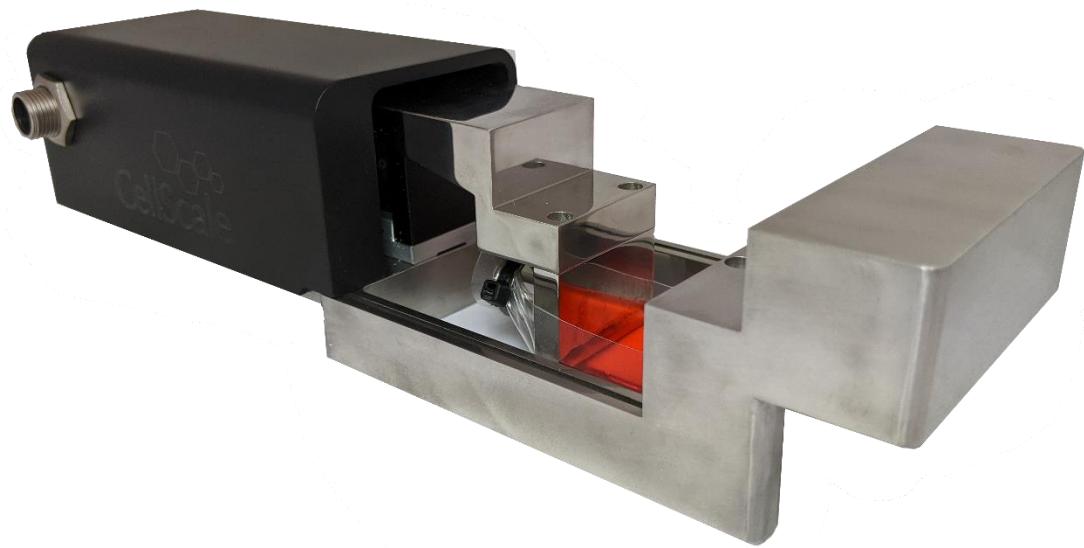




MechanoCulture™

Mechanical Stimulation System

User Manual
version 1.1



CellScale is an industry leader in biomaterial and mechanobiology test systems.

Our mechanical test systems are specifically designed for characterizing the material properties of biomaterials. With integrated features such as image capture and analysis, media chambers, and a range of gripping mechanisms, our biaxial and micro-scale compression test systems are capable of generating high quality test data from day one.

Our mechanobiology technologies help advance research by providing insights into the response of cells to mechanical stimulation. CellScale has systems optimized for high-throughput, real-time imaging, and 3D scaffold use. In addition to our standard products, CellScale can also develop a custom solution for your application.

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1.General Information

The MechanoCulture TM is a precision instrument specifically designed for deforming flexible membranes in a sterile fluid environment. The device has an onboard actuator and a detached control board to enable it to run independently of a computer in an environment-controlled incubator.

Environmental and Electrical Specifications

Electrical Input	24VDC
Current Rating	1.2 Amp
Environmental Conditions	Maximum Operating Temperature 40°C 0% - 100% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree 2
Data Connections	mini USB for programming 3.5mm TRRS Connection Cable to run actuator

System Assembly

The unit requires some initial assembly as well as some assembly/disassembly required between uses. See Appendix C for assembly instructions.

Connections to Supply

Connect power cord into properly grounded 100-240VAC power source to ensure safe operation. 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.

The unit should not be operated without the motor housing in place.

Manual Operating Controls

There is one push-button switch on the front of the control unit. A single push will start and stop the test sequence. A push and hold for 5 seconds will advance the actuator and reset the test sequence to the beginning

General Maintenance

The equipment does not require maintenance other than cleaning. Please see the components overview section for cleaning and sterilization details.

Approvals and Certification

This product conforms to EN61010-1:2001 and EN61326-1.

2.Components Overview



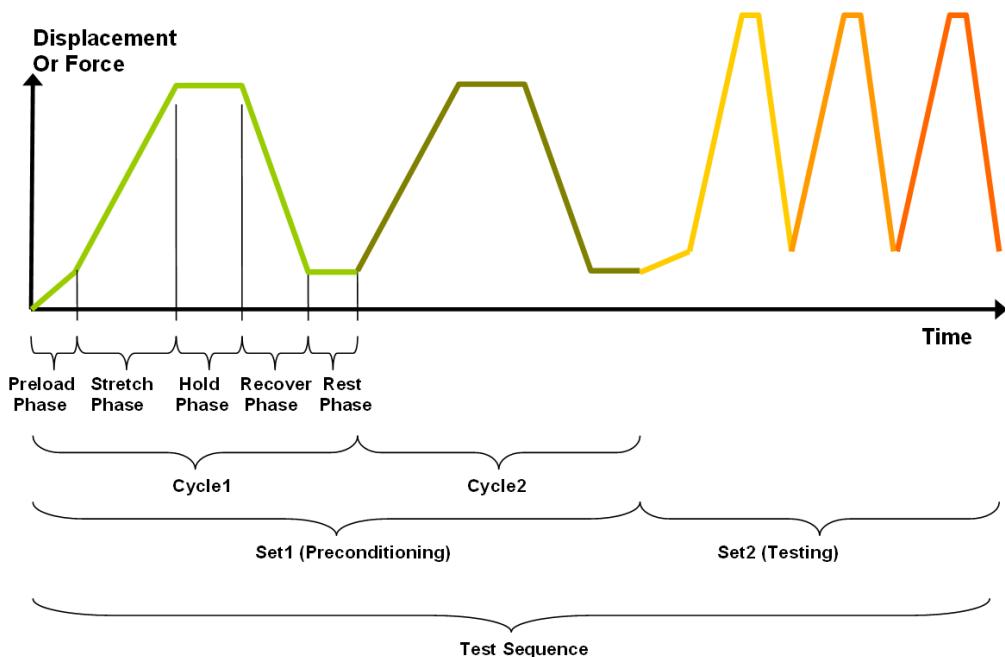
3. Testing Terminology

Multiphase Test Cycles

In order to properly mechanically stimulate/signal cells, it is often necessary to apply more than one load magnitude or frequency during the course of the test.

Phases, Cycles, and Test Sequences

As the following diagram demonstrates, each application and release of the well plate is called a **test cycle**. The same test cycle can be repeated multiple times to achieve a certain goal. 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.
- Finally, the second set is made up of three cycles.

Test Phases: The Smallest Unit of Testing

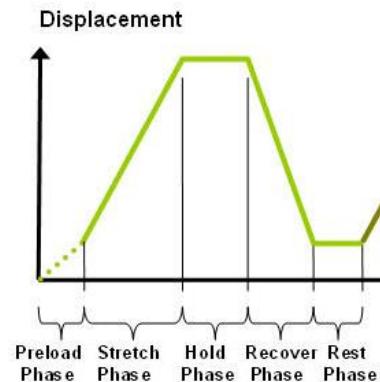
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

Stretching - During the stretch phase, a deformation is applied to the specimen.

Holding - The deformation can be held for a given duration.

Recovering - The recovery phase is the time during which the deformation imposed during the stretching phase is removed.

Resting - Finally, the rest phase is the time between the end of one cycle and the beginning of the next.



Control Functions

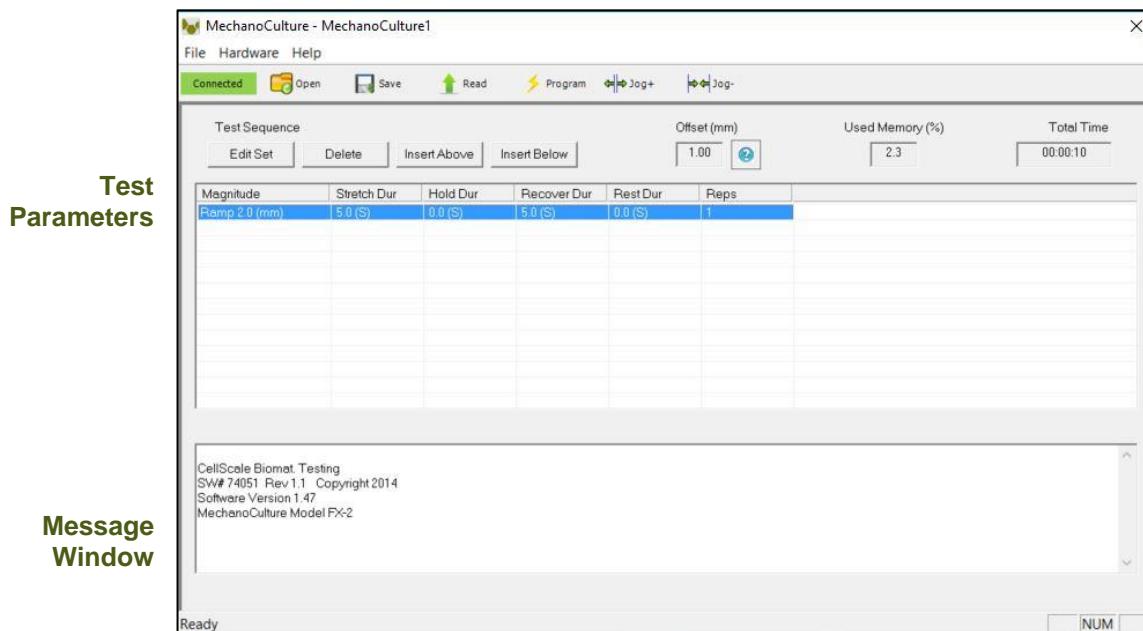
The MechanoCulture makes it possible to stimulate specimens under two control functions:

The **ramp function** applies the displacement at a constant velocity.

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

4. Software Overview

The MechanoCulture software is used to program the device with the phases, cycles, and sets that comprise the test sequence. The screen layout is shown below:



Step 1: Initiate a Test Sequence

Test sequences can be initiated in 3 different ways:

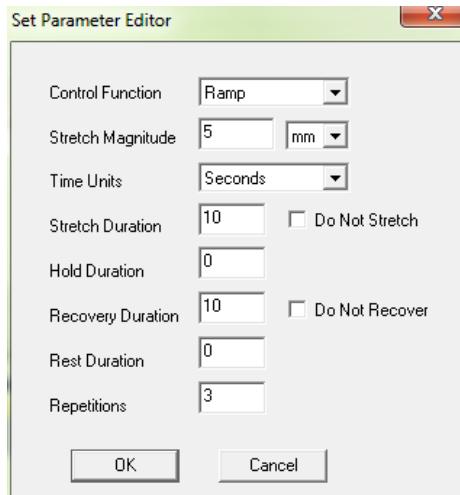
1. A predefined test sequence can be opened from a MechanoCulture Protocol File (*.tmt). From the File menu, select Open, or press the Open button on the toolbar.
2. A currently programmed test sequence on a device can be read. From the Hardware menu, select Read, or press the Read button on the toolbar. To read a MechanoCulture device, it must be connected to the computer's USB port with a USB cable and it must be powered on.
3. A new test sequence can be created by pressing the Insert Above or Insert Below buttons to add sets to the test sequence. Each set will have its own row in the table and sets will be executed sequentially. A test sequence can have a maximum of 8 sets.

Step 2: Modify Testing Parameters (optional)

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

Note that displacements are specified in mm and are relative to the current position. Time units are applied to the entire set but different sets can have different time units.

The Do Not Stretch and Do Not Recover check boxes allow for the stretch or recover phases to be omitted during a testing cycle. This makes the creation of test sequences that include preloading possible.



Step 3: Program a Device with the Specified Test Sequence

From the Hardware menu, select Program, or press the Program button on the toolbar. To program a MechanoCulture device, it must be connected to the computer's USB port with a USB cable and it must be powered on.

MechanoCulture Tip: Saving Test Sequence Protocols

Once you have developed a test sequence, you can save it as a MechanoCulture Protocol file for future use. Select Save from the file menu, or press the Save button on the toolbar. You can then open this test sequence the next time you initiate a test sequence.

Well Plate Pre-Stretch, Device Reset Movement, and Offset Distance

On power-up, the system's actuator will go through a series of moves in order to achieve the correct starting position. These moves involve fully extending the actuator and then retracting to the correct load position. This series of moves is necessary to ensure that the actuator is in the correct global position at the start of the test as all the movements in the test protocol will be relative to this position.

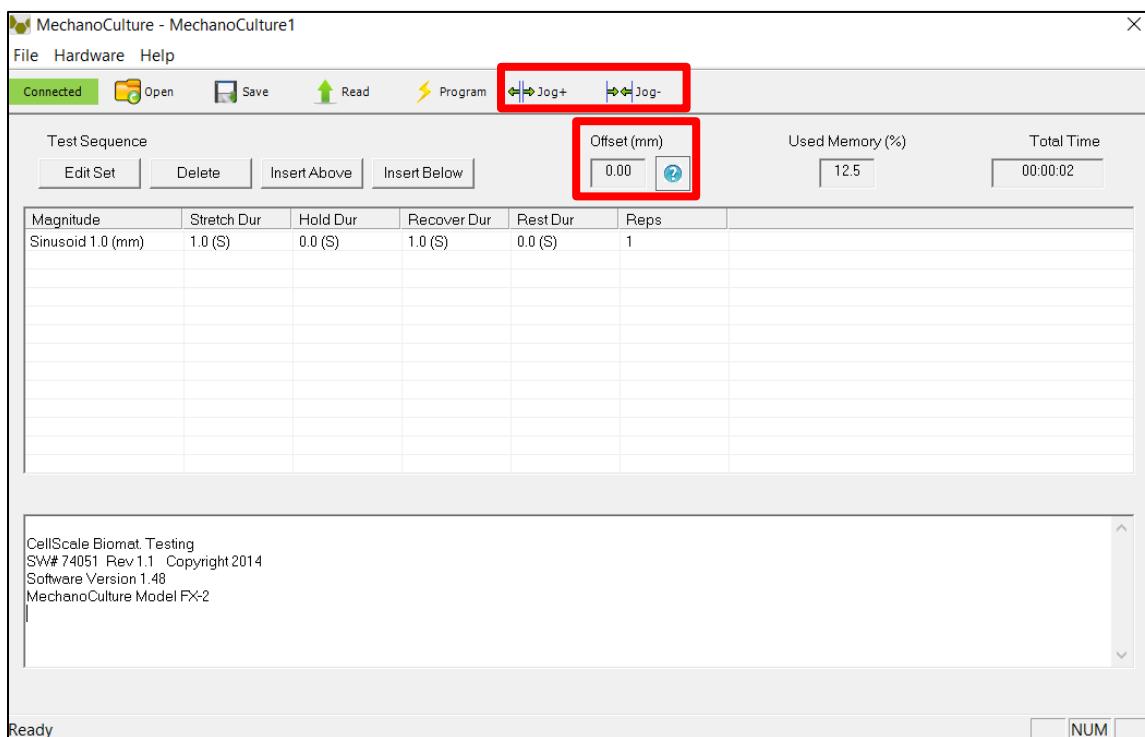
During the reset movements, the distance that the actuator retracts back from its fully extended position is called the offset.

The offset value can be changed by selecting "Hardware", then "Advanced", and then "Settings" from the software menu. Within the dialogue, the offset parameter can be specified and programmed to the device controller. This offset will be applied globally to all test protocols once the "Program" button is clicked.

As a reminder, the offset will be reapplied on every power up of the controller and every time that the controller button is pressed and held for 5 seconds to trigger a reset. The only exception to this is the situation where the device is paused while running a protocol (by pressing but not holding the button). In this case the actuator will not reset on power up so that the protocol can be continued by a second button press.

If it is desired to unplug the controller after the cells have been seeded but before the protocol has been started, it is recommended to take steps to ensure that the reset protocol does not disturb the cells. This can be accomplished by unplugging the actuator cable (TRRS, not the USB cable) from the control box during power up.

Offset and Jog Functions



The **offset** function for the MechanoCulture TM is programmable by the user and how to do this is shown later in the manual.

The **jog buttons** let you extend and retract the actuator outside of executing a test. When the actuator is jogged, the offset value will automatically update and display the current position of the moving pins.

5.Designing Test Sequences

The MechanoCulture software allows for complex test sequences to be generated. The examples in this section illustrate how to generate different types of test protocols.

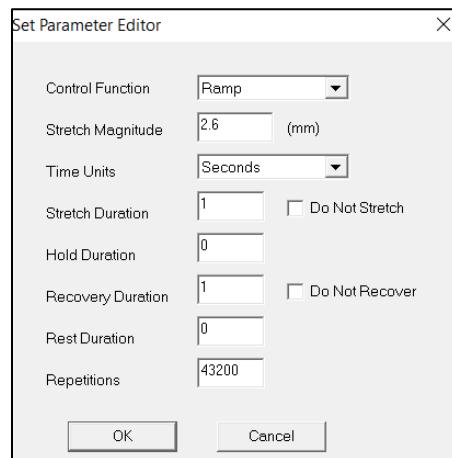
Example 1: Continuous Cycling for Fixed Duration and then Stop

Objective: Create a test sequence that cycles at 0.5 Hz with 2.6mm of stretch for 24 hours and then stops.

Press Insert Above to create a Set. Then double click on that row to modify it.

0.5 Hz is a period of 2 s. In this example, the period is divided into 1 s of stretch and 1 s of recovery with no hold or rest.

To achieve a 24 hr test duration requires 43200 cycles. (24 hr x 3600 s/hr / (2 s/cycle)).

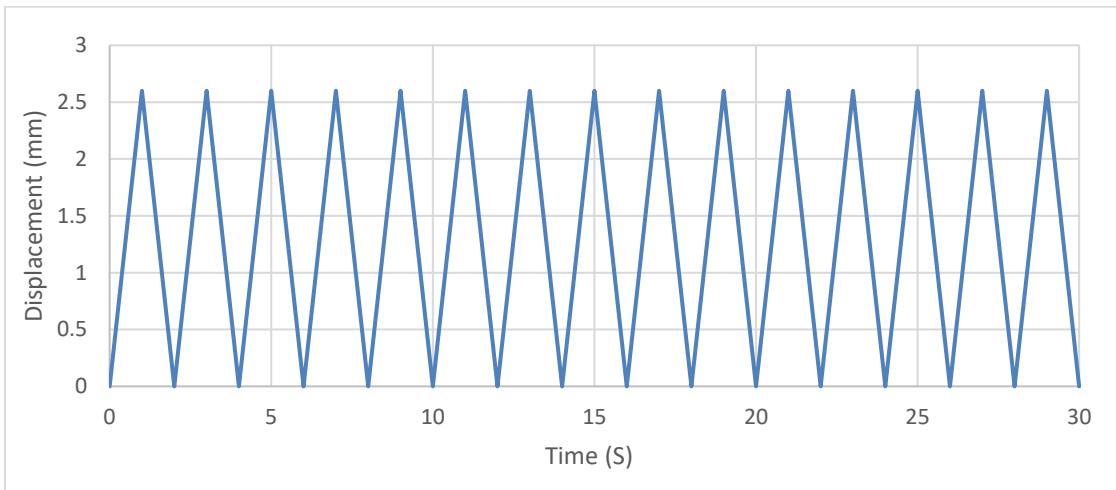


MechanoCulture Tip: Terminating a test

The MechanoCulture software will loop through the test sequence indefinitely. To prevent the sequence from repeating itself, add a test set to the end of the sequence that includes a lengthy rest duration but no stretch or recovery. For example, 21 days of rest could be added to the end of the test sequence (see below for full test sequence).

Test Sequence						Offset (mm)
Edit Set		Delete	Insert Above	Insert Below	0.00	
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Reps	
Ramp 2.6 (mm)	1.0 (S)	0.0 (S)	1.0 (S)	0.0 (S)	43200	
Ramp 0.0 (mm)	None	0.0 (d)	None	1.0 (d)	21	

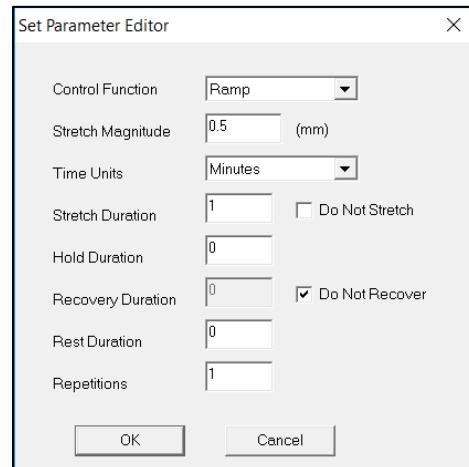
The resulting actuator motion versus time graph for the first 30 seconds:



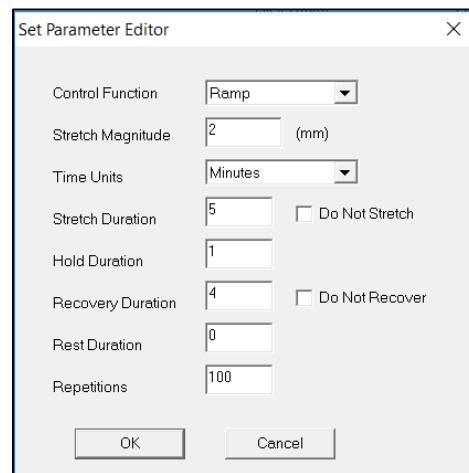
Example 2: Pre-stretch a Single Time, Then Cycle for a Fixed Duration, and Then Stop

Objective: Create a test sequence that starts with a 0.5 mm pre-stretch in 1 minute followed by 100 cycles of an additional 2 mm stretch (5-minute stretch, 1-minute hold and 4-minute recover).

1. Press Insert Above to create a Set. Then double click on that row to modify it.
Check the Do Not Recover box to omit the recovery phase.



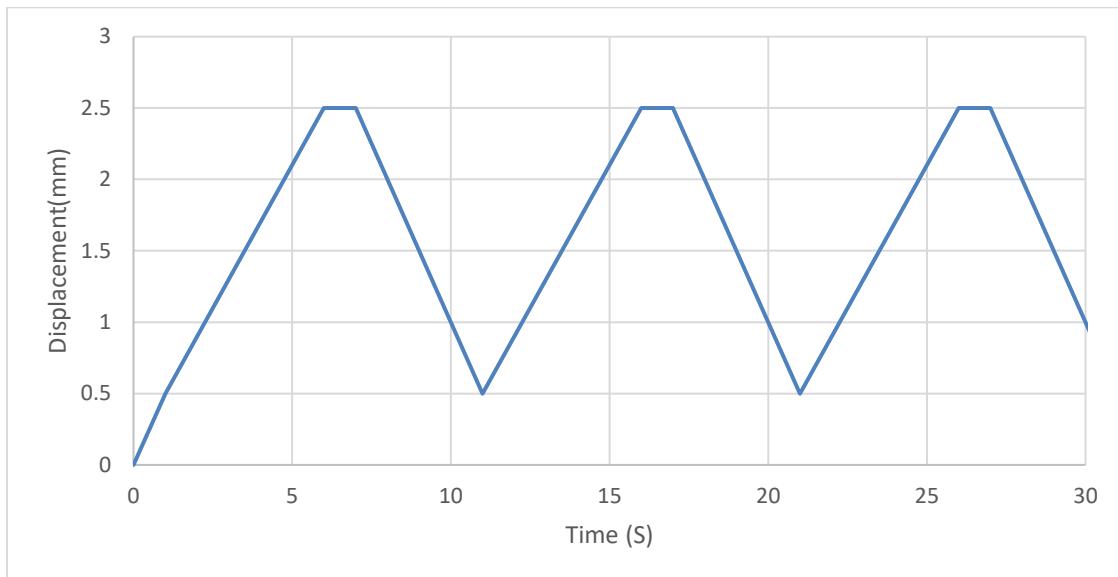
2. Press Insert Below to create a second set. Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the total global stretch will be 6 mm).



3. To terminate the test at the end of 24 hours, insert a lengthy rest set (21 days in this example) to the end of the sequence. If this set is not included, the protocol will automatically restart at the end of the first set (see the MechanoCulture tip in Example 1). The test sequence is shown below:

Test Sequence						Offset (mm)
		Edit Set	Delete	Insert Above	Insert Below	
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Reps	
Ramp 1.0 (mm)	1.0 (m)	0.0 (m)	None	0.0 (m)	1	
Ramp 2.0 (mm)	5.0 (m)	1.0 (m)	4.0 (m)	0.0 (m)	100	
Ramp 0.0 (mm)	None	1.0 (d)	None	0.0 (d)	21	

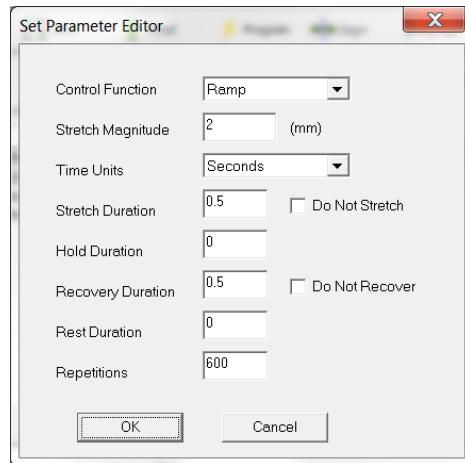
The resulting actuator motion versus time graph for the first 30 minutes:



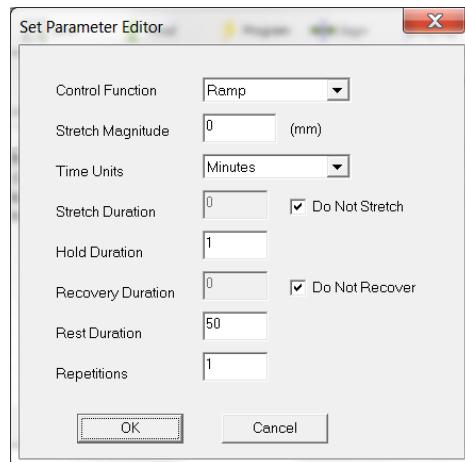
Example 3: Intermittent Stretching

Objective: Create a test sequence that cycles at 1 Hz with 2mm of stretch for 10minutes followed by 50minutes of rest. Repeat this pattern indefinitely.

1. Press Insert Above to create a Set. Then double click on that row to modify it.



2. Press Insert Below to create a second set.



3. In this case, a test set with lengthy rest duration is not required since the test is designed to repeat indefinitely. The test sequence is shown below:

Test Sequence						Offset (mm)	
		Edit Set	Delete	Insert Above	Insert Below	0.00	?
		Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions
Ramp 2.0 (mm)		0.5 (S)	0.0 (S)	0.5 (S)	0.0 (S)	600	
Ramp 0.0 (mm)		None	1.0 (m)	None	50.0 (m)	1	

6. Setting Up & Running a Test

Overview

As a user of the MechanoCulture, you are likely to develop your own test preparation and execution protocol, but there are several elements that are common to most users. This manual will not cover cleaning, disinfecting, sterilization, cell seeding, reagents, and a number of other topics.

The protocol below should be used for running new test sequences. To re-run existing sequences on new specimens, simply power-up the device, ensure the system has been reset, mount specimens, and press the push button to execute the test.

Attach the Sterilized Membrane to the Test Device

See Appendix C for details and images.

Plug in Control Box Power. Connect Control Box and Test System

Program the Device (if needed)

Connect the Device to the PC via USB

Launch the MechanoCulture Software

Generate a Test Sequence

Upload Test Sequence to Device

Reset the system (hold down the button for 5 seconds)

See comments in Section 4 for details on actuator reset, actuator offset, and well plate pre-stretch.

Add Cell Media and Cells. Culture to Develop Adhesion

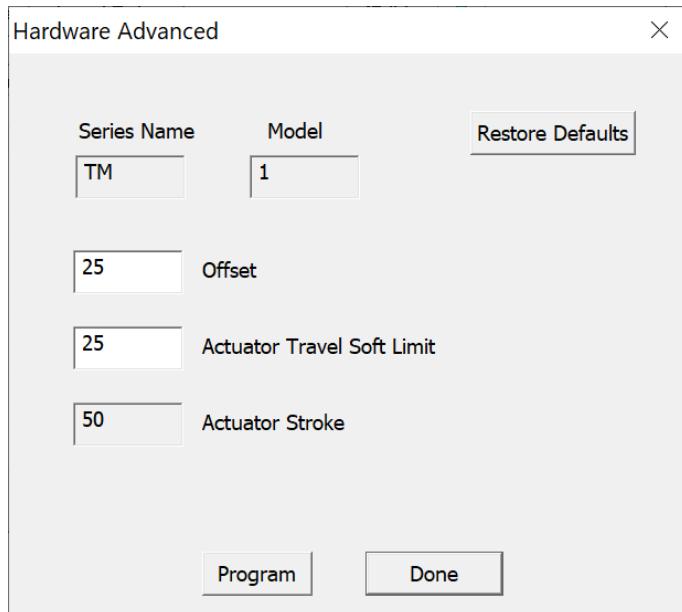
Be sure to set the well plate/carrier assembly on a clean surface so as not to smudge the exterior of the membrane.

Execute Test

To execute the test sequence, simply press the push button once. To pause the sequence, press the button again at any point. Once this has been accomplished, power can be removed and then restored without affecting the test protocol. To restart the test, press the button once more. To reset the device, press and hold the button for 5 seconds.

7. Advanced Parameters

Under the Hardware menu is an Advanced Settings menu containing several different values which should be understood. The menu will appear as below:



Offset: Offset is a distance that controls the starting stretch magnitude of each test. For example, if the offset is set to 1mm, when a stretch of 2mm is programmed it will stretch from 1mm to 3mm. When the system is reset by holding the push button down for 5 seconds it will fully extend the actuator and retract it to the defined offset amount.

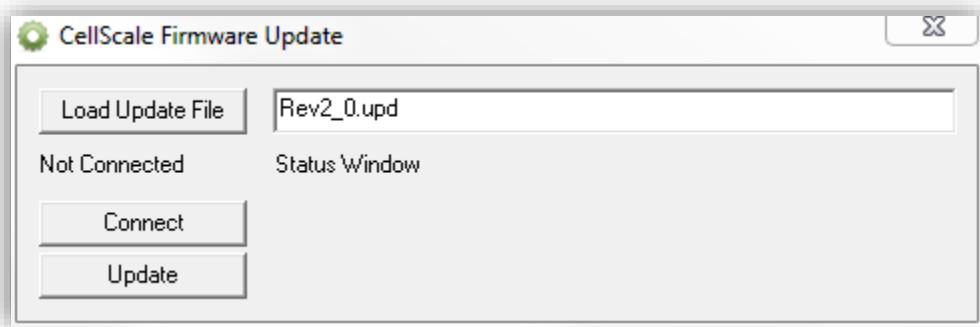
Actuator Travel Soft Limit: The actuator travel soft limit sets the maximum offset that can be achieved via manual jogging (before a test) and maximum stretch magnitude in the protocol editor.

A maximum soft limit of 43mm can be set to prevent the motor from bottoming out on full retraction. If the offset is greater than the actuator travel soft limit, manual jogging will be restricted to only the inward direction until the offset is equal to or smaller than the soft limit.

Actuator Stroke: The TM actuator has 50mm of maximum travel.

8. Appendix A: Updating the Firmware

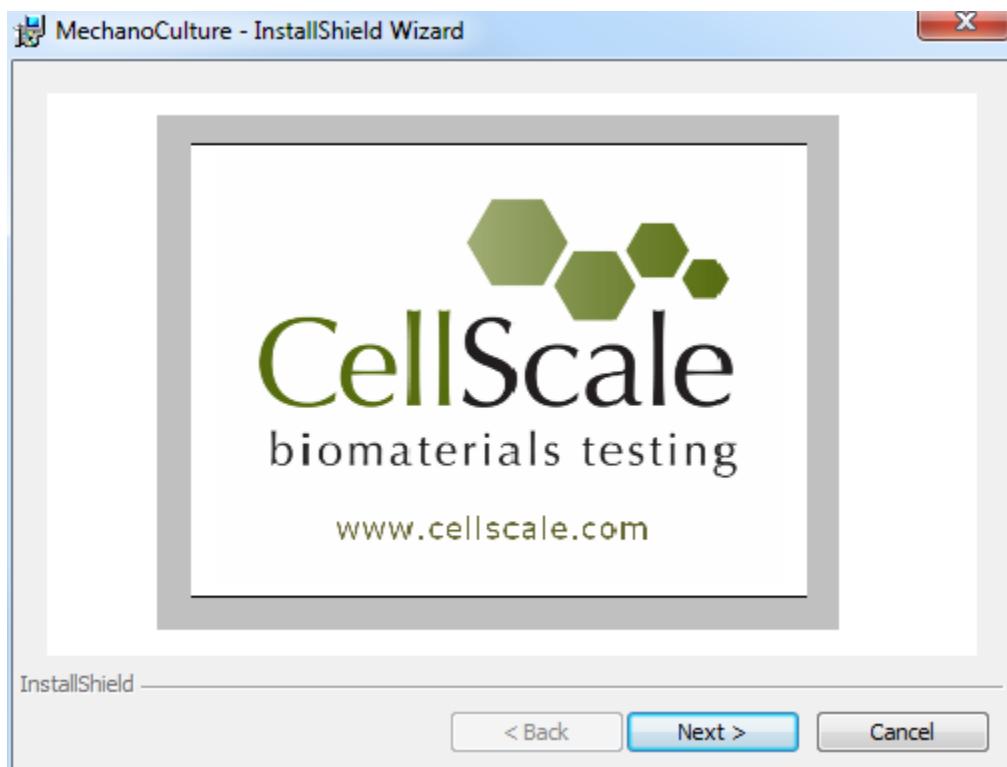
1. With the MechanoCulture connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities.
2. Load the firmware file using the “Load Update File” button.
3. Click the “Connect” button.
4. Execute the update using the “Update” button.

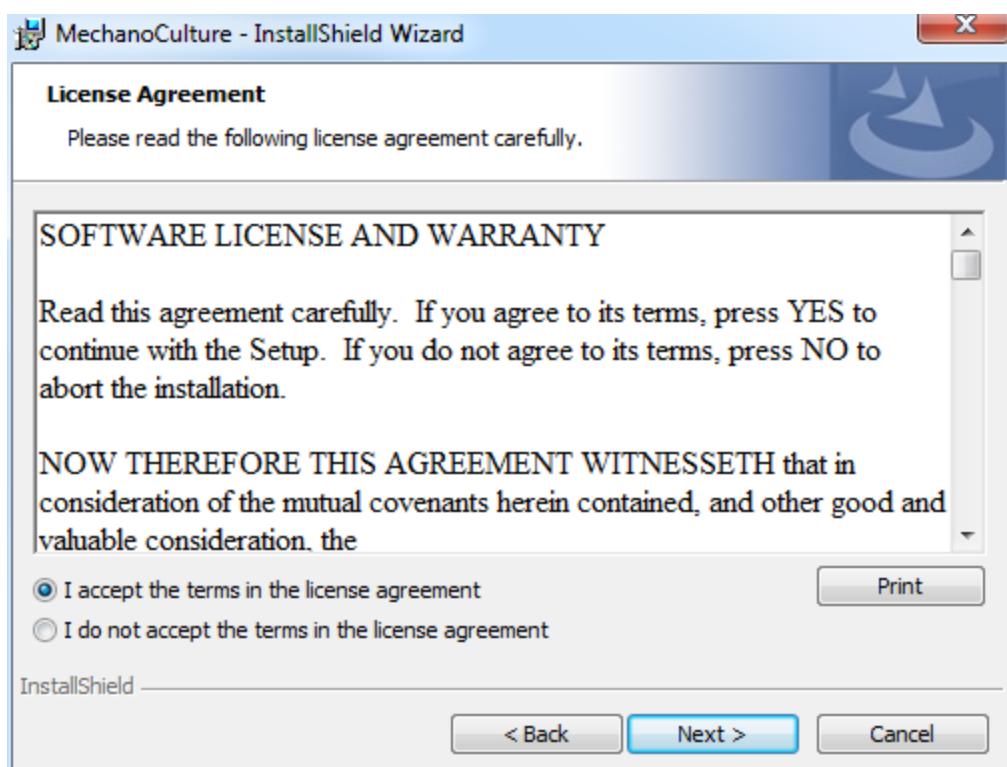
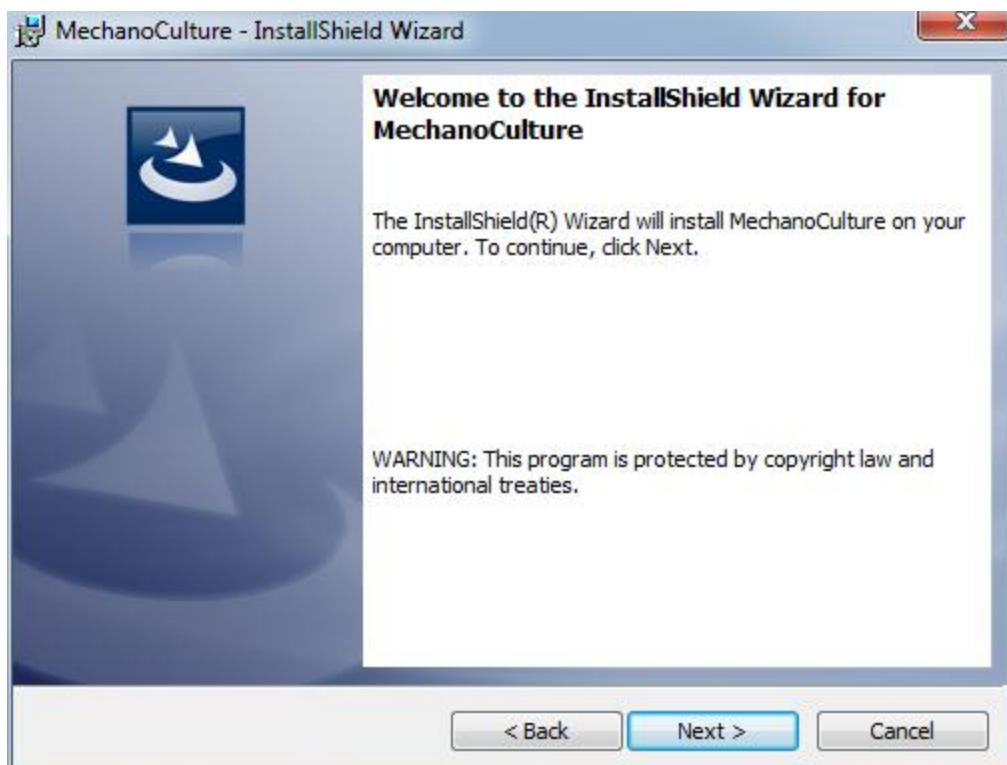


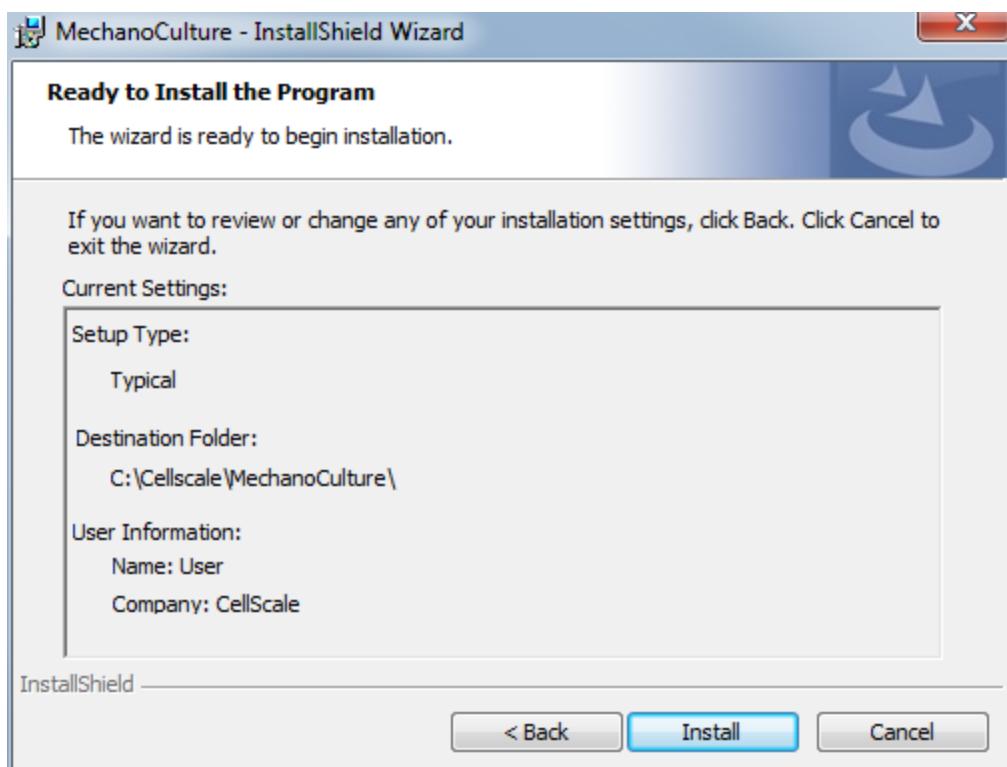
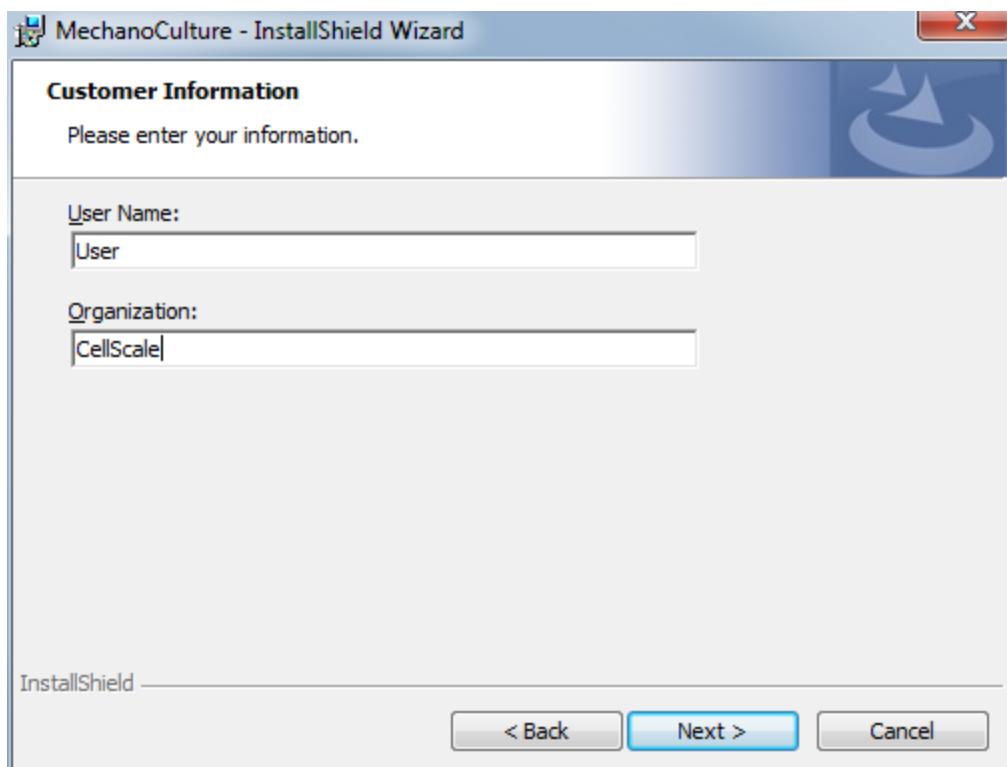
9. Appendix B: Software Installation

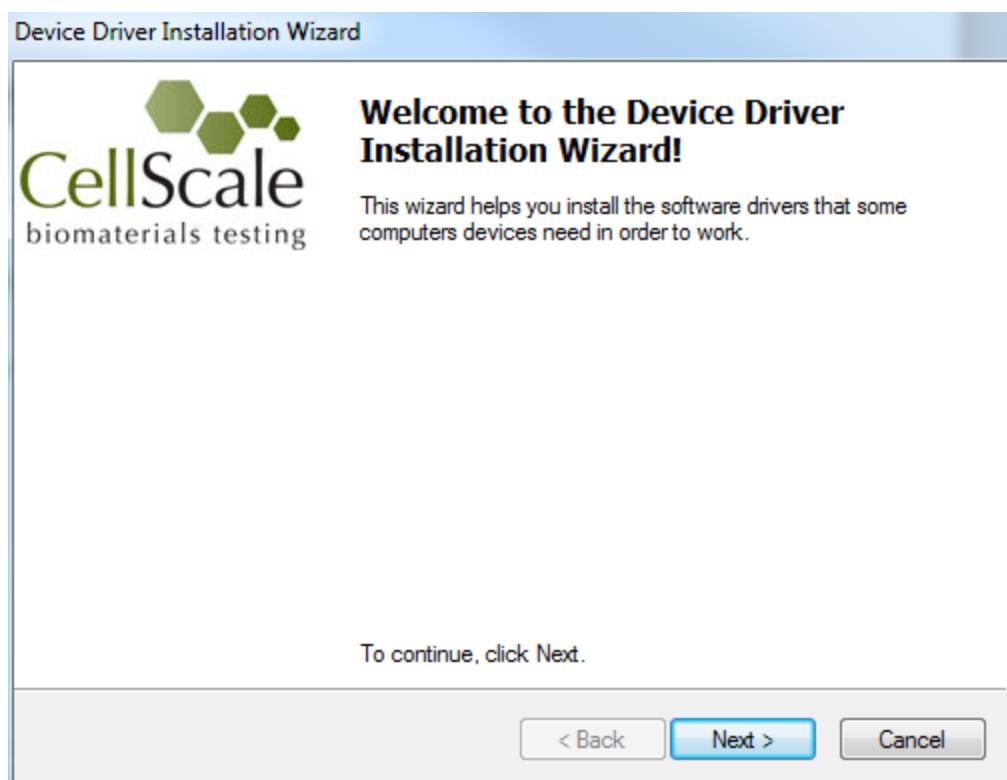
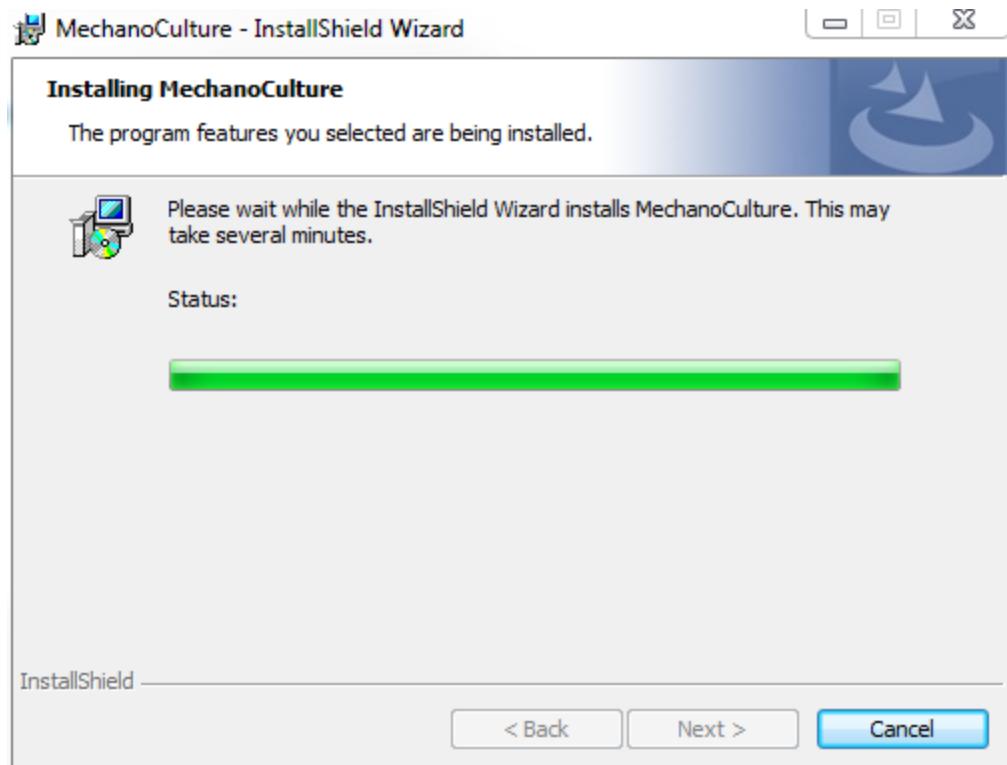
Included in your shipment is a link to install the MechanoCulture software. This installation package contains the installer for the software to program your MechanoCulture devices.

To install the software, go to the provided web address and download the compressed software package. Transfer this file to the computer which will be programming the MechanoCulture. 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.









Device Driver Installation Wizard

Completing the Device Driver Installation Wizard

The drivers were successfully installed on this computer.

You can now connect your device to this computer. If your device came with instructions, please read them first.

Driver Name	Status
✓ FTDI CDM Driver Packa...	Ready to use
✓ FTDI CDM Driver Packa...	Ready to use

< Back **Finish** Cancel

MechanoCulture - InstallShield Wizard

InstallShield Wizard Completed

The InstallShield Wizard has successfully installed MechanoCulture. Click Finish to exit the wizard.

< Back **Finish** Cancel

10. Appendix C: System Assembly

Follow these steps to assemble your MechanoCulture TM system for use:

1. Cut the membrane material to 2.5" wide to the desired length (100mm for the default specimen) and gather the following tools:
 - a. Mounting bar
 - b. Loading rings
 - c. Zip ties
 - d. Zip tie tool



2. Slide the loading rings around the mounting bar and mount the membrane material to the loading rings with zip ties.



3. Use the zip tie tool to ensure the zip ties are pulled taught to secure the membrane material to the loading rings. Cut the excess on the zip ties.



4. Remove the loading rings from the mounting bar and attach the rings to the main device. The photos below show the use of the loading rings.



5. Plug in the controller and main unit and reset the actuator.
6. Mount the specimen and loading rings assembly to the corresponding locations on the main unit.
7. Press the start button on the controller to initiate the test.