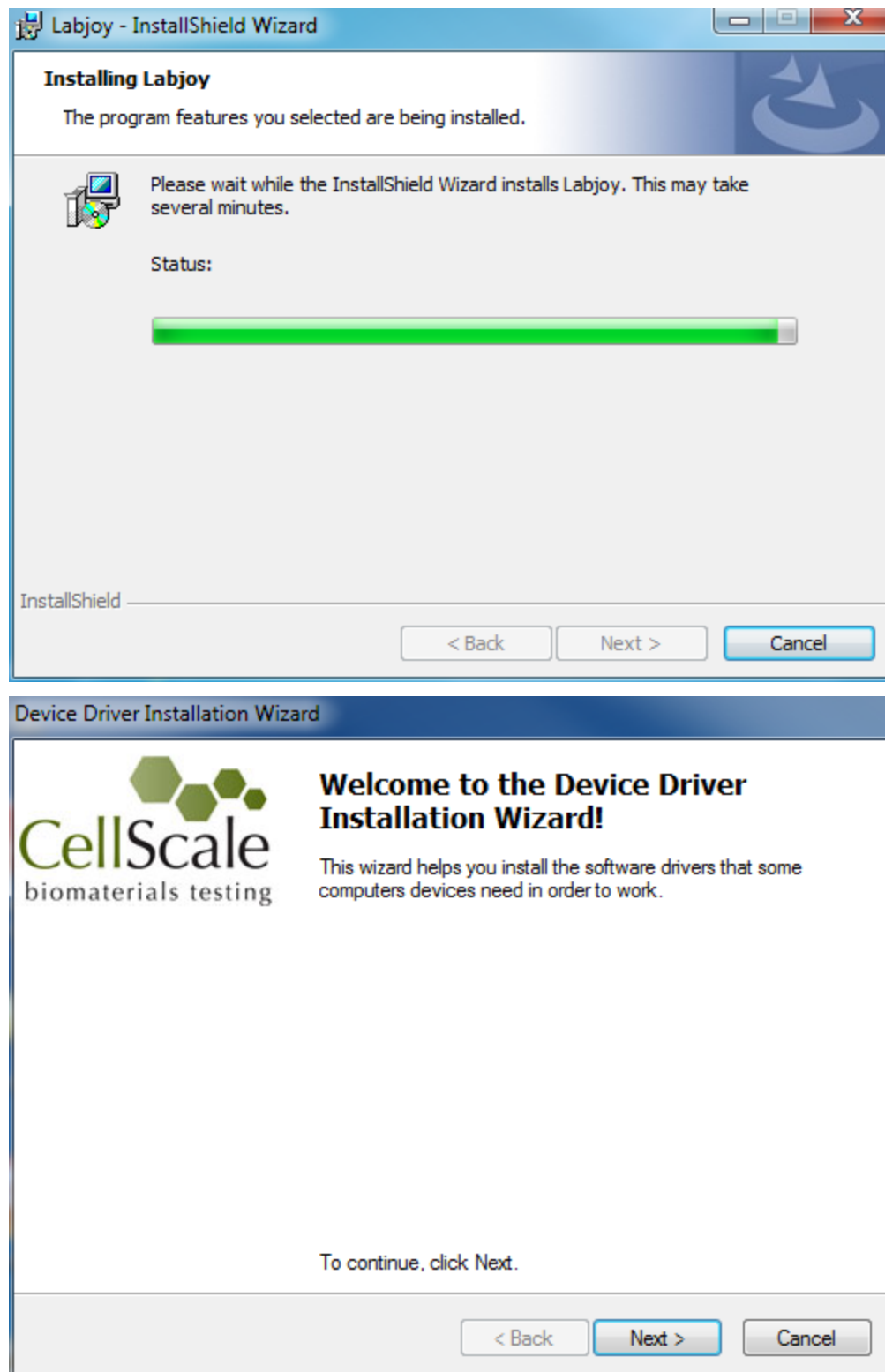
Setup Type:
 Typical

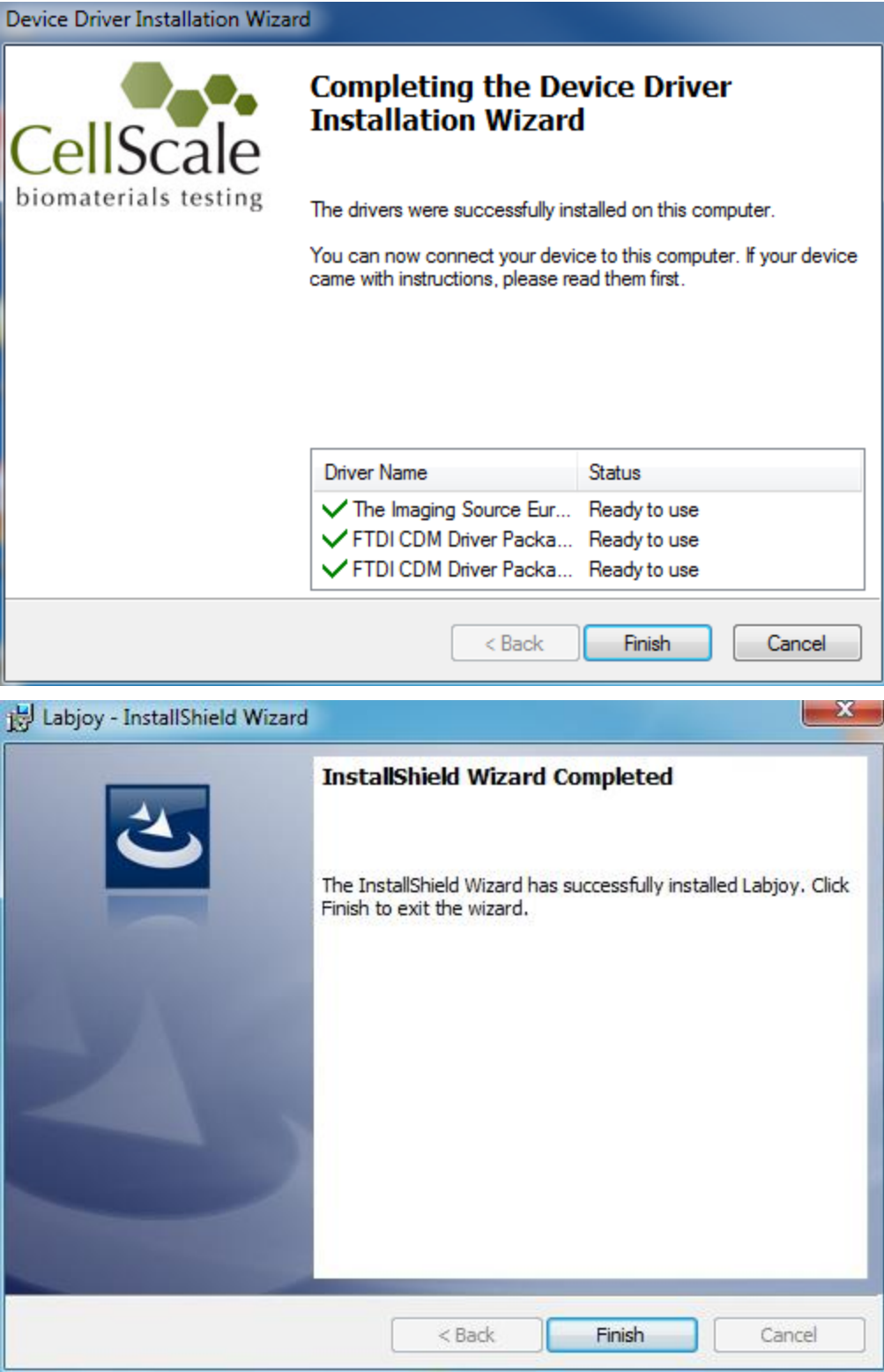
Destination Folder:
 C:\Cellscale\Labjoy\

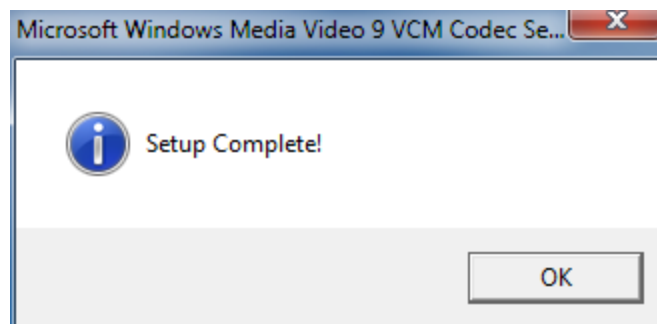
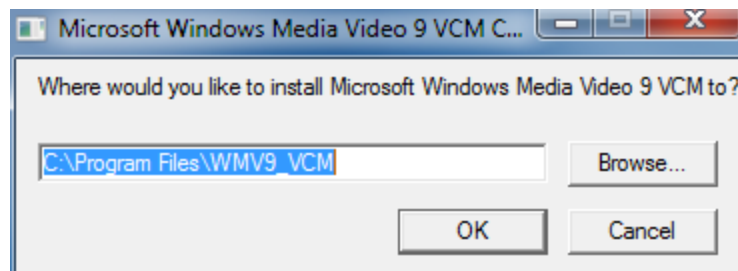
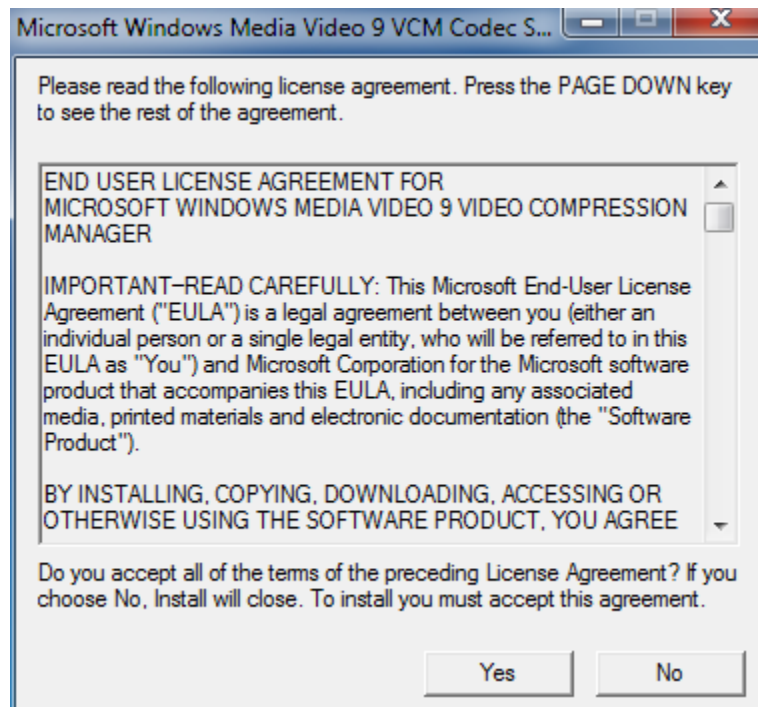
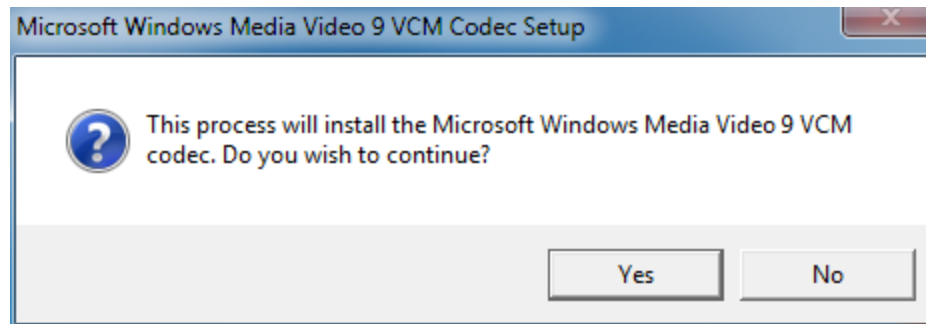
User Information:
 Name: biotester
 Company: Cellscale

InstallShield

< Back Install Cancel







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

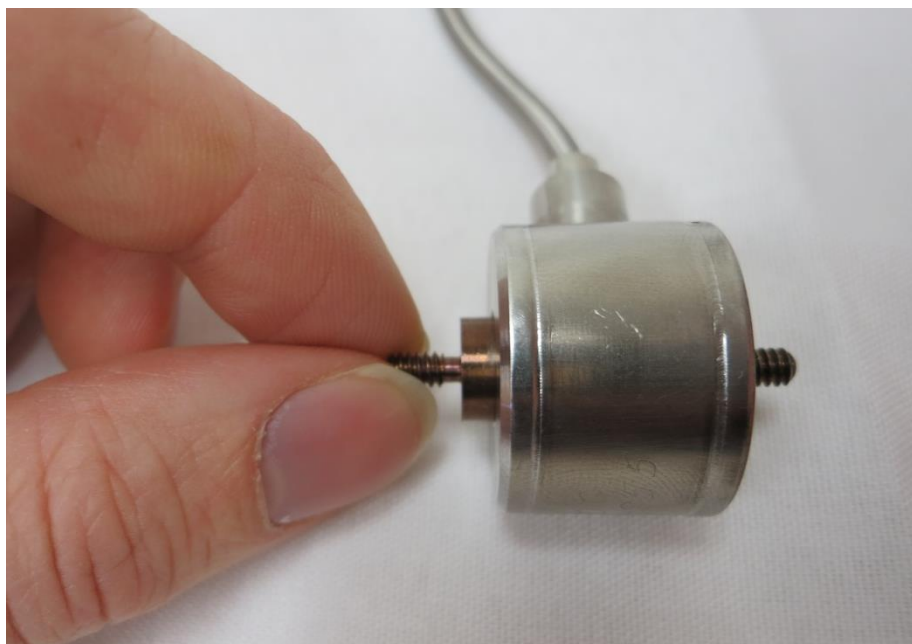
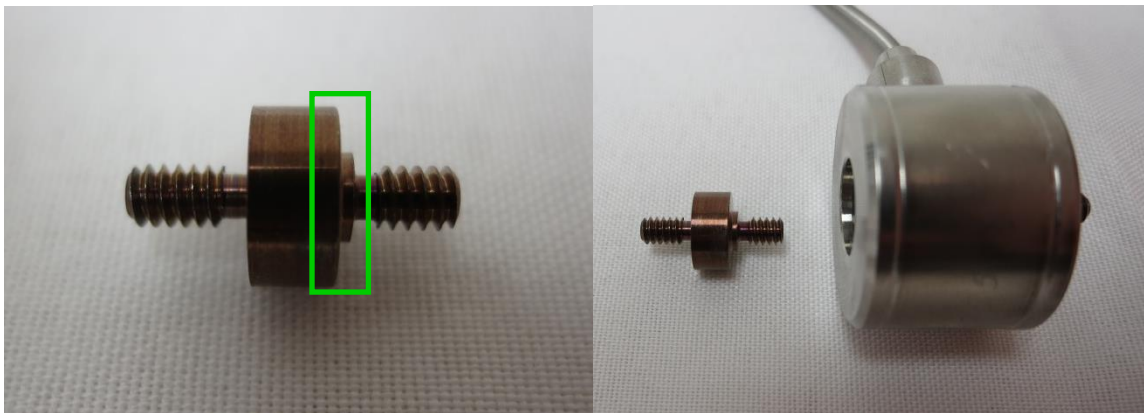
Connect the camera USB cable to a PC USB port. Connect the included USB cable to the main unit and a PC USB port. Plug in the power supply to the BioTester 3000 and then proceed with the following steps. Do not turn the BioTester 3000 on yet.

Attach the Load Cells to the Goosenecks

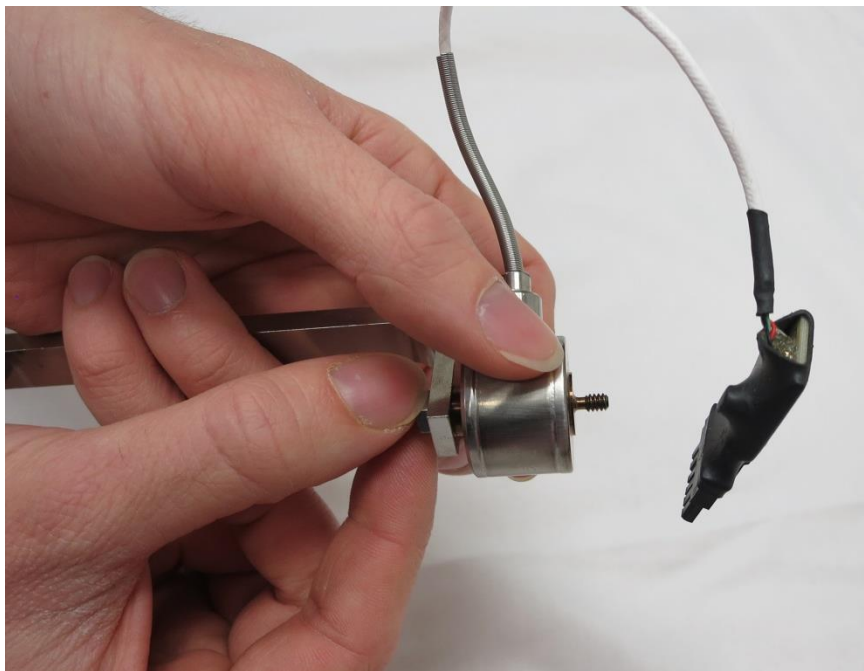
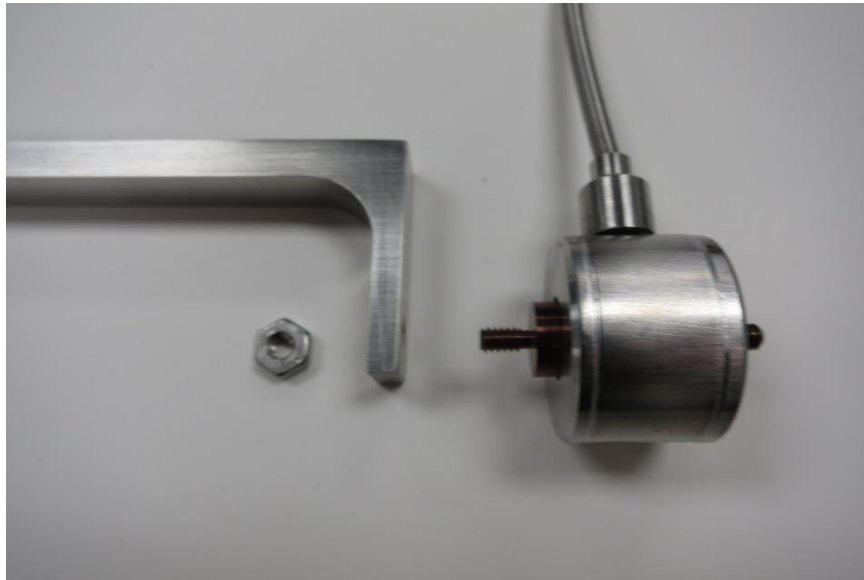
Ensure the correct orientation of the load cell components as shown in the below pictures. Tighten the nuts gently to avoid damaging the load cells (just a little bit tighter than finger tight). **Keep in mind that the load cells are sensitive to excessive torque, excessive overload, transverse loading,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



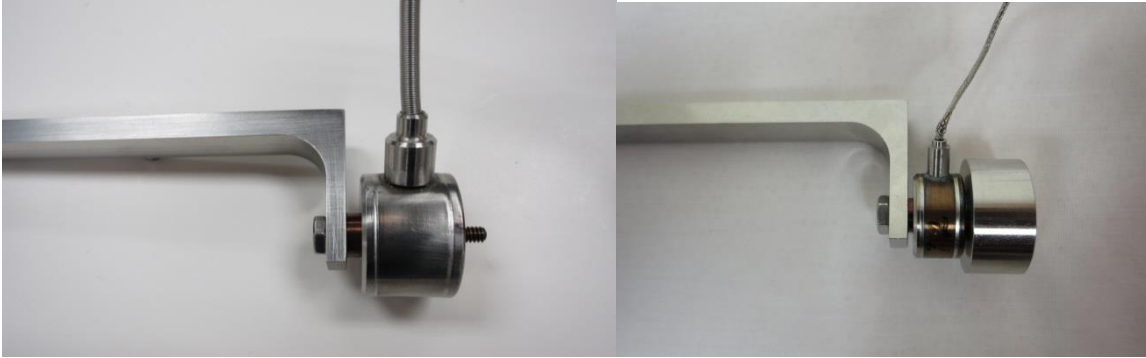
Attach the load cell stud to the load cell using your fingers. The load cell stud has one side with a shoulder below the threads which should be attached to the load cell. The other end is attached to the gooseneck. **If the orientation of the stud is reversed, the load cell may not function properly.**



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.



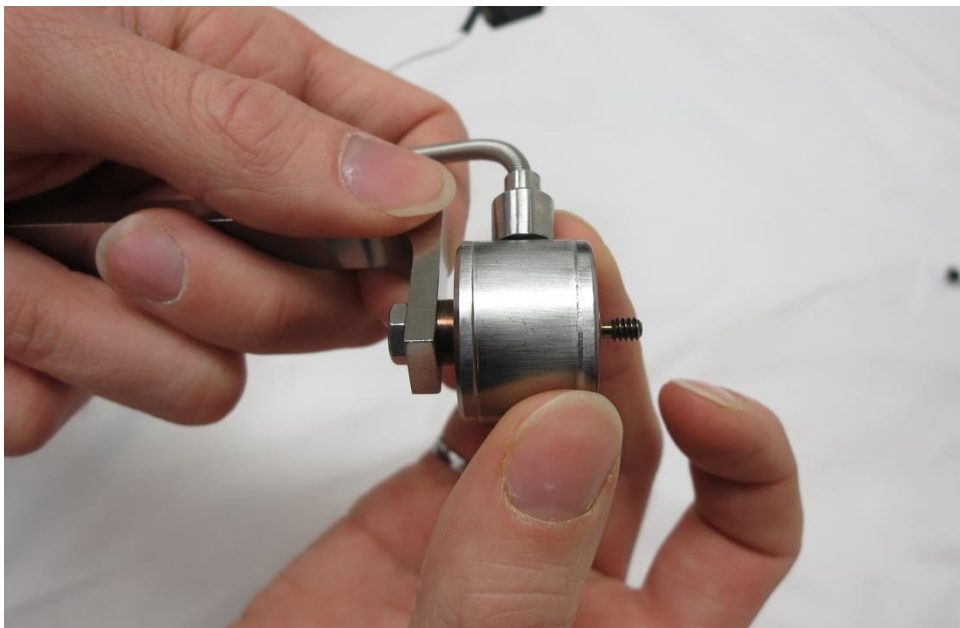
The load cell and gooseneck assembly should look like this when assembled. There are two styles of load cells available (both are shown below).



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 be finger tight while being snug enough so that the components don't freely rotate while using the BioTester 3000.



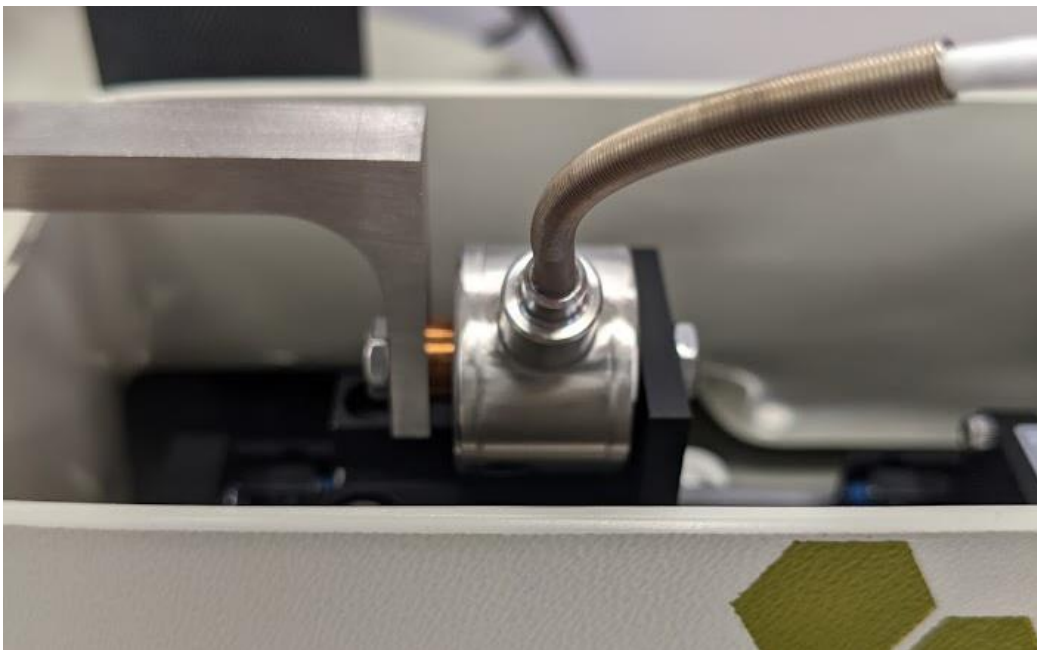
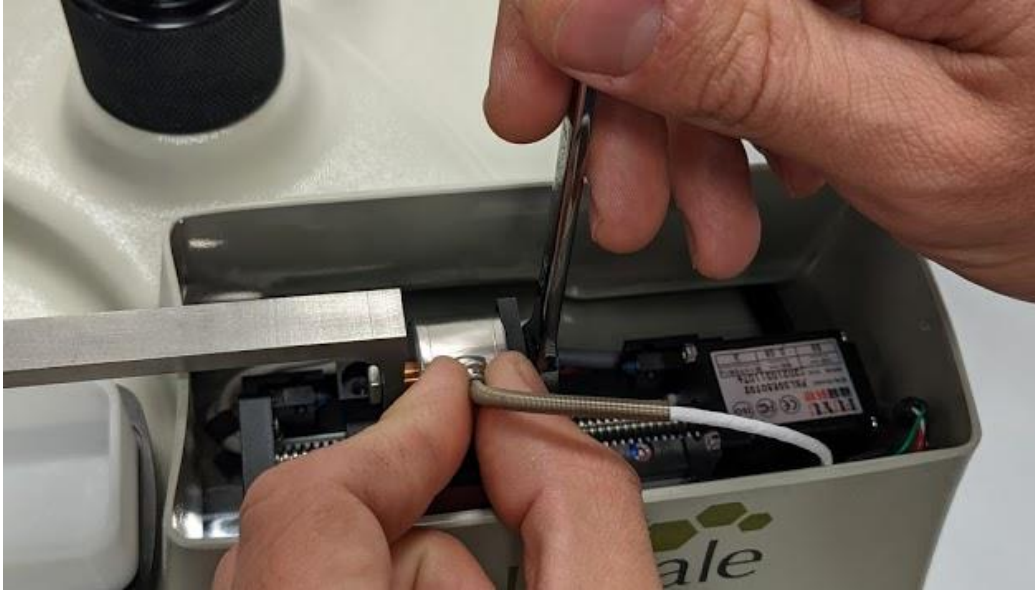
Attach the Load Cell Brackets to the Actuators

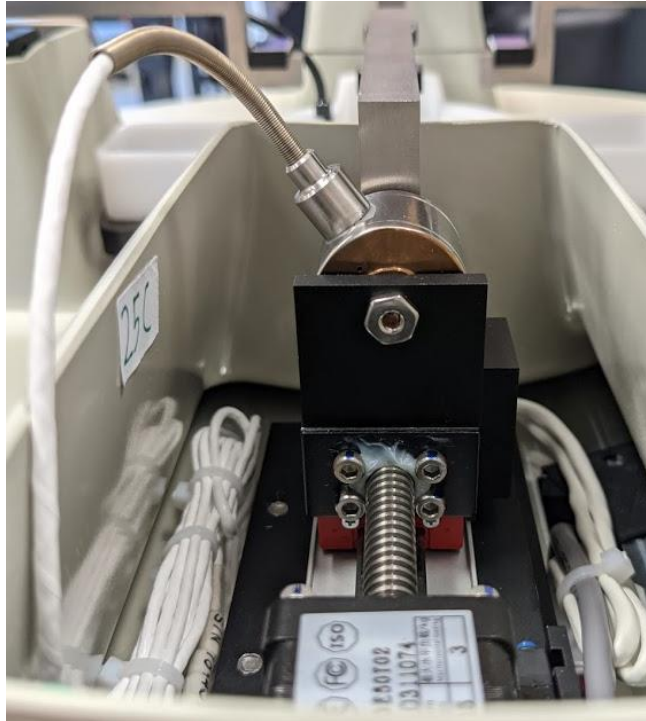
Before attaching the load cell/gooseneck assembly, install the load cell brackets onto the appropriate actuators (these are located on either side of the camera post and will be sitting lower in the actuator housing). Use M4 x 10mm (x4) fasteners provided for each bracket as shown below.



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 M4 x 10mm fasteners (x4 each).





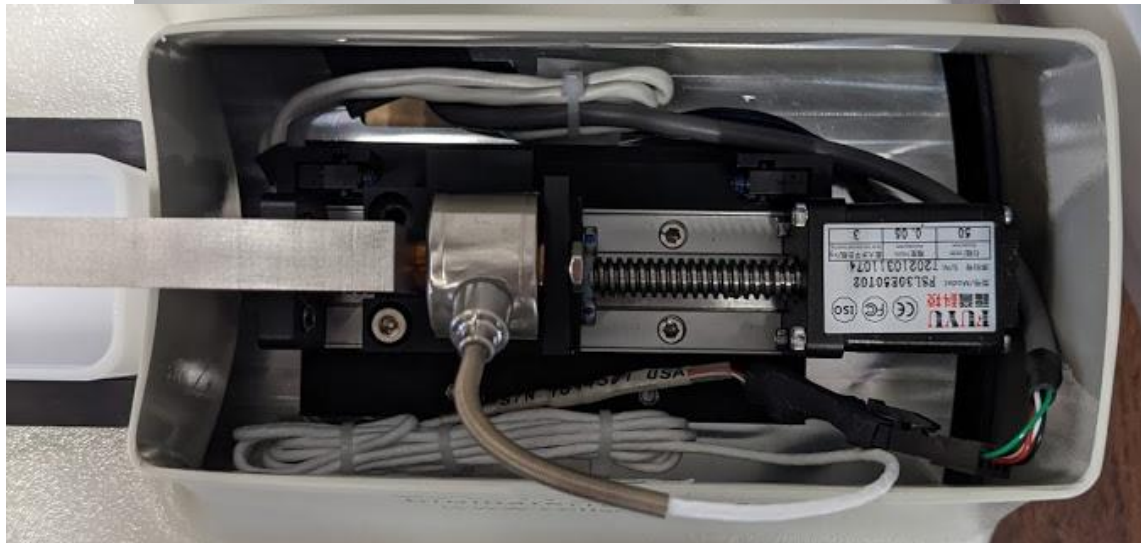
Stow Cables and Plug in the Load Cells

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 3000 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.



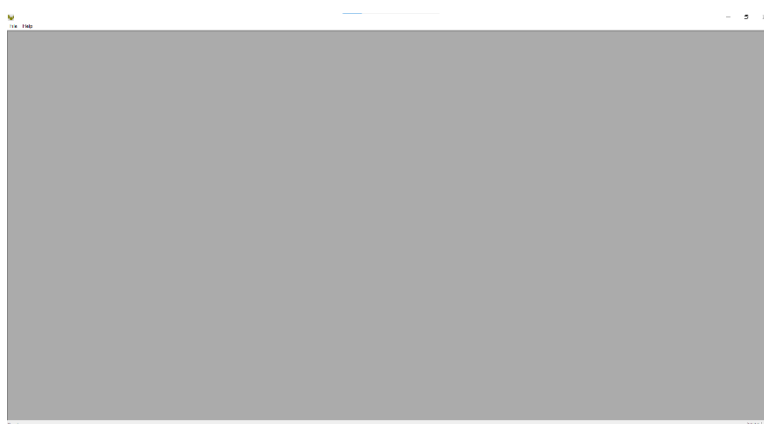


Turn on the BioTester 3000

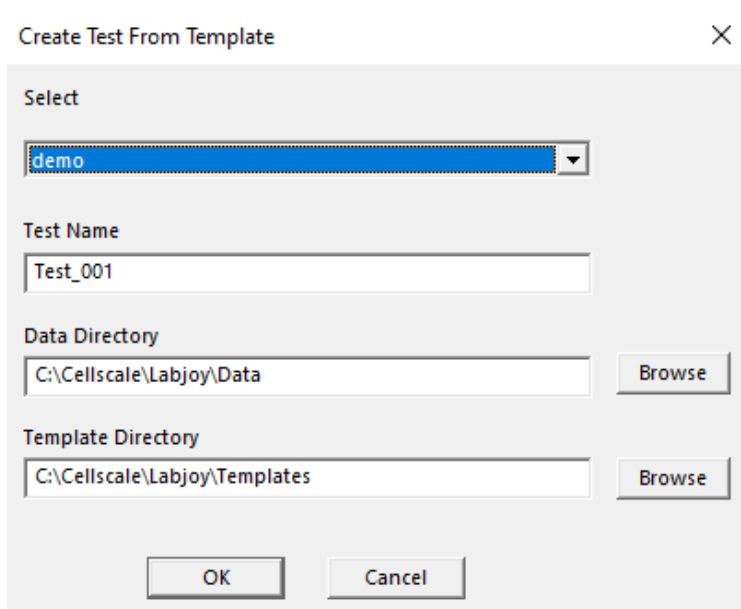
Plug the provided power supply into the BioTester 3000 and a standard electrical outlet. The BioTester 3000 has a main power switch and a heater switch (optional). The “Main” switch turns on the BioTester 3000 main unit. The “Heater” switch (optional) turns on the heater below the stage and may be used 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.

Launch the LabJoy Software

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



Selecting File>Collect New should bring up this window:



Create Test From Template

Select

demo

Test Name

Test_001

Data Directory

C:\Cellscale\Labjoy\Data

Browse

Template Directory

C:\Cellscale\Labjoy\Templates

Browse

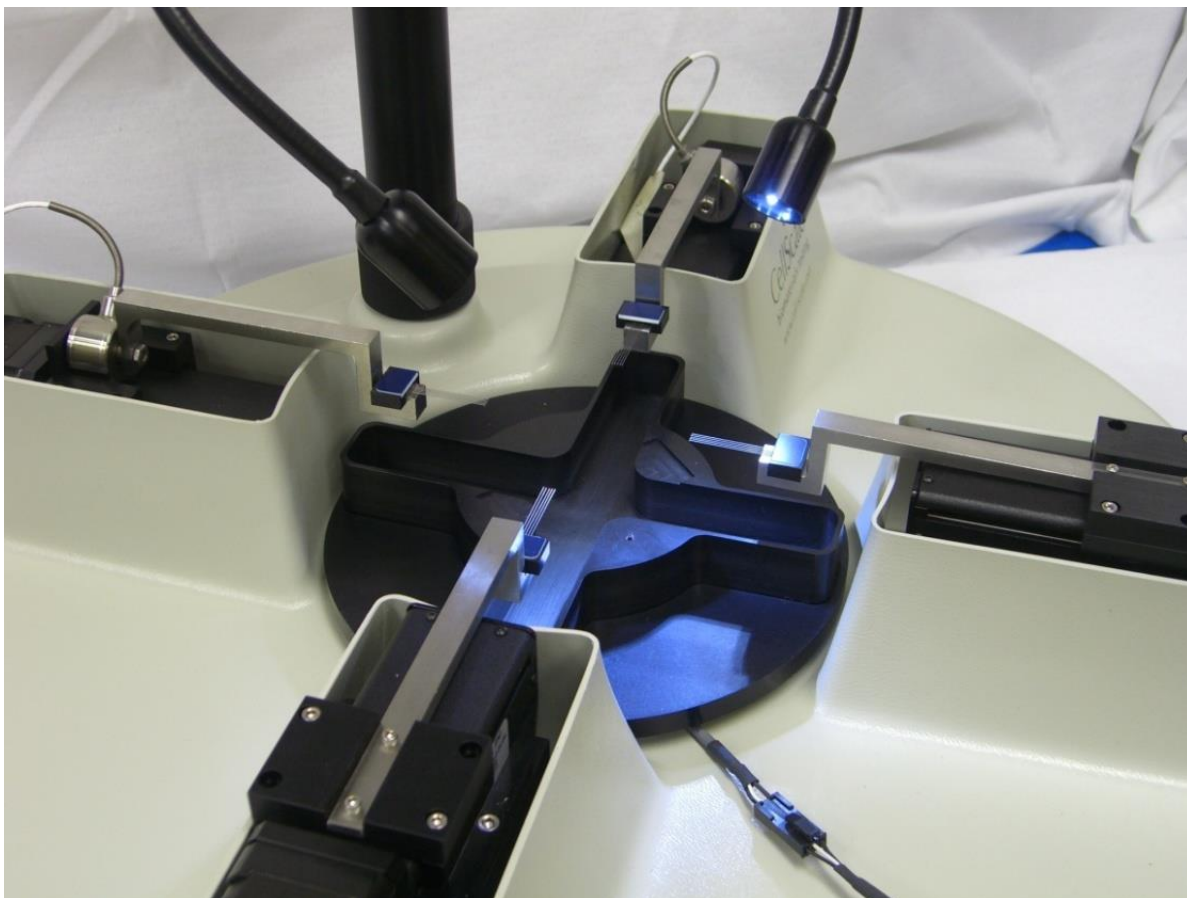
OK Cancel


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.

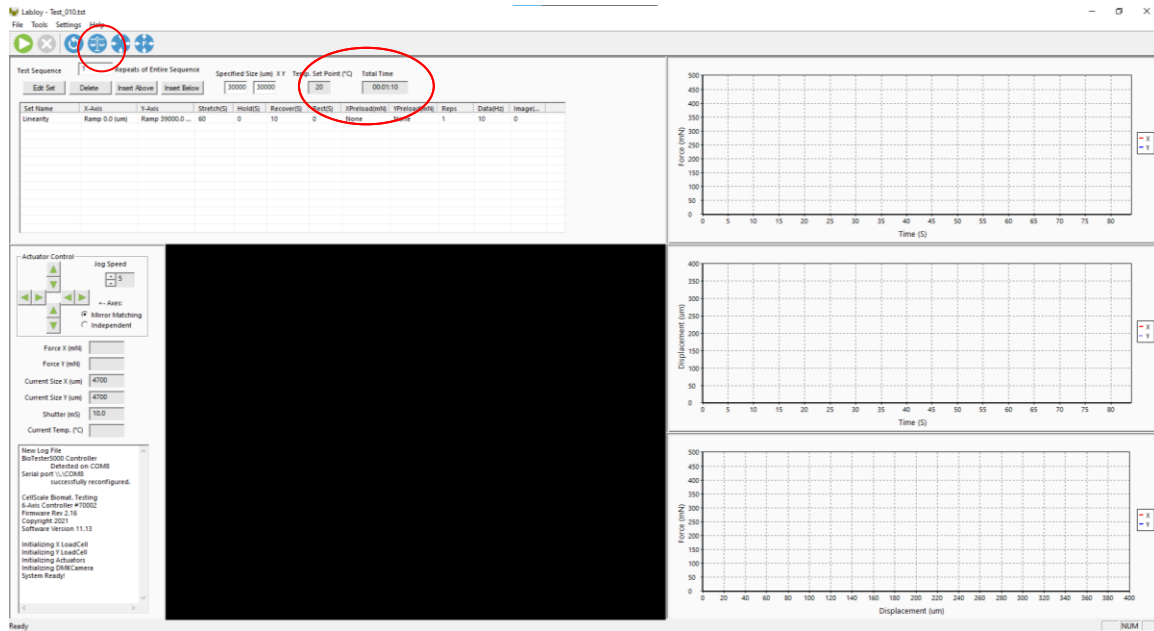
If prompted to Reset Actuators, select "Yes".

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

Next place the BioRakes in each of the 4 goosenecks so that the BioTester 3000 looks like this:



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

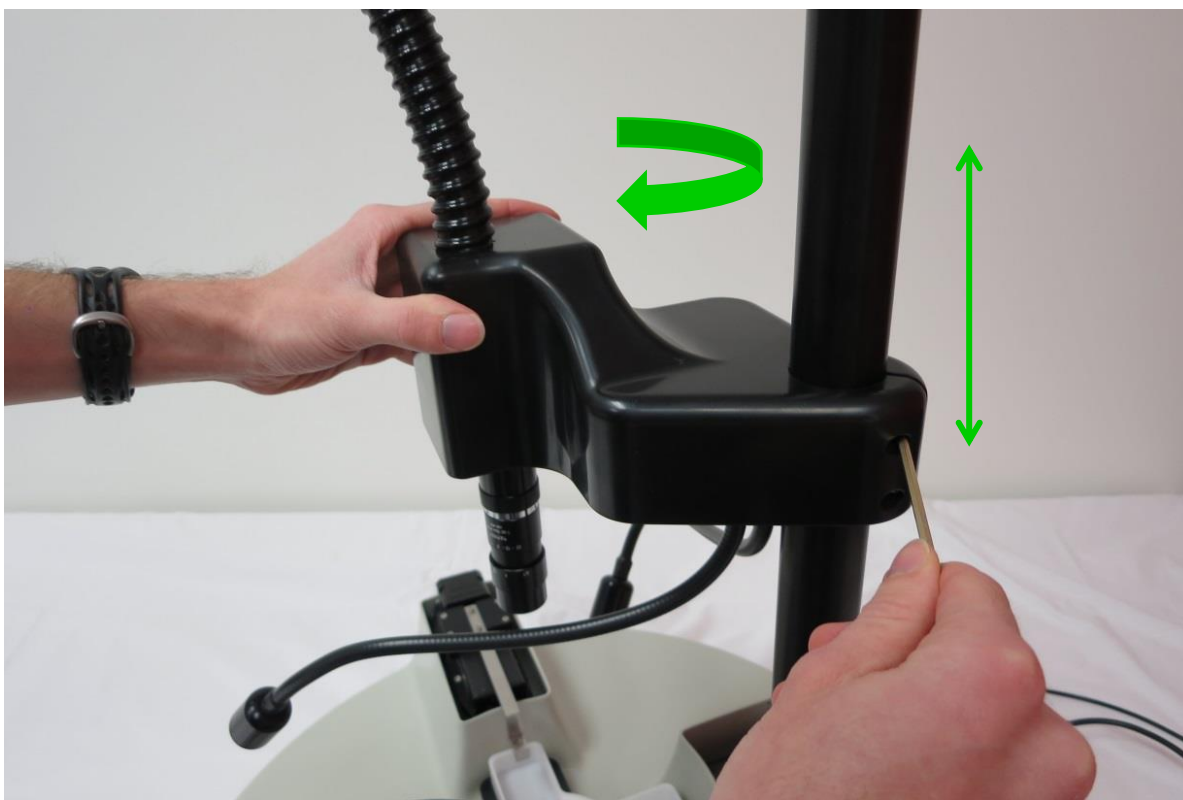


Perform Camera Alignment (optional)

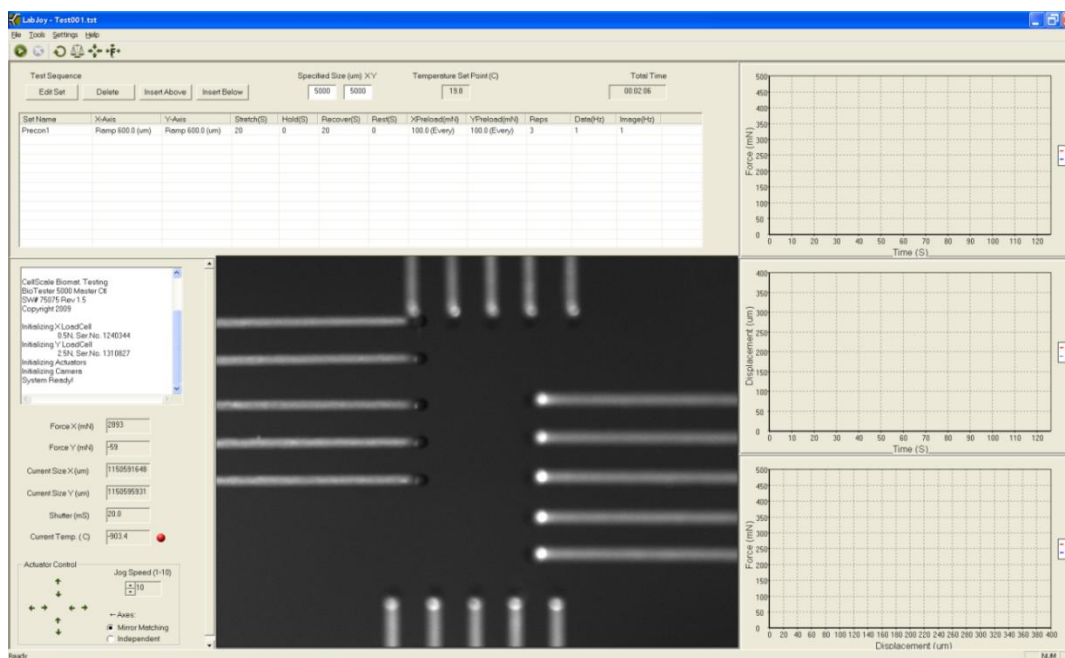
The camera position has both coarse and fine position adjustments and coarse and fine focus adjustments. To start, loosen the fasteners which tighten to camera cover to the mast. Position the camera cover vertically such that the working distance from the lens surface to the BioRakes is **15cm**. Next, 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.

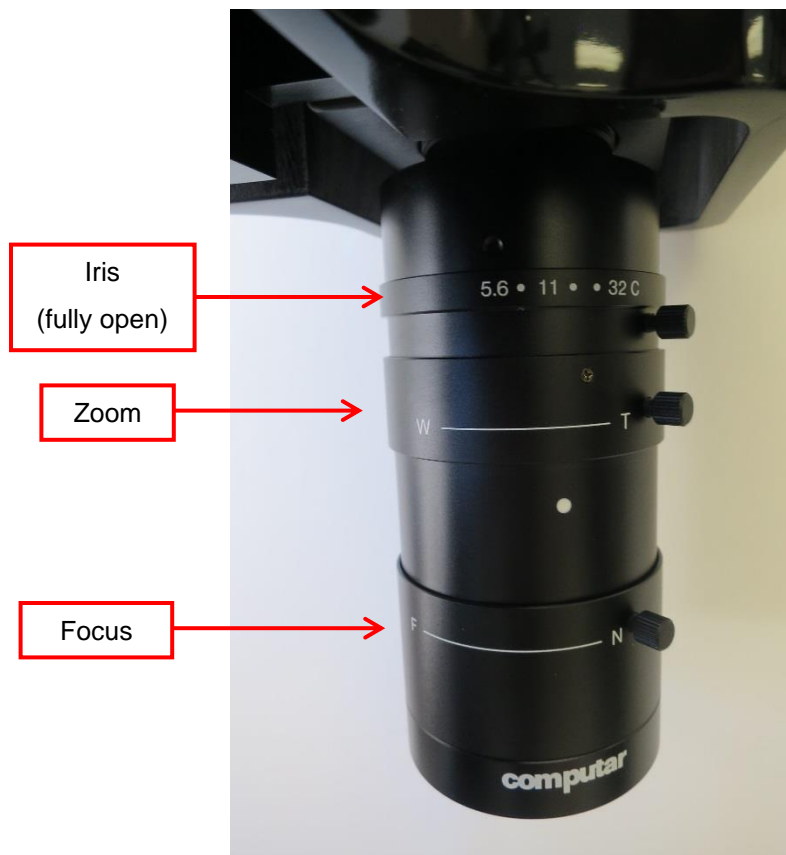


Attempt to bring all of the BioRakes into the image, as shown below:



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.

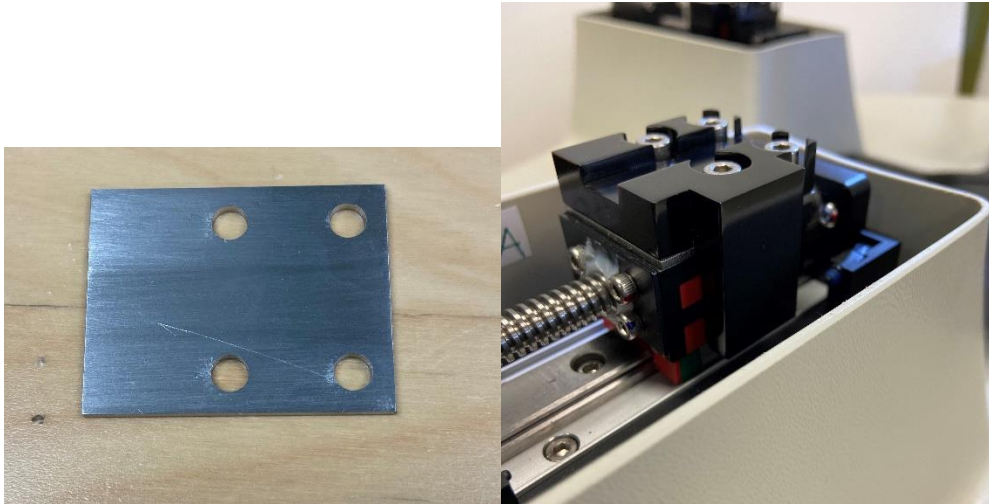




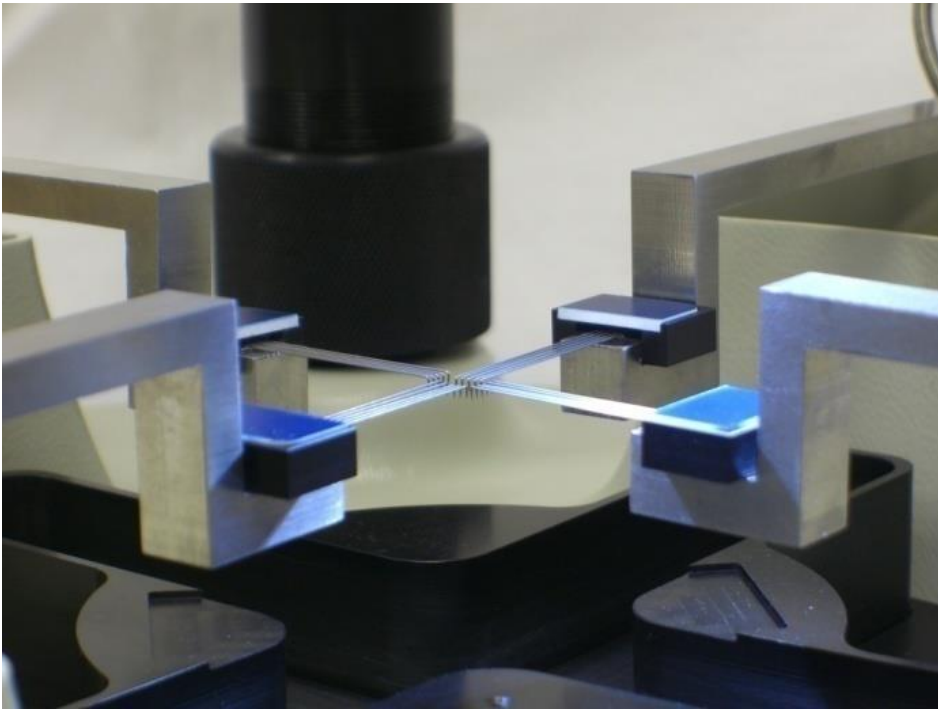
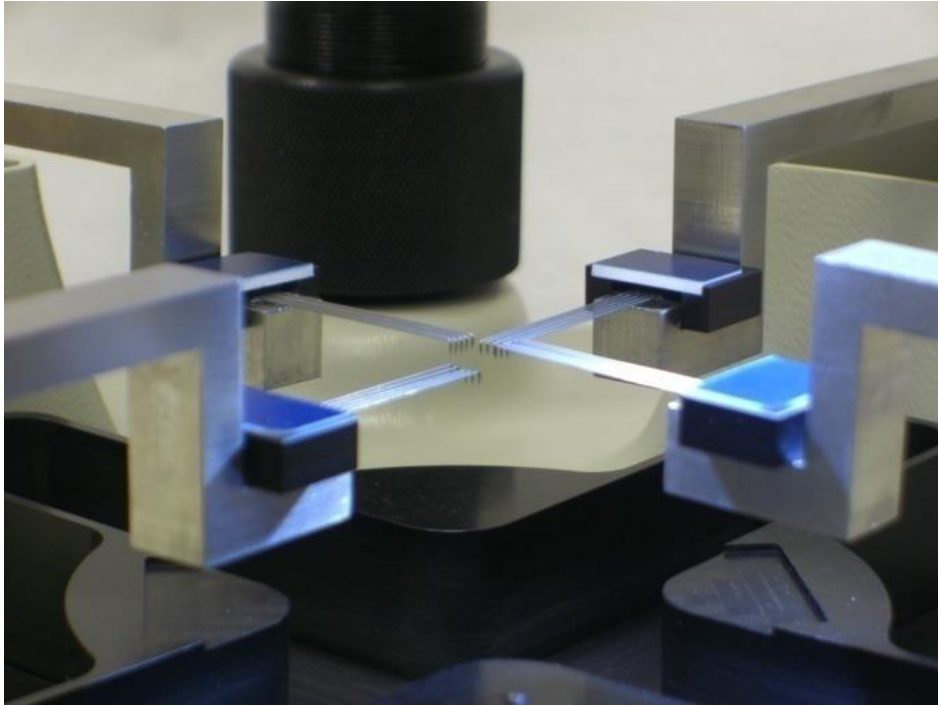
Adjustments to the zoom can be made by adjusting the middle ring on the lens. See the section on “Adjusting the Camera Position and Image Magnification” in section 8 of this manual for more detail.

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.

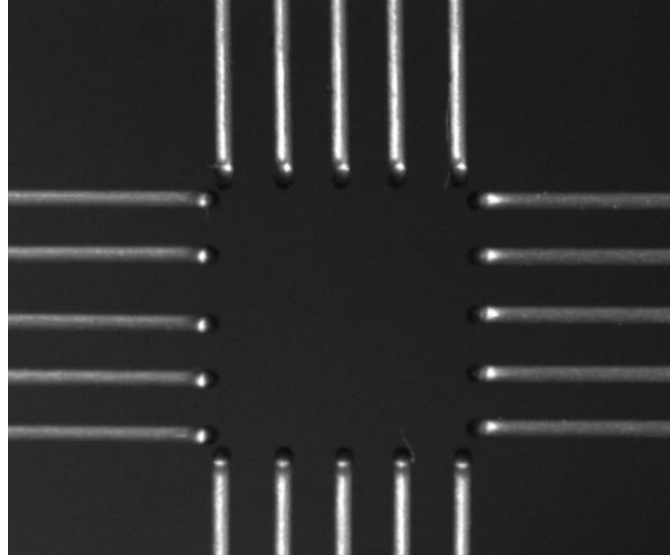


By looking at the BioRakes from the side, it is possible to observe their vertical alignment. Please add or remove shims to vertically align the BioRakes. Here are photos of poor vertical alignment and good vertical alignment:

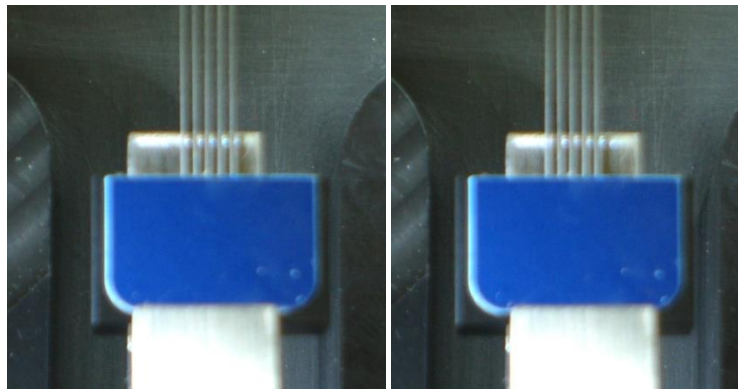


Perform Transverse and Axial Gooseneck Alignment

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

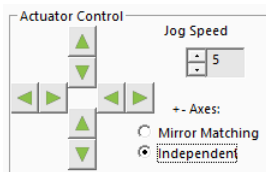


First, make sure the BioRakes are positioned in the transverse center of the gooseneck. The magnetic mounts that hold the BioRakes to the gooseneck fix them in the axial direction, but allow a small amount of adjustment in the transverse direction. This is useful for alignment during regular testing, 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.

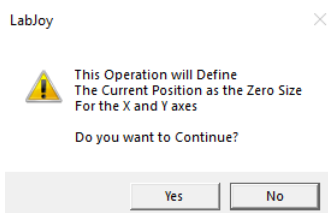
To axially adjust the goosenecks, use the actuator jog icons in the lower left of the screen. Make sure the “**independent**” radio button is selected and **not** the “**mirror matching**” button. The jog speed should be about 7. The goal is to make a square as shown in the figure above.




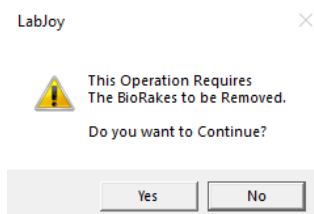
Adjust all 4 goosenecks until you are satisfied with their transverse and axial alignment and the rake tips form a square as shown above. It may also be necessary to make small adjustments to the camera position. The camera alignment should be parallel to the rake alignment and the BioRake tips should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

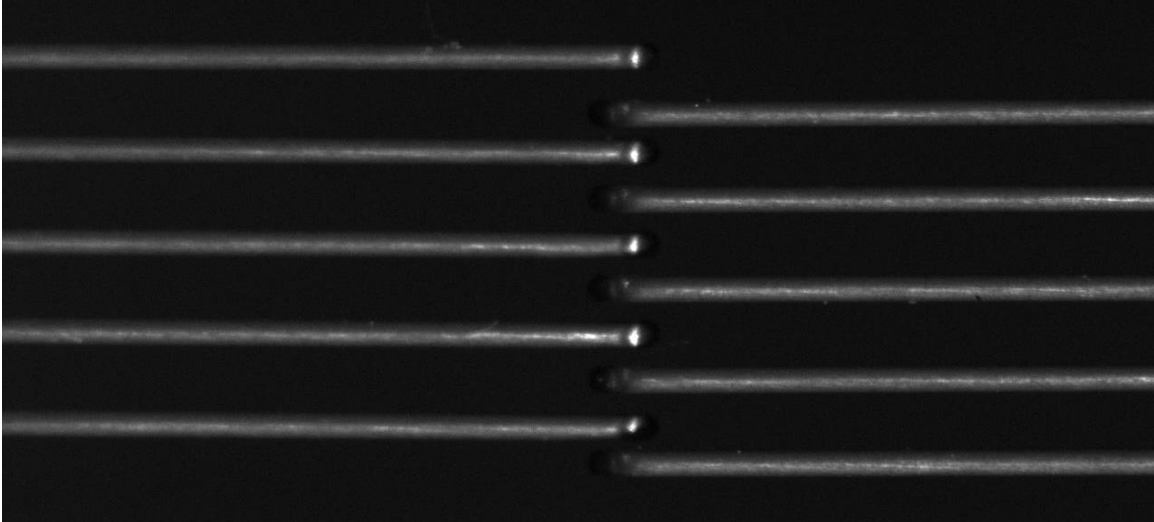
1. Lower the fluid chamber and remove the BioRakes.
2. From the Tools menu select *Advanced* and then select *Centre Position Calibration*. This should bring up this dialogue box:



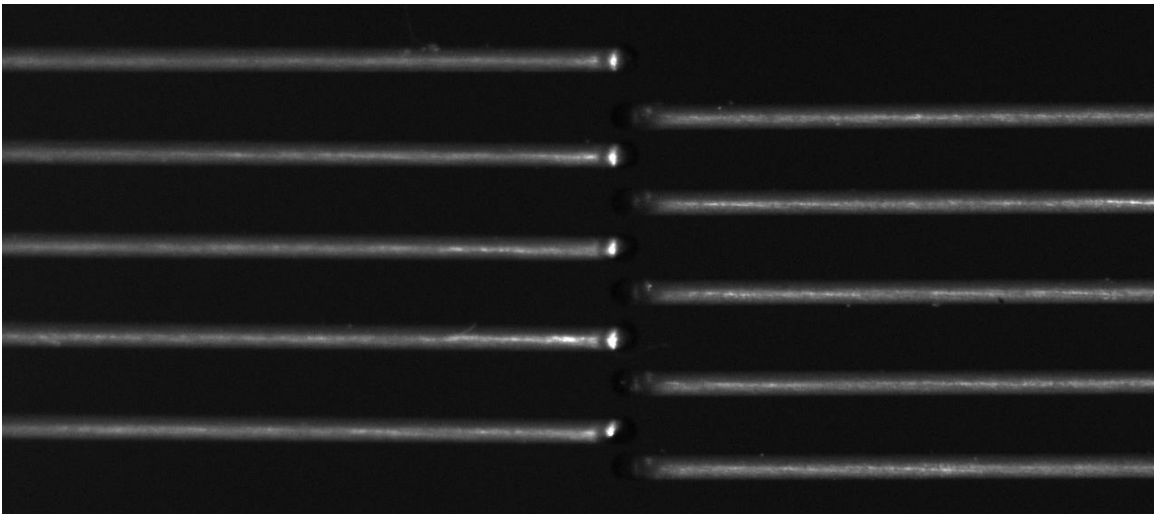
3. Press Yes.
4. Select the *Reset Actuators* button  (or use *Tools>Reset Actuators*).
5. From the Tools menu select *Advanced* then select *Move to Centre*. This should bring up this dialogue box:



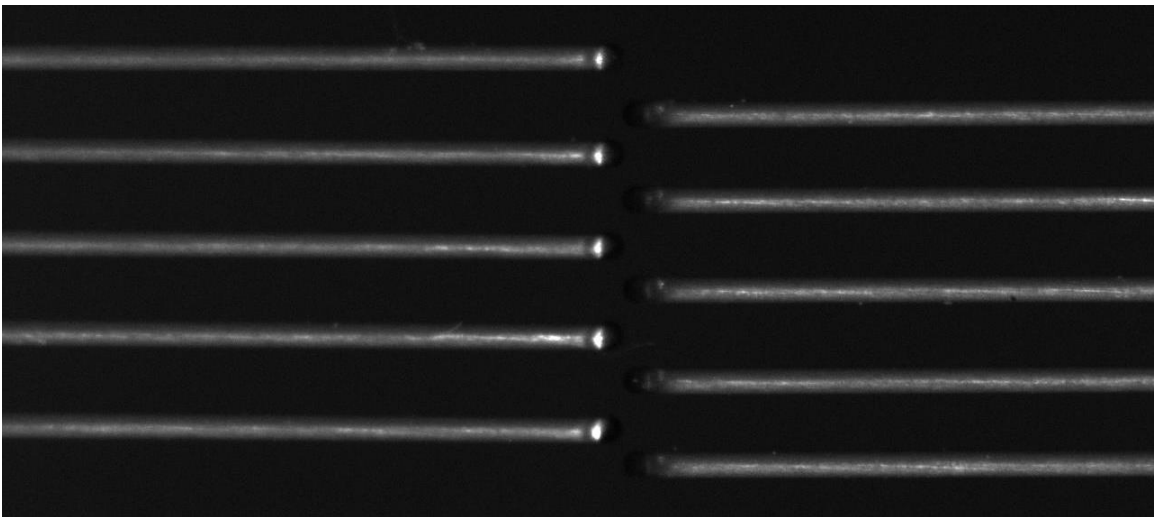
6. Press Yes. Once the actuators have stopped moving, put 2 opposing BioRakes on the X axis.
7. Using the actuator jog control arrows at the lower left of the screen, move the actuators until all 10 BioRake tips are collinear. Make sure the “**mirror matching**” button is selected for this step and **not** 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.

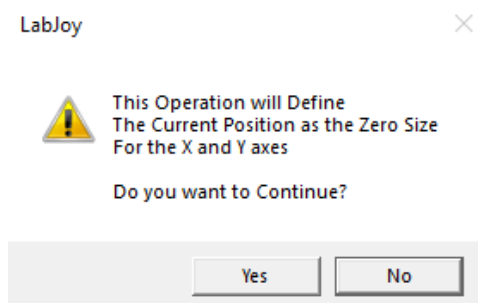


Example 2: BioRakes are correctly positioned.



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

Be careful not to let the cover slip and accidentally apply a transverse load to the gooseneck.



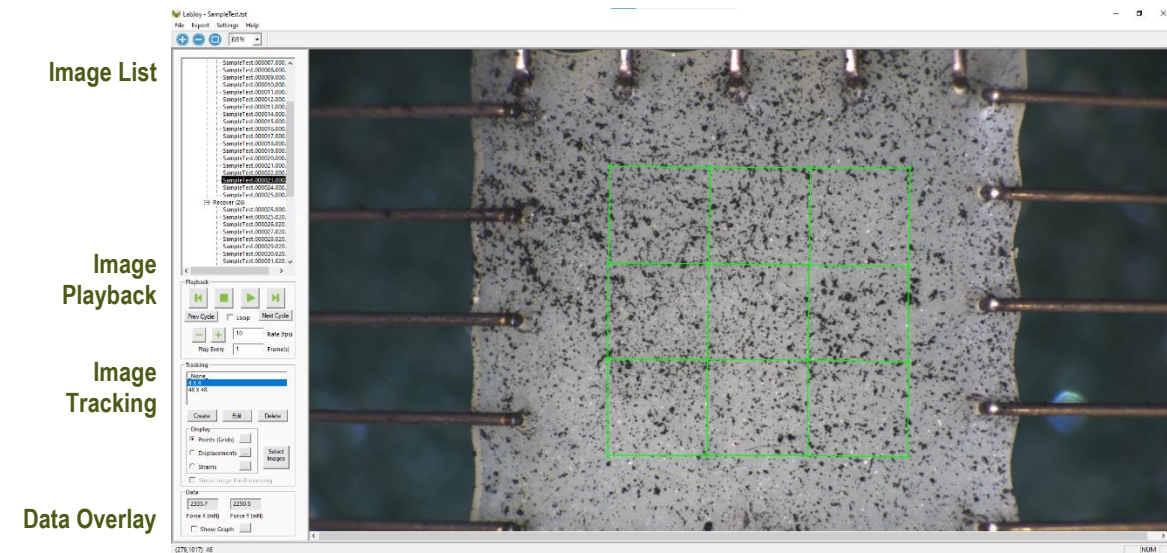
Perform Load Cell Calibration

The final step is to perform a load cell calibration. This procedure is detailed in **System Calibration and Advanced Tools** under **Load Cell Calibration**.

12. Appendix D: Reviewing Test Results

Overview

The BioTester 3000 software has an optional integrated image analysis module which can be accessed by selecting *Analyze and Review Images* from the File menu, then selecting the appropriate test file. The test file is a text file with a .tst extension that contains information about the test parameters so that the software can display the images and data in a useful fashion.



Selecting Images

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

Set (line on the Test Sequence window)

Cycle (iteration of a given Set)

Phase (Preload/Stretch/Hold/Recovery/Rest of each Cycle)

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

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

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

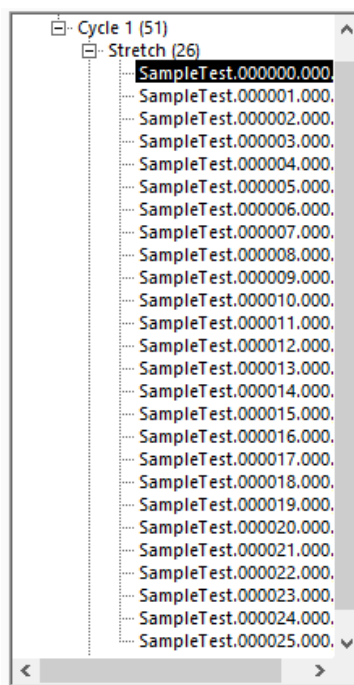
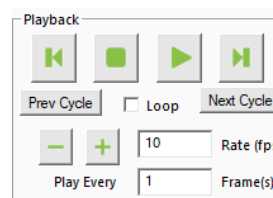


Image Playback Options

The controls on the left of the screen are broken into 2 sections: Playback and Tracking.

The playback buttons allow the user to review the images taken during a test.

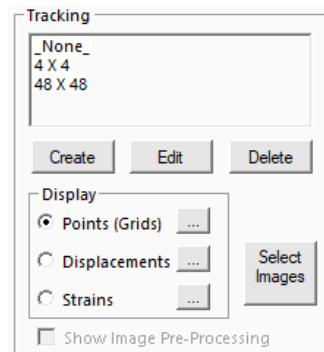


Next/Prev	Display next or previous image (as dictated by "Play Every" parameter).
Play/Stop	Start and stop the playback.
Prev/Next Cycle	Jumps to a corresponding image and phase in the next or previous cycle. Useful when reviewing sets that have multiple cycles.
Loop	Sequences can be played continuously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

Image Tracking: Overview

Image tracking is a function that can be used to quantify the motions of image features (rake lines, specimen texture and fiducial markers). This can be useful for studying localized specimen deformations, verifying strain magnitudes, and comparing the results of one test to another.

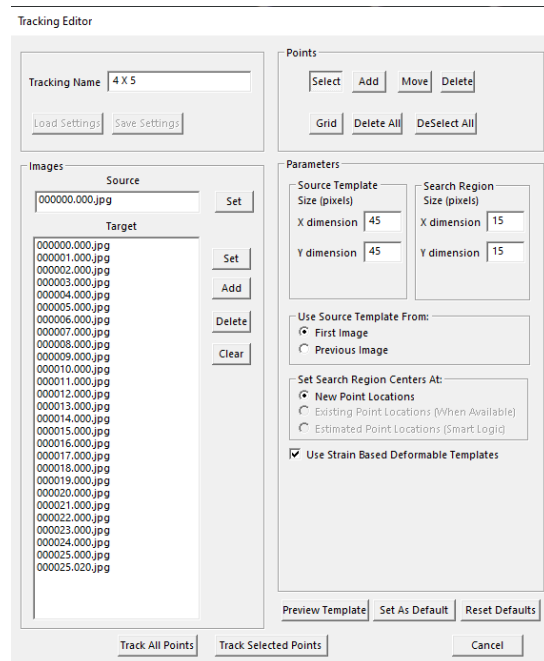
The image tracking engine is based on a template matching algorithm. This algorithm starts by defining a “patch” of pixels (called a template) surrounding the selected source point on the source image. It then determines the optimal location for this template on a target image within a specified search region.



The algorithm defines the location of the tracked point on the target image as the centre of the optimally located template.

To track an image, follow these steps:

1. Click the *Create* button in the tracking window, shown above. This brings up the Tracking Editor dialog box.
2. Enter a Tracking Name.
3. Select the Source Image in the test image list tree, and click *Set* to the left of the Source box.
4. Select the Target Images in the test image list tree, and click *Set* to the left of the Target box. The target image list will automatically be sorted to remove duplicates and be put in sequential order.
5. Generate source points on the source image by performing the following steps:
 - a. Manually click source point locations on the source image with the *Add* button depressed.
 - b. Draw a box on the source image with the *Select* button depressed, then click the *Grid* button and define a grid of points.



The displayed image will automatically change to the source image when either the *Add* or *Grid* buttons are depressed.

6. Modify search parameters:

Source Template	The optimal source template is difficult to predict, but in general it is advantageous for the template to be large enough that it contains at least one feature, but not so large that it contains multiple features. Typical values for this parameter are between 15 and 55, with 35 being a good starting point for most users.
Search Region	The search region needs to be large enough that the optimal location for the template is found. In general, no part of the specimen moves more than the rake tines, so the motion of the rake tines is a good guideline for how large the template needs to be. For example, if the rake tines move 50 pixels over the 10 frames you are interested in, it is unlikely that any part of the specimen is moving more than 5 pixels/frame and so a good value for the search region parameter would be 11 (5 pixels each way, plus the center pixel).
Use Source Template From	<p>First Image tracks all images against the first in the series (1→2, 1→3, 1→4...).</p> <p>Previous Image tracks each subsequent image against the image before it (1→2, 2→3, 3→4...).</p> <p>First Image Tracking tends to have poorer correlation than Previous Image Tracking because the current image is usually closer in content to the previous image than it is to the first image in the sequence. Previous Image Tracking tends to have good correlation for each individual step, but is prone to error accumulation since each step is independent. The best method for a given data set will depend on the number of images and the content of the images. Sometimes it is best to use Previous Image Tracking but to only use every other image or every n^{th} image rather than all the images available. Sometimes it is best to use First Image Tracking and use every image but set the source image in the middle of the image series rather than at the beginning.</p>
Set Search Region Centre	New Point Locations The location of the center of the search region is set to the same location of that point in the previous image (even when the source template is from the first image).
Use Strain Based Deformable Templates	When this option is checked, the source template will be scaled (resized) according to strains calculated from the test Size X and Size Y parameters. Best tracking results are often obtained when using this option. This option may not be the best for uniaxial tests or tests where the specimen deformation is non-uniform.

7. Perform tracking with either Track All Points or Track Selected Points.

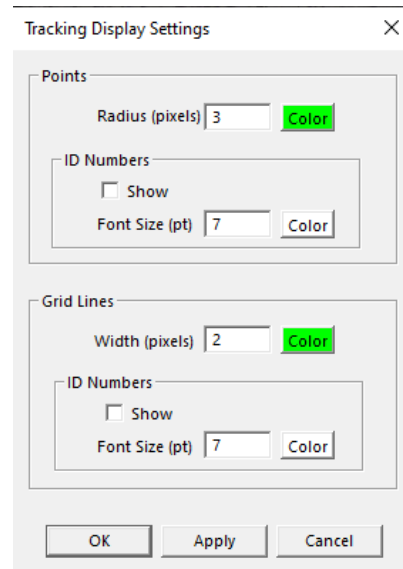
Image Tracking: The Points Display Option

The Points Option displays the points in their current location (i.e. their location on the currently displayed image) with or without gridline connecting points that were generated using the grid function.

Clicking the details icon brings up the window shown to the right. Options include changing the point radius or color, showing ID numbers and showing gridlines with or without ID numbers.

Gridline ID numbers are associated with each grid square (cell), while Point ID numbers are associated with the points themselves.

Tracking display settings default to the last used settings.



The 'Tracking Display Settings' dialog box contains the following options:

- Points Section:**
 - Radius (pixels): 3
 - Color: (Green button)
 - ID Numbers:
 - Show: ☐
 - Font Size (pt): 7
 - Color: (Color button)
- Grid Lines Section:**
 - Width (pixels): 2
 - Color: (Green button)
 - ID Numbers:
 - Show: ☐
 - Font Size (pt): 7
 - Color: (Color button)

Buttons at the bottom: OK, Apply, Cancel.

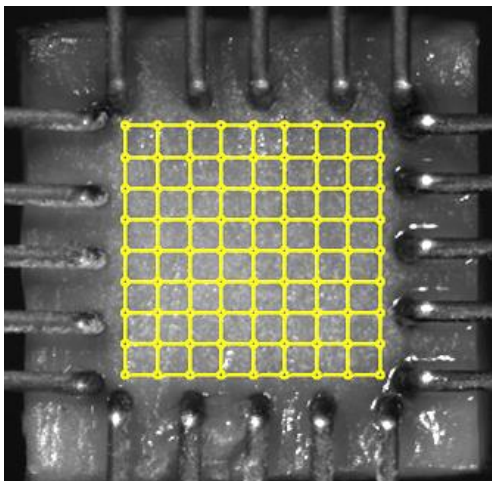


Figure 1: Initial tracking grid displayed as points.

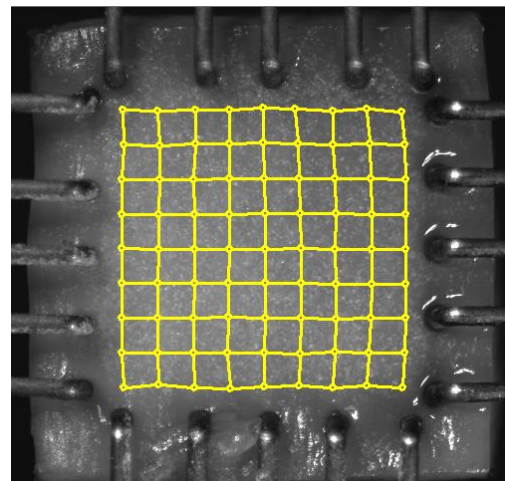


Figure 2: Tracking to 20% strain displayed as points.

Image Tracking: The Displacement Option

The Displacement Option displays the points in their current location as well as graphically representing their motion through time.

Clicking the details icon brings up the window shown to the right. This dialogue allows the user to change the reference image, change the type of connecting line, and change the line appearance.

Displacement connecting lines directly connect the reference and current points via a vector or a path. The path option provides more information but can result in slow playback speeds for sets with many points and/or many images.

The Flow option generates a direction vector based at the current location and oriented in the direction of motion relative to the reference image. This can be useful because the length of this vector can be scaled to better visualize small displacements.

Tracking displacement settings default to the last used settings.

Tracking Displacement Settings

Reference Image

Display As
☒ Displacements (Tail at Reference Location)
 ☒ Vector (single line segment) (fast)
 ☐ Path (many segments) (slow)
☐ Flow (Tail at Current Location)
 Scale Factor

Appearance
 Line Width (pixels)
 Head Radius (pixels)
☐ Show Points (Grids)

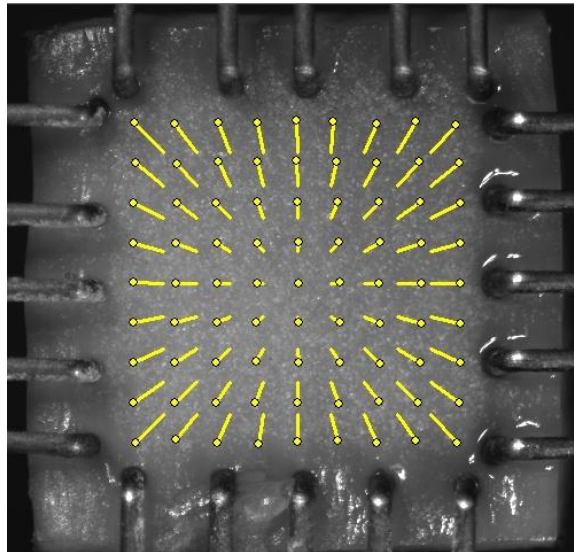


Figure 3: Tracking to 20% strain displayed as displacement.

Image Tracking: The Strains Option

The Strains Option can only be used in conjunction with a grid of points (as opposed to individually placed points). This option calculates the regional strains inside every grid box and displays this data as an ellipse. One can imagine this is what would happen to a grid of circles drawn on the surface of the specimen at the beginning of the test.

Clicking the details icon brings up the window shown to the right. This dialogue allows the user to change the reference image as well as the appearance of the circles and text.

Showing text for E1, E2 will result in the text showing the strains along the major and minor axis of the ellipse. Showing text for Ex, Ey will result in text showing the strains along the X and Y directions regardless of the ellipse orientation.

Tracking strain settings default to the last used settings.

Tracking Strain Settings

Reference Image

000000.000.jpg

Set

Options

(E1)

4 Point Average

16 Point Average

E1,E2 = Principal Strains

Ex,Ey = Coordinate Strains

R = Principal Angle (Deg)

Show Points (Grids)

Color Grid By Strain Values

Show

Auto

Mir

1

Max

2

Strain Ellipses

Show

Scale Factor

1

Line Width (pixels)

1

Color

Text (% Strain)

Show

Font Size (pt)

10

Color

OK

Apply

Cancel

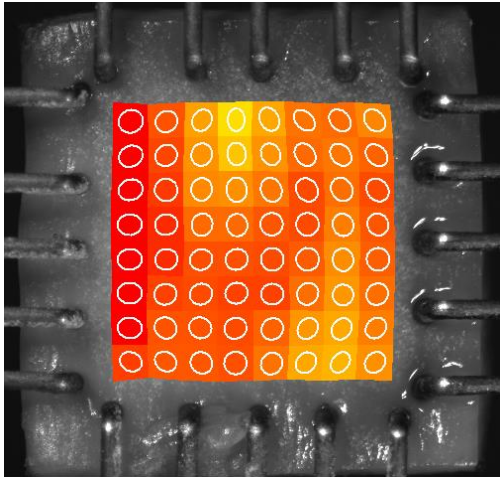


Figure 4: Tracking to 20% strain displayed as strain ellipses.

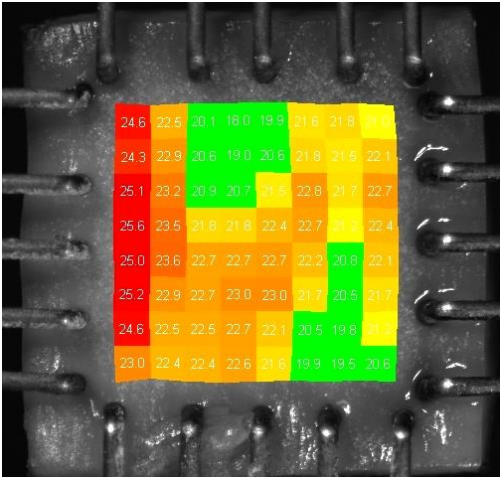


Figure 5: Tracking to 20% strain with text showing x% strain.

Data Overlay

Data associated with each image is displayed below the tracking controls on the left side of the main window. Checking the *Show Graph* box displays this data as a graph overlaid on the images

Data

10

-20

Force (mN)

Force Y (mN)

☒ Show Graph

...

Clicking the details icon displays the window shown to the right. The dialogue allows the user to change the size and appearance of the overlaid graph. The data points associated with the current image are marked on the graph with green circles. The overlaid data graph will be drawn on top of any image tracking displays (when they overlap).

Data Overlay settings default to the last used settings.

Data Output Settings

Data

Force

Graph Overlay

Range

☒ Current Set

☐ Current Cycle

Size (Pixels)

Width

400

Height

100

Location (Pixels)

Left

400

Top

400

X Data

☒ Show

Line Width (pixels)

2

Color

Y Data

☒ Show

Line Width (pixels)

2

Color

☐ Show Solid Background

Color

☒ Show Border

OK

Apply

Cancel

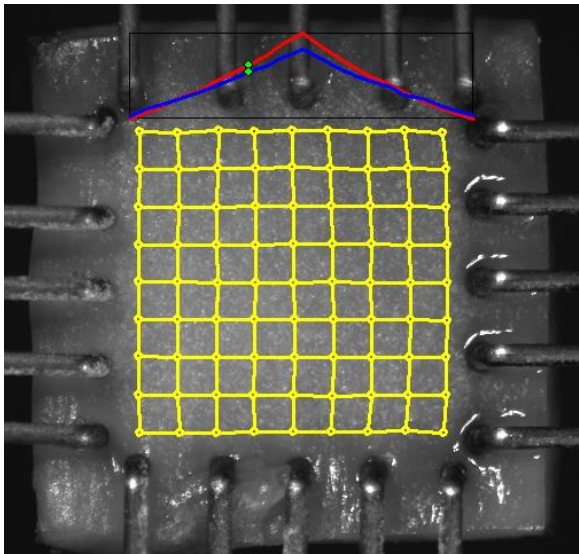


Figure 6: Tracking to 20% strain with overlaid x-y force graph.

Exporting Tracked Data

Tracked grid point coordinates and calculated grid strains can be exported to a .csv file. From the Export menu, select *Data Points* to display the dialogue shown on the right. The image list is automatically set to the currently selected images in the test image list tree. This image list can be changed by selecting new images in the test image list tree and pressing the set button. When exporting Strains data from Selected Tracking Points, all four points of a grid cell need to be selected for data from that cell to be exported.

Exported data is labeled with associated ID numbers (grid point ID numbers for exported coordinates and grid cell ID numbers for exported strain values). To display ID numbers on tracking grids see Image Tracking: The Points Display Option. In the following example (Figure 9), grid point ID numbers are shown in yellow and grid cell ID numbers are shown in white. When exporting data for the selected points as shown in red, coordinates are exported for points with ID numbers 5, 6, 7, 8, 9, 10, 11 and 12, and strains are exported for grid cells with ID numbers 4, 5, and 6.

The exported .csv file is written to the test directory.

Export Points (Grids) Coordinates

Image List

000000.000.jpg

Set

Add

Delete

Clear

Export

☒ All Tracking Points

☐ Selected Tracking Points

☒ Grid Point Coordinates (Pixels)

☒ Principal Strains (E1, E2, Angle)

☒ Coordinate Strains (Ex, Ey)

☐ Deformation Tensor (F)

Output Filename

C:\Cellscale\LabJoy\Data\SampleTest\Coords1.csv

Browse

OK

Cancel

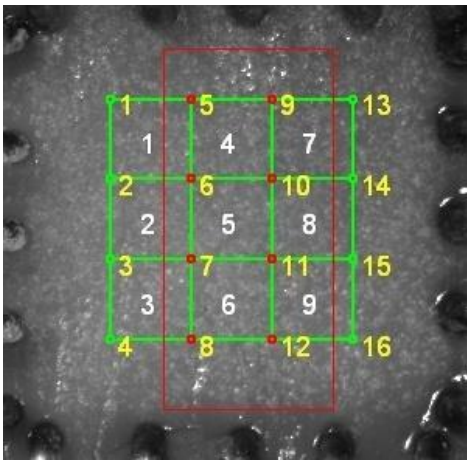


Figure 7: Tracking Grid showing ID Numbers.

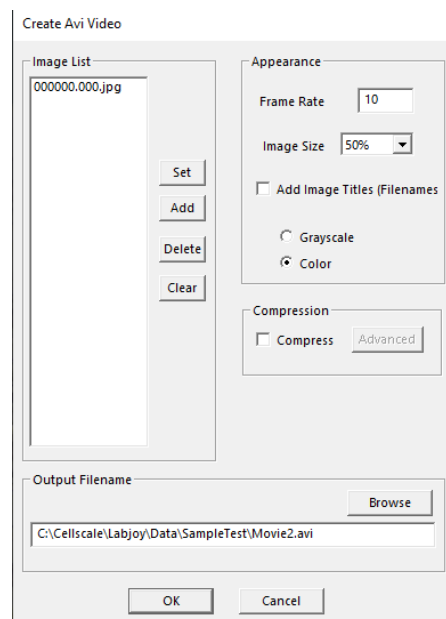
Grid Point	Coordinates (pixels)									
FileName	Time(S)	X5	Y5	X6	Y6	X7	Y7	X8	Y8	X9
2nd Goos	181.4	588	323	588	426.667	588	530.333	588	634	69
2nd Goos	182.4	587.125	318.75	586.75	424.75	586.875	531.625	586.625	636.875	692.62
2nd Goos	183.4	586.5	314.75	586.25	423.5	586.125	532.375	585.375	639.25	693.62
2nd Goos	184.4	585.375	310.5	585.25	421.625	585.375	532.625	584.375	642	694.37
2nd Goos	185.4	584.375	306.875	584.125	420	584.125	533.125	583.125	645.625	695.12
2nd Goos	186.4	583.625	303.125	583.125	418.875	583.25	534.75	582.5	649	695.87
2nd Goos	187.4	582.5	299.125	582.5	417.5	582.125	535.75	581.25	652	696.7
2nd Goos	188.4	581.5	295.25	581	416	581	536.25	578.625	655.875	697.62
2nd Goos	189.4	580.5	291.125	579.875	414.875	579.75	537.5	577.375	658.875	698.62
2nd Goos	190.4	579.375	286.625	578.75	413.25	578	539	576.5	662	699.
2nd Goos	191.4	578.25	283.5	577.5	412	577.25	539.75	574.25	665.75	700.
2nd Goos	191.5	578.25	283	577.5	412	577.125	540.125	576.875	665.125	700.62
Coordinate Strain X - Values (16 point least squares average) (Grid Cells are numbered from the top left corner.										
FileName	Time(S)	StrainX_4 StrainX_5 StrainX_6								
2nd Goos	181.4	0	0	0						
2nd Goos	182.4	0.02448	0.02448	0.02512						
2nd Goos	183.4	0.04696	0.04739	0.0492						
2nd Goos	194.4	0.06915	0.06963	0.07204						
2nd Goos	185.4	0.08994	0.09044	0.09307						
2nd Goos	186.4	0.10994	0.11052	0.11394						
2nd Goos	187.4	0.13117	0.13203	0.13574						
2nd Goos	188.4	0.15236	0.15373	0.15853						
2nd Goos	189.4	0.17472	0.17701	0.18233						
2nd Goos	190.4	0.19704	0.19955	0.20549						
2nd Goos	191.4	0.21951	0.222	0.22825						
2nd Goos	191.5	0.22055	0.2223	0.22813						
Coordinate Strain Y - Values (16 point least squares average) (Grid Cells are numbered from the top left corner.										
FileName	Time(S)	StrainY_4 StrainY_5 StrainY_6								
2nd Goos	181.4	0	0	0						
2nd Goos	182.4	0.01942	0.02171	0.02187						
2nd Goos	183.4	0.04246	0.04261	0.03932						

Figure 8: Sample Exported Data.

Exporting Images and Movies

The current Image view, including all displayed tracking grids, tracking data and overlaid data can be exported as a metafile by selecting *Current Image* from the Export menu. Alternately, the current image view can be copied to the clipboard by selecting Ctrl-C from the keyboard.

To export a series of image views, including all displayed tracking grids, tracking data and overlaid data as an .avi movie, select *Movie* from the Export menu. The dialogue shown on the right will be displayed. The image list is automatically set to the currently selected images in the test image list tree. This image list can be changed by selecting new images in the test image list tree and pressing the set button. Options are available for setting the frame rate or image size (resolution), adding filenames, and choosing color or grayscale output. Compression options are also available.



Importing and Analyzing Non- BioTester 3000 Images

The LabJoy software can be used to perform feature displacement tracking and strain tracking on .jpg images using the "Import Images" function located in the File menu. To use this feature, select the desired images using the shift/ctrl keys in the dialogue box or else select the directory to import all of the contained images.

Once the images have been imported, the software works much the same as with BioTester 3000-generated images.

13. Appendix E: Mechanical Grip Usage

Installing Grips

In addition to BioRakes, the BioTester 3000 can optionally be equipped with mechanical grips for sample attachment.

The mechanical 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.

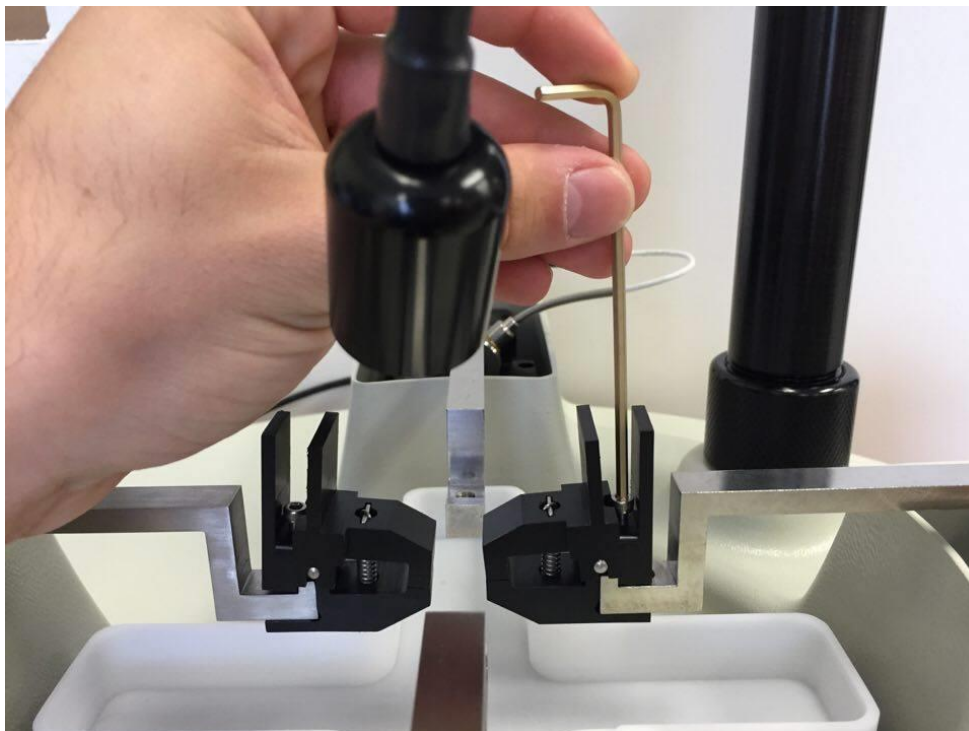
The light, medium and heavy springs will exert **1.5N**, **6N**, and **15N** of gripping force respectively.

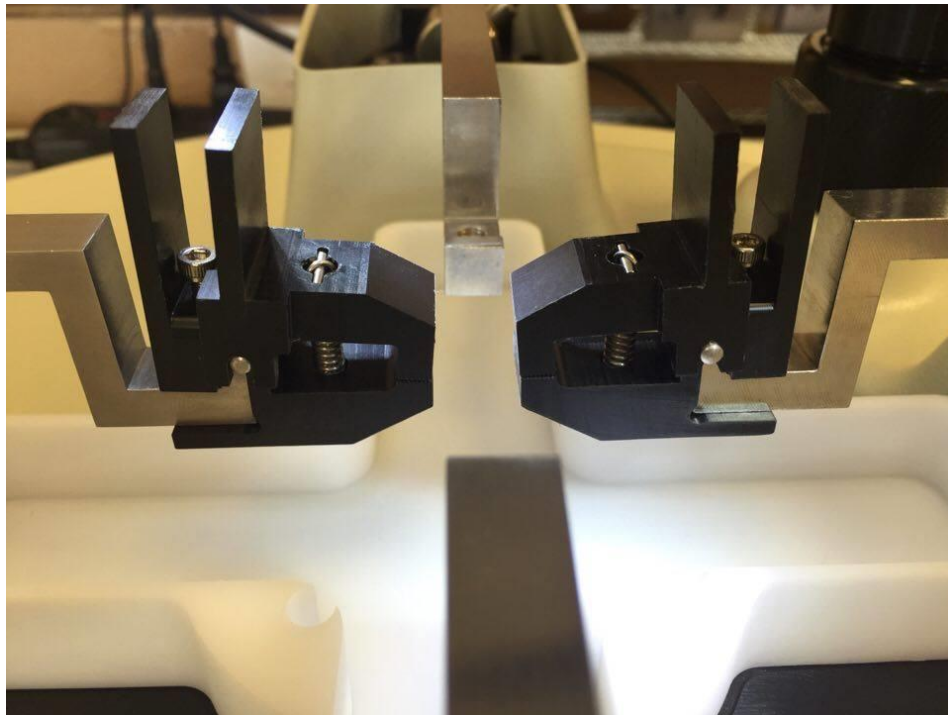
System Alert

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:

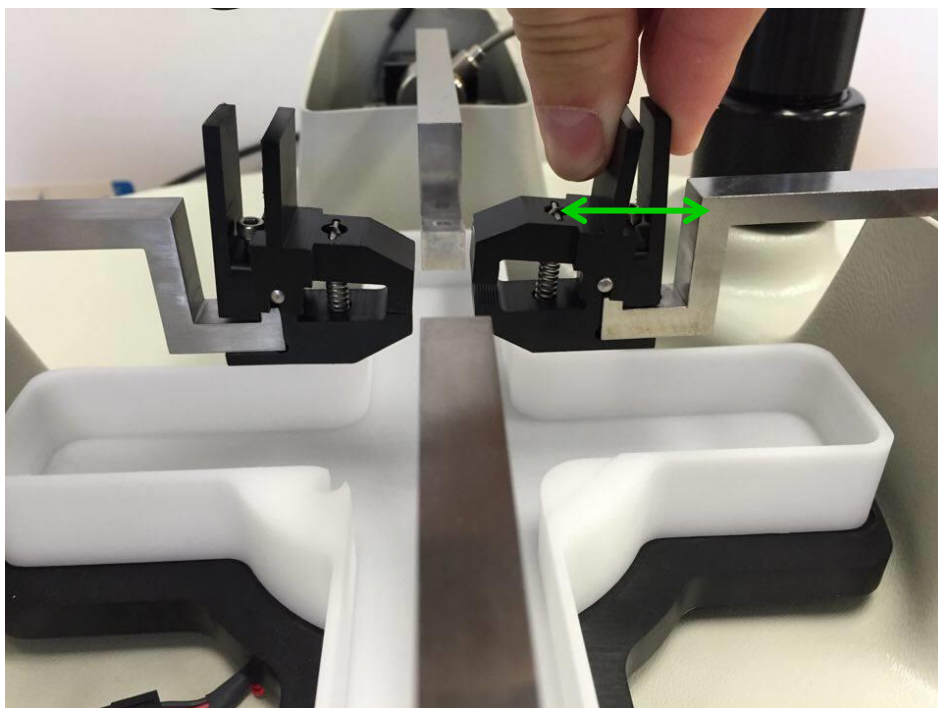
1. Slide the grips onto the goosenecks as pictured below.
2. Tighten the locking fastener to hold the grips in place on the gooseneck. Do not over-tighten the locking fastener as this may push the tip of the grip out of alignment.





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.

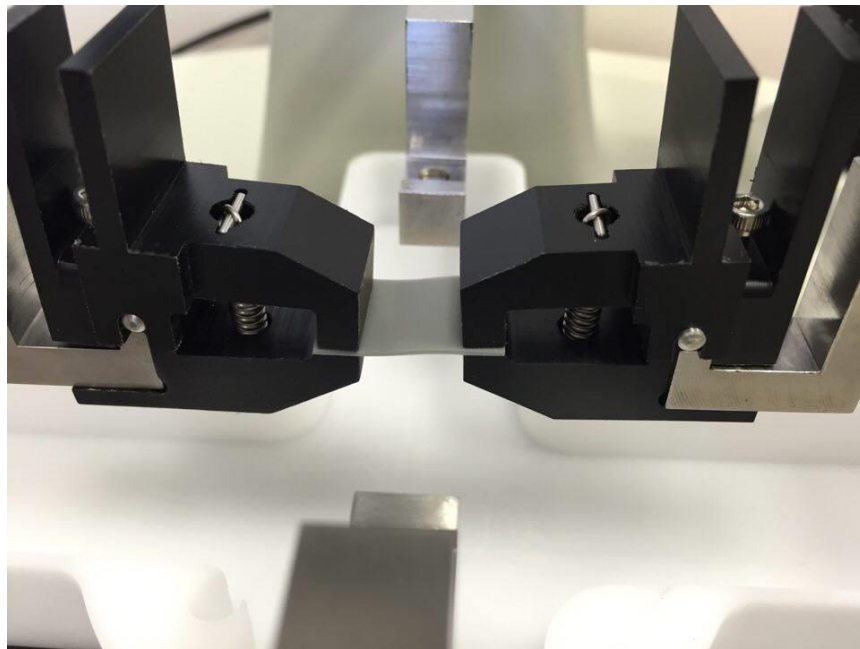


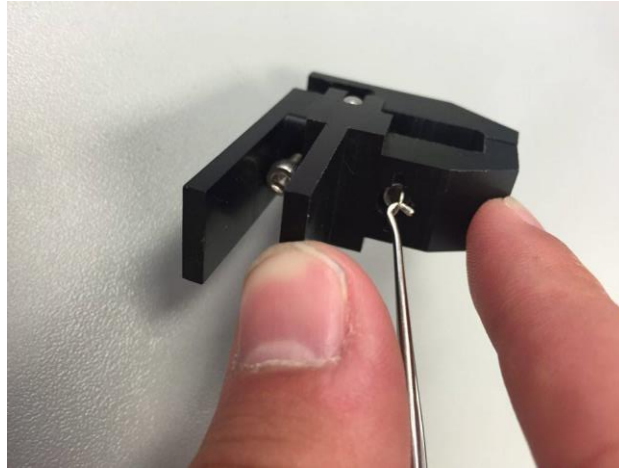
Figure 9 - Uniaxial Test Setup



Figure 10 - 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.



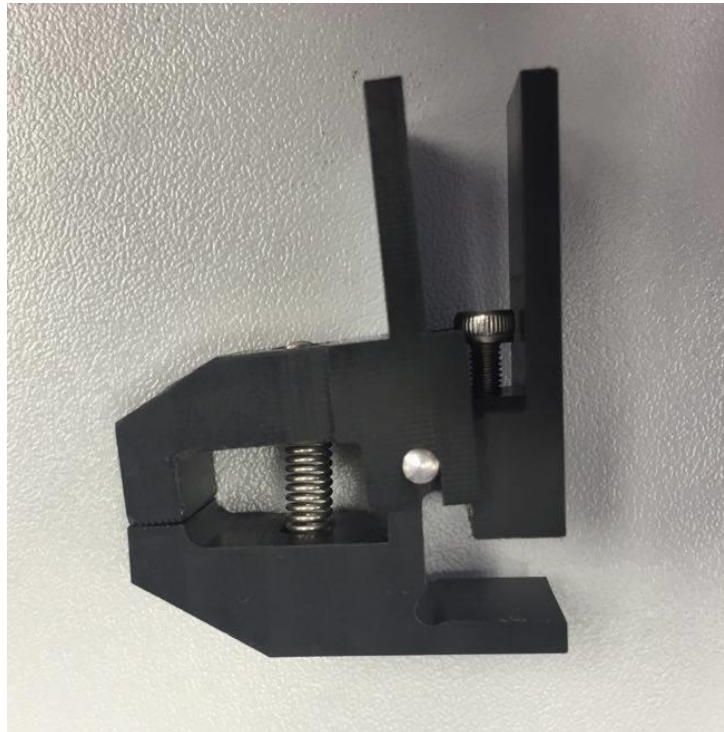
System Alert

*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).

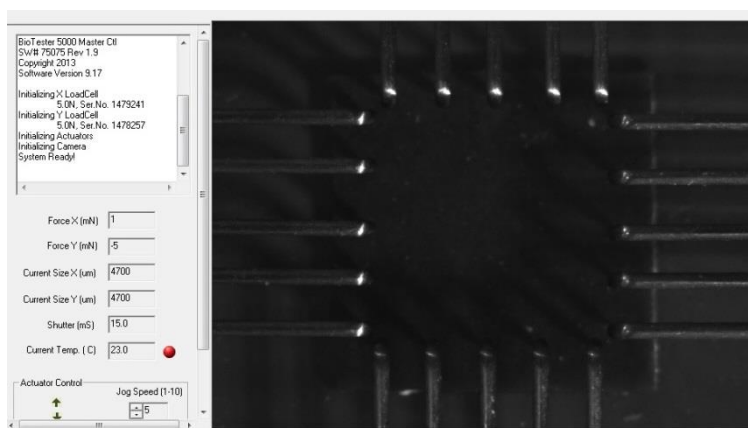


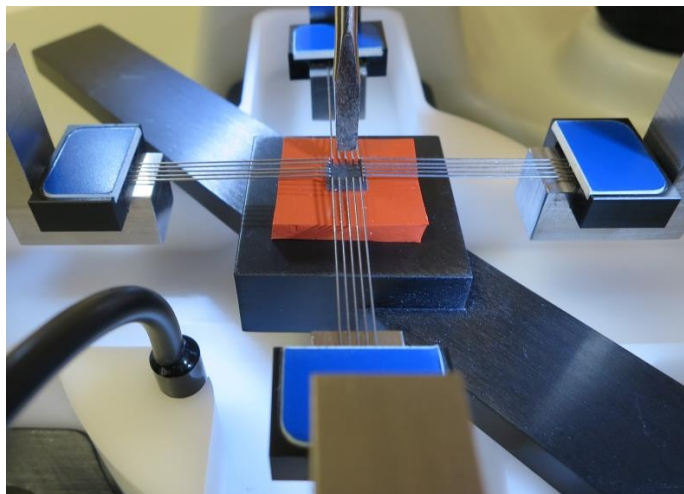
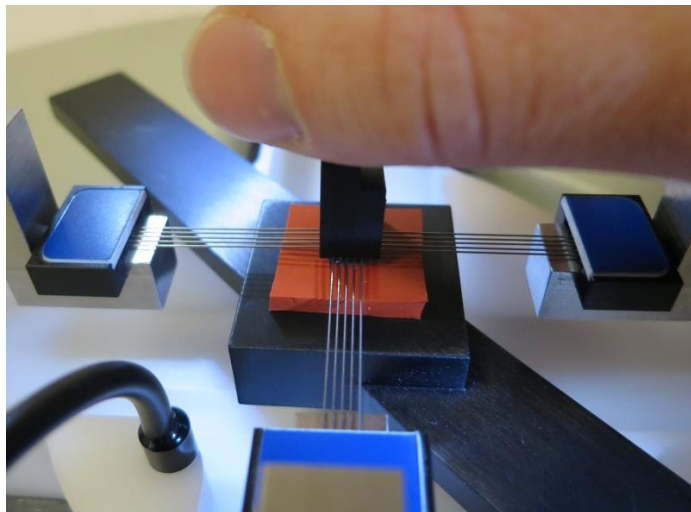
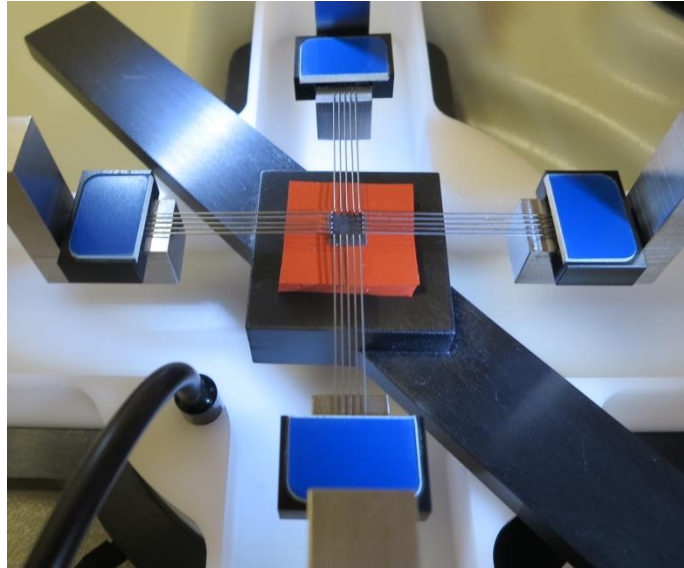
14. Appendix F: Specimen Mounting with Temperature Controlled Fluid Bath

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 soft silicon or 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.





Adjusting the Camera Position and Image Magnification

The optional 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.

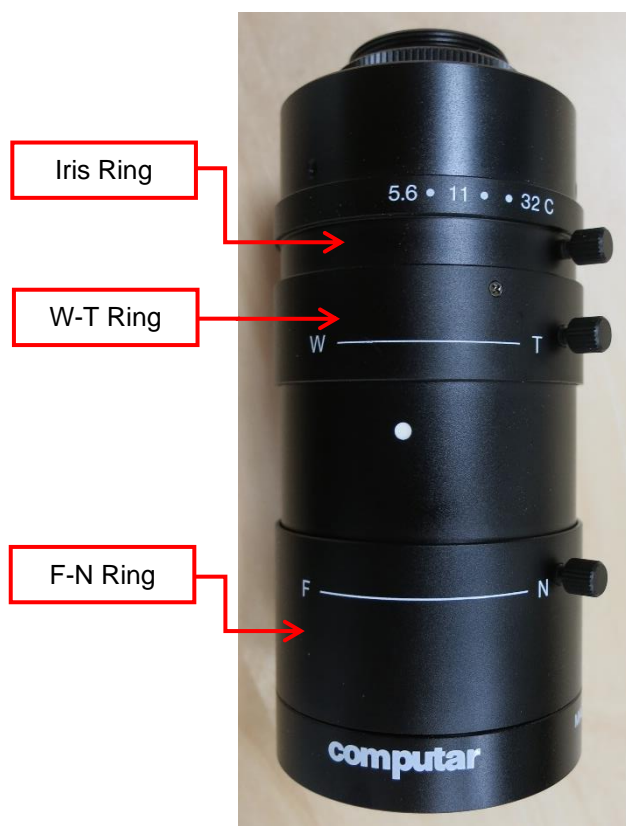
The BioTester 3000 comes equipped with a 10X zoom lens as shown below. Other versions of the BioTester 3000 may be equipped with a fixed magnification 75mm focal length lens (see Appendix E).

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:

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.

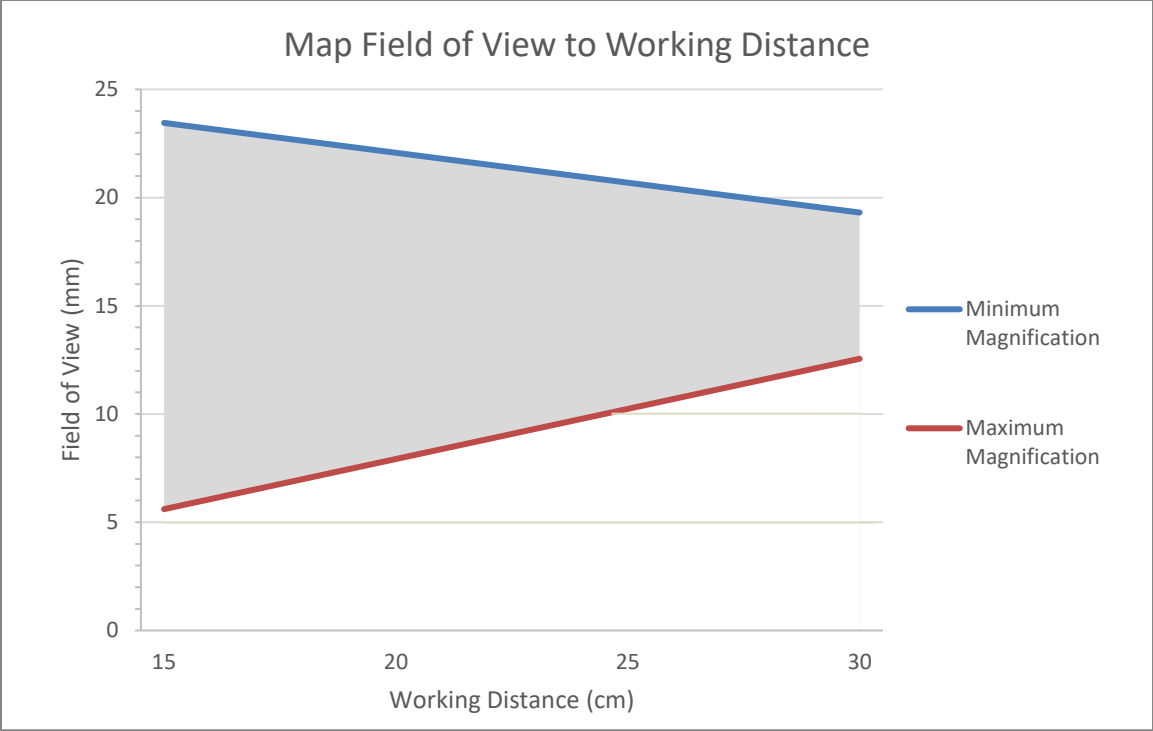
The W – T ring changes the lens zoom.

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.

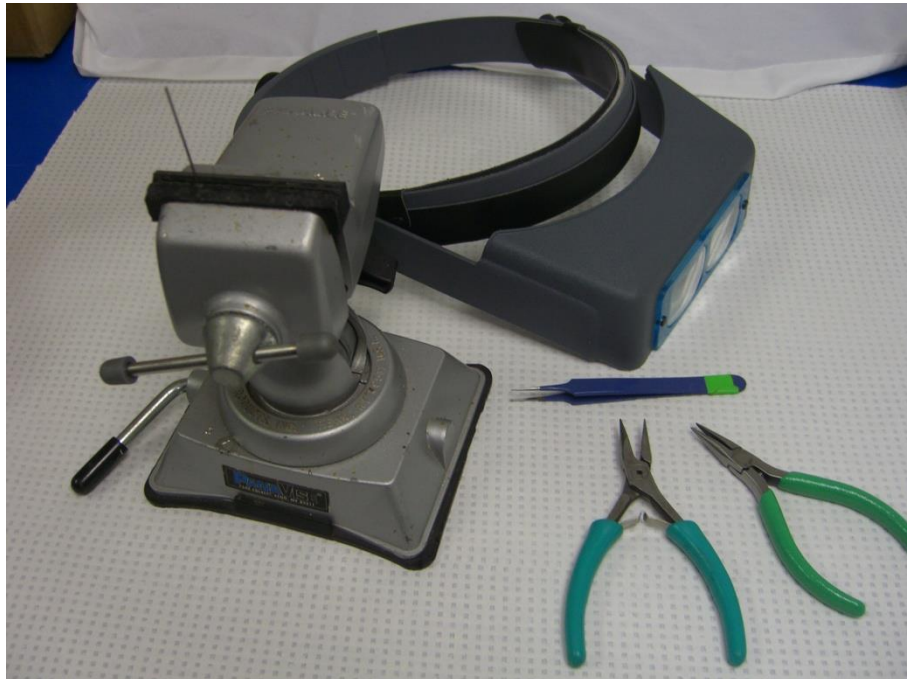


15. Appendix G: Protocol for Tying and Cutting Sutures for Biaxial Testing

In addition to BioRakes and grips, the BioTester 3000 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.

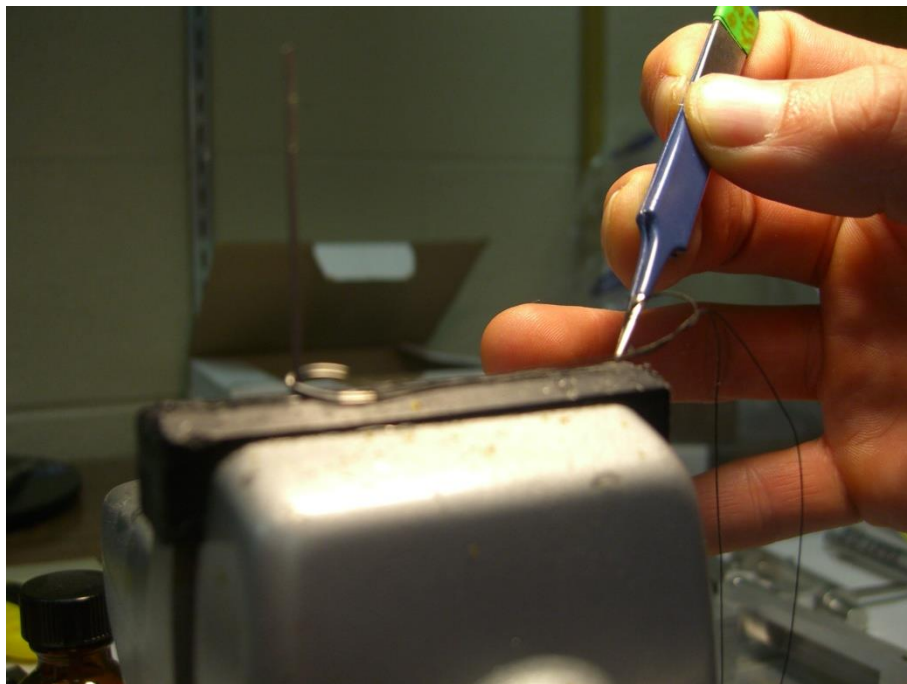
1. Assemble equipment: small vice, magnifying goggles, 2 needle nose pliers, small forceps.

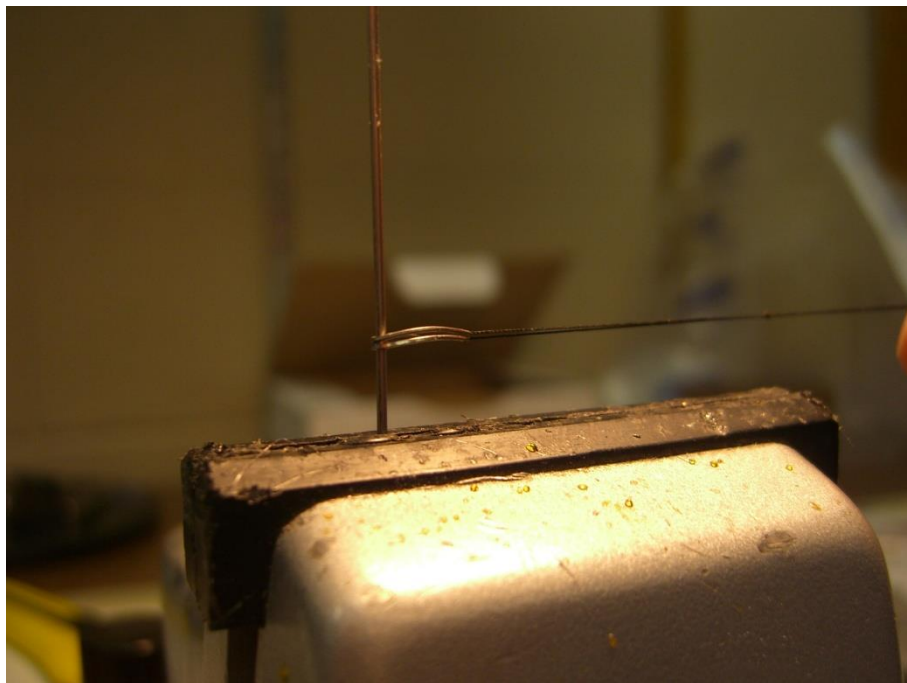
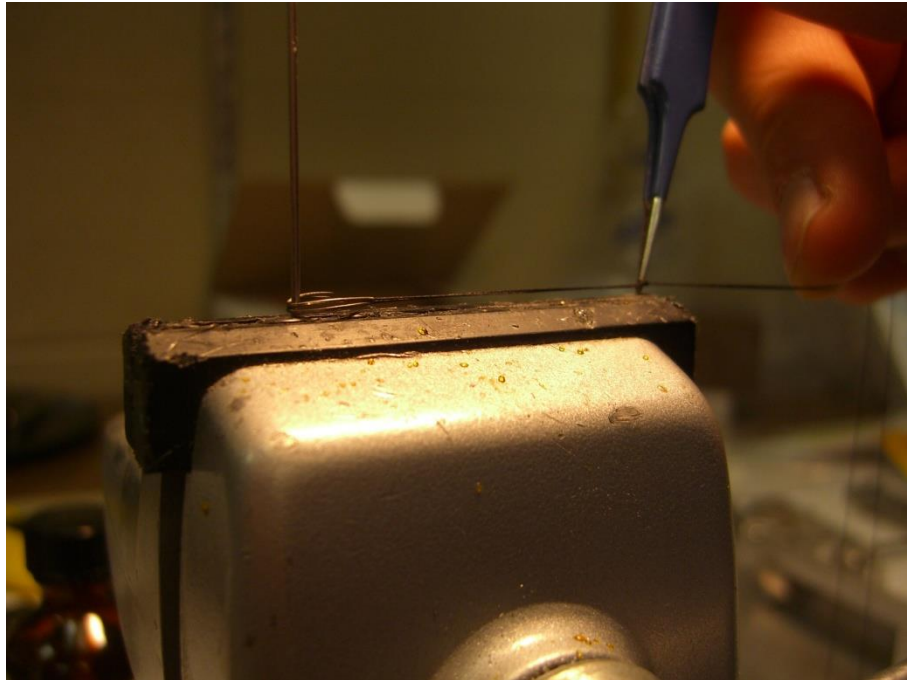


2. Bend suture hooks

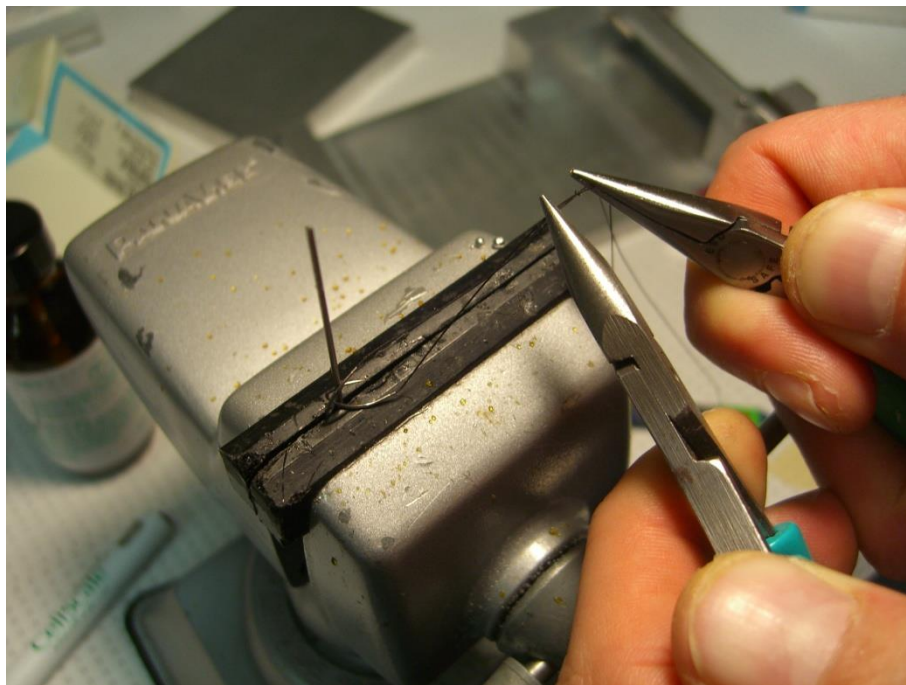


3. Tie off sutures. First hook each bent hook around a secured post held in the vice. Then tie a standard knot in the suture and use forceps to guide the location of the knot so that it tightens at the correct location.





4. Use pliers to tighten knot



5. Cut off excess.



The resulting mounted specimen should ideally look something like this:

