



MechanoCulture FX

Mechanical Stimulation System

User Manual
version 2.0



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 FX2 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 D 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 and 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 advance the actuator and reset the test sequence to the beginning.

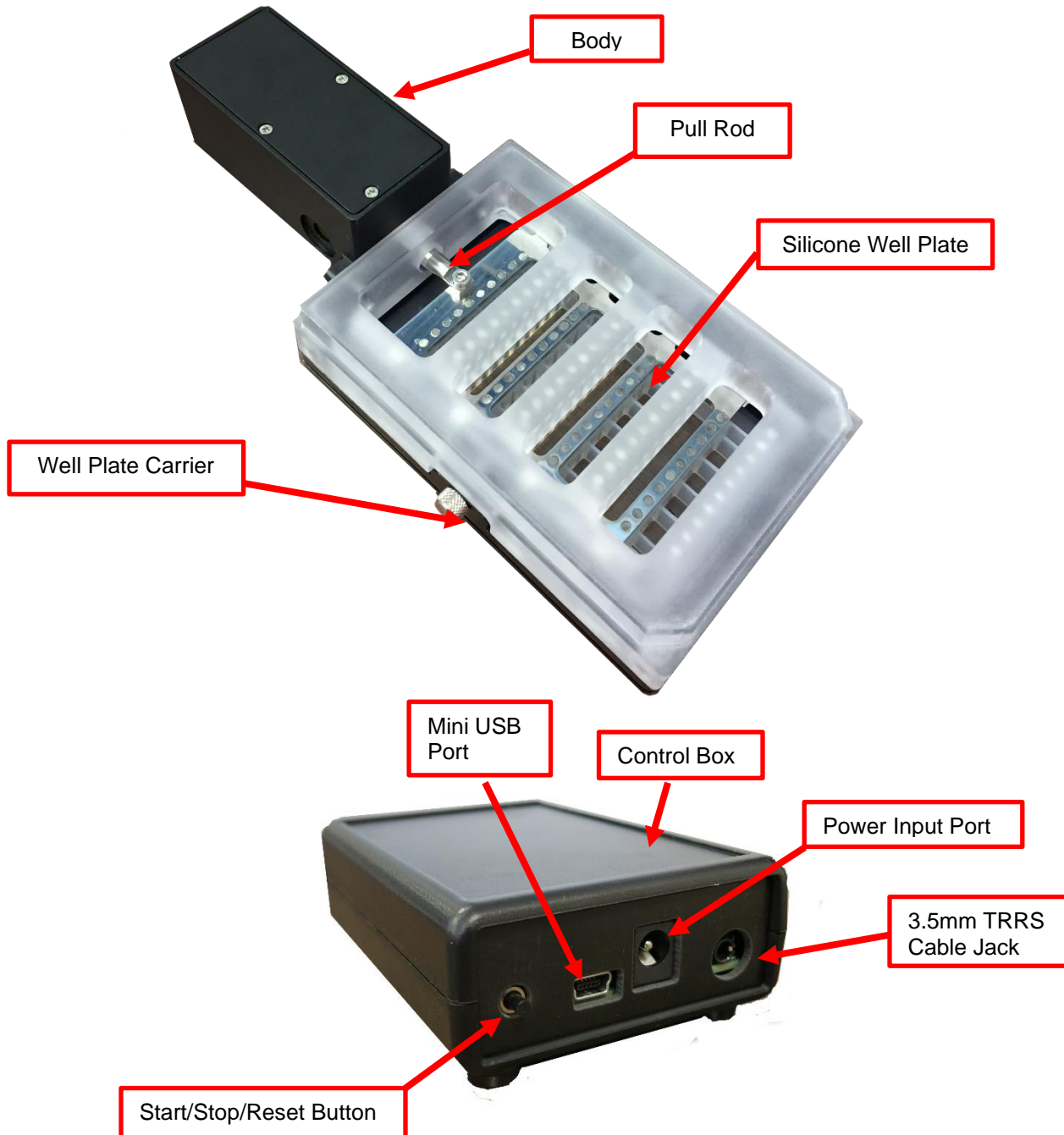
General Maintenance

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>Silicone Well Plate</p> <p>Material: Silicone Rubber</p> <p>Cleaning: Ethanol, UV, Autoclave, EtO</p>
	<p>Well Plate Carrier Assembly</p> <p>Material: Anodized Aluminum</p> <p>Cleaning: Ethanol</p>
	<p>Main Body</p> <p>Material: Polycarbonate, 316 Stainless Steel pins</p> <p>Cleaning: Ethanol</p>
	<p>Actuator Enclosure</p> <p>Material: Anodized Aluminum</p> <p>Cleaning: Ethanol</p>

	<p>Moving Pins</p> <p>Material: 316 Stainless Steel</p> <p>Cleaning: Ethanol, UV, Autoclave</p>
	<p>Polycarbonate Stand</p> <p>Material: Polycarbonate</p> <p>Cleaning: Ethanol</p>
	<p>Control Box</p> <p>Cleaning: None</p>
	<p>Power Supply</p> <p>Cleaning: None</p>



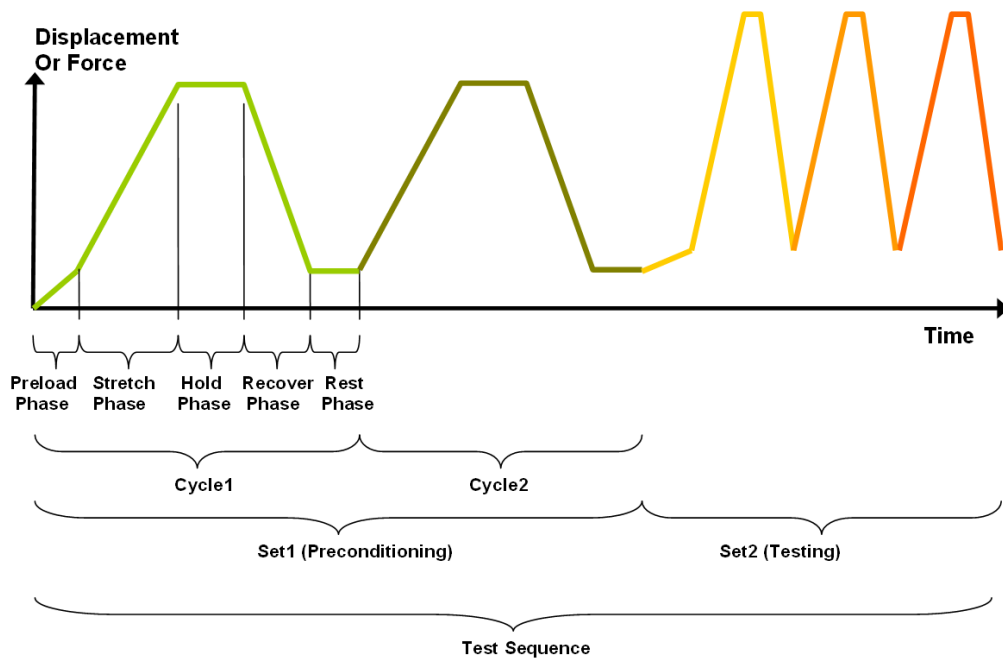
3. Testing Terminology

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

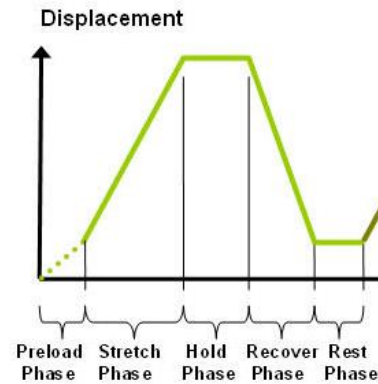
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

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

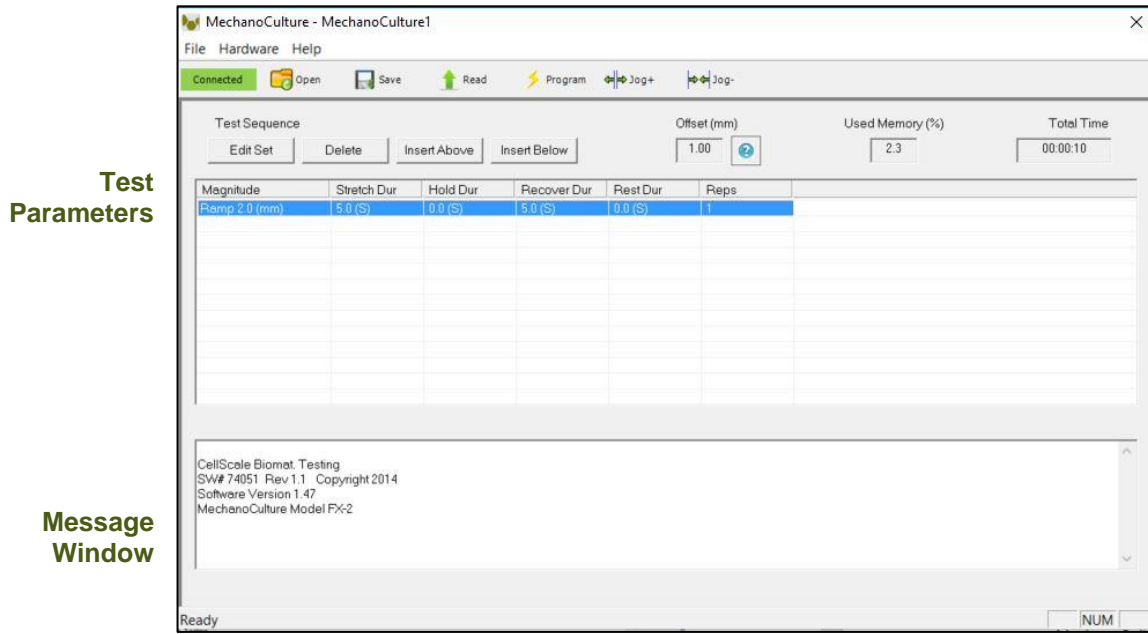
The MechanoCulture makes it possible to stimulate specimens under two control functions:

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:

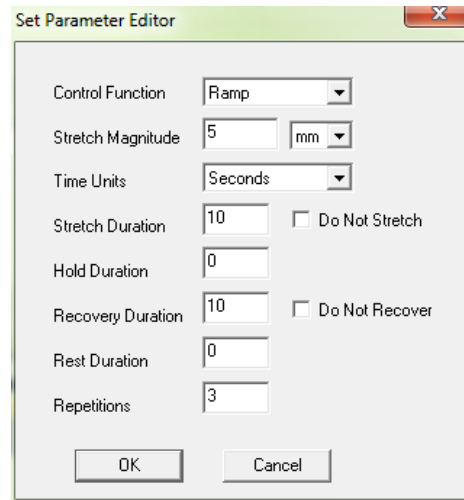
1. A predefined test sequence can be opened from a MechanoCulture Protocol File (*.tmt). From the File menu, select Open, or press the Open button on the toolbar.
2. A currently programmed test sequence on a device can be read. From the Hardware menu, select Read, or press the Read button on the toolbar.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



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

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

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.

MechanoCulture Tip: Axial Strain in Well Plate

When creating a test sequence, it is good to keep in mind that the axial strain in each of the 16 wells is 4.83 %/mm

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.

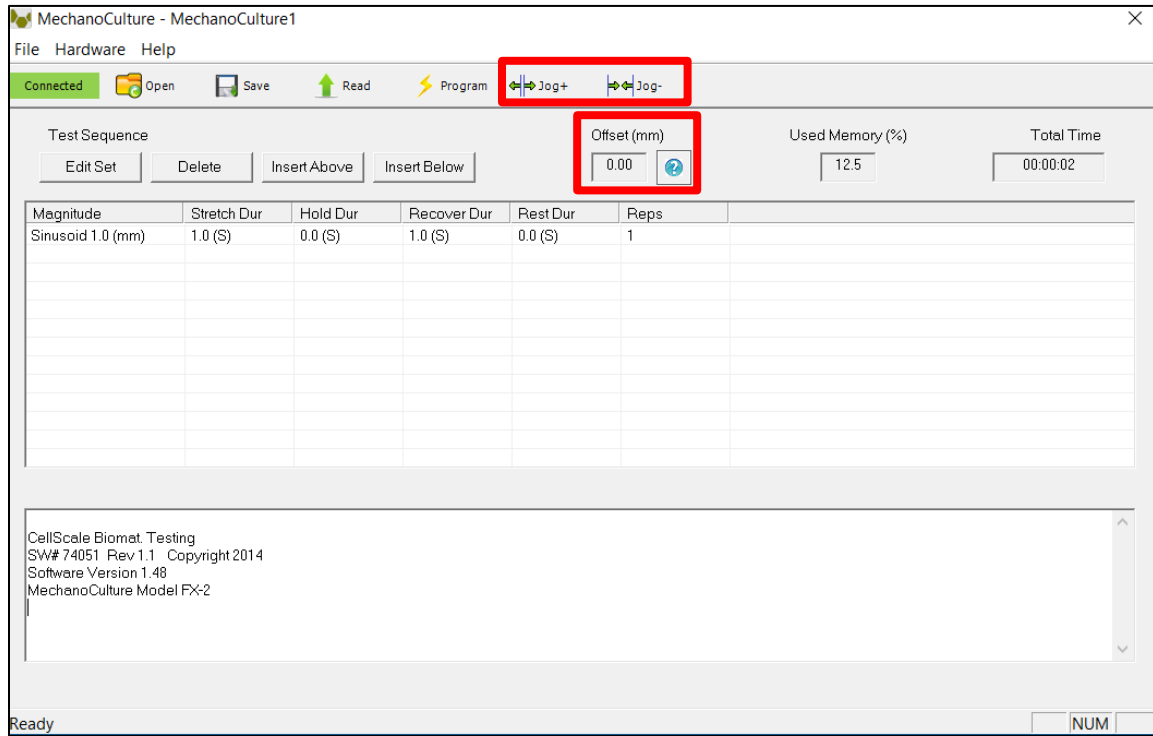
During the reset movements, the distance that the actuator retracts back from its fully extended position is called the offset. At the fully extended position, the system is designed such that the pins are aligned with the holes in the well plate. If the tests are started from this position, the bottom membranes in each well will start to sag during cyclic stretching due to relaxation of the silicone material. To stop this sagging from happening, it is necessary to apply an offset that pre-stretches the well plate prior to seeding the well plate with cells. The value of the offset should be nominally 0.6mm.

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 FX2 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: Maximum Actuator Travel

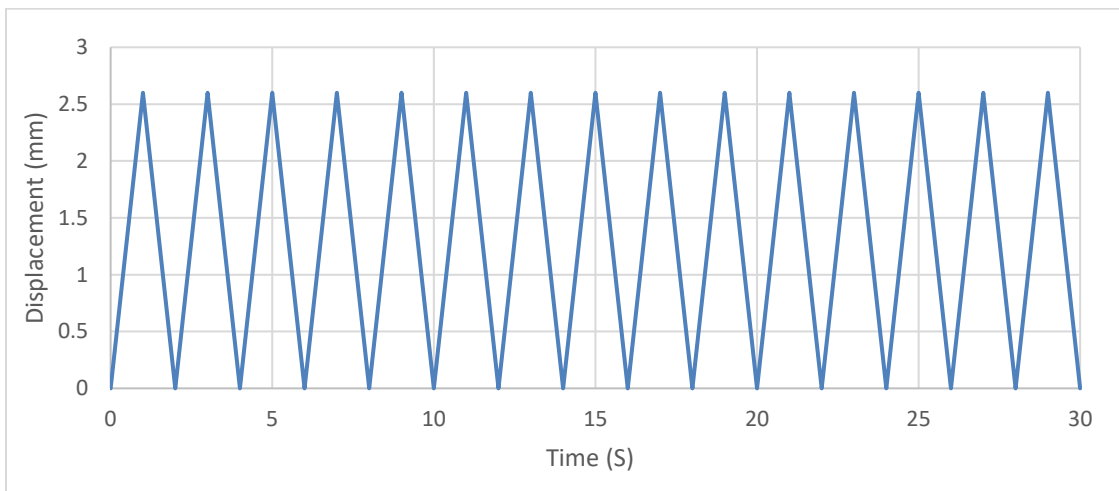
When developing a test sequence, it is good to keep in mind that the strain is 4.83 %/mm. The system is rated to a maximum strain of 9.5%, making the maximum displacement 2.0mm before the actuator may start to skip. It is recommended to keep your test displacement under 2.0mm.

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)
<div> <div>Edit Set</div> <div>Delete</div> <div>Insert Above</div> <div>Insert Below</div> </div>						<div>0.00</div> <div>?</div>
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.

Set Parameter Editor

Control Function: Ramp

Stretch Magnitude: 0.5 (mm)

Time Units: Minutes

Stretch Duration: 1

Hold Duration: 0

Recovery Duration: 0

Rest Duration: 0

Repetitions: 1

☐ Do Not Stretch

☒ Do Not Recover

OK Cancel

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).

Set Parameter Editor

Control Function: Ramp

Stretch Magnitude: 2 (mm)

Time Units: Minutes

Stretch Duration: 5

Hold Duration: 1

Recovery Duration: 4

Rest Duration: 0

Repetitions: 100

☐ Do Not Stretch

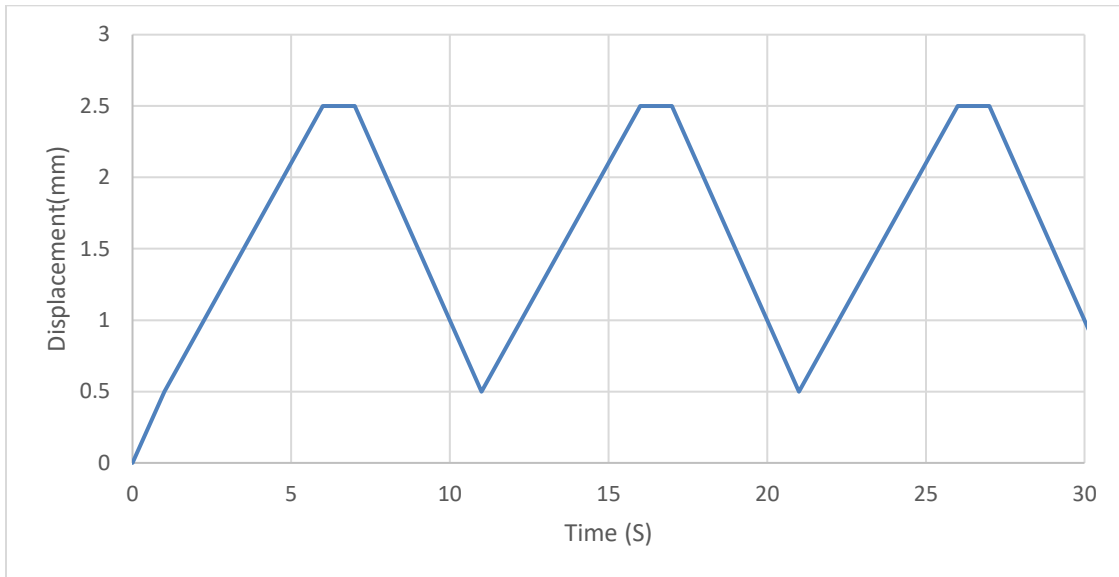
☐ Do Not Recover

OK Cancel

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)
<div> Edit Set Delete Insert Above Insert Below </div>						0.00 ?
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.

The 'Set Parameter Editor' dialog box shows 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 shows 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) <div>0.00 ?</div>	
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.

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.

Attach the Sterilized Well Plate/Well Plate Carrier to the test Device

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

Note: Axial strain across all 16 wells is 4.83%/mm

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

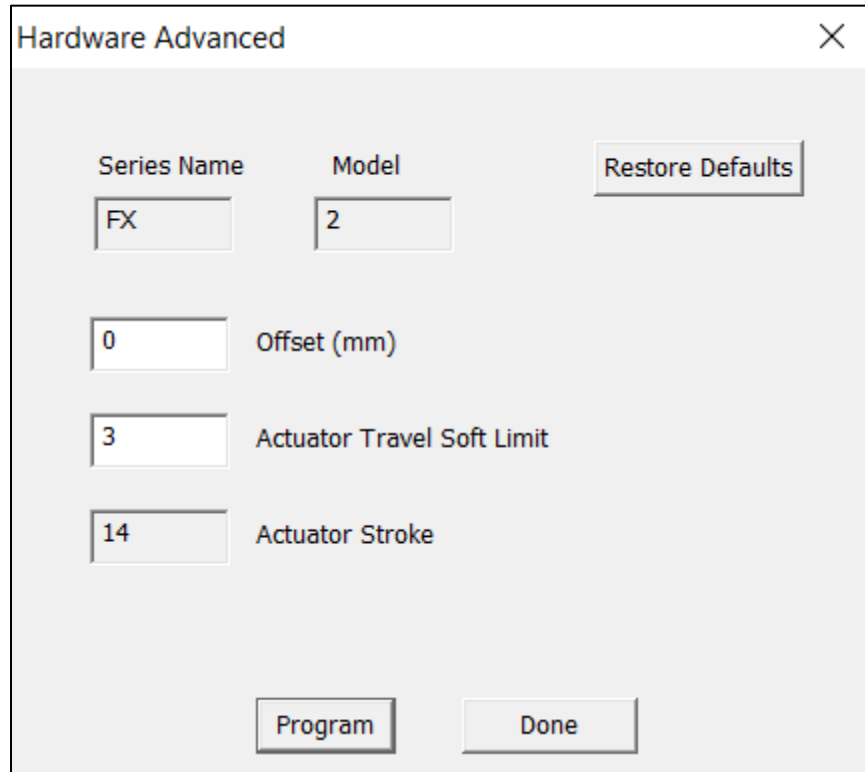
Be sure to set the well plate/carrier assembly on a clean surface so as not to smudge the exterior of the optically clear bottom of the wells.

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:



The screenshot shows a window titled "Hardware Advanced" with a close button (X) in the top right corner. Inside the window, there are several input fields and buttons. At the top, there are two input fields: "Series Name" with the value "FX" and "Model" with the value "2". To the right of these fields is a button labeled "Restore Defaults". Below these are three more input fields, each with a label to its right: "Offset (mm)" with the value "0", "Actuator Travel Soft Limit" with the value "3", and "Actuator Stroke" with the value "14". At the bottom of the window, there are two buttons: "Program" and "Done".

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 a small amount, defined by the offset.

Actuator Travel Soft Limit: The FX2 actuator has 14mm of travel. The soft limit should be kept at a much lower value. By default it is set to 5mm, but since the system has an axial strain of 4.83%/mm it is suggested that its soft limit be set to under 2mm to prevent the actuator from slipping.

Actuator Stroke: This is the physical travel capability of the stepper motor to drive the lead screw. For the FX2, this value is 14mm and is programmed at the factory.

8. Appendix A: Strain Analysis Summary

The FX2 well plate was designed to deform in such a way that provides uniform strain across all 16 individual wells. Using our strain tracking software the average value of axial strain was found to be 4.83 %/mm. The transverse and shear strains were also found to be negligible and were equal to less than 10% of the axial strain.

In all 16 wells between 0 and 2mm of actuator travel:

- The **axial** strain is 4.83 %/mm
- The **transverse** strain is less than 10% of the axial strain.
- The **shear** strain is less than 10% of the axial strain.
- The strains are uniform and show no spatial patterns within the well.

Method

A 16-well plate was dusted with graphite powder and loaded into a standard MCFX instrument. The system was programmed to execute a series of 0.2mm moves with 10 second holds until a total travel of 2mm was achieved. During each hold period, images of a single well were collected for analysis. This process was repeated 3 times for each well.

The strains within each well were analyzed by CellScale's proprietary digital image correlation software. A 9 X 9 virtual grid was overlaid on the unstretched image of each well. These grids were tracked across all 10 images from 0 to 2mm actuator displacement to determine the axial, transverse, and shear strains.

Results

As an example, unstretched and stretched images from a typical well are shown below. Further below is a graph of the average strain values measured for this well between 0 and 2mm actuator displacement.

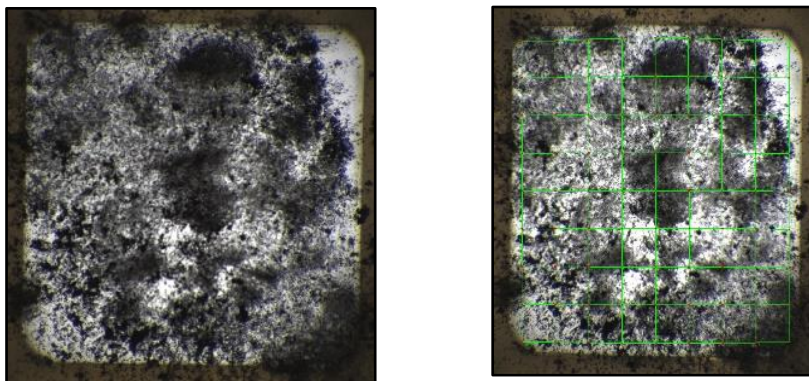
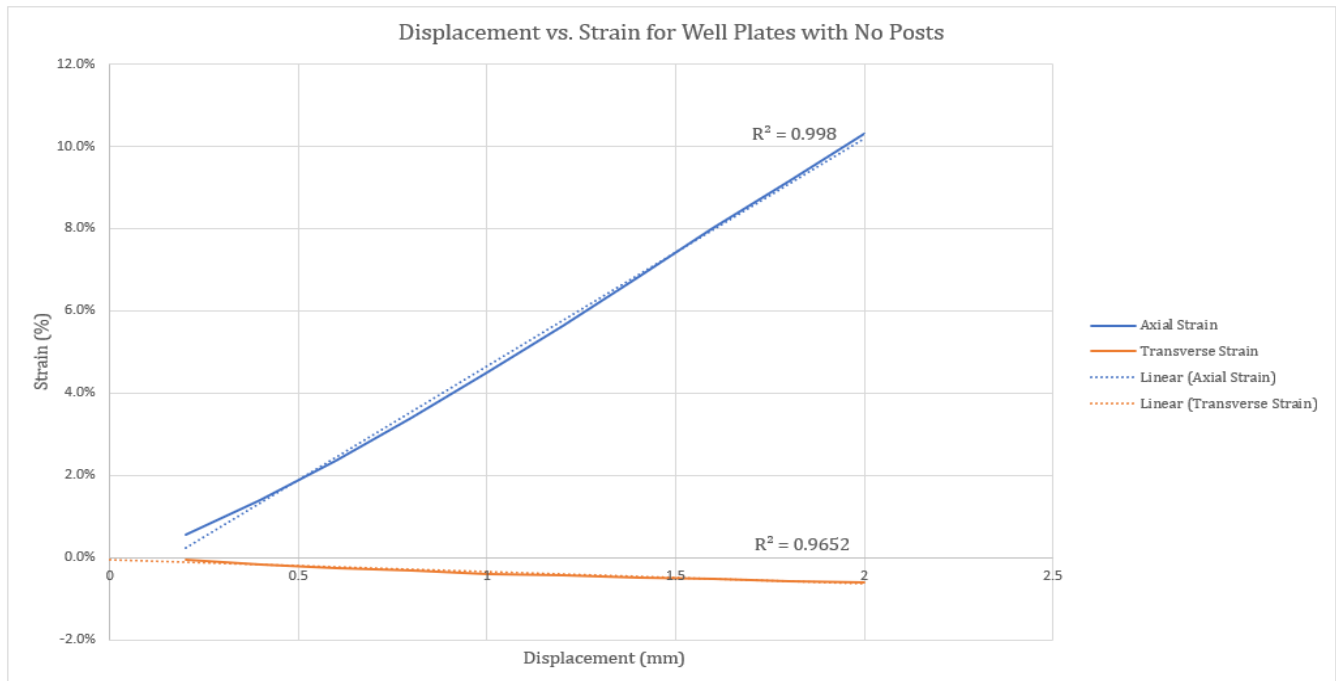


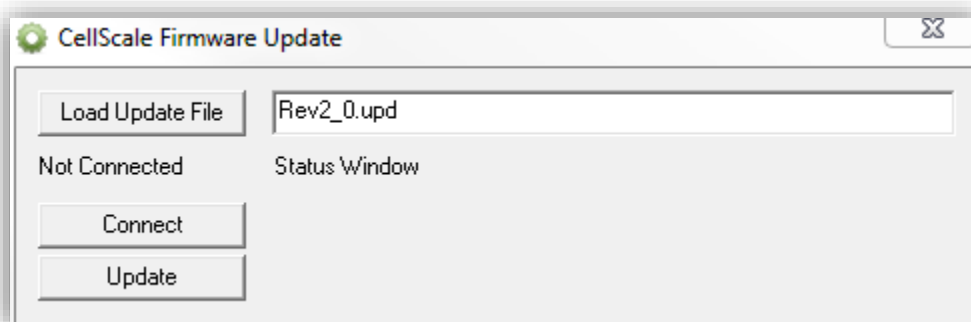
Figure 1: Unstretched well (L) and stretched well with 9 X 9 grid overlay (R)



Graph 1: Axial And Transverse Strain as a function of Axial Displacement

9. Appendix B: Updating the Firmware

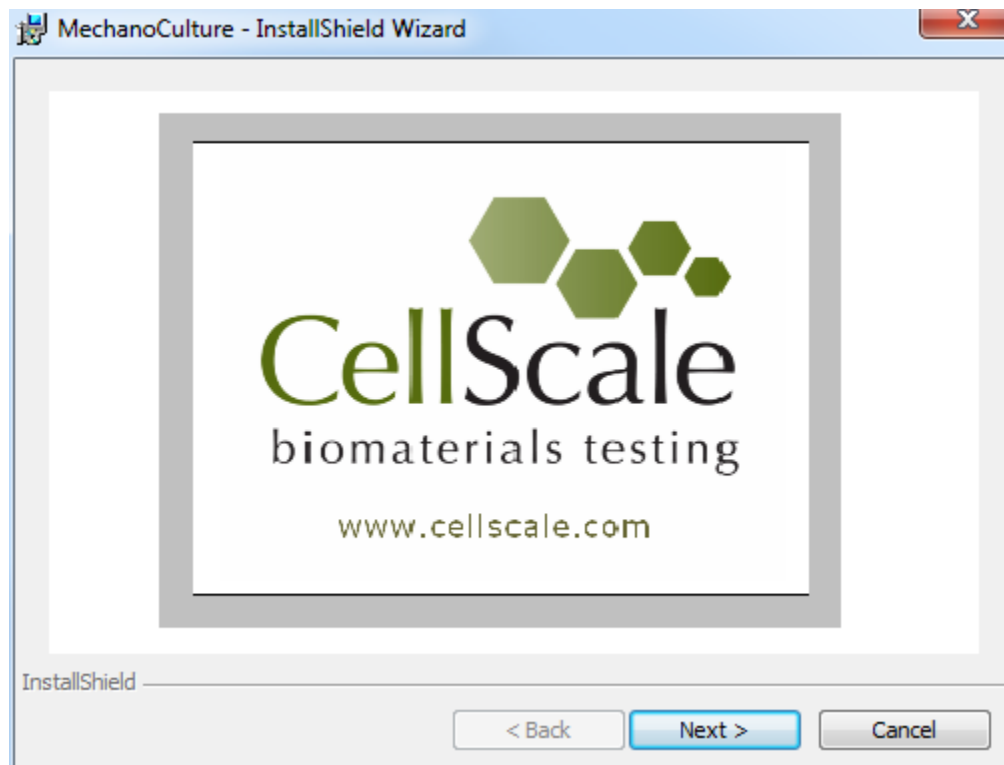
1. With the MechanoCulture connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities.
2. Load the firmware file using the “Load Update File” button.
3. Click the “Connect” button.
4. Execute the update using the “Update” button.

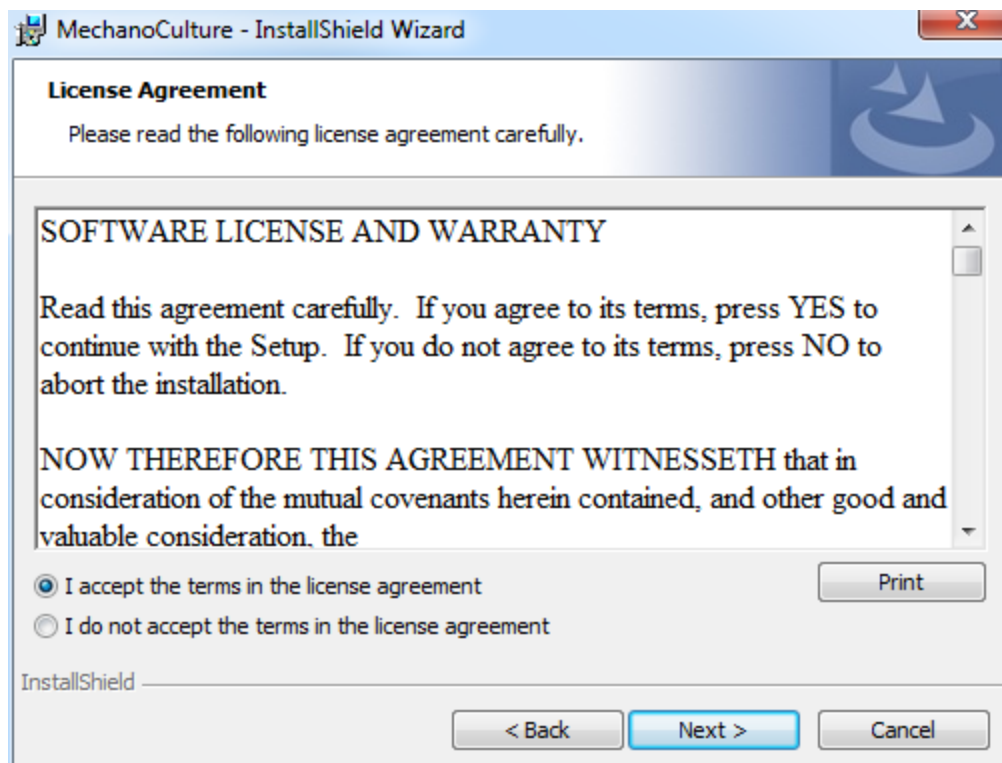
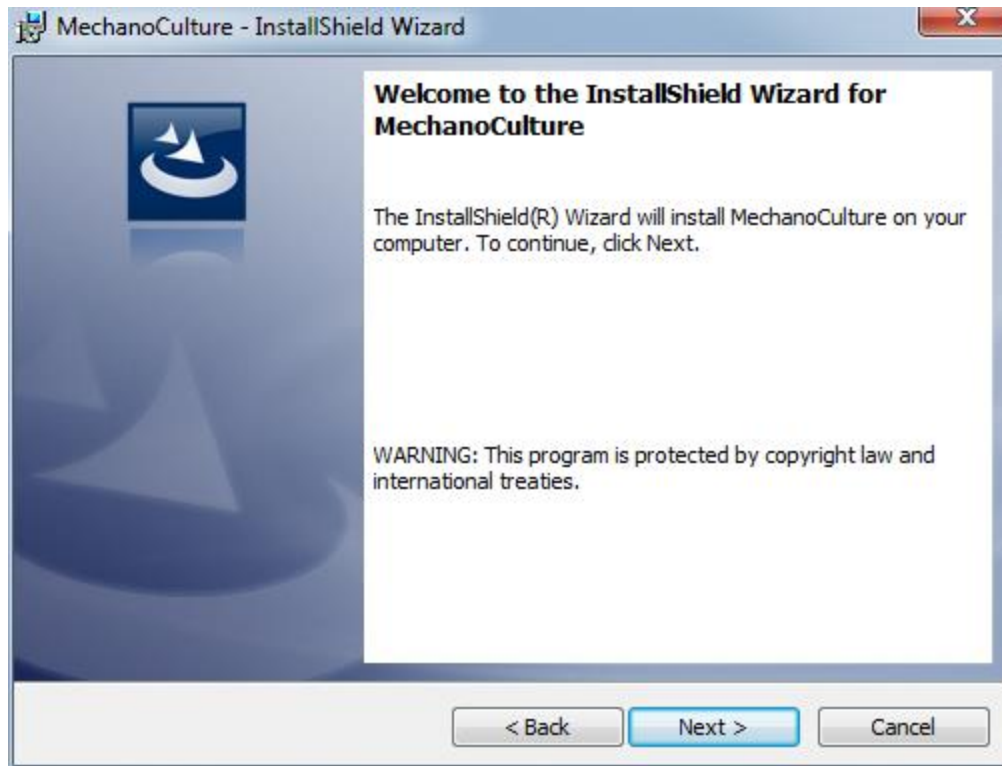


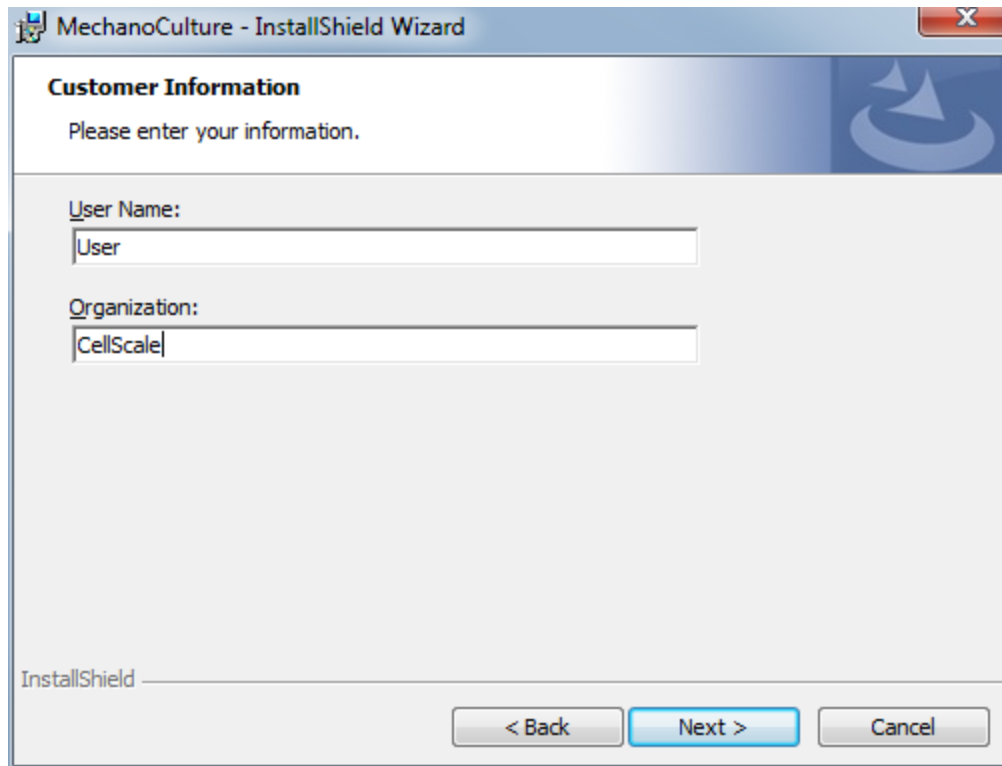
10. Appendix C: Software Installation

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.







MechanoCulture - InstallShield Wizard

Customer Information

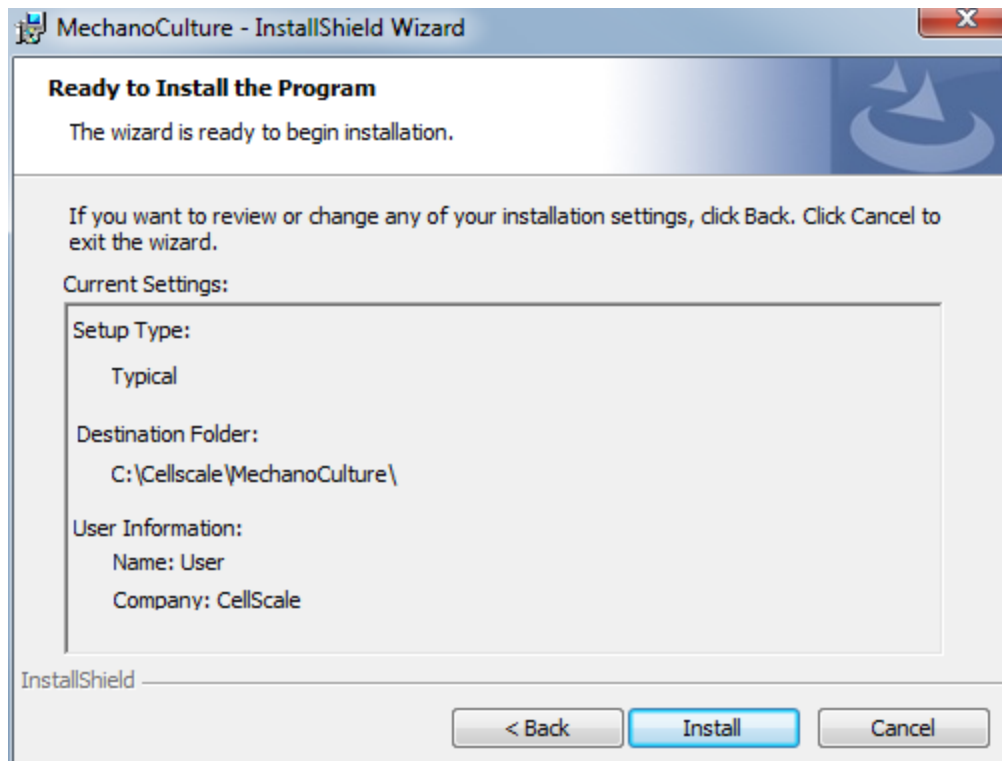
Please enter your information.

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:

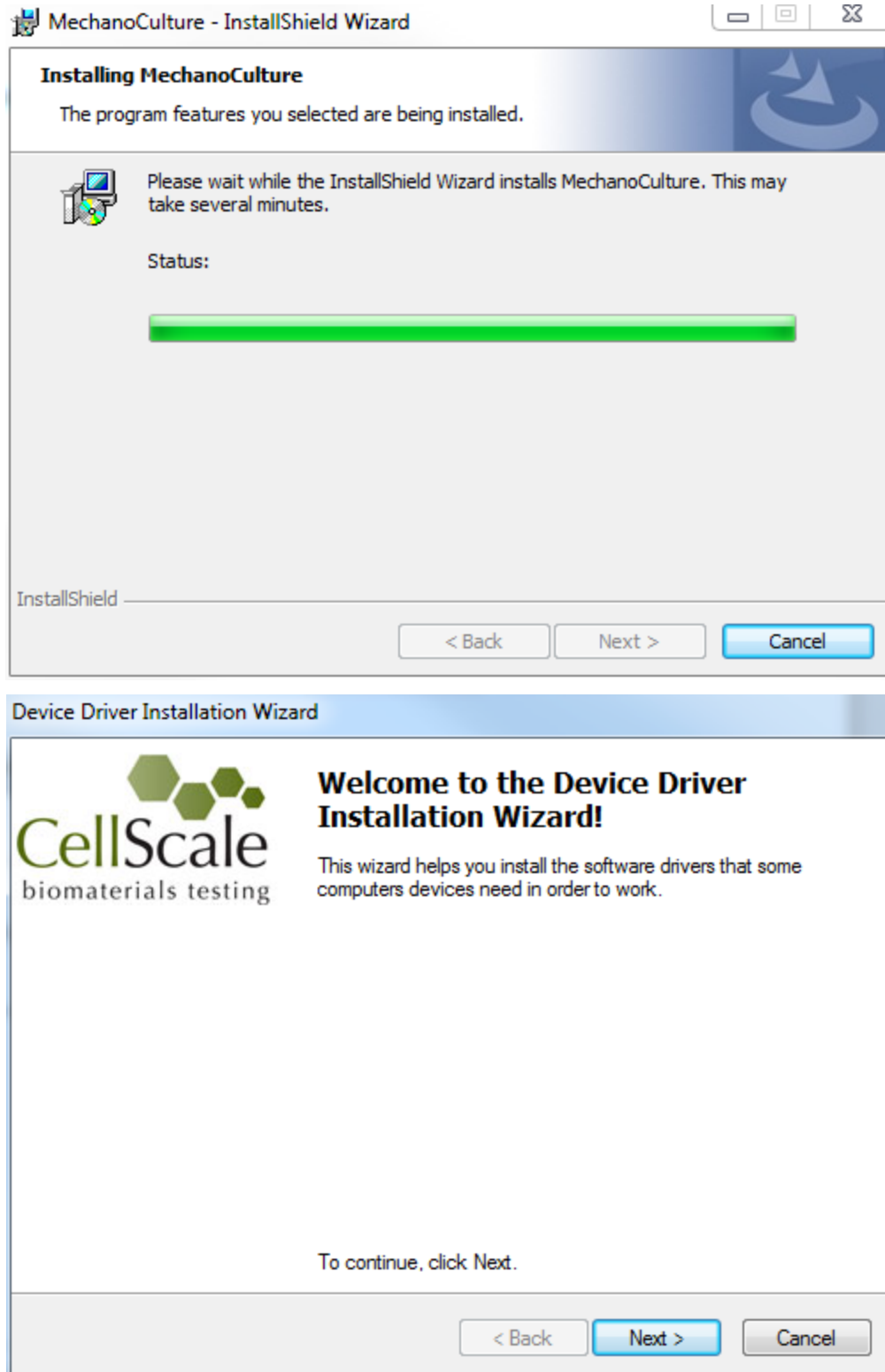
Setup Type:
Typical

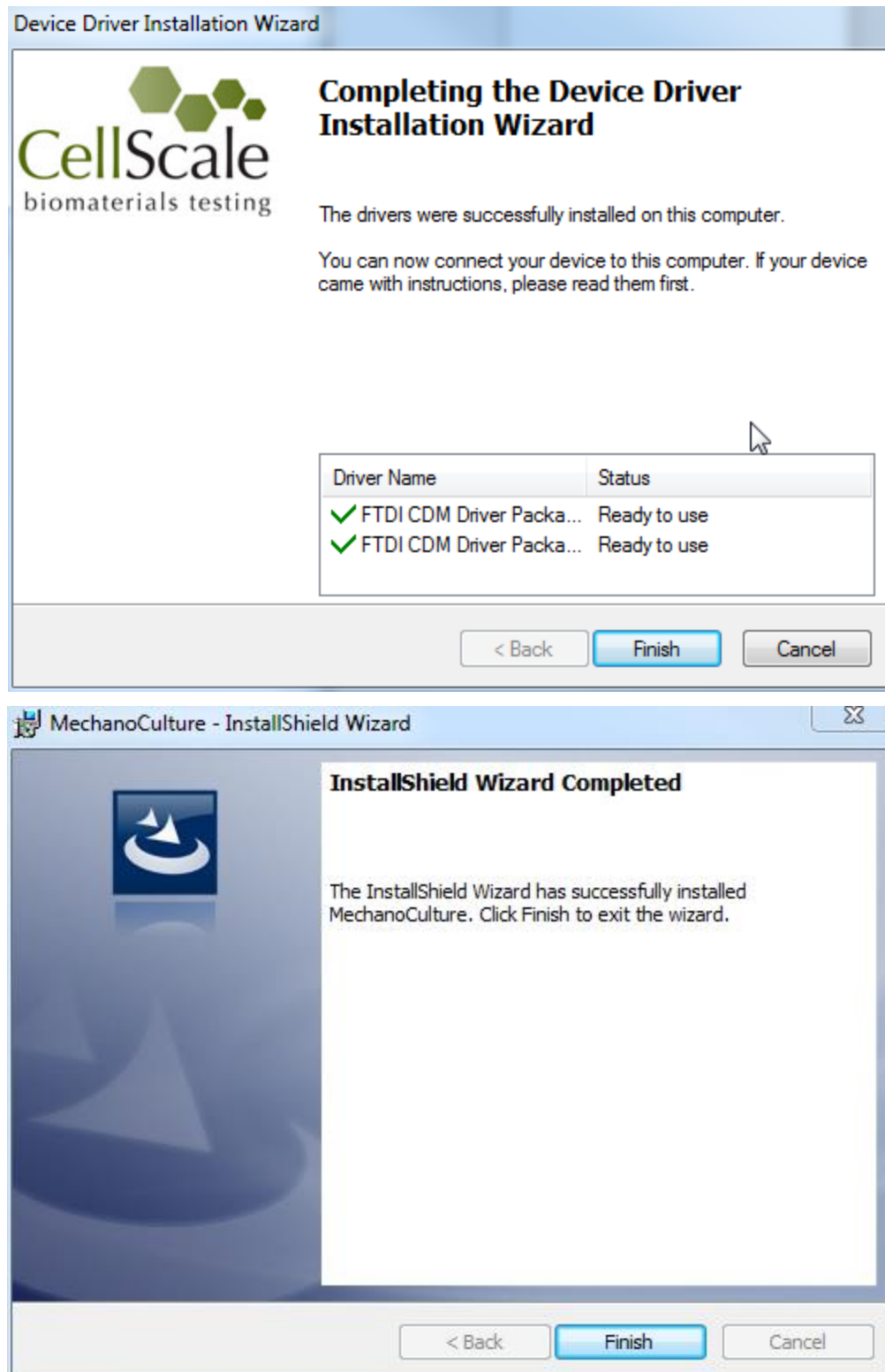
Destination Folder:
C:\Cellscale\MechanoCulture\

User Information:
Name: User
Company: CellScale

InstallShield

< Back Install Cancel

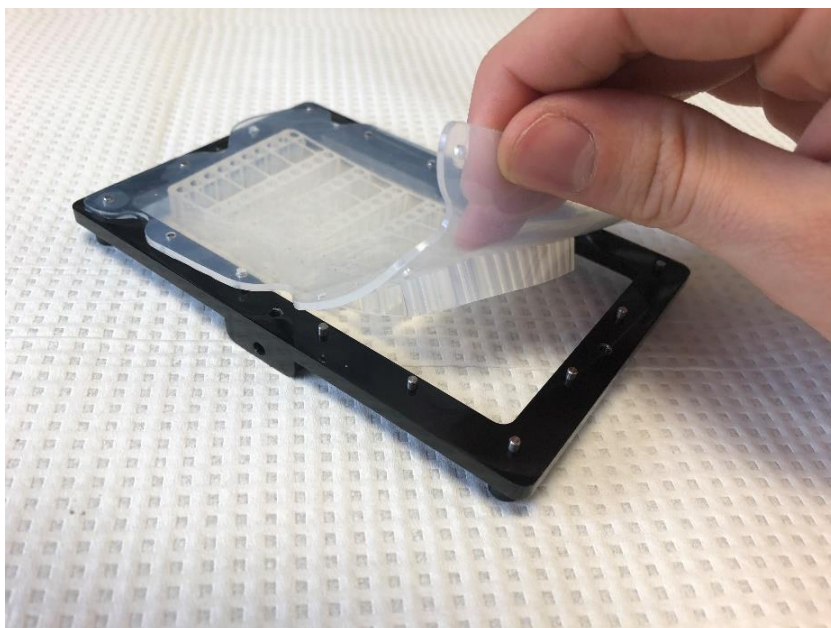




11. Appendix D: System Assembly

Follow these steps to assemble your MechanoCulture FX system for use.

1. Place the top plate of the well plate carrier assembly such that the metal pegs are facing upwards. Place the silicone well plate on the pegs such that the wells are facing downwards.



2. Attach the bottom of the well plate carrier assembly to secure the well plate in place. There are two options available to do this.

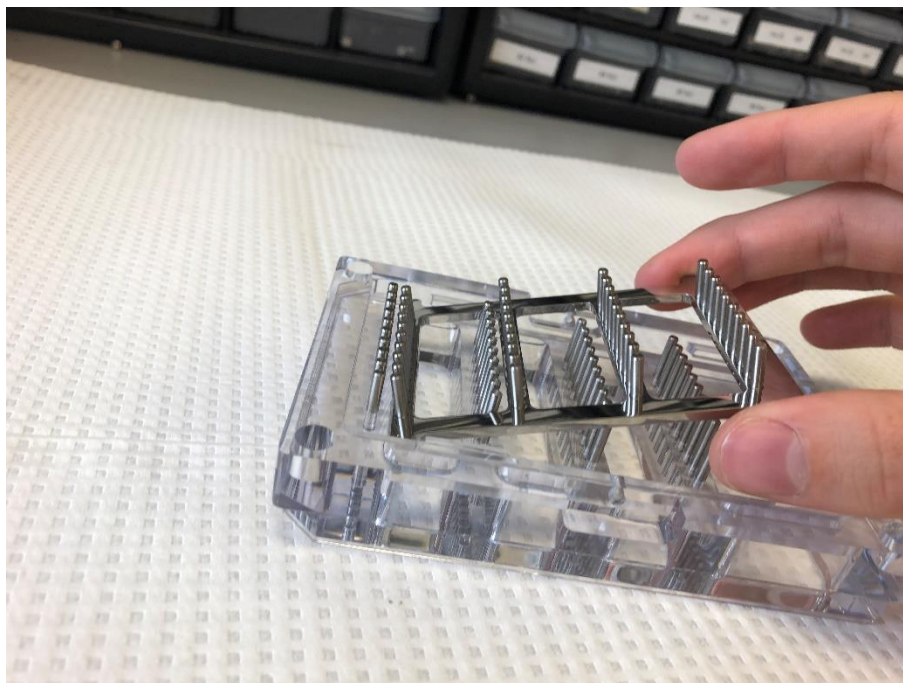
OPTION 1: Use the black manual thumb screws to secure the well plate carrier assembly.



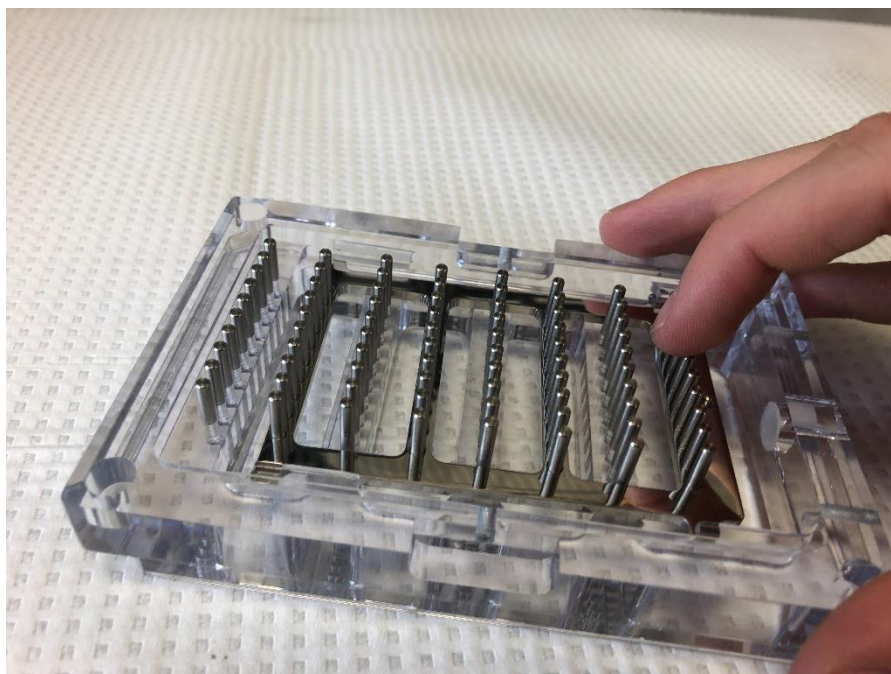
OPTION 2: Secure the well plate carrier assembly with the provided M3 flat-head screws. Using these fasteners reduces the working distance to the well bottoms when placing the MechanoCulture FX on an inverted microscope.



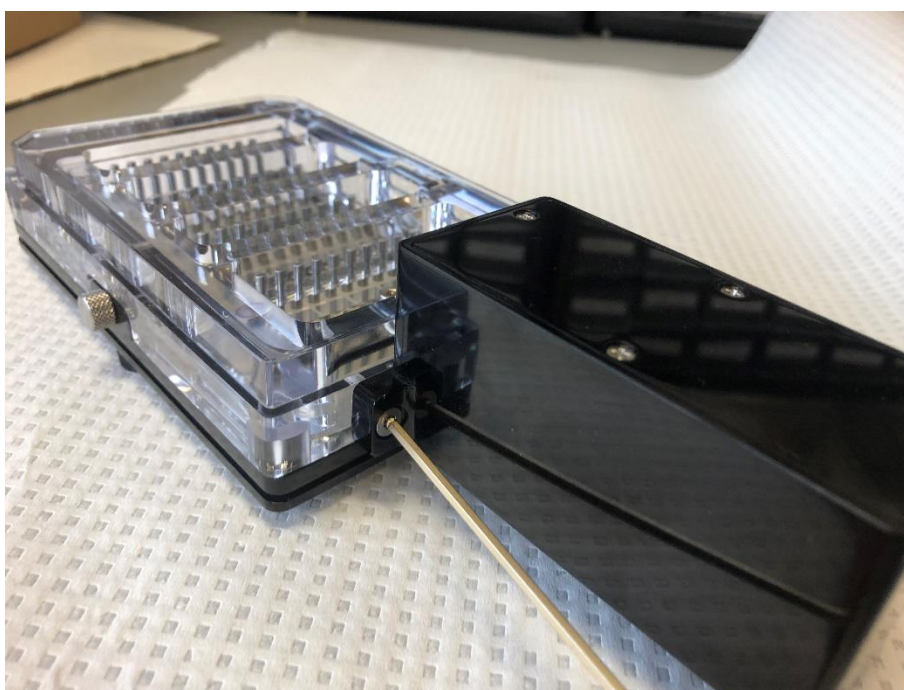
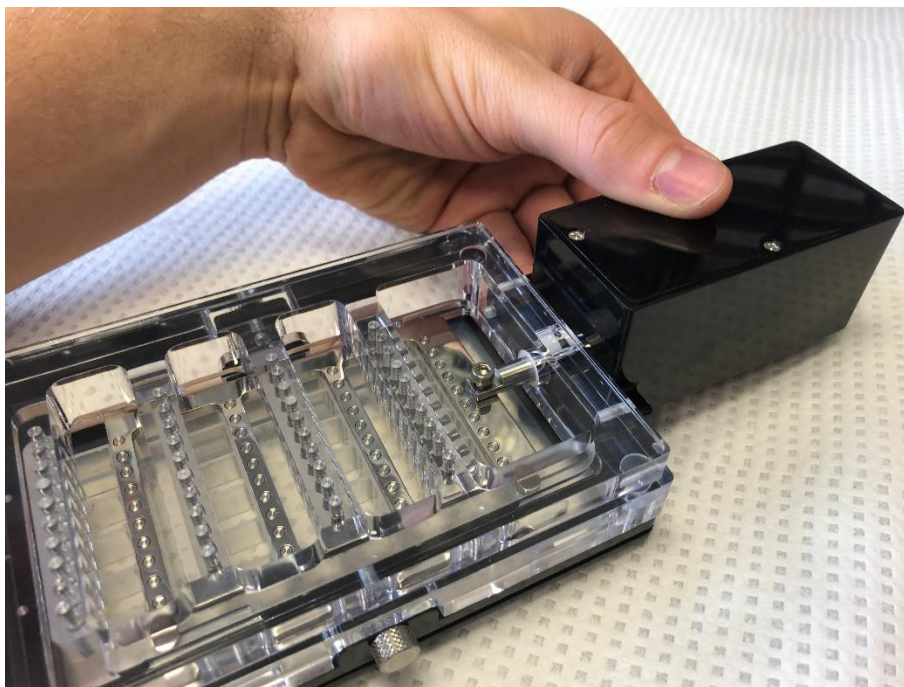
3. Place the moving pins in the polycarbonate main body in the provided slots.



4. Slide the moving pins such that there is roughly even spacing between each row of pins.



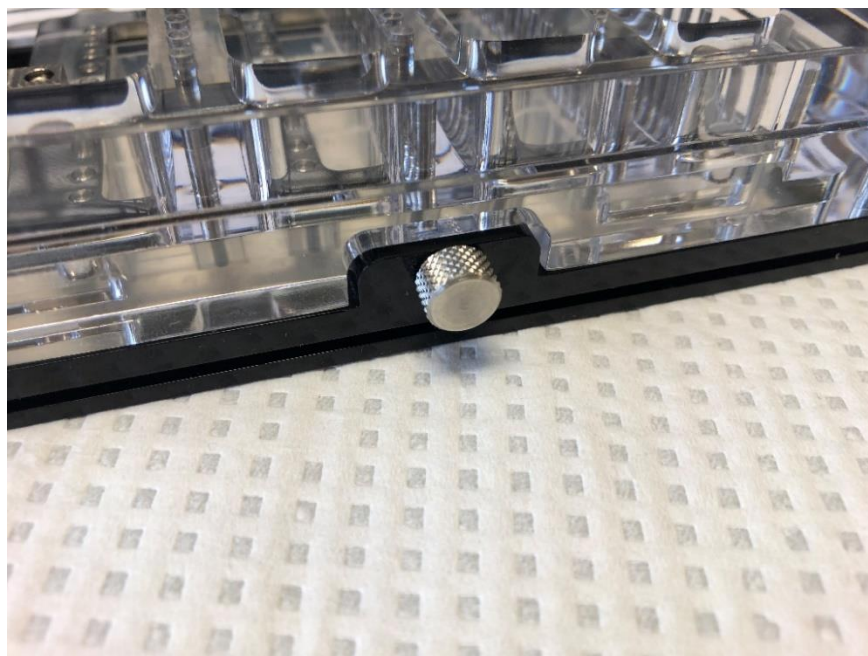
5. Slide the actuator arm through the hole in the main body. Align the actuator enclosure and secure it to the main body with the provided M3 flathead screws. Secure the actuator arm to the moving pins with the provided M3 screw.



6. Power on the controller and reset the actuator by pressing and holding the start/stop/reset button for 5 seconds. This will extend the actuator arm fully thus evenly spacing out the rows of pins.
7. Align the well plate carrier assembly on the main body and slide the well plate holes onto the pins. Make sure each pin is well seated in the well plate.



8. Fasten the two steel thumb screws on either side, securing the well plate carrier assembly to the main body.



9. The assembled system can be set in the polycarbonate stand for stability.

