



MechanoCulture B1

Cell Mechanical Stimulation System

User Manual
version 1.6

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.

Our products are used by researchers around the globe to study everything from tissue strength and stiffness to cellular response. Please visit our website for more information and a list of publications. Also, please contact us to find out how our test systems can help you.

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1. General Information

The MechanoCulture is a precision test instrument specifically designed for deforming flexible membranes and 3D matrices in a sterile fluid environment. This device is capable of equibiaxial, deformation patterns on 36-50mm diameter flexible cell-seeded substrates or scaffolds. The device has an onboard actuator and a separate control unit to enable it to run independent of a computer in an environment-controlled incubator.

Environmental and Electrical Specifications

Electrical Input	24V DC
Current Rating	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 does not require initial assembly. Some assembly/disassembly required between uses.

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.

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 double push will cause the actuator to advance to the load position before pausing the test sequence. A push and hold for 5 seconds will advance the actuator and reset the test sequence to the beginning.

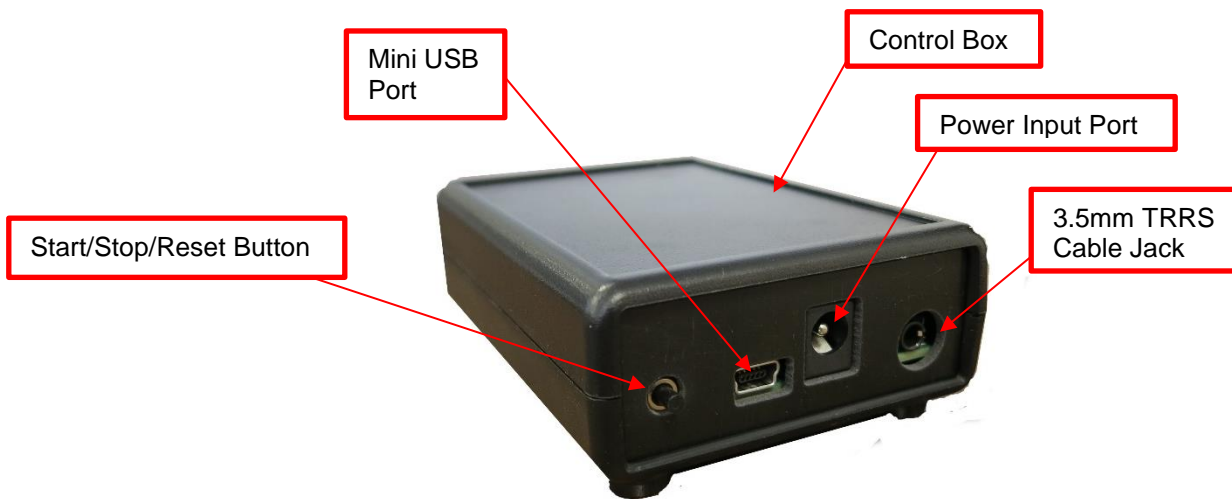
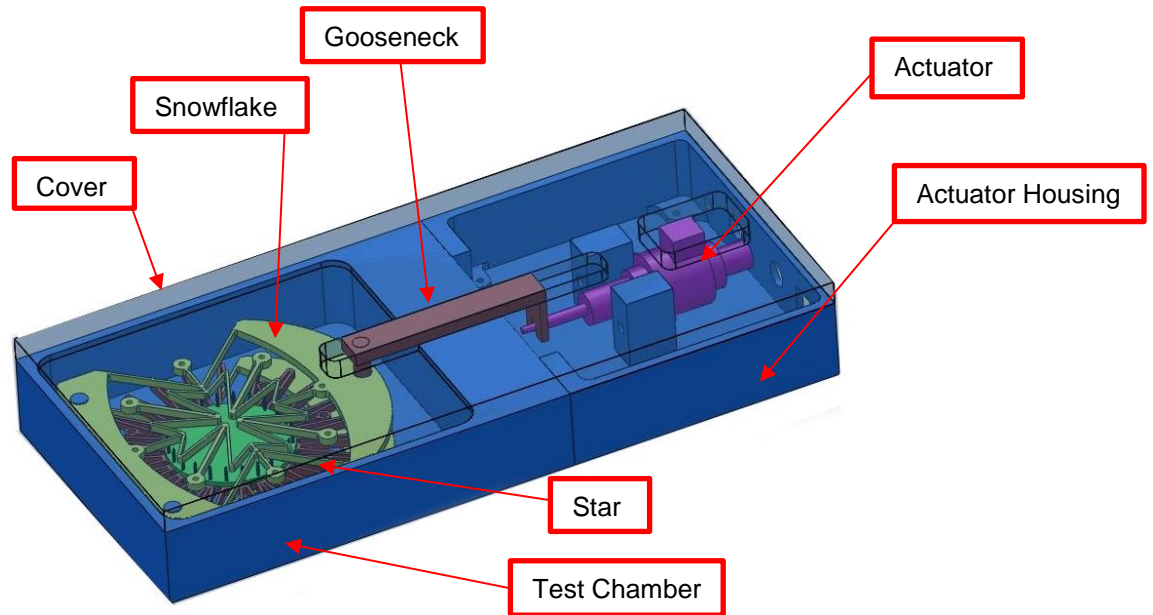
General Maintenance

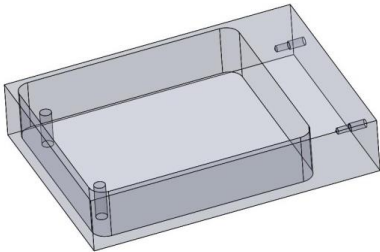
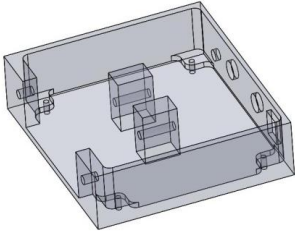
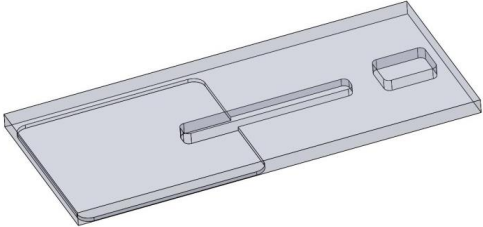
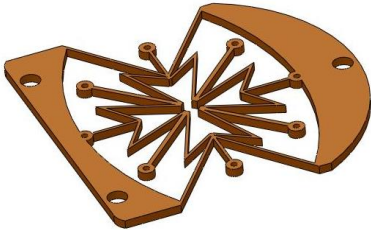
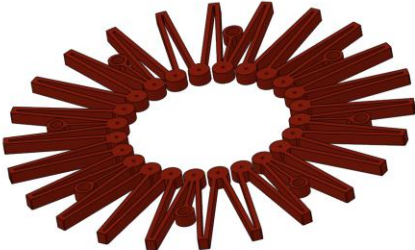
The equipment does not require maintenance other than cleaning. Please see the components overview section for cleaning and sterilization details.

Approvals and Certification

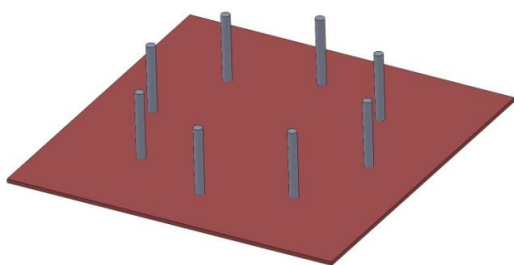
This product conforms to EN61010-1:2001 and EN61326-1.

2.Components Overview



	<p>Test Chamber</p> <p>Material: Polycarbonate</p> <p>Cleaning: Ethanol, UV, Autoclave</p>
	<p>Actuator Housing</p> <p>Material: Polycarbonate</p> <p>Cleaning: UV only (contains circuitry)</p>
	<p>Cover</p> <p>Material: Polycarbonate</p> <p>Cleaning: Ethanol, UV, Autoclave</p>
	<p>Snowflake</p> <p>Material: PEEK</p> <p>Cleaning: Ethanol, UV, Autoclave</p>
	<p>Star</p> <p>Material: PEEK</p> <p>Cleaning: Ethanol, UV, Autoclave</p>

	<p>Gooseneck</p> <p>Material: 316 Stainless Steel</p> <p>Cleaning: Ethanol, UV, Autoclave</p>
	<p>Power Supply</p>
	<p>USB Cable</p>
	<p>3.5mm TRRS Cable</p>
	<p>Control Box</p> <p>Cleaning: None</p>



Assembly Plate

Material:

PEEK and 316 Stainless Steel

Cleaning:

Ethanol, UV, Autoclave

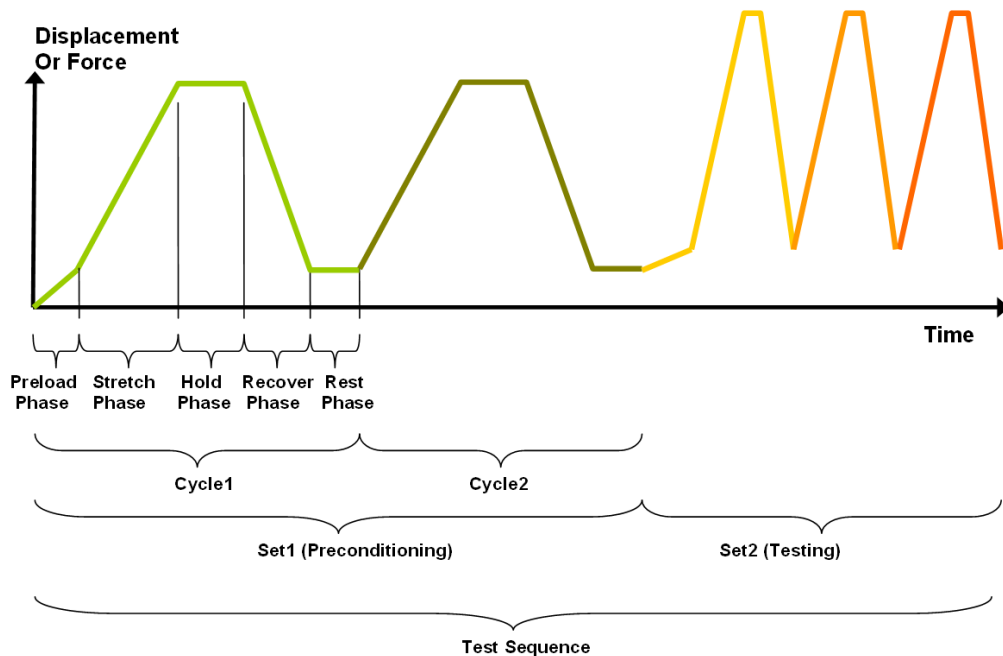
3. Testing Terminology

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 specimen 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*: 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

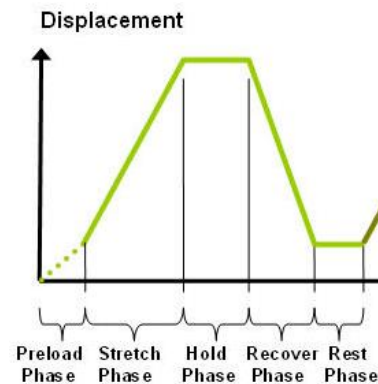
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

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

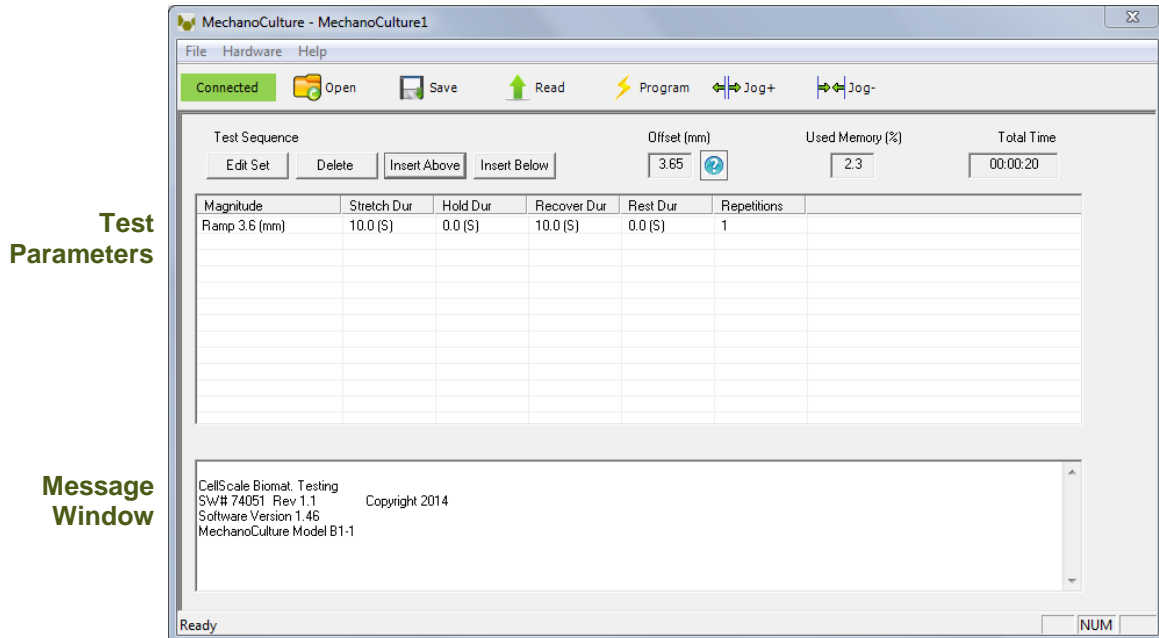
The MechanoCulture makes it possible to test specimens under two control functions:

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. This sinusoid is approximated as a series of linear segments.

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:

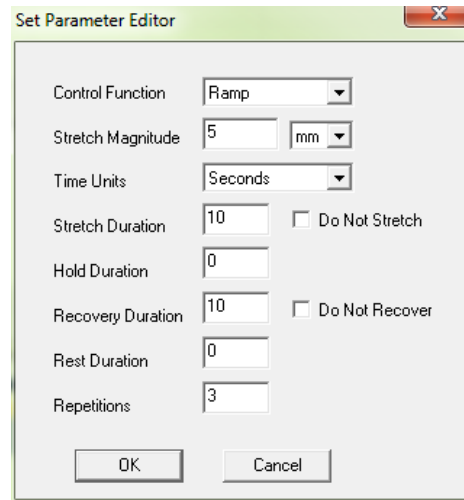
1. A predefined test sequence can be opened from a MechanoCulture Protocol File (*.tmt). From the File menu, select Open, or press the Open button on the toolbar.
2. The currently programmed test sequence on a device can be read. From the Hardware menu, select Read, or press the Read button on the toolbar. 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.



The Set Parameter Editor dialog box contains the following fields and controls:

- Control Function: Ramp (dropdown)
- Stretch Magnitude: 5 (text input) mm (dropdown)
- Time Units: Seconds (dropdown)
- Stretch Duration: 10 (text input)
- Hold Duration: 0 (text input)
- Recovery Duration: 10 (text input)
- Rest Duration: 0 (text input)
- Repetitions: 3 (text input)
- Do Not Stretch: ☐ (checkbox)
- Do Not Recover: ☐ (checkbox)
- OK button
- Cancel button

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.

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.

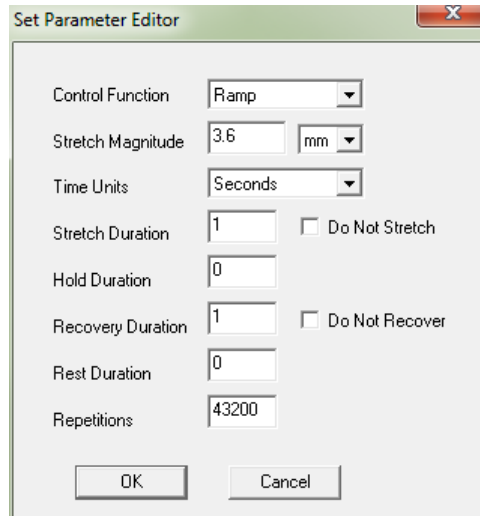
Example 1: Continuous Cycling for Fixed Duration and Then Stop

Objective: Create a test sequence that cycles at 0.5 Hz with 3.6mm 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 requires 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)).



The Set Parameter Editor dialog box contains the following fields and controls:

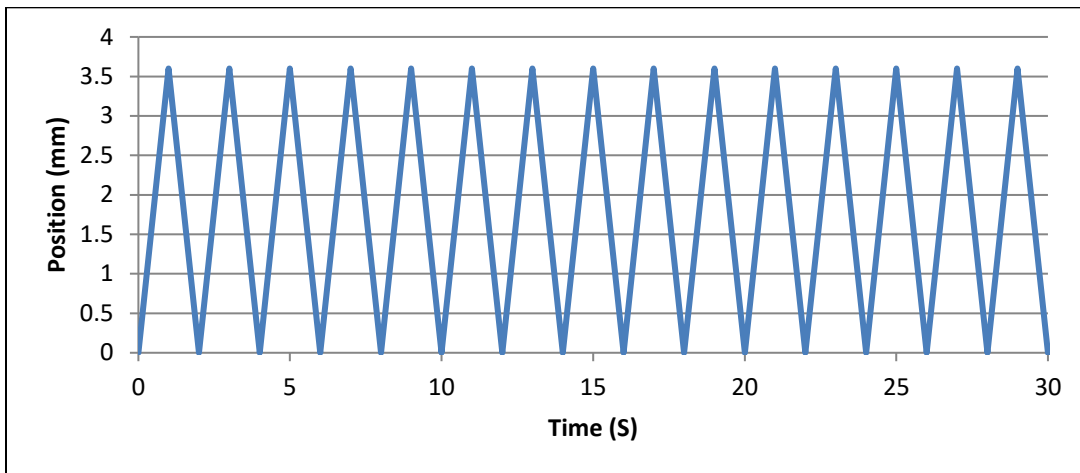
- Control Function: Ramp (dropdown)
- Stretch Magnitude: 3.6 (text input), mm (dropdown)
- Time Units: Seconds (dropdown)
- Stretch Duration: 1 (text input), Do Not Stretch (checkbox)
- Hold Duration: 0 (text input)
- Recovery Duration: 1 (text input), Do Not Recover (checkbox)
- Rest Duration: 0 (text input)
- Repetitions: 43200 (text input)
- OK and Cancel buttons at the bottom.

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	Repetitions
Ramp 3.6 (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 1 mm pre-stretch in 75 seconds followed by 100 cycles of an additional 5 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.

The 'Set Parameter Editor' dialog box shows the following settings:

- Control Function: Ramp
- Stretch Magnitude: 1 mm
- Time Units: Seconds
- Stretch Duration: 75
- Hold Duration: 0
- Recovery Duration: 0
- Rest Duration: 0
- Repetitions: 1
- Do Not Stretch: ☐
- Do Not Recover: ☒

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).

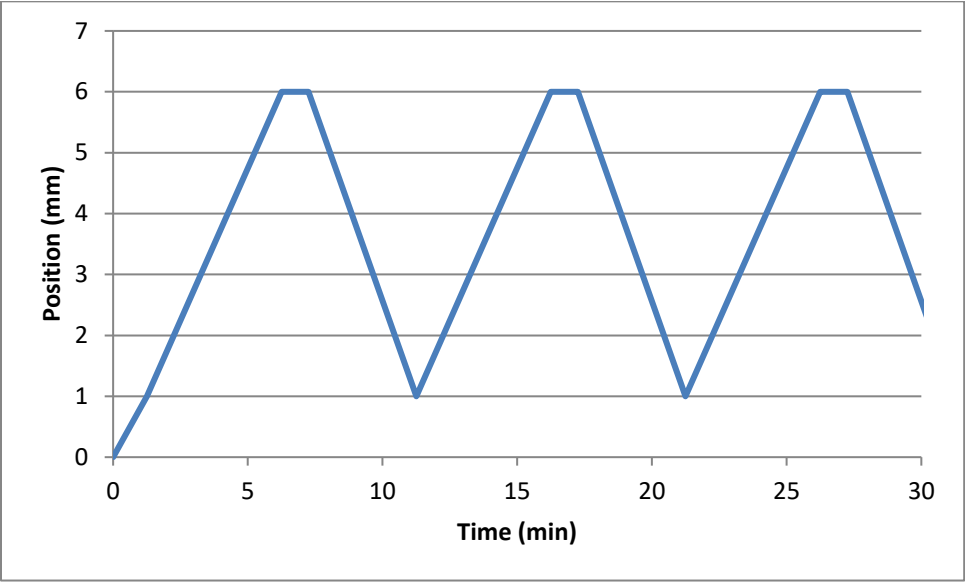
The 'Set Parameter Editor' dialog box shows the following settings:

- Control Function: Ramp
- Stretch Magnitude: 5 mm
- Time Units: Minutes
- Stretch Duration: 5
- Hold Duration: 1
- Recovery Duration: 4
- Rest Duration: 0
- Repetitions: 100
- Do Not Stretch: ☐
- Do Not Recover: ☐

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					
<div> Edit Set Delete Insert Above Insert Below </div> <div> Offset (mm) 0.00 ?</div>					
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions
Ramp 1.0 (mm)	75.0 (S)	0.0 (S)	None	0.0 (S)	1
Ramp 5.0 (mm)	5.0 (m)	1.0 (m)	4.0 (m)	0.0 (m)	100
Ramp 0.0 (mm)	None	0.0 (d)	None	21.0 (d)	1

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 10 minutes followed by 50 minutes of rest. Repeat this pattern indefinitely.

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

The 'Set Parameter Editor' dialog box is shown with the following settings:

- Control Function: Ramp
- Stretch Magnitude: 2 (mm)
- Time Units: Seconds
- Stretch Duration: 0.5
- Hold Duration: 0
- Recovery Duration: 0.5
- Rest Duration: 0
- Repetitions: 600
- Do Not Stretch: ☐
- Do Not Recover: ☐

2. Press Insert Below to create a second set.

The 'Set Parameter Editor' dialog box is shown with the following settings:

- Control Function: Ramp
- Stretch Magnitude: 0 (mm)
- Time Units: Minutes
- Stretch Duration: 0
- Hold Duration: 1
- Recovery Duration: 0
- Rest Duration: 50
- Repetitions: 1
- Do Not Stretch: ☒
- Do Not Recover: ☒

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					
<div>Edit Set Delete Insert Above Insert Below</div>				Offset (mm)	
				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.

Before beginning your first test, please visit the CellScale Website (www.cellscale.com) and review the video posts to observe the operation of this device.

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.

Set up MechanoCulture Device

See Appendix A for a sample guide to setting up the MechanoCulture, including the star and snowflake assembly.

Plug in Control Box

Connect Control Box and Test System

Program the Device (if needed)

Connect the Device to the PC via USB

Launch the MechanoCulture Software

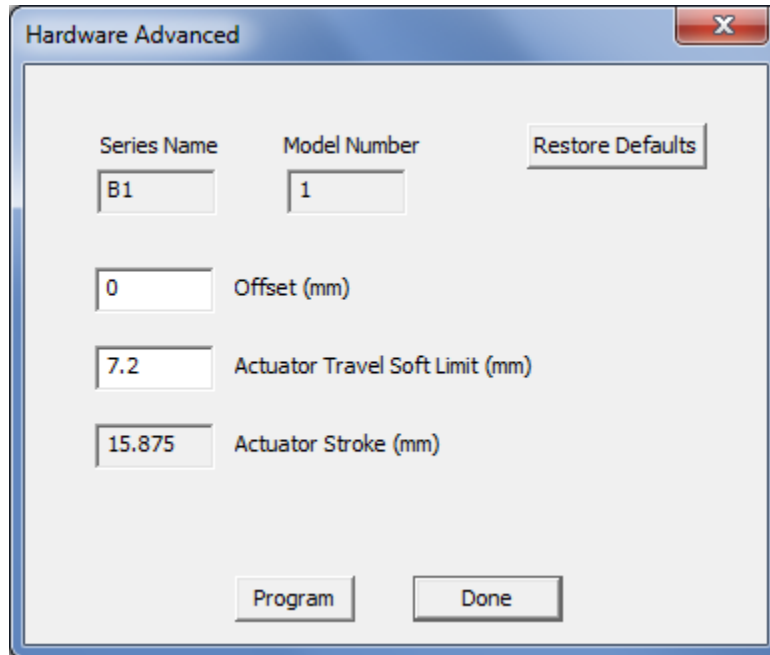
Generate a Test Sequence

Upload Test Sequence to Device

Execute Test

7. Advanced Parameters

Under the Hardware menu is an Advanced Settings menu containing a number of values which should be understood. The menu appears as below:



The screenshot shows a software window titled "Hardware Advanced" with a standard Windows-style title bar (blue with a red close button). The window contains several input fields and buttons. At the top, there are two labels: "Series Name" and "Model Number". Below "Series Name" is a text box containing "B1". Below "Model Number" is a text box containing "1". To the right of these is a button labeled "Restore Defaults". Below the "Series Name" field is another text box containing "0", followed by the label "Offset (mm)". Below the "Model Number" field is a text box containing "7.2", followed by the label "Actuator Travel Soft Limit (mm)". Below the "Offset (mm)" field is a text box containing "15.875", followed by the label "Actuator Stroke (mm)". At the bottom of the window are two buttons: "Program" and "Done".

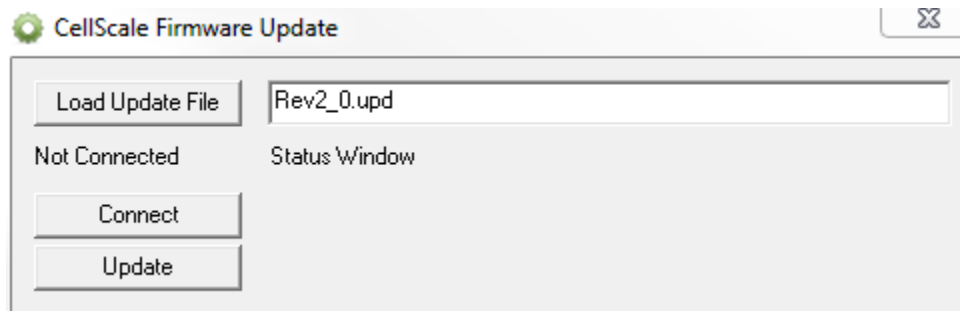
Offset: Offset is a distance that controls the starting stretch magnitude of each test. For example, a stretch of 2mm will stretch from 5mm to 7mm when the offset is 5mm. Click Program and then reset the MechanoCulture using the pushbutton to change the offset. During a reset the actuator fully extends and then retracts a small amount, defined by the offset. The default value for offset is 0mm.

Actuator Travel Soft Limit: The B1 actuator has 15.875mm of travel. The soft limit should be kept slightly less than this to prevent over-travel during manual jogging of the actuator. If desired, this value can be set even lower to prevent accidental damage to specimens. To change the soft travel limit, click program and then reset the MechanoCulture using the pushbutton.

Actuator Stroke: The physical travel capability of the stepper motor to drive the lead screw. For the B1, this value is 15.875mm.

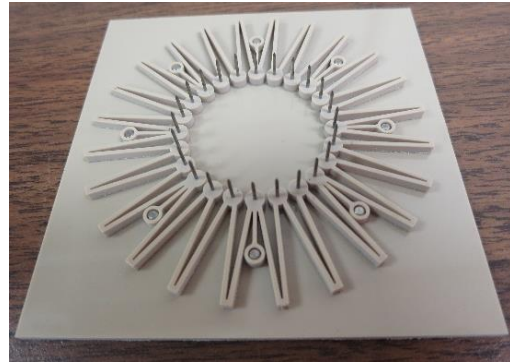
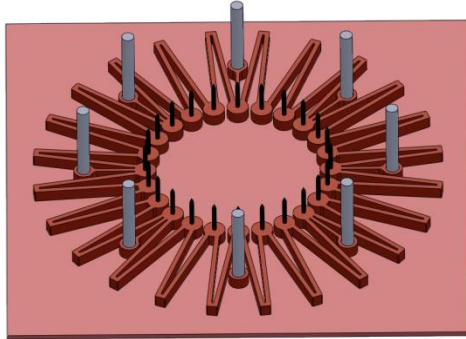
8.Updating the Firmware

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.

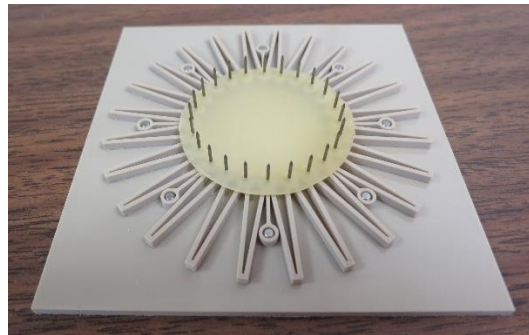
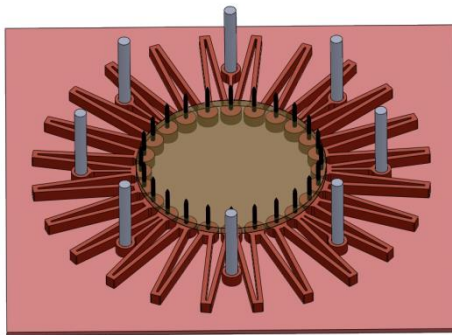


9. Appendix A: Sample Test Setup

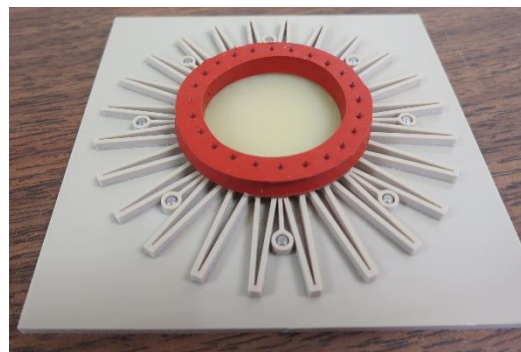
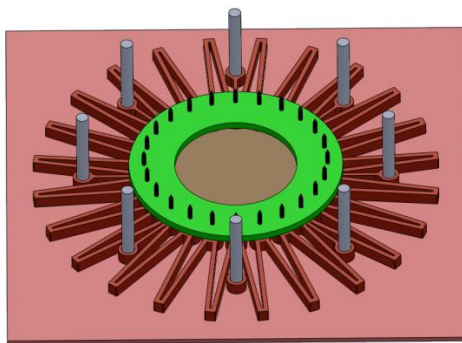
1. Place lower star on assembly plate



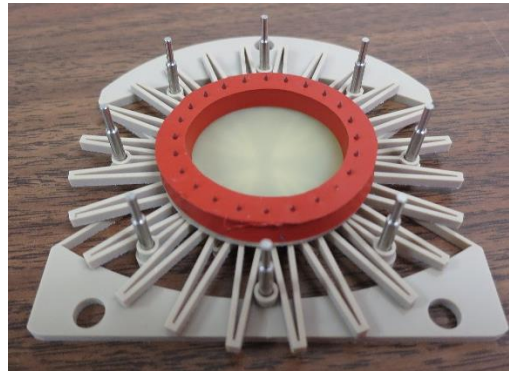
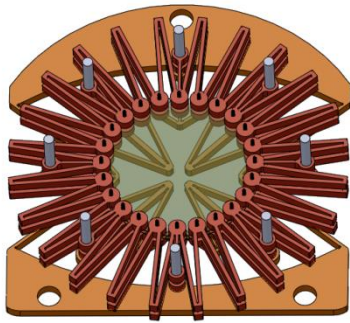
2. Place membrane on top of puncture pins and press to mount specimen



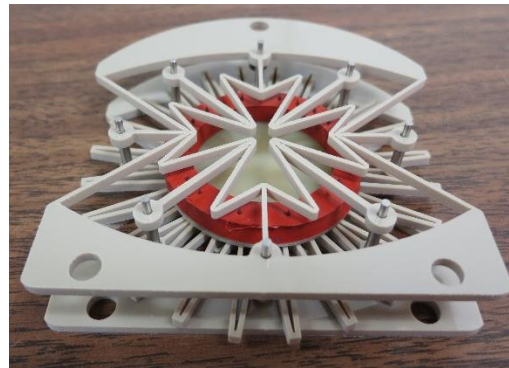
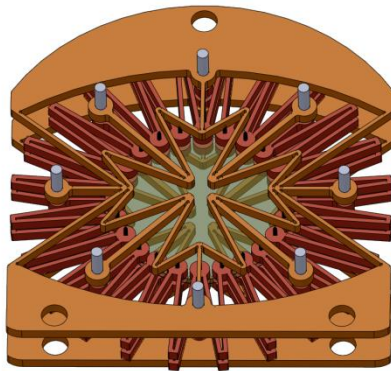
3. OPTIONAL: Place flexible containment ring on top of pins and press to mount. It may be necessary to use a silicone paste to ensure a good seal between the containment ring and the substrate.



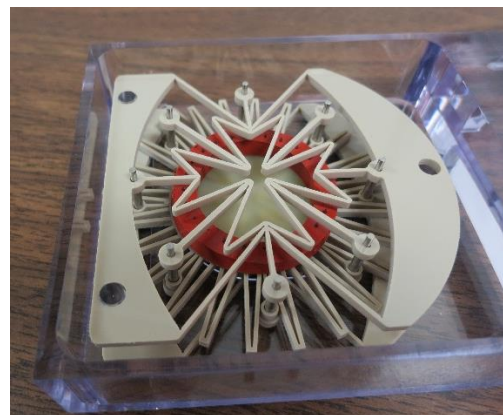
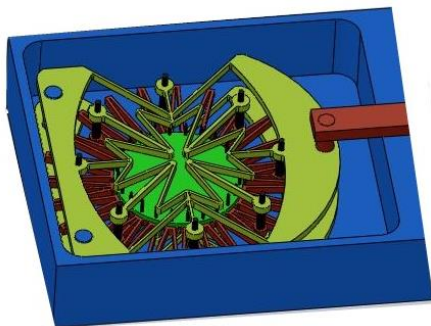
4. Lift the star/specimen assembly off of the assembly plate and lower on to the lower snowflake. Optionally, first place star/specimen assembly in Petri dish and perform cell seeding.



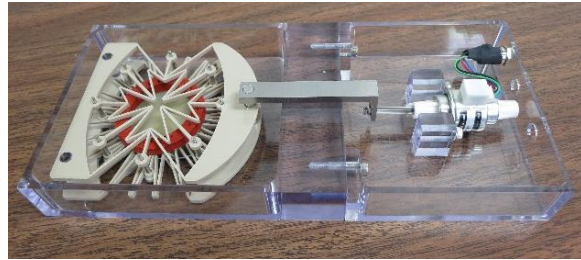
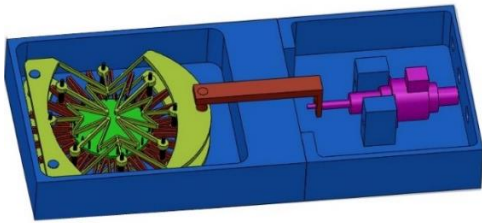
5. Place upper snowflake on top of lower snowflake



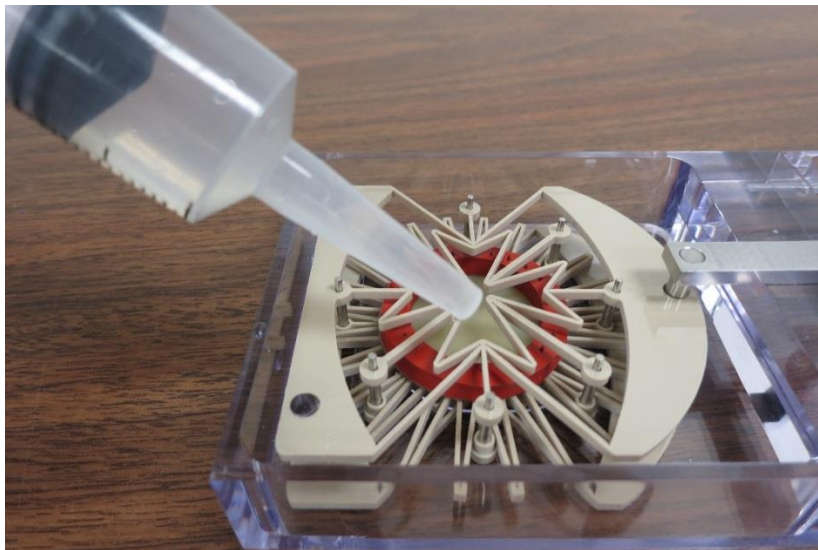
6. Place snowflake/star assembly in the MechanoCulture test chamber



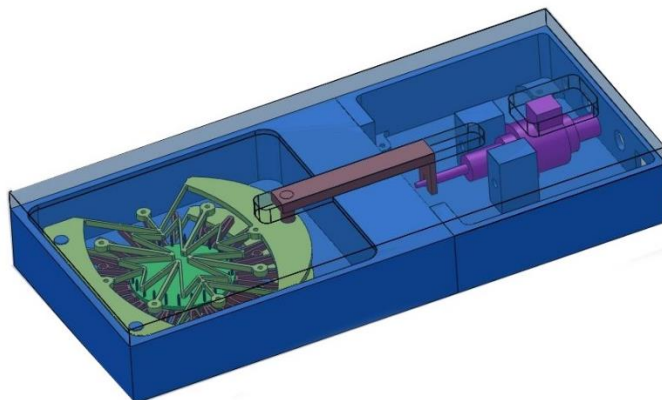
7. Place gooseneck in position

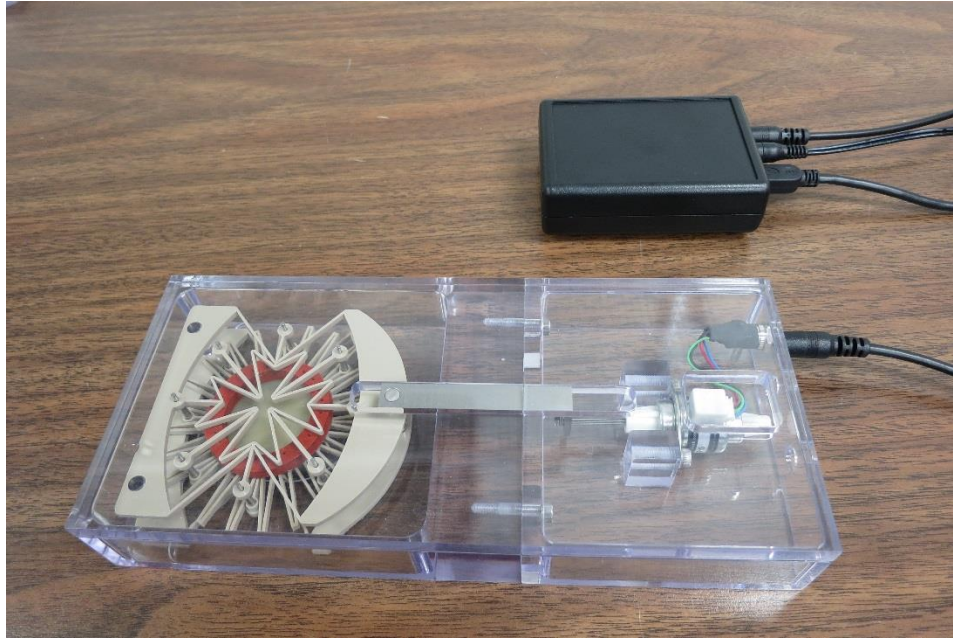


8. Fill test chamber with media OR fill test region inside containment ring with media



9. Place top covers in position



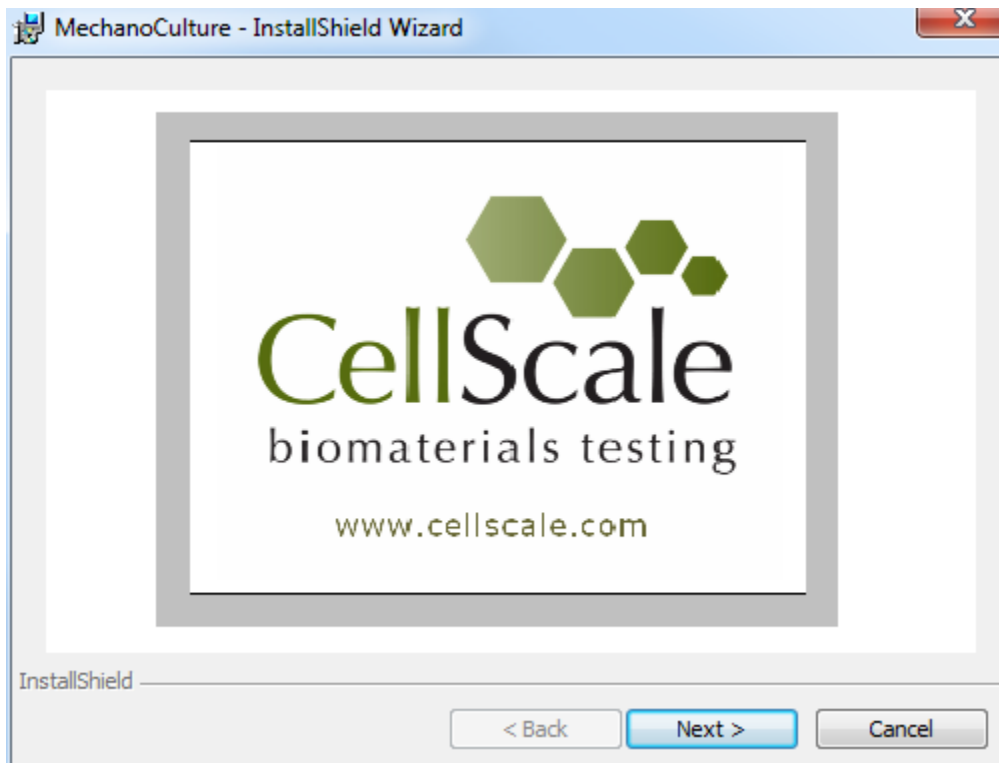


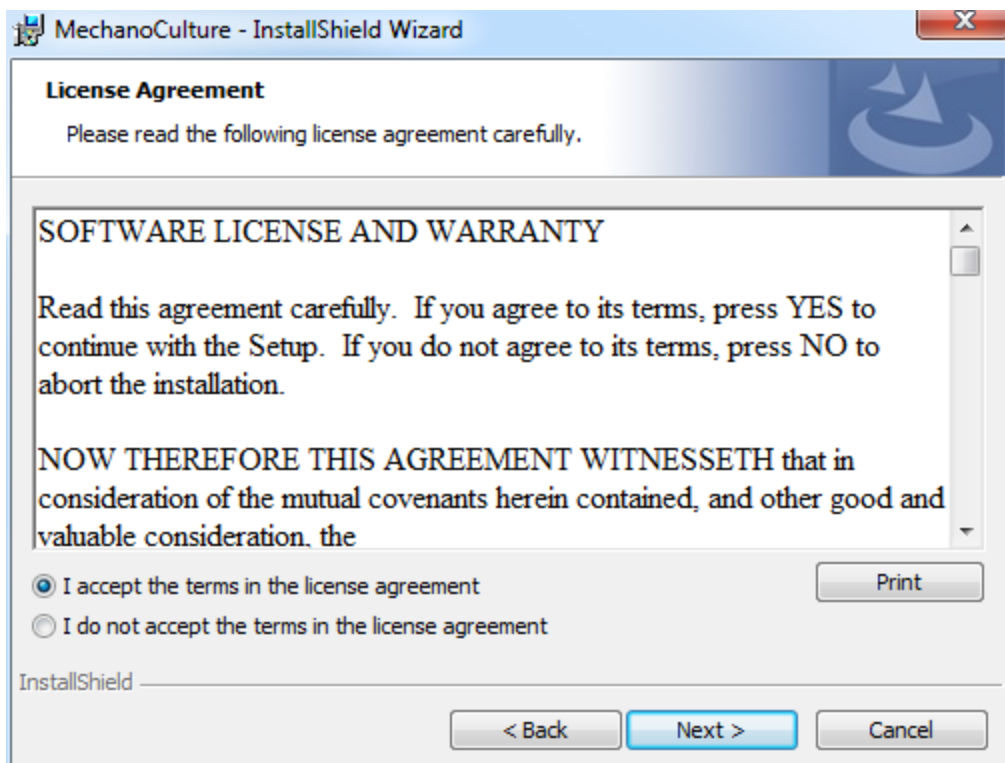
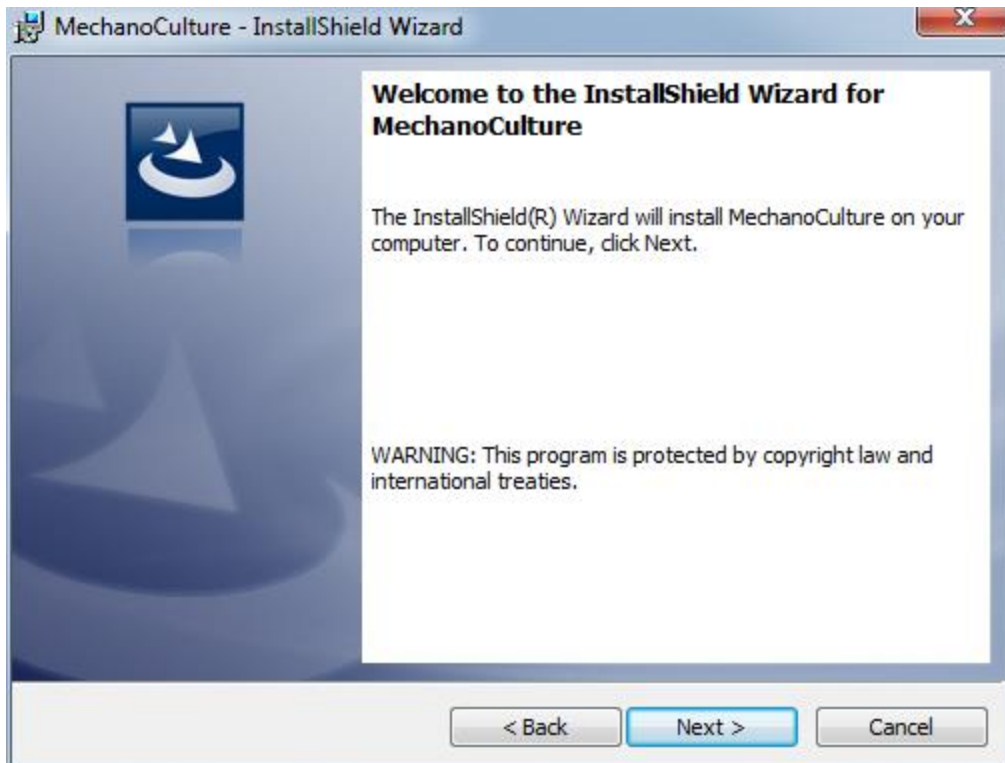
10. Place unit in incubator and begin test by using the push-button switch on the front of the control unit. A single push will start and stop the test sequence. A double push will cause the actuator to advance to the load position before pausing the sequence. A push and hold for 5 seconds will advance the actuator and reset the test sequence to the beginning.

10. 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 installation 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.





MechanoCulture - InstallShield Wizard

Customer Information

Please enter your information.

User Name:
User

Organization:
CellScale

InstallShield

< Back Next > Cancel

MechanoCulture - 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\MechanoCulture\

User Information:
Name: User
Company: CellScale

InstallShield

< Back Install Cancel

