

MicroTester LT

Micro-scale Tension-Compression Test System

User Manual version 4.5



Mechanical measurement and analysis of biomedical materials

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Table of Contents

1. General Information	1
System Assembly	1
Connections to Supply	1
Safety Warnings	1
Operating Controls	1
General Maintenance	1
Approvals and Certification	2
2. Parts	3
Tools	3
Basic Components	3
3. Testing Terminology	4
Multiphase Test Cycles	4
Phases, Cycles, and Test Sequences	4
Test Phases: The Smallest Unit of Testing	5
Control Modes	6
Control Functions	6
4. System Overview	7
Microbeam	7
Microbeam Actuators	9
Imaging System	9
Fluid Bath	9
Software Overview	11
Output Files and Data Structures	13
5. Setting Up & Starting a Test	14
Overview	14
Step 1: Start a New Test	15
Step 2: Modify Testing Parameters (optional)	16
Step 3: Specify the Microbeam Properties (Occasional)	19
Step 4: Load the Specimen	20
Step 5: Set the Optics Zoom Level	21
Step 6: Adjust the Initial Size Crosshairs	21
Step 7: Select a Tracking Point	22
Step 8: Execute the Test	23
Step 9: Terminate the Test Prematurely (optional)	23
6. Additional Settings	
Camera Shutter and Gain	
Grid Overlay	24
Temperature	25

Configuring the Real-Time Graphs	26
Data Output Settings	27
Advanced Settings	28
7. Reviewing Test Results	29
Overview	29
Selecting Images	30
Image Playback Options	30
Data Overlay	31
Exporting Images and Movies	32
Image Tracing	33
Exporting Traced Data	35
8. System Calibration and Advanced Tools	36
Optics Calibration	36
Snap Image	37
9. Mounting a MicroBeam	38
10. Force Calculation Formulas	45
11. Appendix A: System Setup	46
Unpack Main Unit and Accessories	46
Foam Removal from Beam Stages	46
Attach Camera in Clamshell Mount	48
Set Test Chamber on the Chamber Holder	49
Place Temperature Sensor in the Test Chamber	49
Connect Cables	50
System Calibration Check	50
12. Appendix B: Software Installation	51
13. Appendix C: Software Troubleshooting	57

1. General Information

Electrical Input to Power Supply	100-240VAC, 50-60Hz
Input Current Rating of Power Supply	0.6 Amp
Electrical Output from Power Supply	24 VDC
Output Current Rating of Power Supply	1.75 Amp
Recommended Computer OS	Windows 10
Environmental Conditions	Maximum Operating Temperature 25°C
	20% - 80% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree II
Data Connections	1 – USB 3.0 for camera/PC communication
	1 – USB for actuator controller/PC communication

System Assembly

Please see instructions in Appendix A for details on system assembly. See Appendix B for installing the software, and Appendix C for details on setting up the host computer.

Connections to Supply

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

Safety Warnings

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

Operating Controls

Both the camera and microbeam assembly contains motorized X, Y and Z adjustment stages to position and focus the camera view. In addition, the camera brightness can be controlled by setting the software interface. The only manual control on this unit is the zoom level, it is set using the twist knob.

General Maintenance

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

Approvals and Certification

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

EN 55011: 2008 Class A

EN 61000-3-3: 2008

EN 61000-4-2: 2009

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

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

EN 61000-4-5: 2006

EN 61000-4-6: 2009

EN 61000-4-8: 2010

EN 61000-4-11: 2004

2. Parts

Tools

Small flat head screwdriver

Metric hex wrenches: 1.5mm, 2 mm, 2.5mm, 3mm

SAE hex wrenches: 5/64"

Basic Components

Main Assembly – Houses control board used for operating motorized stages for beam and camera, temperature controller, heater and ring light.

3.0 USB Camera

3. Testing Terminology

The MicroTester LT is designed to apply compressive loads to small test specimens 80 to 2000 μm in size. Typical specimens include biological tissue samples, scaffolds, cell aggregates, or hydrogel microspheres.

Multiphase Test Cycles

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

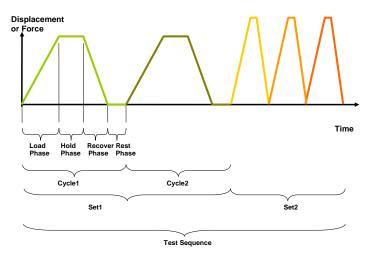
Preconditioning - The goal of preconditioning is to restore a specimen to its physiological or *in vivo* state. During the process of specimen storage and preparation, a specimen may swell, dry out, have its material fibers realign, or its molecules reorganize. It may take multiple preconditioning cycles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

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

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

Phases, Cycles, and Test Sequences

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



The above example describes the following:

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

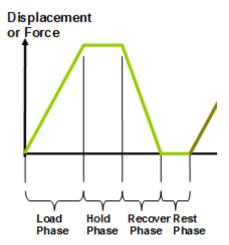
Test Phases: The Smallest Unit of Testing

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

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

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

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



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

Control Modes

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

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

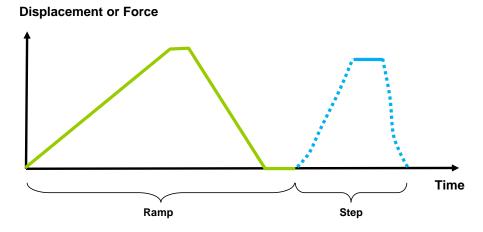
Under **force control**, the force applied to the specimen is predefined. The MicroTester LT compresses the specimen until the predefined force is achieved. The displacement required to achieve the force is an output of the test.

Control Functions

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

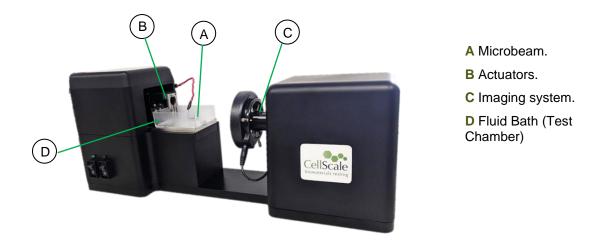
The **ramp function** applies the displacement at a constant nominal rate. This is equivalent to a constant engineering or nominal strain rate.

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



4. System Overview

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



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

Microbeam

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

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

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

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

Conversely, if the microbeam is too flexible, then the deflection will be too large. This can have a negative influence on the reaction time of the device and on the parallelism of the compression plates. With more deflection of the microbeam, the actuator must travel further to achieve the desired tip displacement. Since the velocity of the actuator is limited to about 2mm/s, this can cause unwanted delays. Also, as the beam

deflects, the top compression plate can become slightly less parallel to the lower compression plate. In a test with a moderate microbeam deflection of 100µm, this effect is very small, since the beam is typically over 30mm long. In a test with a large microbeam deflection of 2mm, this effect becomes more significant.

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

Diameter (mm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588	1.0160	1.5748
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (µN)	0.012	0.038	0.191	0.604	3.056	9.658	34.52	330.54	1907.88
Recommended Max Force (µN)	4	11	57	181	917	2,897	10,536	99,162	572,363



Microbeam with Platen

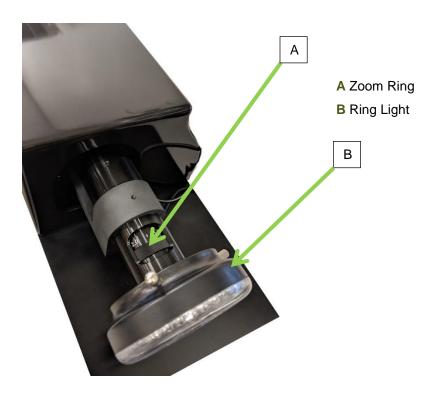
Microbeam Actuators

There are 2 actuation systems integrated to the microbeam assembly. The first system is comprised of the 3 stepper motors that are used to position the microbeam in plane before the start of the test. The actuators are controlled by the user, using on-screen controls in an open loop jog mode. There is no absolute measurement of how far they have been moved since feedback is based on user interpretation of the live on-screen image.

The second actuator system is the closed-loop, stepper motor actuator that is used to move the microbeam vertically. It is driven in a user-controlled jog mode during setup and then under computer control during the test. The actuator used for this purpose is identical to the other 2 axes. This motion constitutes the movement of the base of the microbeam. The displacement of the base and tip of the microbeam are needed in order to determine the deflection of (and hence the force applied by) the microbeam.

Imaging System

The imaging system is comprised of a USB digital camera, a zoom lens, and a motorized XYZ stage assembly to allow position adjustment and focusing. The camera has a resolution of 1536 X 1536 pixels and can match the control frequency of 5Hz. The zoom optics allow for a field of view ranging from approximately 0.8mm to 5.5mm.



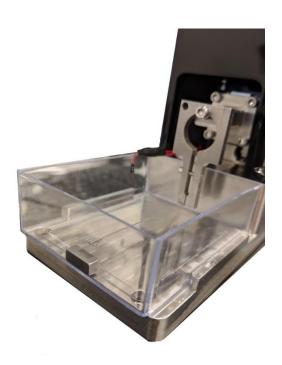
Fluid Bath

The fluid bath contains approximately 45mL of fluid and is heated by a resistance foil heater in the base of the bath. The fluid temperature is controlled by an onboard temperature controller that communicates with the PC via the USB connection. The temperature of the bath is measured by a solid-state temperature probe with a temperature resolution of 0.1°C. To function the MicroTester LT software will log the actual temperature of the fluid. To enable the controller to heat the fluid, the "HEATER" switch beside the main power switch must be turned on to illuminate the amber heater light.

MicroTester LT Tip: Temperature Probe

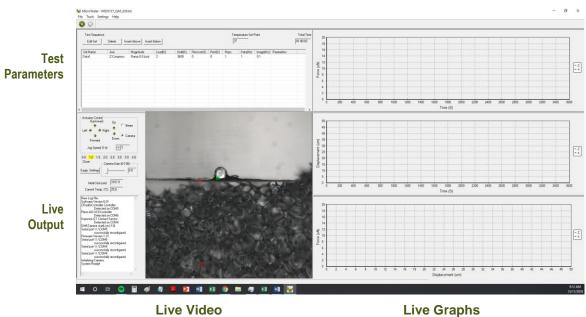
The temperature controller works best if the temperature probe tip is completely submerged and **not** touching the side of the fluid chamber.

The bath is a clear polycarbonate dish which allows for visibility from all sides, the main imaging system is setup to image the front side of the dish, where the compression platform is setup in the image below. The compression plate is typically made of a stainless-steel block and is flush with the front window.

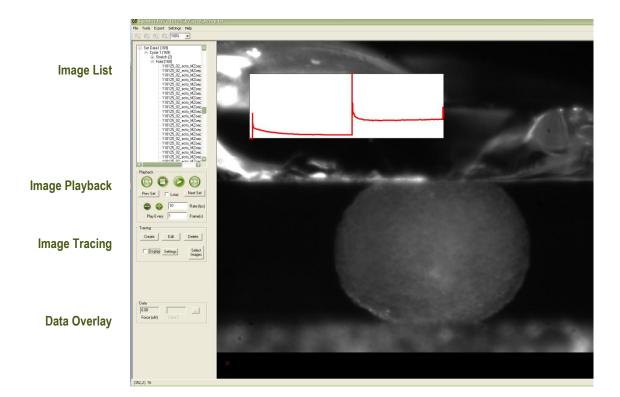


Software Overview

The software included with the MicroTester LT system is called MicroTester. 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 modules are used to playback accelerated or decelerated test images, perform image analysis and tracking, and output movie files for presentation purposes. The screen layout for this module is shown below:



Output Files and Data Structures

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

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

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

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

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

5. Setting Up & Starting a Test

Overview

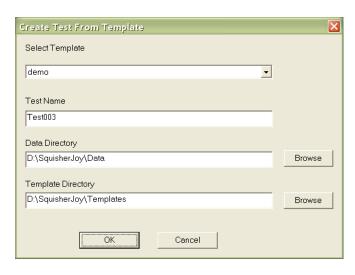
Setting up and running a new test is a simple process made up of both mandatory and optional steps. The following list presents all the steps, while the rest of this chapter describes each of the steps in detail.

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

Step 1: Start a New Test

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

- Select a template that matches the type of test you wish to perform. See the *MicroTester LT Tip* on how to select and use a template. You can modify the template parameters in Step 2, below.
- Name your test. The dialog will have a default test name. You can use the default or rename the test. Each time you start a new test, the default name will continue to increment the number at the end of the name.



3. If desired, you can change the location of the output data. The location of your output data and images is determined by specifying a Test Name and Data Directory. The template and data directories are user specific (computer login name). Each system user can store their files to a different default location. The system uses the last location selected as the default.

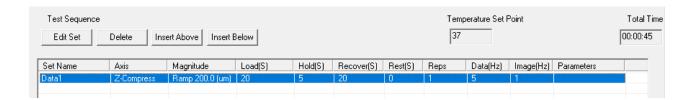
MicroTester LT Tip: Selecting and Using Templates

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

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

Step 2: Modify Testing Parameters (optional)

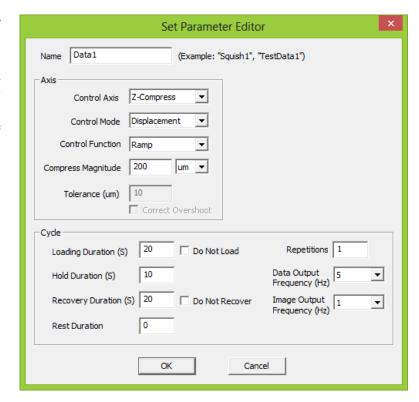
You can select and modify test parameter sets by clicking on their row in the Test Sequence table and then pressing the Edit Set button (or by double clicking on their row).



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 μN , and durations are specified in seconds.

The following table describes each of the parameters.



Test Parameter	Description
Control Axis	Loading is normally applied in the vertical Z-Compress axis.
Control Mode	Compression tests are typically performed under displacement control. However, you can select the following control modes to achieve specific testing objectives:
	For creep testing, use force control with a long hold duration.
	For relaxation testing, use displacement control with a long hold duration.
Control Function	Ramp functions are most commonly used although step functions are also used.
Compress Magnitude (Load Magnitude when in	Compression magnitude is specified as an absolute position or force relative to the initial state of the microbeam. Positive magnitudes are compressive (the beam moves down).
Force Control mode)	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 1000µm Initial Size specimen can be expressed as either 100µm or 10%.
	If you are using Force control mode, you can only specify the force magnitude in μN .
	Watch the test results carefully to determine which magnitude setting best achieves your test goals.
Tolerance	For step functions only.
	A step compression is considered achieved when the current microbeam position (or force) is at the specified magnitude +/- the tolerance. A tolerance that is too small may result in unacceptably long compression durations and/or instability.
	Recommended values are at least 10 times the force resolution of the beam. (see chapter: Force Calculation Formulas for more details).
Correct Overshoot	For step functions only.
	An overshoot is defined as a compression magnitude that is larger than the specified magnitude plus the tolerance. When checked the control software will move the microbeam up and/or down until the desired step magnitude is achieved (i.e. recover from overshoot). When unchecked, the control software will consider overshoot as the successful completion of the step function. Not correcting overshoot is important for relaxation testing.
Loading Duration	For evenly spaced images, it is recommended to choose a number that is an integer multiple of the Image Output Frequency.
Hold Duration	Hold Duration is typically set to 0, however it is useful for creep or relaxation testing.
Recovery Duration	Recovery Duration is often set to the same value as the Stretch Duration.
Do Not Recover	Select this to skip the recovery phase.
Rest Duration	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.
Repetitions	Apply enough repetitions until the force deformation curves from one repetition to the next start to overlie each other.
Data Output Frequency	Typically set to the same frequency as the image output frequency.
Image Output Frequency	Typically set to 1Hz for cycles > 30 seconds and 5 Hz for cycles < 30 seconds.

MicroTester LT Tip: Multi-Stage Compression Testing

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

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

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

MicroTester LT Tip: Testing Tissues that Relax Quickly

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

MicroTester LT Tip: Applying Preloads

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

MicroTester LT Tip: Tension Testing

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

Step 3: Specify the Microbeam Properties (Occasional)

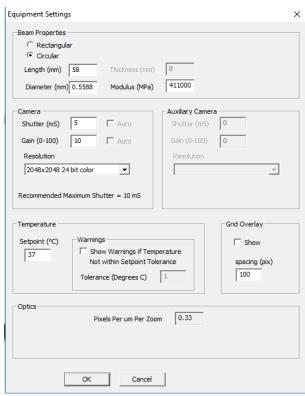
Microbeam properties including dimensions and modulus must be correctly specified to obtain correct force outputs. You can check and set Beam properties by selecting Equipment from the Settings menu or by pressing the Equipment Settings button in the Live Output section of the main display window. The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

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

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

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

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



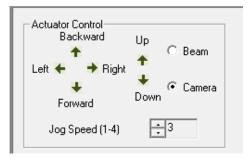
Diameter (mm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (μN)	0.012	0.038	0.191	0.604	3.056	9.658	34.521
Recommended Max Force (μN)	4	11	57	181	917	2,897	10,536

MicroTester LT Tip: Update Templates After Changing Beam Properties

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

Step 4: Load the Specimen

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



MicroTester LT Tip: Viewing the Specimen in the Live Video Window

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

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

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

MicroTester LT Tip: Microbeam Position

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

Step 5: Set the Optics Zoom Level

A zoom specification grid is located below the Actuator Control interface. The "Zoom" **MUST** be set correctly (at the same value as the zoom indicator on the lens) so that the software can convert image tracking data from pixel values to µm values.



MicroTester LT Tip: Setting a Default Software Zoom Level

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

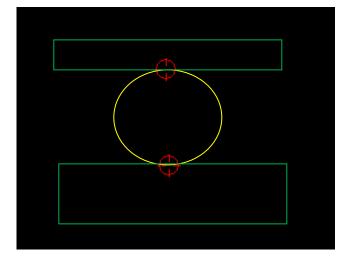
Step 6: Adjust the Initial Size Crosshairs

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

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

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

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



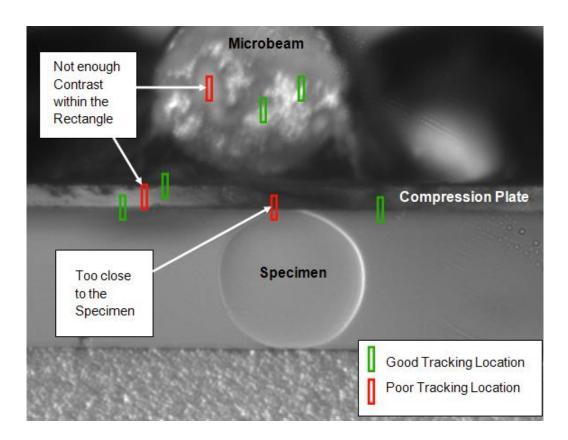
Step 7: Select a Tracking Point

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

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

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

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



Step 8: Execute the Test

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

MicroTester LT Tip: Check Settings

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

Step 9: Terminate the Test Prematurely (optional)

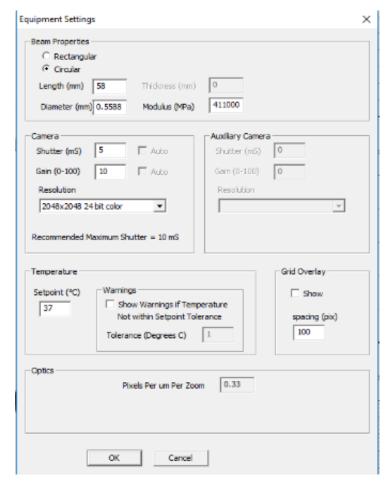
You can stop a test at any time by clicking Son the toolbar, or by selecting Stop from the File menu.

6. Additional Settings

Camera Shutter and Gain

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

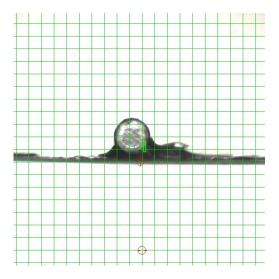
Increasing the camera shutter duration or the camera gain makes the image brighter. Decreasing either of these parameters makes the image darker. If the gain is too large, the image may appear grainy. If the shutter is too long, the image may appear blurry. These settings are stored with the test template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



Grid Overlay

Within the Equipment Settings menu, a grid overlay option can be implemented during a trial. The spacing can be manually inputted (in pixels). Checking the Show check box will display a green grid with the specified spacing.

Applying a grid overlay can provide many benefits during a trial such as distance referencing, beam alignment checking, and quadrant mapping.

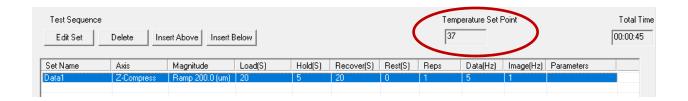


Temperature

To activate the fluid chamber heater the "HEATER" ON/OFF switch must be turned on and the amber light should be illuminated. At this point, the heater will warm the fluid to the last used temperature set point. The temperature set point can be changed by selecting Equipment from the Settings menu.

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

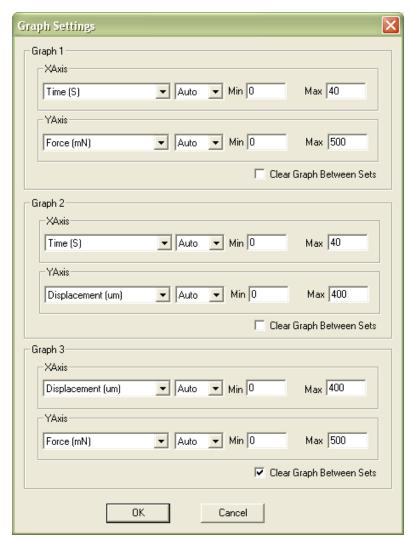
When Show Temperature Warnings is enabled, and the Current Temperature is not at the Temperature Set Point plus or minus the specified tolerance -- a red indicator button will be displayed next to the Current Temperature live output and a warning dialog will appear when you execute the test.



Configuring the Real-Time Graphs

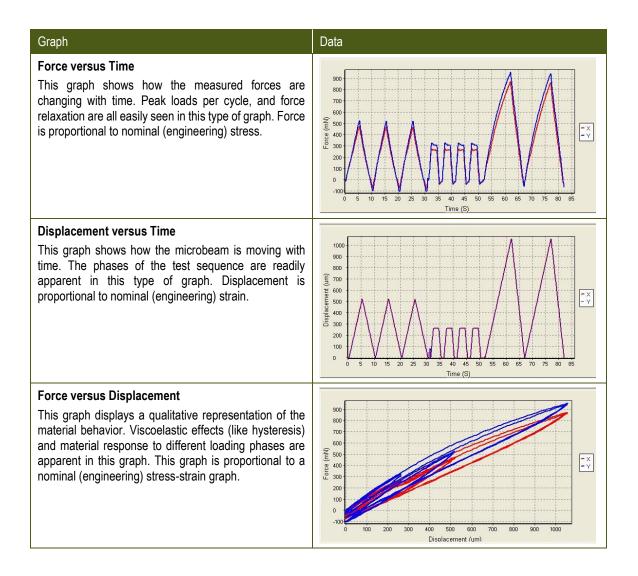
The three graphs on the right side of the screen provide real-time user feedback during a test. These graphs are intended to be used for qualitative feedback, not for detailed analysis.

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



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

Force (μN)	Displacement (um)	Time (S)
Force (mN)	Displacement (mm)	Temperature



Data Output Settings

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

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



Advanced Settings

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

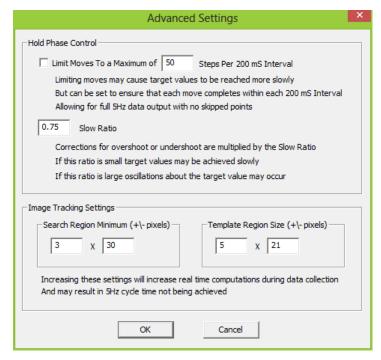
Hold Phase Control allows the user to set some limits on actuator movement, in order to improve system stability.

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

The second parameter ("Slow Ratio") is like a safety factor. For example, if a move of 10µm is desired in each time step, the actuator will attempt to move 10µm X the slow ratio. See the tip below for more information.

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

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



MicroTester LT Tip: Slow Ratio

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

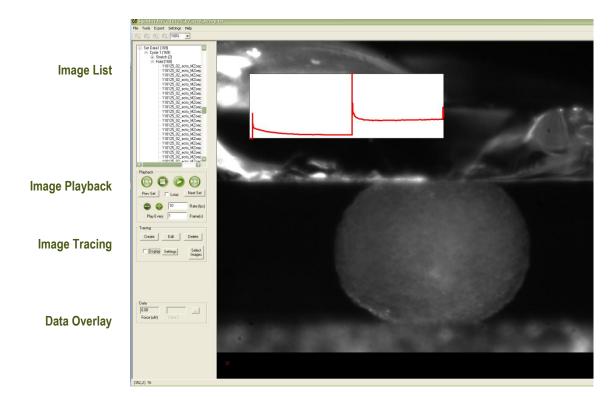
7. Reviewing Test Results

Overview

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

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

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



Selecting Images

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

Set (line on the test parameter window)

Cycle (iteration of a given Set)

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

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

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

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

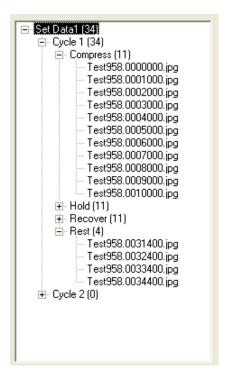


Image Playback Options

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

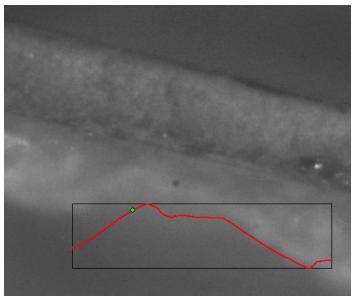


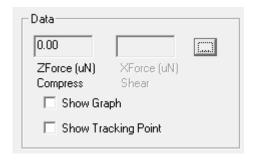
Next/Prev	Display next or previous image (as dictated by "Play Every" parameter).
Play/Stop	Start and stop the playback.
Prev/Next Cycle	Jumps to a corresponding image and phase in the next or previous cycle. Useful when reviewing sets that have multiple cycles.
Loop	Sequences can be played continuously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

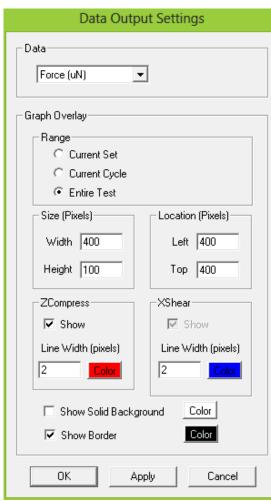
Data Overlay

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

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







Exporting Images and Movies

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

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

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

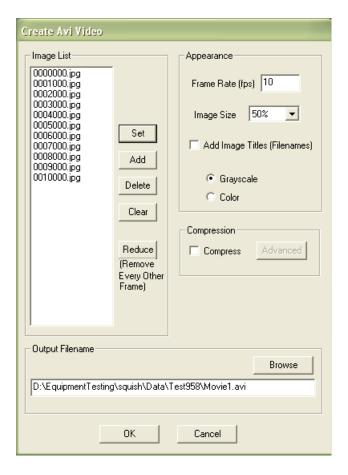


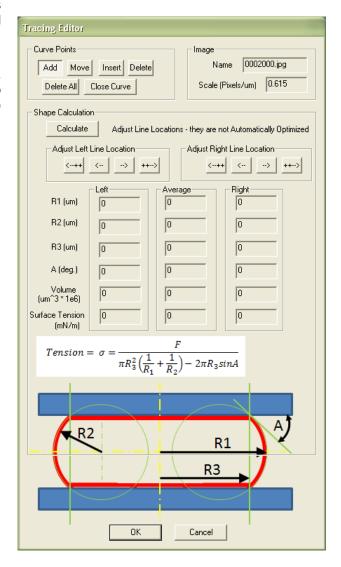
Image Tracing

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

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

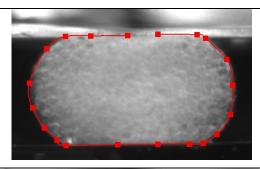
Clicking "Create" (or if an existing trace exists, clicking "Edit") brings up the following dialog. This dialog floats so that it can be positioned to allow the user to visualize the image and the dialog simultaneously.



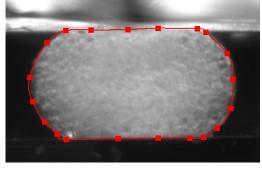


The general procedure for creating a specimen tracing is as follows:

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

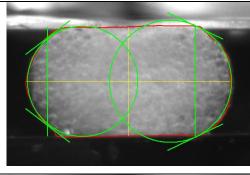


2. Click the "Close Curve" button to attach the first and last point.

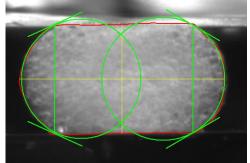


3. Use the "Move" and/or "Insert" buttons to modify the points as needed. There is an image zoom feature available on the top toolbar if needed.

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



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

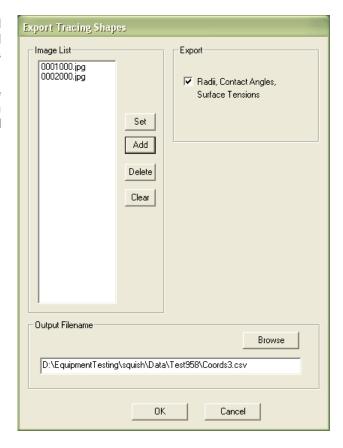


6. Click "OK"

Exporting Traced Data

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

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

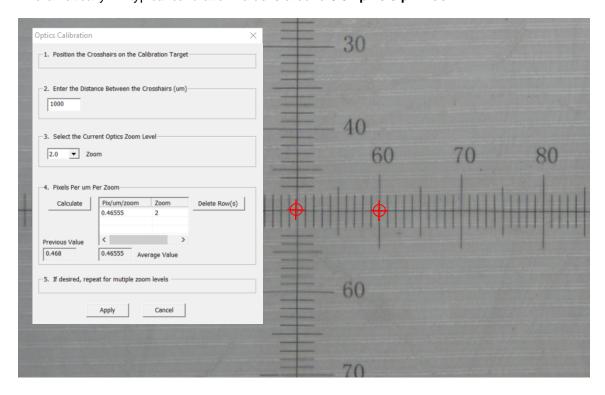


8. System Calibration and Advanced Tools

Optics Calibration

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

- 1. Launch the MicroTester LT software and launch a new test in order to see the live video window.
- 2. Bring up the Optics Calibration Dialog by selecting Optics Calibration from the Calibrations submenu of the Tools menu.
- 3. Position a calibrated target with features of known size on the front of the fluid chamber. A glass target with 10 lines/mm (1mm = 1000 µm) is provided with your equipment for this purpose. Note that this means 10 lines/mm. This target can be positioned with the provided holder. Focus the Image using the three axis stages on which the optics and camera are mounted.
- 4. Drag the crosshairs on the live video window such that they are positioned a known distance apart. Be sure that the crosshairs are **aligned with each other** as the computer measures the **absolute distance** between them, not just the horizontal or vertical distance.
- 5. Enter the distance between the crosshairs in the dialog field.
- 6. Select the optics zoom level.
- 7. Click the "Calibrate" button in the dialog box.
- 8. Selecting the "Apply" button will cause the software to use the current calibration value. It is useful to compare the current calculated value with the previous value as the value should not change dramatically. A typical calibration value is around **0.32 pixels/µm/zoom**.



Snap Image

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

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



9. Mounting a MicroBeam

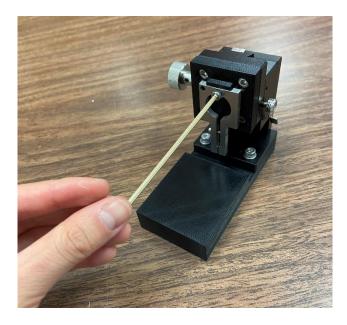
The exact details of how to best adhere the upper compression plate and mount the microbeam to the grips may vary depending on your specific application, but a general protocol is outlined here.

- 1. Gather the following materials:
 - a. Vertical mounting stage
 - b. 5/64" hex key
 - c. 2.5mm hex key
 - d. Cyanoacrylate (i.e. Crazy Glue or Loctite 422)
 - e. Glue Accelerator
 - f. Cotton Swab
 - g. Tweezers
 - h. Microbeam clamp with M3 fastener
 - i. Desired microbeam and compression plate



2. Bring the vertical stage to its highest point by rotating the knob on the left side. If the stage does not move, release the stopper on the right side by rotating and loosening the side screw.

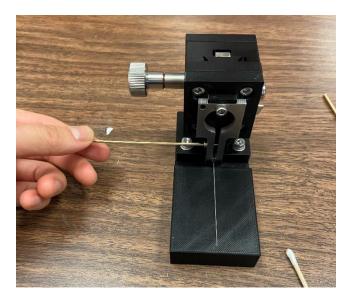
3. Align the microbeam clamp to the lip on the face of the stage, and fasten it using a 2.5mm hex key and M3 fastener



4. Loosen the side screw of the microbeam clamp using a 5/64" mm hex key



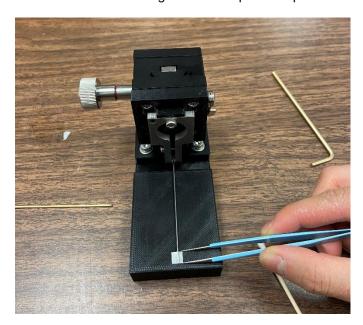
5. Place microbeam below the clamp opening and lower the stage until the beam is just above the tips of the clamp. Tighten the clamp using a 5/64" hex key. This is to ensure the microbeam is horizontal. Lift the stage slightly to ensure it is secure affix in the clamp



MicroTester G2 Tip: Microbeam Position

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

6. Place the desired compression plate below the tip of the microbeam. Use tweezers to help ensure the tip of the microbeam is in line with the edge of the compression plate



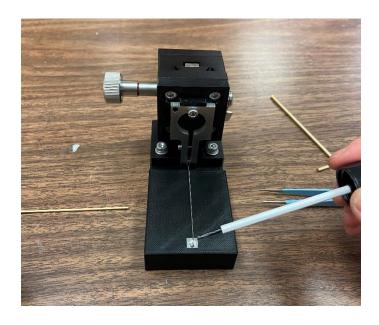
7. Place a small dab of glue on the tip of a cotton swap, and place this on the tip of the microbeam



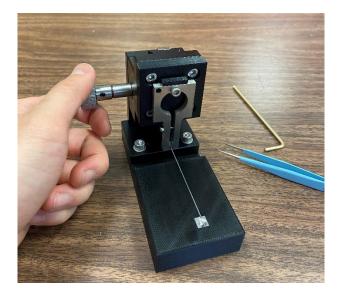
MicroTester G2 Tip: Cyanoacrylate Adhesive

Cyanoacrylate adhesive is provided complimentary with the MicroTester G2 system to bond the microbeam and compression platen. This adhesive will best retain its properties if it is **refrigerated** when not being used.

- 8. Lower the microbeam until it touches the plate
- 9. Cure the glue by brushing a small amount of glue accelerator



10. Lift the stage and remove the clamp from the stage using a 2.5mm hex key to loosen the M3 fastener



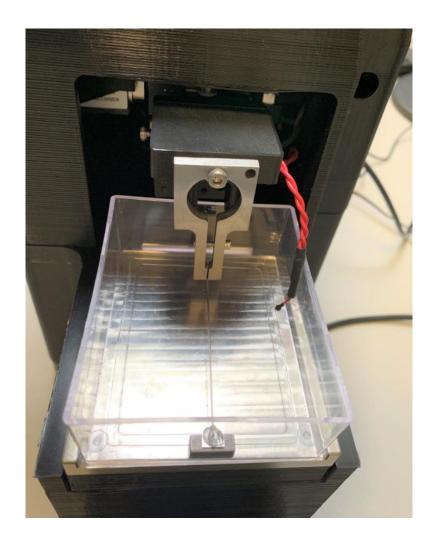
11. The clamp and microbeam assembly is ready to be mounted to the MicroTester system using the M3 fastener and 2.5mm hex key

MicroTester G2 Warning: Adhesive and Accelerator

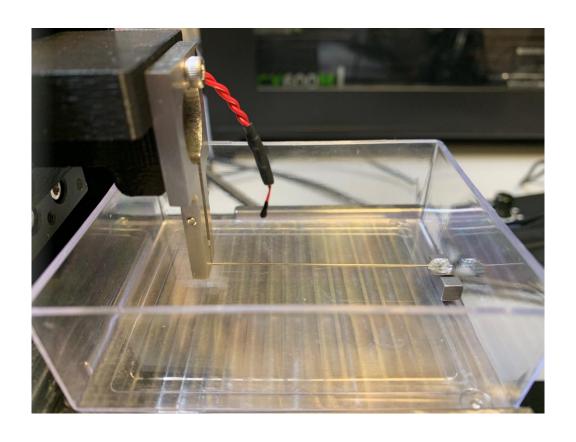
Cyanoacrylate adhesive and accelerator are provided with the MicroTester G2 system to bond the microbeam and compression platen. These are eye, skin, and respiratory irritants. Cyanoacrylate will instantly bond skin. The use of gloves is recommended.

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

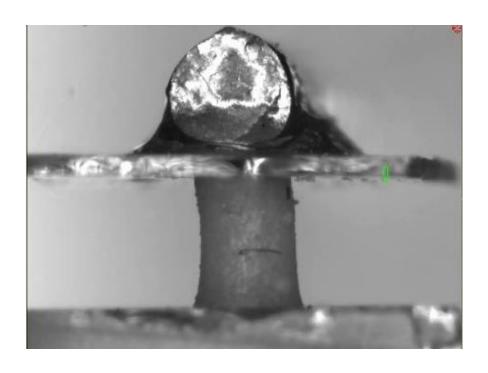
The microbeam length must be measured and the dimensions and modulus correctly specified before a test. See Section 3 - Step 5 for more details.



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



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



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

10. Force Calculation Formulas

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

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

where

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

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

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

Diameter (mm)	0.0762	0.1016	0.1524	0.2032	0.3048	0.4064	0.5588	1.0160	1.5748
Modulus (MPa)	411000	411000	411000	411000	411000	411000	411000	411000	411000
Typical Force Resolution (µN)	0.012	0.038	0.191	0.604	3.056	9.658	34.52	330.54	1907.88
Recommended Max Force (µN)	4	11	57	181	917	2,897	10,536	99,162	572,363

11. Appendix A: System Setup

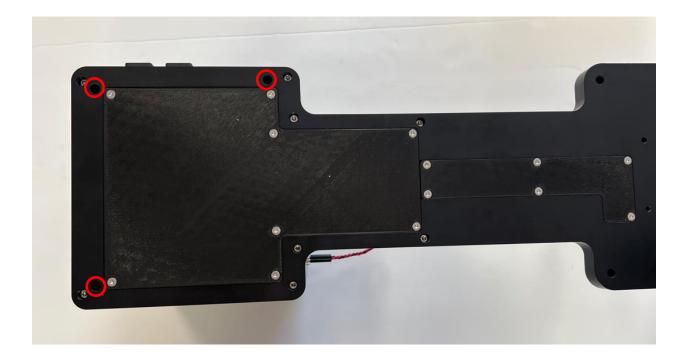
Unpack Main Unit and Accessories

Remove the main unit from its packing material. A foam "clamshell" encases the main unit inside a cardboard box for shipping. This material must be carefully removed.

Foam Removal from Beam Stages

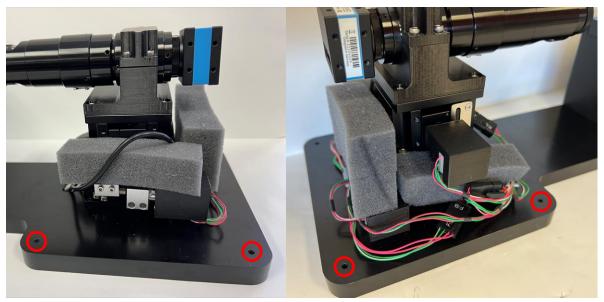
The beam positioning actuator stages are protected by a plastic cap. Foam surrounds these components inside the cap during shipping and the stages are locked in place. The protective foam will need to be removed before operation and the stages will need to be unlocked.

To do so, remove use the long 2.5 mm T-shape hex key to remove the cover from the riser. There are 3 screws which are recessed in the riser body. You will be able to access the stages to remove the foam and loosen the thumbscrews with a flat-heat screwdriver.





The camera positioning stages will also be packed with foam for protection during shipping, to remove this foam the camera cover will need to be removed using a 2.5mm hex key provided and removing the 4 M3x 10 fasteners, pictured below.



Attach Camera in Clamshell Mount

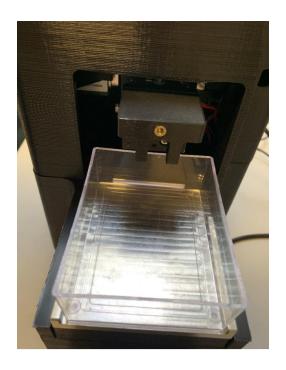
Once the foam is removed and the thumbscrews are loosened the camera can be mounted in its clamshell mount. The 4 x M3 fasteners must be removed and the camera lens placed into the mount as seen below:



Once the camera is installed the cover can be put back on using the M3x10 fasteners provided. Do not forget to place the cable for the ring light out of the from hole making it accessible when the ring light is installed.

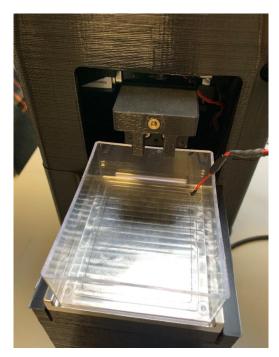
Set Test Chamber on the Chamber Holder

Set the fluid bath (test chamber) in the chamber holder on the main unit.



Place Temperature Sensor in the Test Chamber

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



Connect Cables

Few power and communication cables need to be connected between the main unit, a power supply, and a computer:

- 20V power supply to power input jack on the main unit
- LED ring light
- USB Cable to back of camera (do not yet connect to PC)
- USB Cable for Main Unit

NOTE: At the completion of this step, there should be 2 USB connections ready to connect to the PC USB ports

- Black USB cable from camera
- Black USB cable from Main Unit





LED Ringlight power connector and plug

System Calibration Check

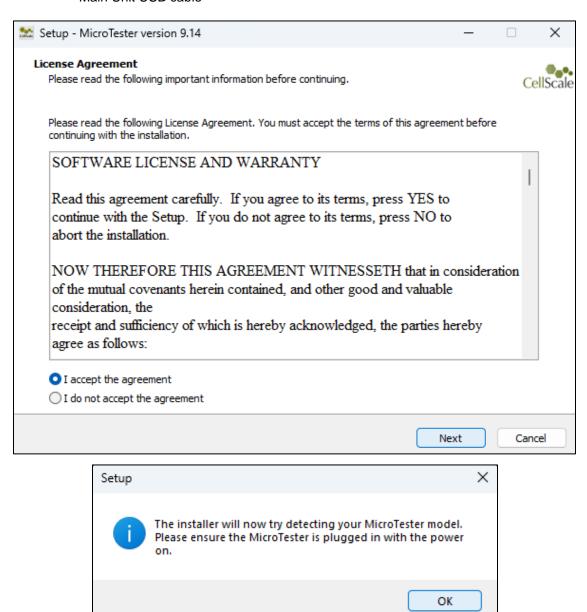
After the MicroTester LT is fully assembled and set up, the MicroTester LT software must be installed (see Appendix B). The system can then be switched on and the computer connectivity may be tested by opening an initial test. However, before any tests are run, it is recommended to check the optics calibration.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

12. Appendix B: Software Installation

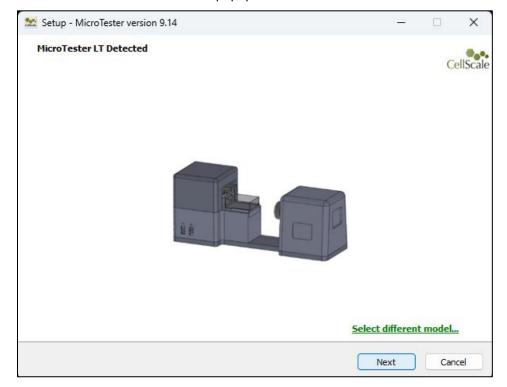
A Windows based PC (Windows 10 or above) with at least 2 USB ports is required to operate the MicroTester LT. Go to www.cellscale.com/downloads to download the latest MicroTester LT software package. The package contains the software installer for the user interface and control software for the MicroTester LT.

To install the software:

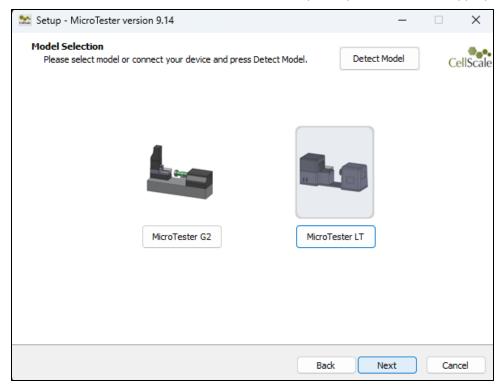
- 1. Go to the provided web address and download the compressed installation package.
- 2. After this is complete, go to the uncompressed installation folder and select *MicroTester Installer.exe*. Details of typical dialogue boxes are shown below.
- 3. Once the installer is finished, plug the USB cables into a USB port for the following components:
 - Camera (black USB cable)
 - Main Unit USB cable

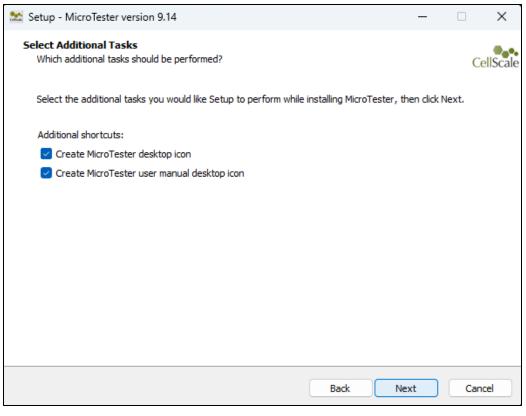


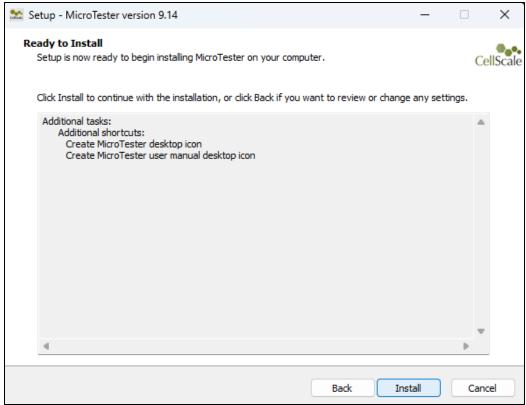
If your MicroTester is detected this screen will popup:

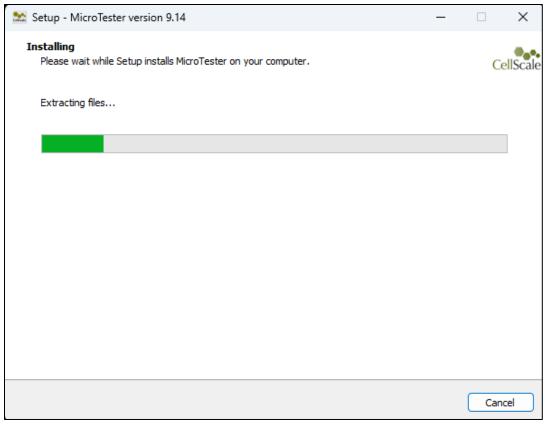


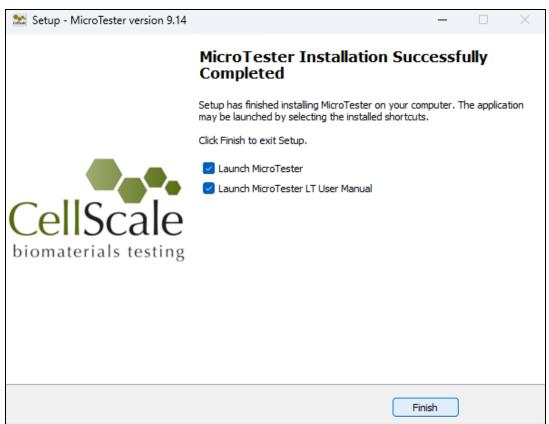
If MicroTester is not detected, then you will be taken to a model selection screen where you can select your MicroTester model. To install the correct user manual and templates please select the appropriate model.











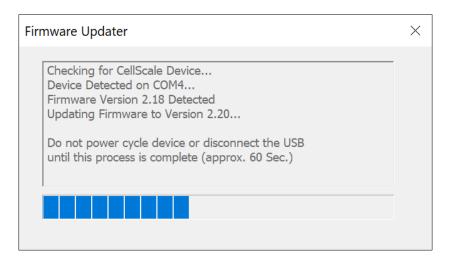
Update Firmware

Periodic firmware updates may be issued by CellScale for the MicroTester. When a new firmware or software is available, you may download it from our website (www.cellscale.com/downloads).

To update the firmware:

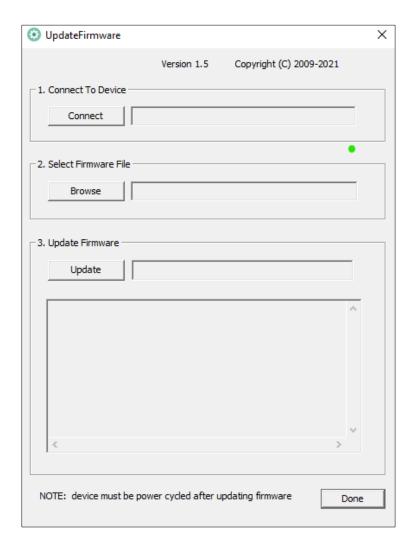
- 1. Connect the MicroTester to the PC via USB and turn on power to the MicroTester
- 2. Open the *UpdateFirmware.exe* program located in *MicroTester>Software>Utilities>Update Firmware*
- 3. The program will automatically update the firmware of the MicroTester
- 4. Power cycle the system after the firmware is successfully updated before using the MicroTester software again

The firmware version is checked and the automatic updater is also run when installing a new version of the software.



If the automatic firmware update software does not work, please follow the manual steps below:

- 1. With the MicroTester connected to the PC and turned on, launch the manual firmware update software located in *MicroTester>Utilities>Update Firmware>Advanced*.
- 2. Click the Connect button.
- 3. Load the firmware file using the *Browse* button. The file will be a .bin extension.
- 4. Execute the update using the *Update* button.
- 5. Power cycle the system after the firmware is successfully updated before using the MicroTester software again.



13. Appendix C: Software Troubleshooting

This software is recommended for use with a Windows 10 operating system. Use on Windows 7 systems may result in some hardware communication errors with the MicroTester, such as the following:

Issue: MicroTester software cannot communicate with camera and/or controller



Solution: This is often caused by turning off the device while the software is still running on Windows 7, then trying to reopen the software. If you encounter this error, try closing and turning everything off, removing and reinserting the USBs, then try again. Make sure both USBs and the power supply are plugged in and the device is on before you launch the software. You can also try manually renumbering the port numbers for the USB port being used by the MicroTester controller in your computers Device Manager > Ports. Finally, try restarting your computer.

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