CellScale
biomaterials testing

MechanoCulture FX

Mechanical Stimulation System

User Manual

version 1.4

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.

CellScale
biomaterials testing

Sales and Support
CellScale Biomaterials Testing
11 – 564 Weber St. N.
Waterloo, Ontario, Canada N2L 5C6
Phone: 519.342.6870

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1. General Information

The MechanoCulture FX is a precision instrument specifically designed for deforming flexible membranes in a sterile fluid environment. The device has an onboard actuator and 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 40ºC 0% - 100% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree 2
Data Connections	mini USB for programming

System Assembly

The unit 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.

The unit should not be operated without the motor housing or media chamber lid 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 reset the test sequence to the beginning and disable the motor to allow the carriage to move freely.

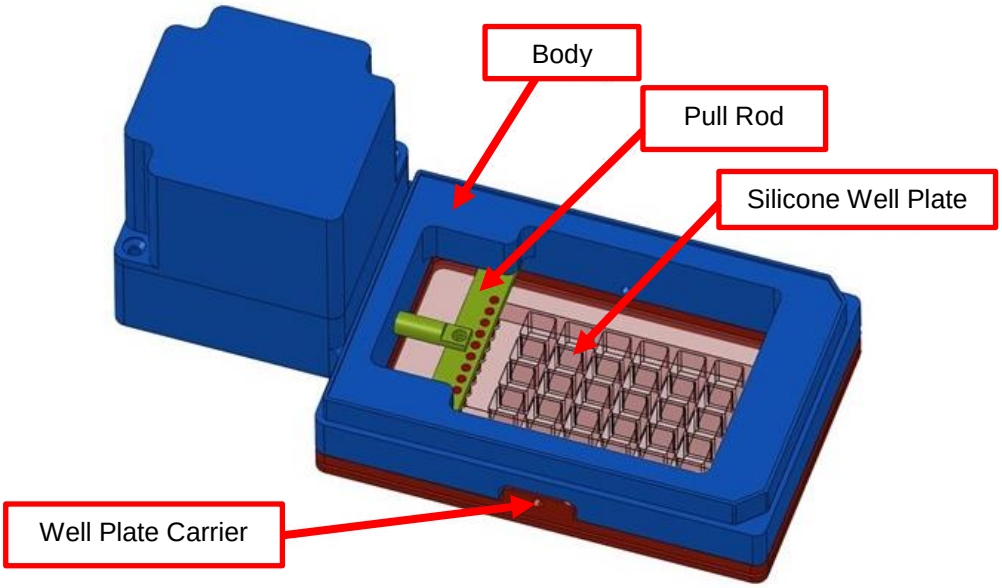
General Maintenance

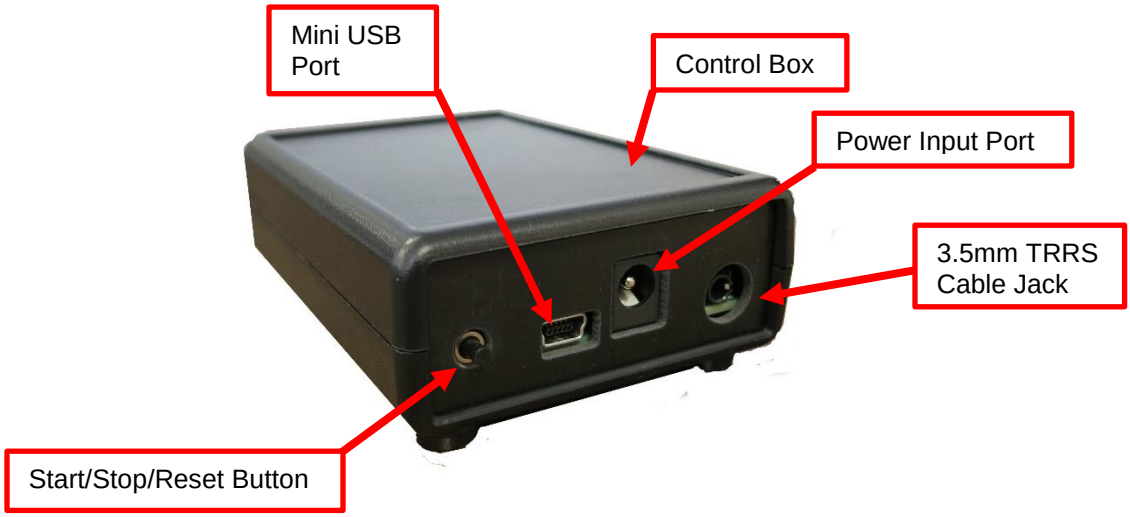
The equipment does not require maintenance other than cleaning. Please see the components overview section for cleaning and sterilization details.

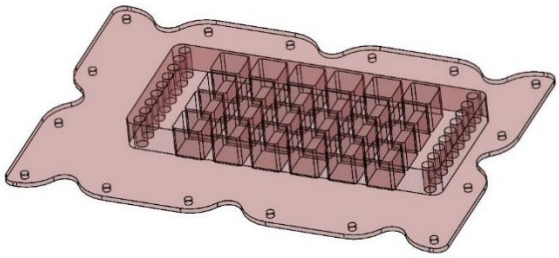
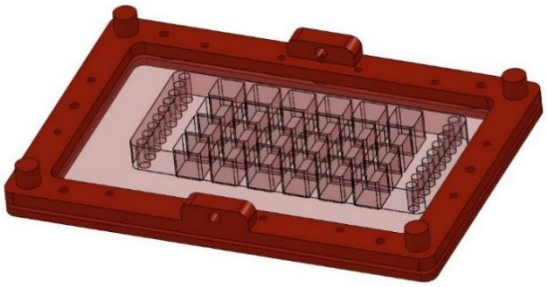
Approvals and Certification

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

2. Components Overview





	**Silicone Well Plate** **Material:** Silicone Rubber **Cleaning:** Ethanol, UV, Autoclave, EtO
	**Well Plate Carrier Assembly** **Material:** Anodized Aluminum
	**Power Supply**
	**USB Cable**

	**Control Box** **Cleaning:** None

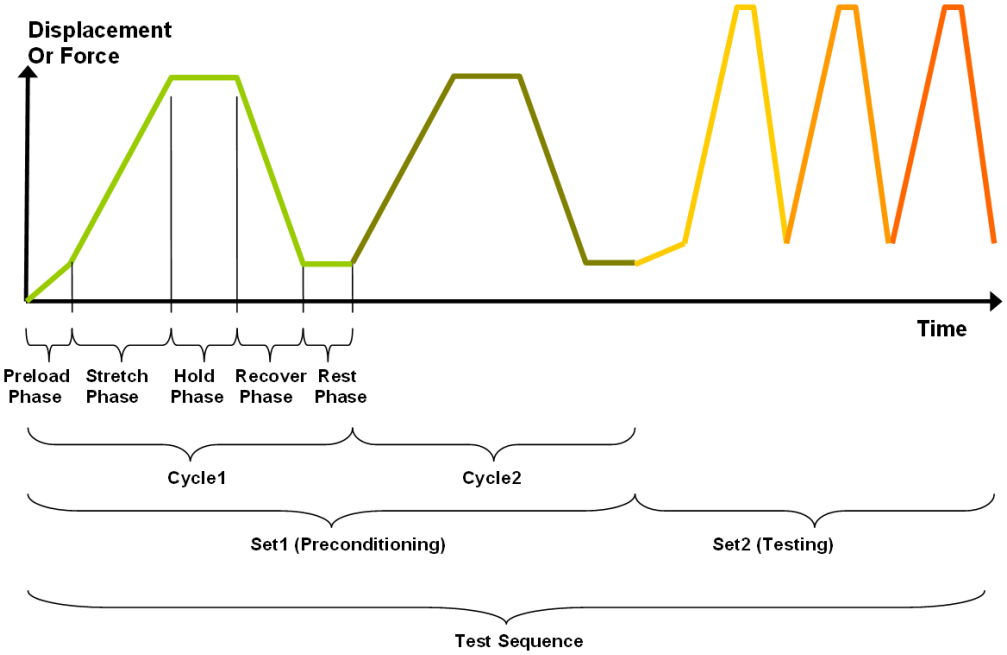
3. Testing Terminology

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*: 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

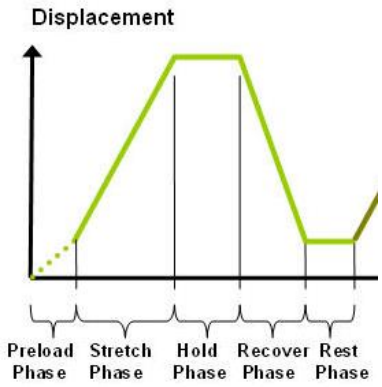
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

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

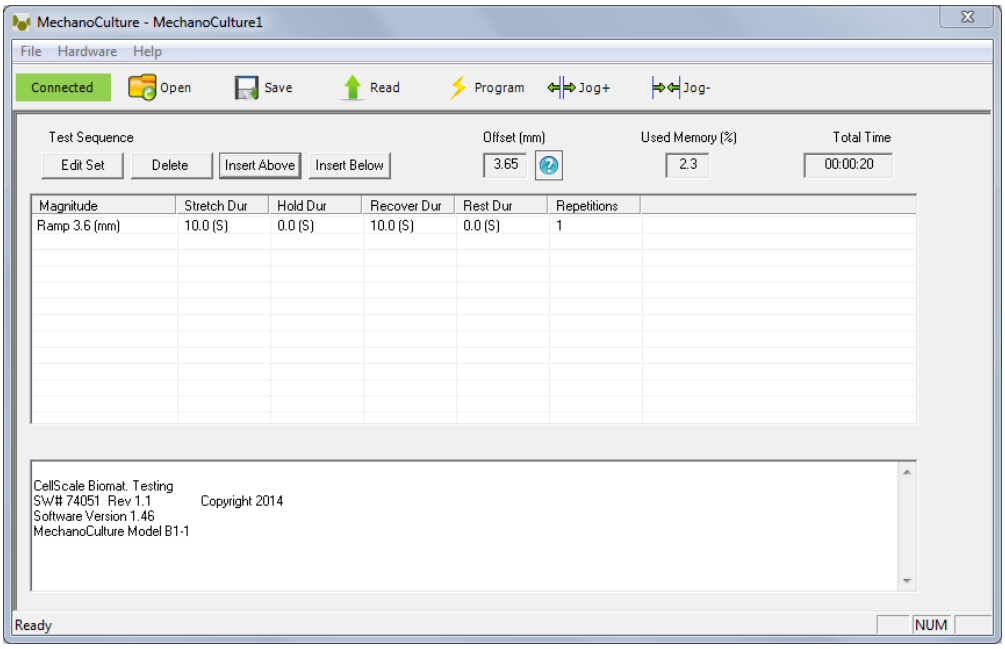
The MechanoCulture makes it possible to stimulate specimens under two control functions:

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:



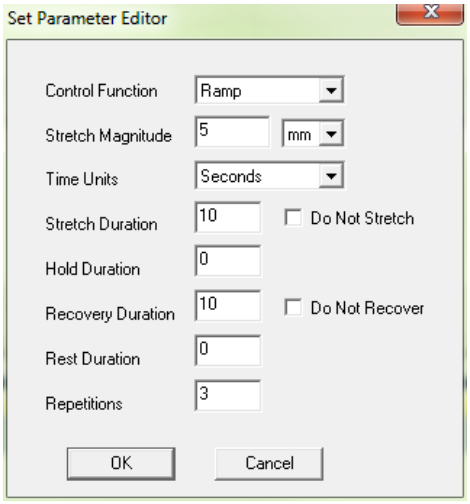
Step 1: Initiate a Test Sequence

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.

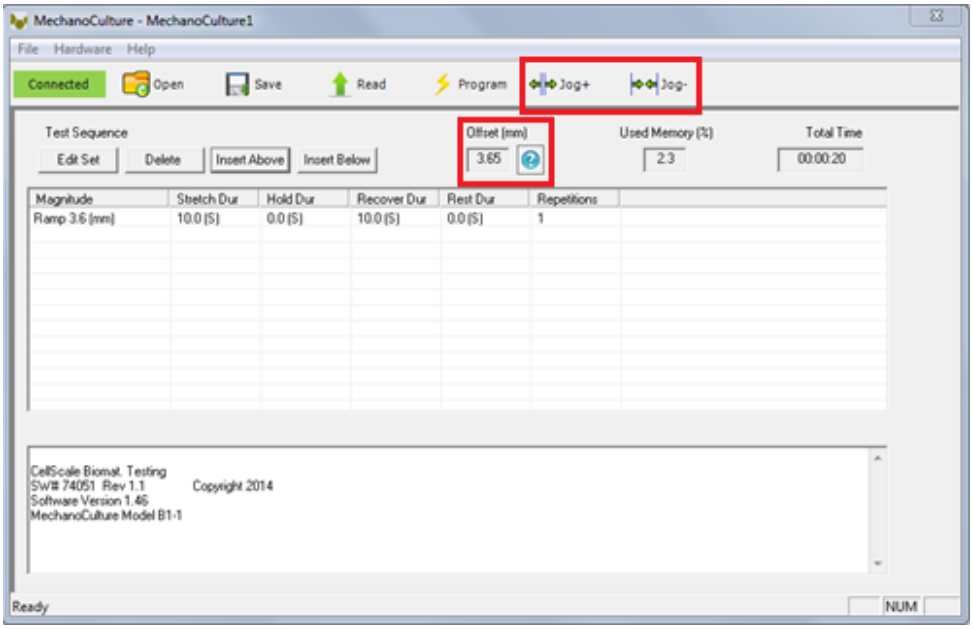
The Do Not Stretch and Do Not Recover check boxes allow for the stretch or recover phases to be omitted during a testing cycle. This makes the creation of test sequences that include preloading possible.

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.*

Offset and Jog Functions



The offset and jog functions for the MechanoCulture FX are not useful and these functions are not enabled. In the software interface, these can be ignored.

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.

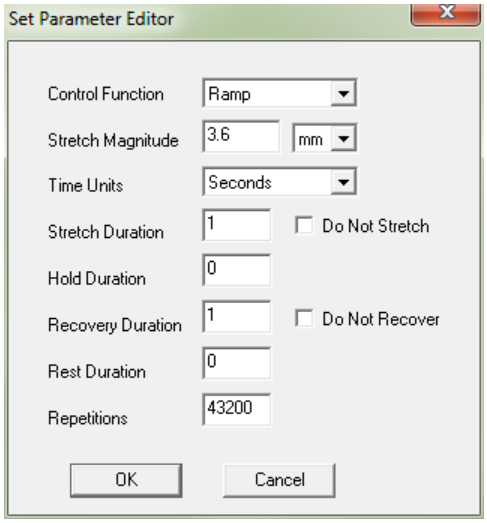
Example 1: Continuous Cycling for Fixed Duration and then Stop

Objective: Create a test sequence that cycles at 0.5 Hz with 3.6mm of stretch for 24 hours and then stops+.

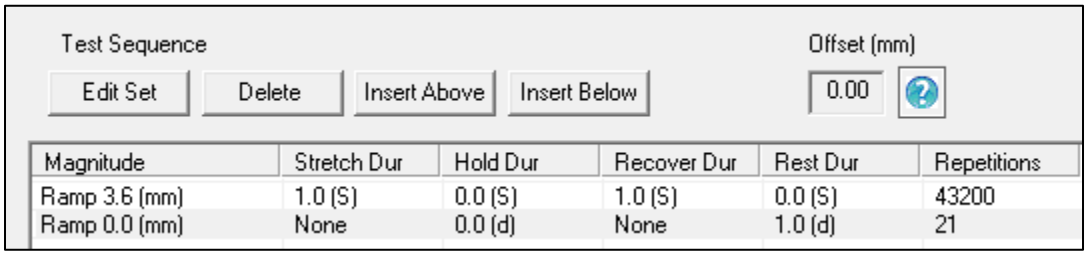
Press Insert Above to create a Set. Then double click on that row to modify it.

0.5 Hz 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)).

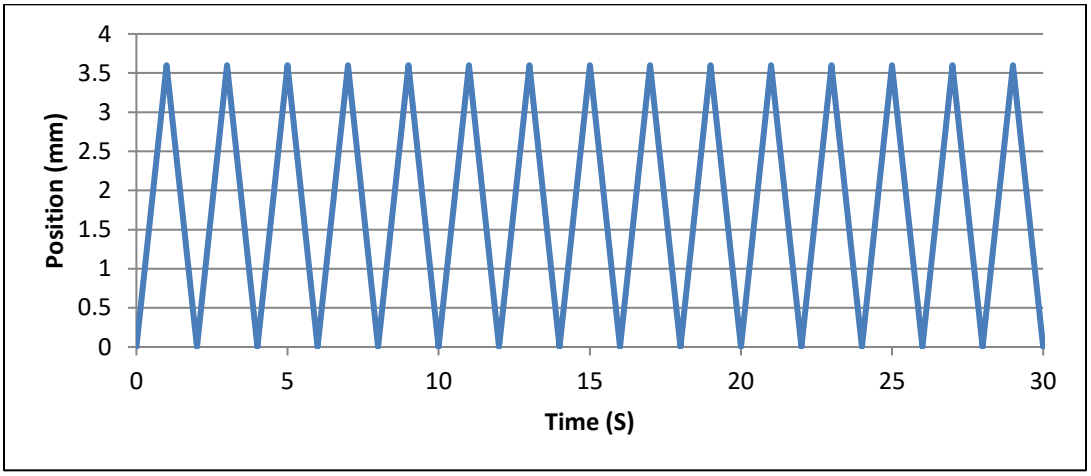


> ***MechanoCulture Tip: Terminating a test***
>
> *The MechanoCulture software will loop through the test sequence indefinitely. To prevent the sequence from repeating itself, add a test set to the end of the sequence that includes a lengthy rest duration but no stretch or recovery. For example, 21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



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

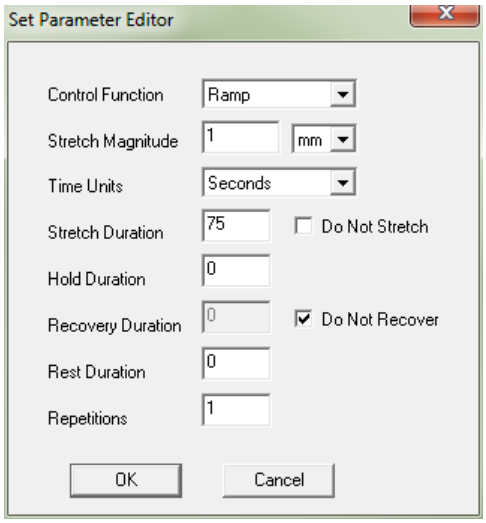
The resulting actuator motion versus time graph for the first 30 seconds:



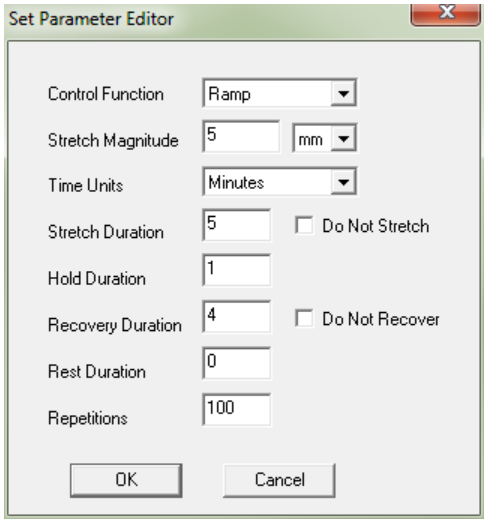
Example 2: Pre-stretch a Single Time, Then Cycle for a Fixed Duration, and Then Stop

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).

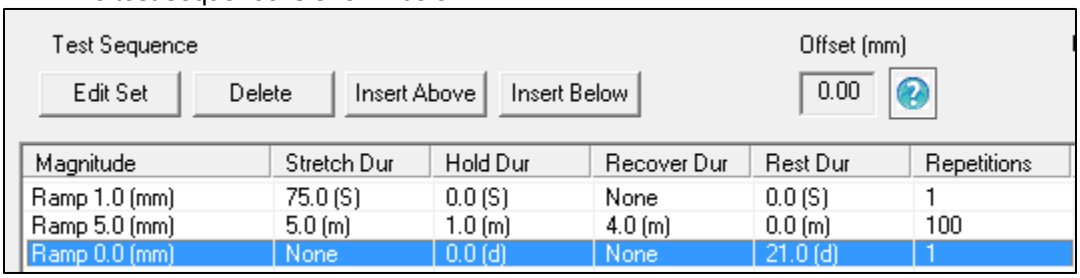
1. Press Insert Above to create a Set. Then double click on that row to modify it.
   Check the Do Not Recover box to omit the recovery phase.



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).

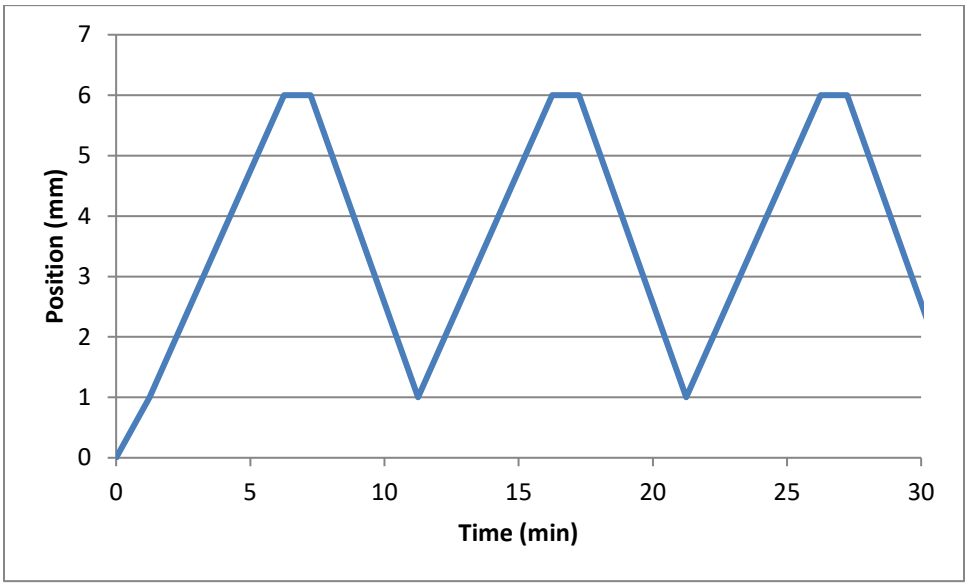


3. To terminate the test at the end of 24 hours, insert a lengthy rest set (21 days in this example) to the end of the sequence. If this set is not included, the protocol will automatically restart at the end of the first set (see the MechanoCulture tip in Example 1). The test sequence is shown below:



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

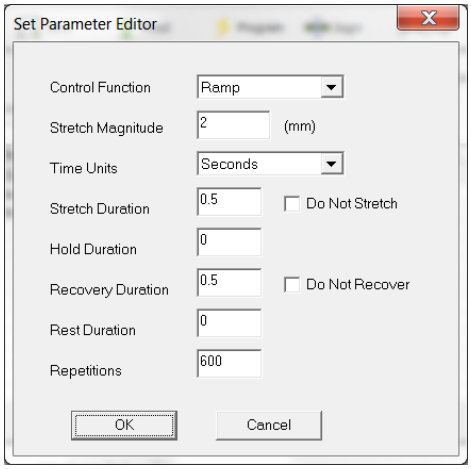
The resulting actuator motion versus time graph for the first 30 minutes:



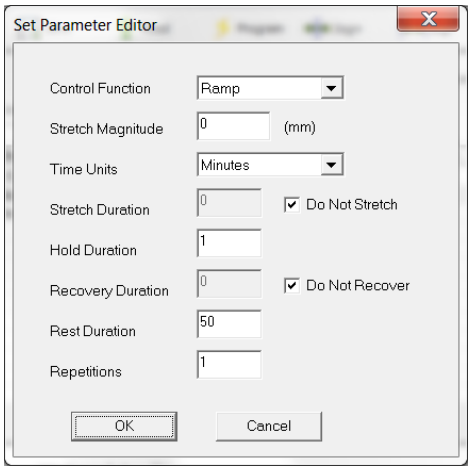
Example 3: Intermittent Stretching

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

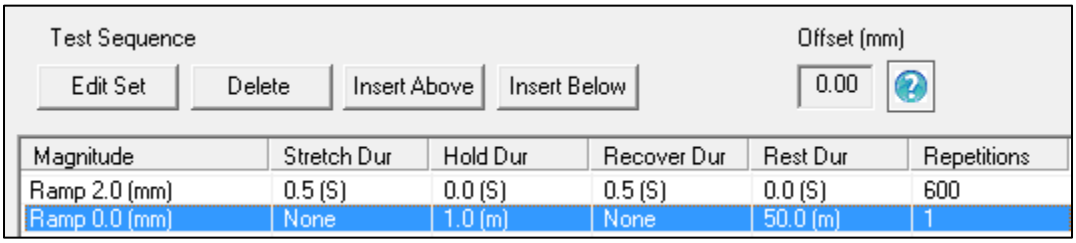
1. Press Insert Above to create a Set. Then double click on that row to modify it.



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:



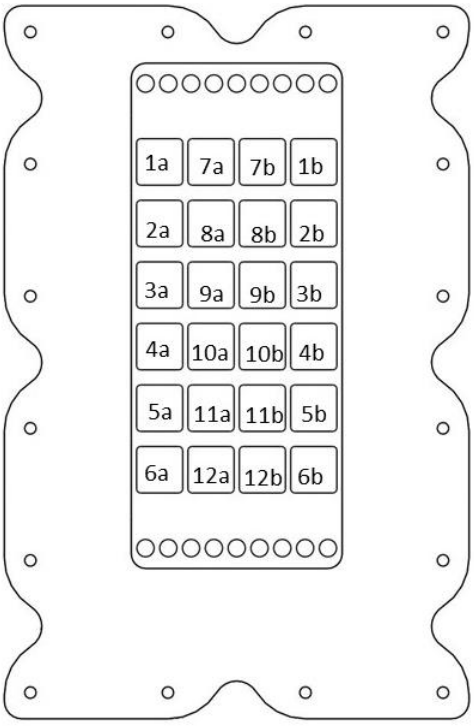
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions
Ramp 2.0 (mm)	0.5 (S)	0.0 (S)	0.5 (S)	0.0 (S)	600
Ramp 0.0 (mm)	None	1.0 (m)	None	50.0 (m)	1

6. Strain Patterns

There are two ways to use the silicone well plate: unmodified or modified. Used in the unmodified condition, the system is optimized for minimizing contamination of the test wells. Modifying the well plate by adding two longitudinal slits (shown in red on next page) creates a configuration that is optimized for strain uniformity in each of the 24 wells.

Unmodified Well Plate

The well plate deforms in such a way as to create a range of strain patterns amongst the 24 individual wells. There is a line of symmetry along the axis of stretch, so there are two instances each of 12 strain states captured by the well plate. These strain states vary in their degree of axial strain and also their degree of shear strain. The transverse strains are negligible (<10% of the axial strains.



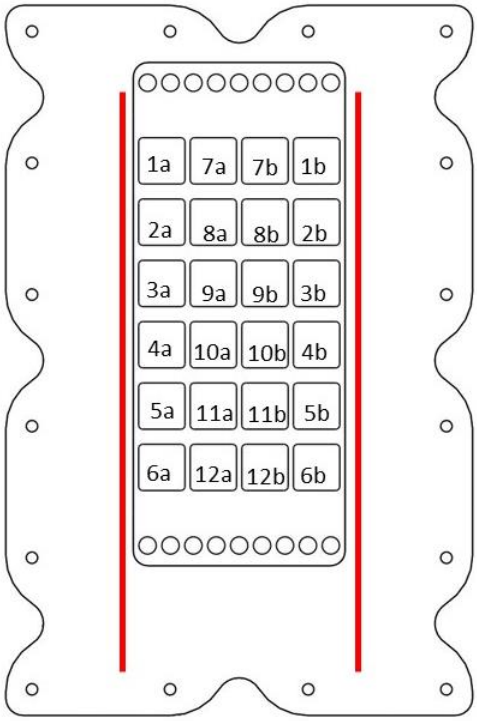
0.7129 0.2923	0.9712 0.1267	Symmetry with Left Side
0.7936 0.7841	1.0744 0.2781	
1.0034 1.2653	1.2177 0.4313	
1.3702 1.8057	1.5354 0.6099	
1.866 2.3452	1.9007 0.8730	
2.9632 2.3313	2.2217 0.5136	

% axial strain, % engineering shear strain per mm of actuator travel with an unmodified well plate.

Modified Well Plate

The well plate can be modified by slitting the specimen using the provided jig. When modified, the well plate deforms in such a way as to create relatively uniform axial strains amongst the 24 individual wells with negligible shear (engineering shear strains <10% of axial strains) and minimal transverse strains.

The average axial strain is 1.5% per mm of actuator travel



1.5462 -0.4173	1.4439 -0.2180	Symmetry with Left Side
1.5521 -0.4540	1.4908 -0.3009	
1.5835 -0.4660	1.4901 -0.3194	
1.6041 -0.4608	1.5137 -0.3401	
1.4850 -0.4327	1.4823 -0.2927	
1.3735 -0.4051	1.4021 -0.2653	

% axial strain, % transverse strain per mm of actuator travel with a modified well plate.

7. 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 rerun 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.

Load the Sterilized Well Plate in the Well Plate Carrier

The use of intermediate layers in the surface of the wells may be required to promote adhesion between the silicone and the cells.

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 optically clear bottom of the wells.

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

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

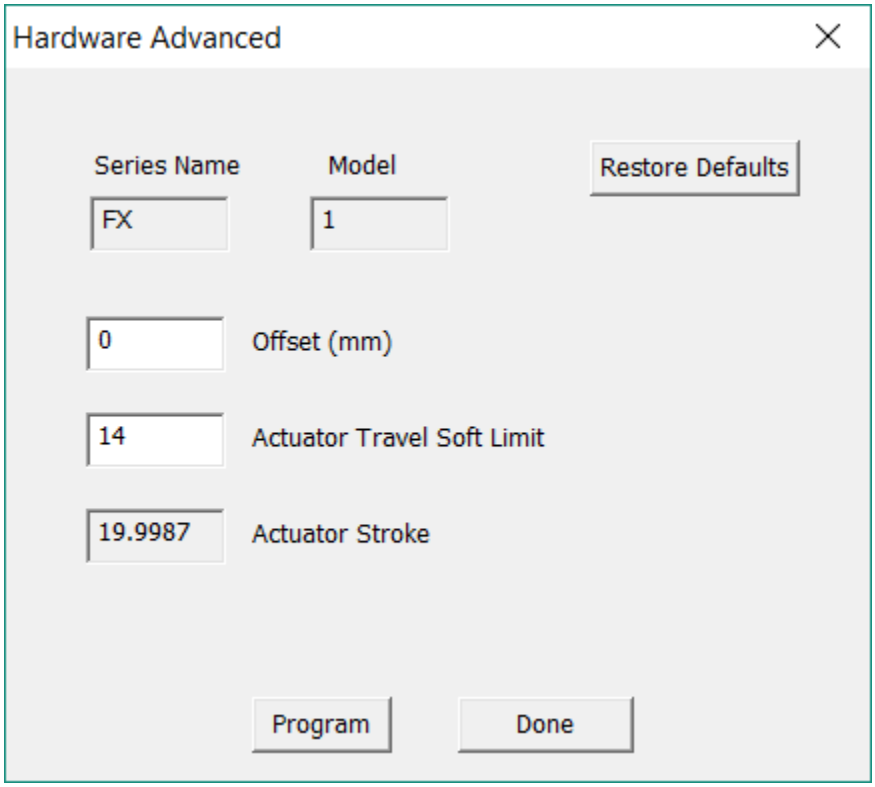
Load Specimen Well Plate and Carrier

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 the sequence is paused, **a screw on the side of the device must be tightened to lock the actuator shaft in place if you wish to remove power**. Once this has been accomplished, power can be removed and then restored without affecting the test protocol. Be sure to release the locking screw before restarting the test. To restart the test, press the button once more. To reset the device, press and hold the button for 5 seconds.

8. Advanced Parameters

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



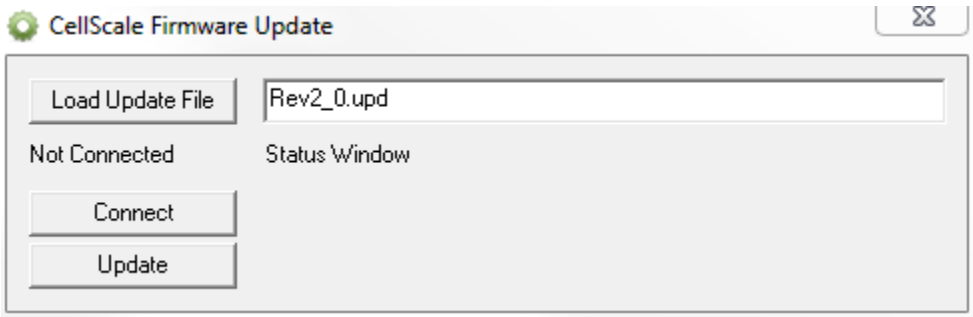
Offset: Offset is a setting that is not applicable to the MechanoCulture FX. The default value for offset is 0mm and this should not be altered.

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. 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 FX, this value is 15.875mm.

9. Appendix A: Updating the Firmware

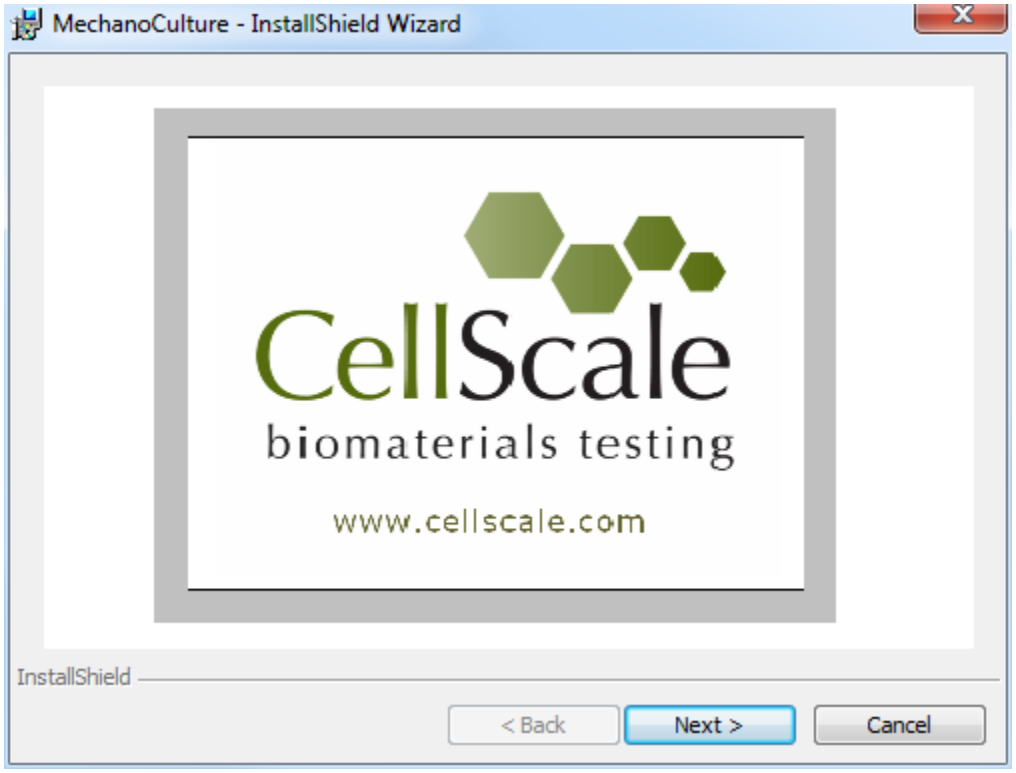
1. With the MechanoCulture connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities.
2. Load the firmware file using the “Load Update File” button.
3. Click the “Connect” button.
4. Execute the update using the “Update” button.

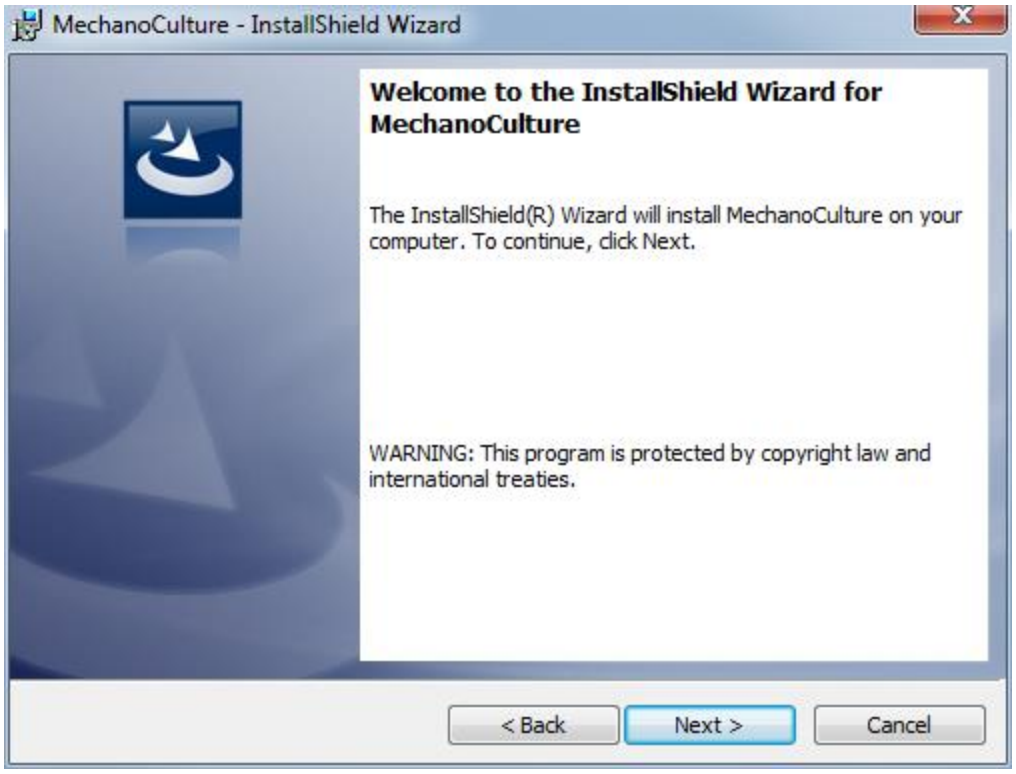


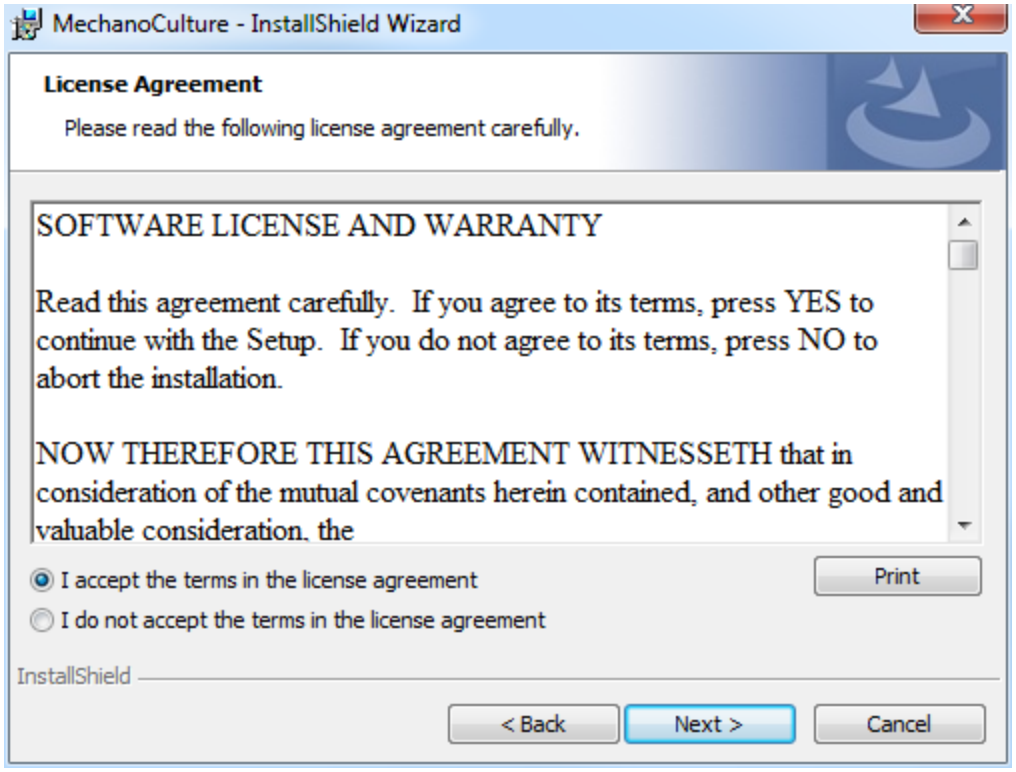
10. Appendix B: Software Installation

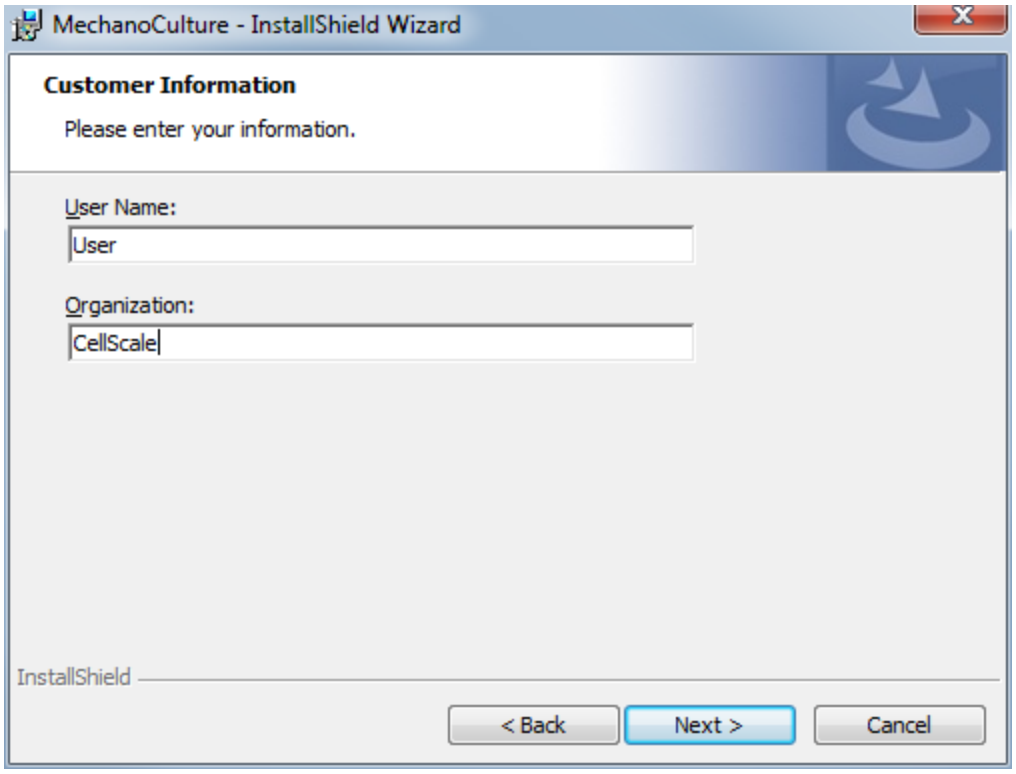
Included in your shipment is a link to install the MechanoCulture software. This installation package contains the installer for the software to program your MechanoCulture devices.

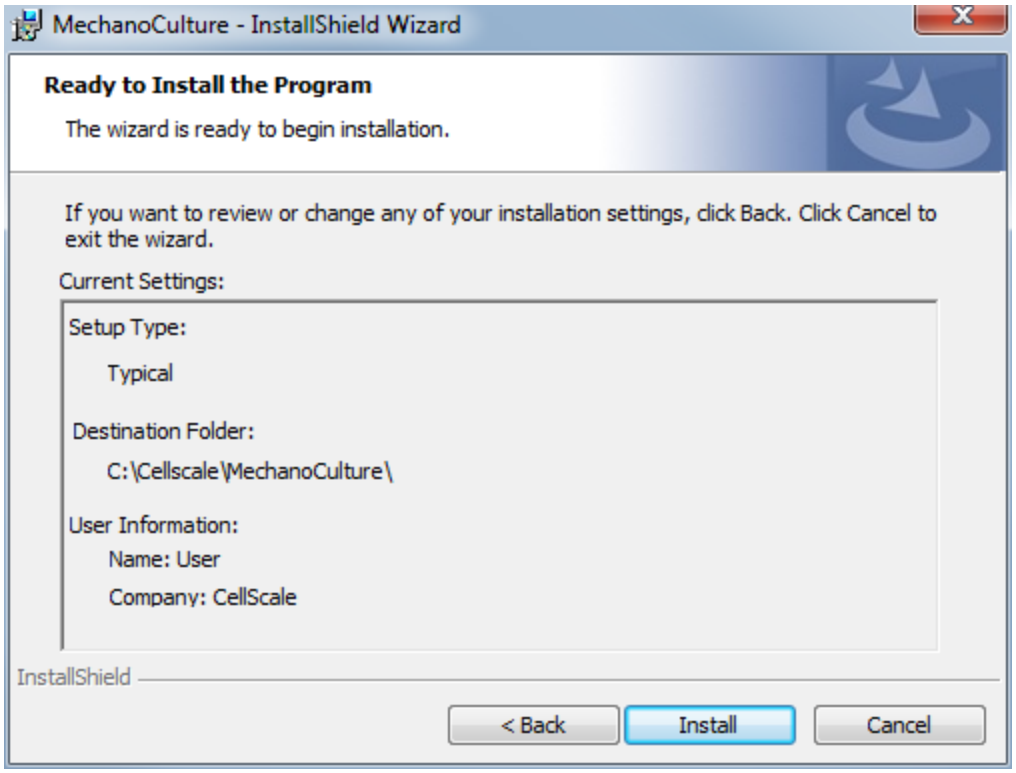
To install the software, go to the provided web address and download the compressed installation package. Transfer this file to the computer which will be programming the MechanoCulture. Right click on the file and select “Extract All…” to unzip. After this is complete, go to the unzipped installation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

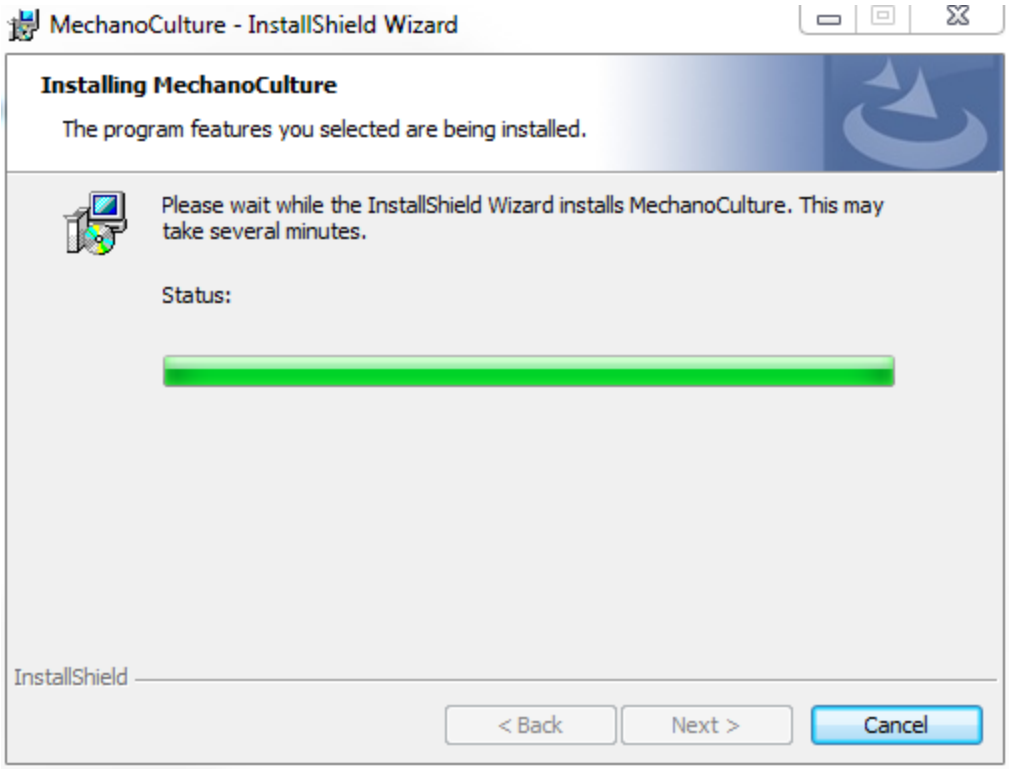


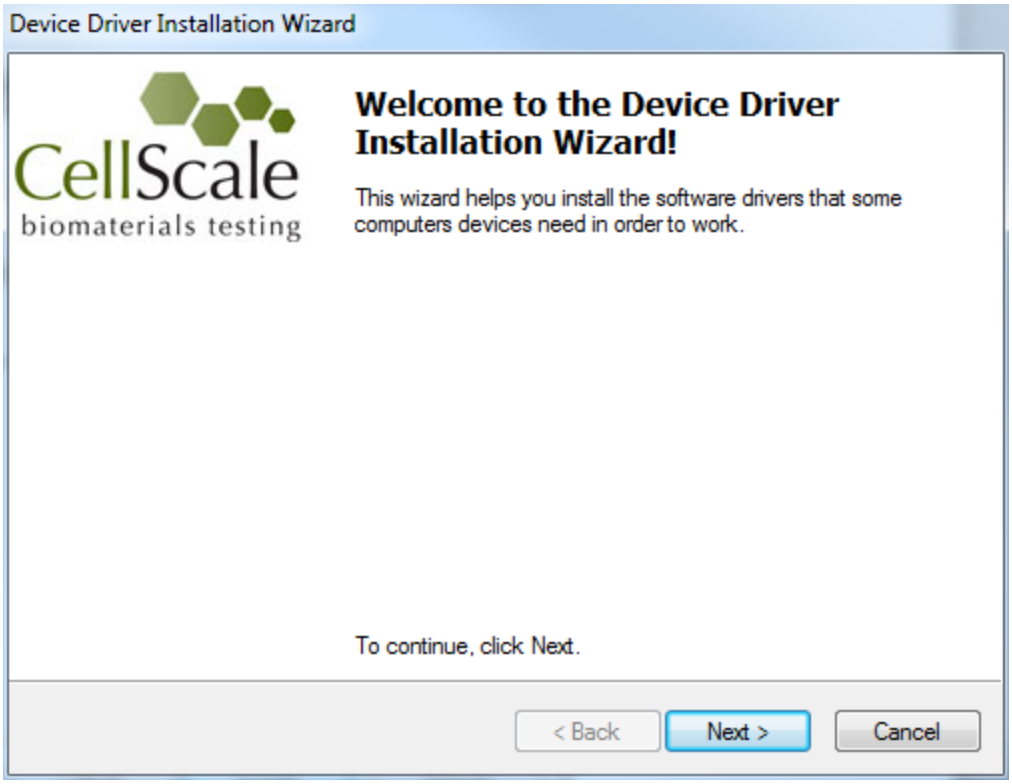

MechanoCulture - InstallShield Wizard
Welcome to the InstallShield Wizard for MechanoCulture
The InstallShield(R) Wizard will install MechanoCulture on your computer. To continue, click Next.
WARNING: This program is protected by copyright law and international treaties.
< Back
Next >
Cancel


MechanoCulture - InstallShield Wizard
License Agreement
Please read the following license agreement carefully.
SOFTWARE LICENSE AND WARRANTY
Read this agreement carefully. If you agree to its terms, press YES to continue with the Setup. If you do not agree to its terms, press NO to abort the installation.
NOW THEREFORE THIS AGREEMENT WITNESSETH that in consideration of the mutual covenants herein contained, and other good and valuable consideration, the
I accept the terms in the license agreement
I do not accept the terms in the license agreement
Print
InstallShield
< Back
Next >
Cancel


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


MechanoCulture - InstallShield Wizard
Installing MechanoCulture
The program features you selected are being installed.
Please wait while the InstallShield Wizard installs MechanoCulture. This may take several minutes.
Status:
InstallShield
< Back
Next >
Cancel


Device Driver Installation Wizard
CellScale
biomaterials testing
Welcome to the Device Driver Installation Wizard!
This wizard helps you install the software drivers that some computers devices need in order to work.
To continue, click Next.
< Back
Next >
Cancel