CellScale
biomaterials testing

MechanoCulture TX

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.

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 TX is a precision instrument specifically designed for compression testing biomaterials in a sterile fluid environment. This device is capable of uniaxial deformations on flexible cell-seeded substrates or scaffolds. It has a control board to enable it to run independently of a PC in an environment-controlled incubator.

Environmental and Electrical Specifications

Parameter	Specification
Electrical Input	24 VDC
Current Rating	2.71 A
Environmental Conditions	Maximum Operating Temperature 40 ˚C 0 – 100% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree 2
Data Connections	USB-B for programming, Micro SD for data storage

System Assembly

The MechanoCulture TX unit requires initial assembly and disassembly/reassembly between uses. See the Device Assembly and Loading Specimens section for detailed instructions.

Connections to Supply

Connect the power supply to a 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 top cover in place.

General Maintenance

The main device does not require maintenance other than cleaning. Please see the components overview section for cleaning and sterilization/disinfection details. The peristaltic pump requires you to change the section of tubing inside the pump head after approximately 50hrs of accumulated operation, how to do this can be found in Appendix B.

2. MechanoCulture TX Components

The MechanoCulture TX consists of a cell culture assembly and a control box housing the actuators and force measuring load cells. As shown in the following table, the cell culture assembly consists of a well plate, O-ring, and membrane. The control box contains the master controller, the linear actuators, load cells and plungers.

Component	Description
Well Plate	The well plate contains 6 wells. The well plate is made from polycarbonate and can be sterilized using an autoclave.
1 Large O-Ring	Placed in the grove surrounding all the wells to completely seal the sterile culture area from the plungers that apply the forces to the specimens.
Plungers	A plunger is attached to the end of each load cell and is used to compress the specimens in the wells. These are made from Delrin.
Flexible Membrane	Covers and seals the culture area from plungers. When the force is applied, the membrane will deform and compress the specimens.
Control Box	The top control box houses all of the linear actuators which apply the force and the load cells which measure the force applied as well as the electronics to control these items independent of a computer. The control box has inputs for the power supply and USB to program and read data.
M5/M6 Socket Nuts	Used to secure the Control Box to the Well Plate
Peristaltic Pump	This system is liquid cooled using a reservoir of approximately 10L of room temperature water and the peristaltic pump. The tubing in this pump must be changed after every 50 hours of accumulated use to prevent hose failure and leaks. Detailed instructions on how to change the tubing can be found in Appendix B.

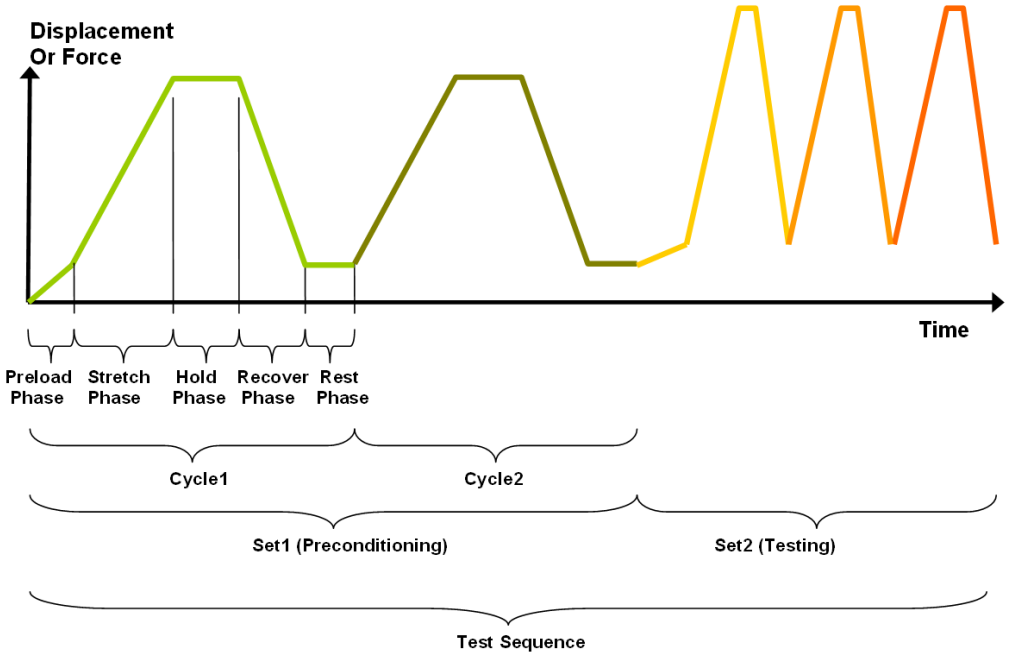
3. Testing Terminology

Multiphase Test Cycles

To properly mechanically stimulate/signal cells, it is often necessary to apply more than one load magnitude or frequency during 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

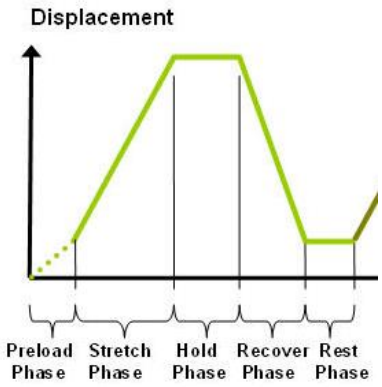
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

Compressing - During the Compress 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 compress phase is removed.

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

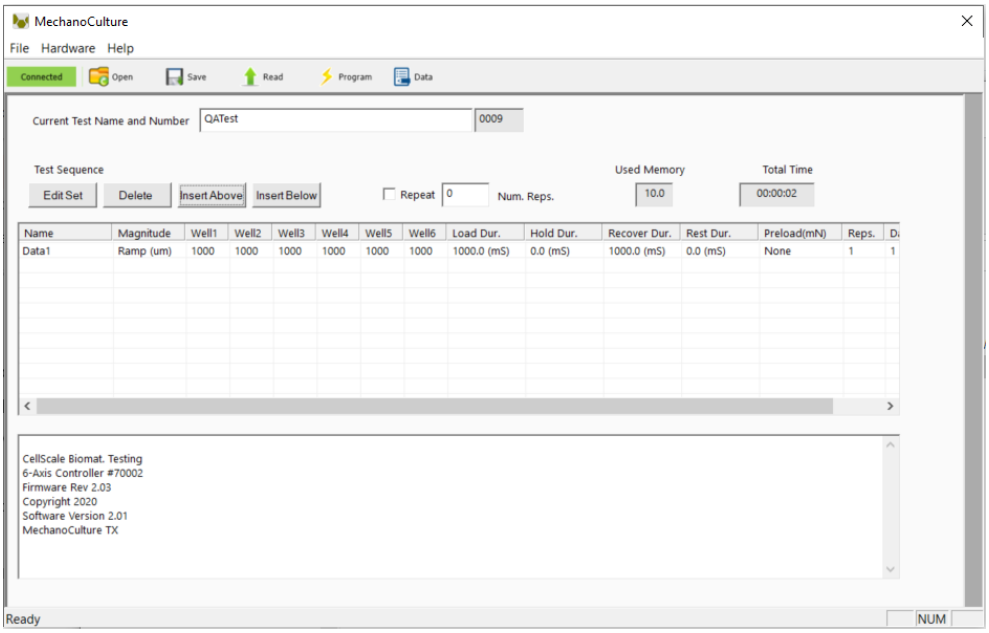


4. Software Overview

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

Test Parameters

Message Window



The test parameters section of the main window shows the current testing protocol. Errors and other information are displayed in the message window section.

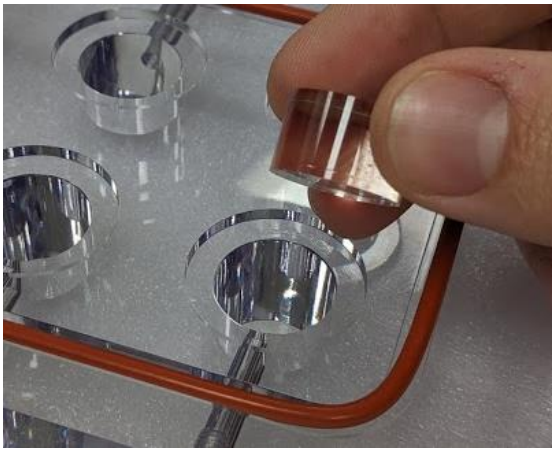
5. Setting Up and Starting a Test

Setting up a test involves loading specimens and generating a test sequence protocol. Data generated during testing can be accessed after the test is completed. Details of this process are described below:

Step 1: Specimen Loading

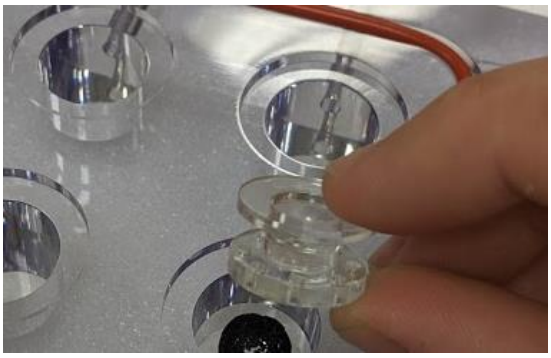
Disassembly

1. Begin by removing the M5 and M6 socket nuts and lifting the control unit off the well plate.

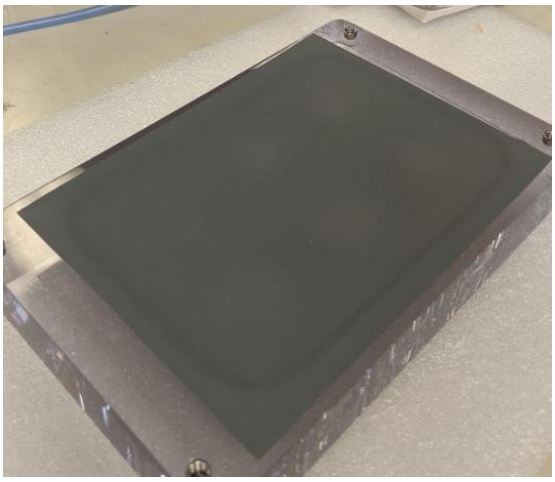


2. Remove the membrane and sterilize the spacers and membrane or well plate if needed. Next insert the spacers provided for your specimens.

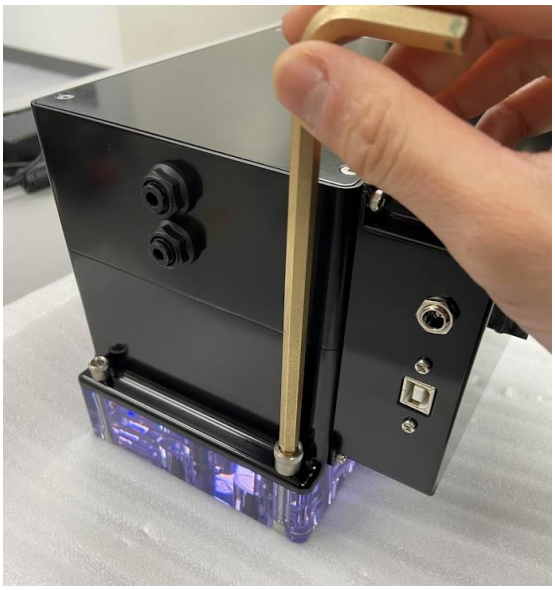
Note: Due to the compression between the top cover and the specimen holder the membrane may stick to the top cover as it is lifted off. Carefully peel the membrane off the top cover and continue with disassembly.



3. Insert the sample base that corresponds to your sample size into each of the 6 wells, insert the sample, and then place the hourglass shaped plunger on top. Ensure that they are flush with the top of the well plate. If not, additional spacers or a larger specimen may be required.



4. Once all samples are set into the wells, the membrane is placed overtop to create a barrier between the shuttles and the samples.



5. Next, place the control unit on top and fasten it to the well plate using the M5 and M6 Socket Nuts. The diagram below shows a top view of how the wells are numbered.

Step 2: System Setup

System setup includes connecting all power and data cables (electrical connections) and connecting the peristaltic pump for the liquid cooling.

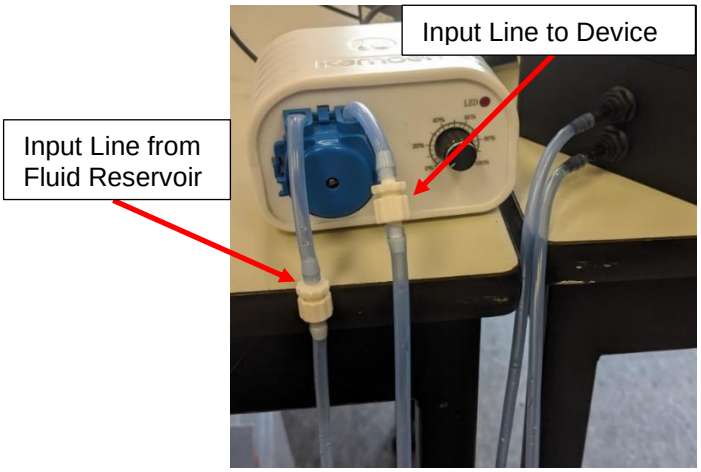
Electrical Connections

1. Connect the USB cable. This cable connects the system to the host computer. This cable is necessary for programming testing protocols and accessing collected data. It does not need to be connected while running a test.
2. Connect the 24V DC power supply cable. Plug it into an appropriate 100-240VAC power source and the other end into the system.

Fluid Connections

The actuators within the device generate heat over time. As a result, fluid tubes and a peristaltic pump have been provided to cool the system during operation. This is to ensure the heat from the actuators will not affect the temperature of the specimens. The pump is only required when using the device in an incubator or temperature-sensitive environment.

A large reservoir of water will need to be supplied by the user. This will act as a heat sink where the cooling water will circulate and will need to be large enough to stay cool during long tests. The long end coming from the pump must be submerged in the water reservoir, the other end with the black barbed connection must be inserted into either of the fluid ports. The tubing with one free end and one barbed end must be put into the other fluid port and the other end will drain into the water reservoir.



MechanoCulture TX Note: Clearing Cooling Lines

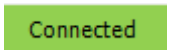
Once you are finished with your test you must clear the cooling tube lines, this is done by running the pump dry. To do this you leave the pump running and remove the feed line from the cooling reservoir and leave the system to pump air through the tubing. It is complete once no water is coming out the outlet line.

MechanoCulture TX Note: Changing Cooling Lines

The rubber hoses in the peristaltic pump head must be changed every 50hrs to prevent leaks. How to change these tubes can be found in Appendix B

Step 3: Generate a Test Sequence

Generating a test sequence includes creating or loading a protocol and programming it to the controller.

Before generating a test sequence, please launch the MechanoCulture TX Software from the host computer. See Appendix B for more details on Software Installation. The software should show a green “connected” box in the upper left corner 

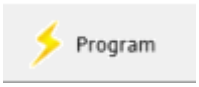
and the hardware and software versions should be listed in the message window.

Creating or Loading a Test Sequence

Test sequences 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 10 sets.

Alternatively, a predefined test sequence can be opened from a MechanoCulture TX Protocol or Template File (*.tmt). From the File menu, select Open, or press the Open button on the toolbar.

Programming a Test Sequence

From the Hardware menu, select Program, or press the 

button on the toolbar. To program a MechanoCulture TX device it must be connected to the computer’s USB port and connected to power.

To view the test sequence that is currently programmed on the device – select Read from the Hardware menu or press the 

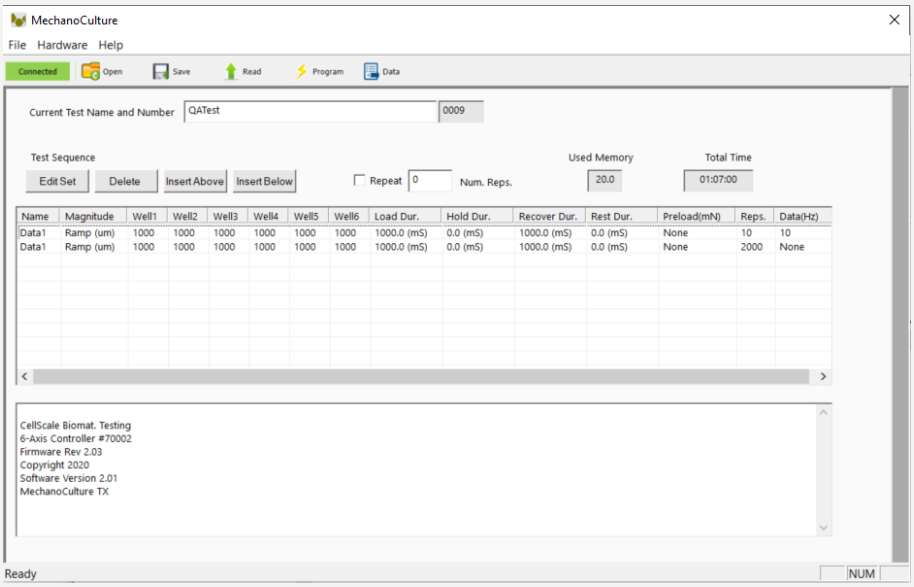
button on the toolbar.

MechanoCulture TX Note: Maximum Speed

The Maximum speed of the system is 4mm/s, programming a protocol with a speed faster than this will result in an error message. For example: 2mm at 1Hz or 1mm at 2Hz is possible but 2mm at 2Hz will not be achieved.

MechanoCulture TX Note: Long Tests

The MecahnoCulture TX is capable of running extremely long tests, however the data collection should not be used for the entirety of the test. A good way to avoid large data files is to run a test with 2 lines, the first line will be a short test and collect data, the second line will be a longer cycle with no data collection. The example below has 1 line of 10 cycles collecting data followed by the same testing protocol for 2000 cycles collecting no data: If you encounter a large data file the Micro-SD card can be removed and plugged directly into the PC for faster downloading.



Modify Testing Parameters

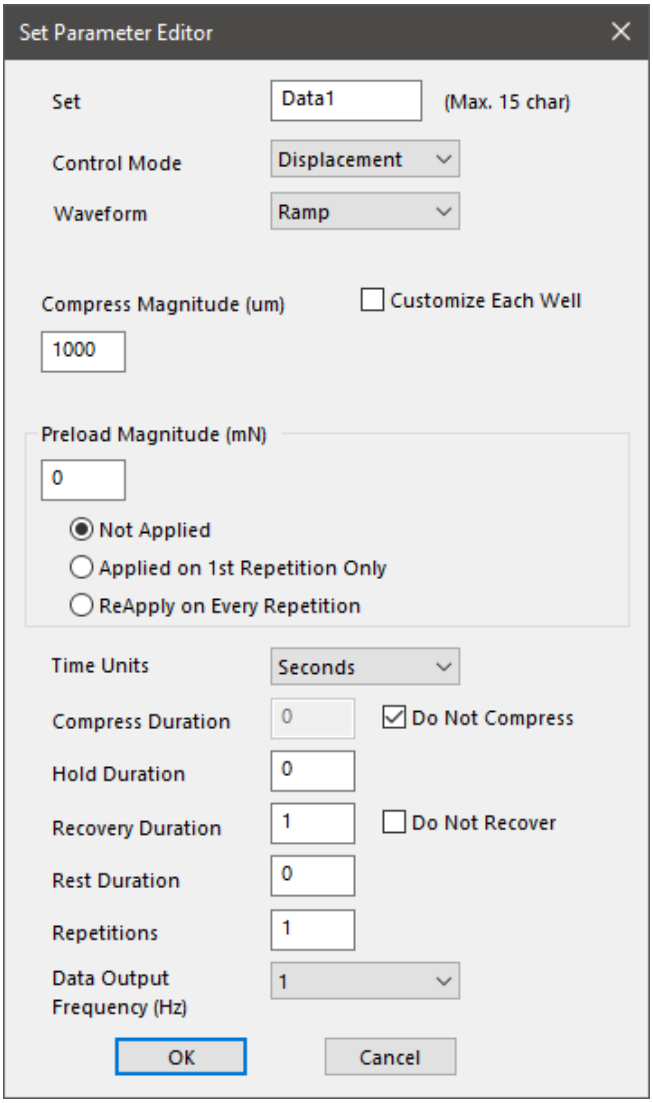
You can select and modify parameter sets by clicking on a row in the Test Parameter Specification table and then pressing the Edit Set button (or by double clicking on the row). When you do so, the Set Parameter Editor Dialog will appear.

The test parameters editor allows you to modify:

- Set Name
- Control Mode
- Waveform
- Compress Magnitude
- Preload Magnitude
- Duration of each phase
- Number of repetitions
- Data output frequency

The load and preload magnitude of each well can be customized by selecting the Customize Each Well check box.

The following table describes each of the parameters.



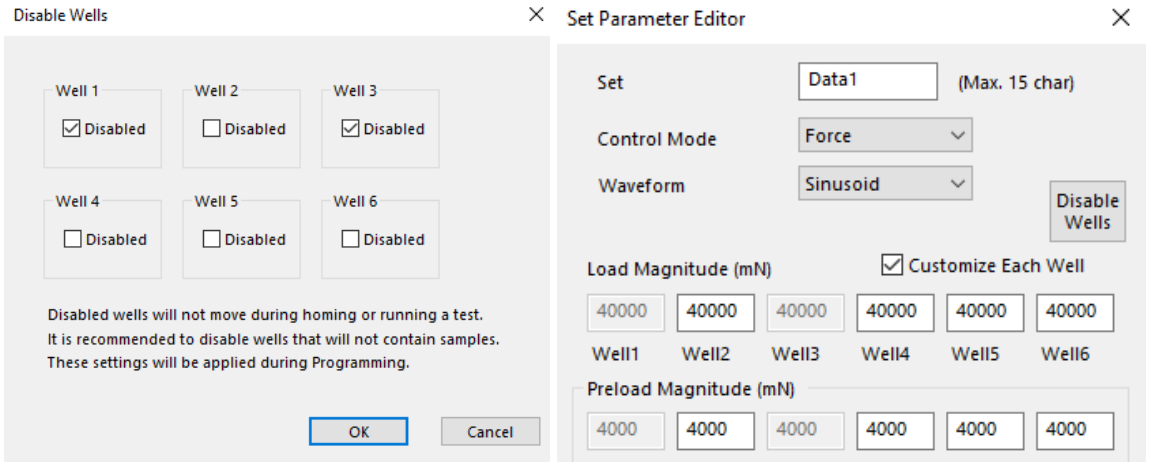
Test Parameter	Description
Set Name	User defined name of each set
Control Mode	Tests can be controlled by actuator displacement or force readings. The mode selected will change the units of the load magnitude.
Waveform	Ramp waveforms will conduct linear compress and recoveries. Whereas sinusoid waveforms will have smoother transitions between phases.
Load Magnitude	The displacement magnitude is in um and force magnitude is mN. The maximum displacement magnitude is 2000um. The maximum force magnitude will depend on the selected load cells for the device.
Time Units	Time units are applied to the entire set, but different sets can have different time units.
Compress Duration	The time over which the compress phase of a cycle is applied. The total duration of a cycle is the sum of the Compress, Hold, Recovery, and Rest durations. The frequency of a cycle is then the inverse of this total duration.
Hold Duration	Hold Duration is typically set to 0, however it is useful for creep testing.
Recovery Duration	Recovery Duration is typically set to the same value as the Stretch Duration.
Rest Duration	Rest duration is typically set to 0, however a non-zero value may be used to mimic *in vivo* conditions or for specialized testing.
Repetitions	The number of repetitions of a given test cycle.
Data Output Frequency	This menu allows the user to select how much data is recorded by the device. The options are in Hz, from 0 to 100Hz. The greater the data frequency the larger the data file will be and the longer it will take to download at the end of the test.

MechanoCulture TX Note: Do Not Compress or Recover

For certain specimens or protocols, it may be useful to not compress and/or recover. Click the check box next to the corresponding duration field to skip that phase. This is especially useful if a manual position is set to observe creep or to create a step compress pattern. See the Additional Settings section for more information about Move to Position and Disable Homing.

Customizing and Disabling Wells

Inside the Set parameter Editor you can customize each well to move a certain displacement or force. You can also disable certain wells so that they do not move during the programmed test. Wells can be disabled when they do not have a sample loaded or if a loadcell has become damaged. To disable wells you can click on the Disable Wells button inside the Set Parameter Editor and select which wells you would like to be disabled. The below example shows wells 1 and 3 are disabled.



Step 4: Running a Test

Once the device is assembled with specimens and a test sequence is programmed using the MechanoCulture TX software, the device can be unplugged from the host computer.

Controller Push Button

There is one push-button switch on the front of the control unit. A single push will begin the initialization zeroing sequence, once this is complete a green flashing light can be seen. Pushing the button once this sequence is complete will begin the test, a solid green light can be seen when the system is operating.

Pushing the button during a test will pause and can be restarted with another single push. A force-controlled test will be paused after the current test sequence is completed; a displacement-controlled test will pause in place. Once the test is complete the green LED will flash. To perform another test, simply press the button to start the zeroing sequence and a second press after that is complete will begin a new test.

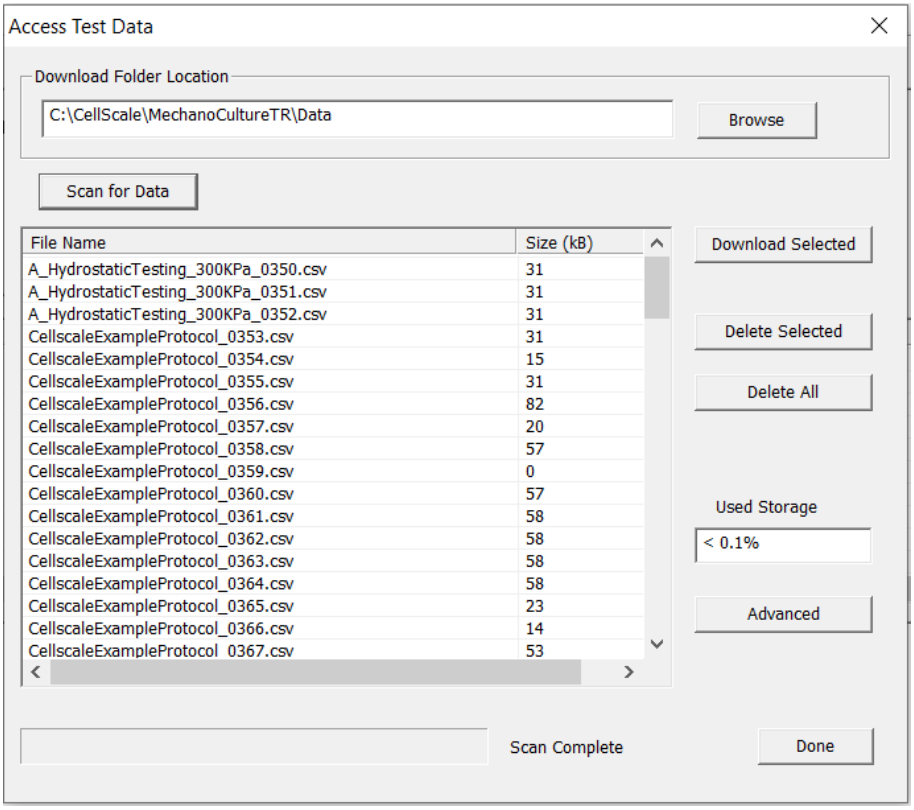
Step 5: Accessing Data

Data collected during a test protocol is saved directly onto the device and can be accessed from the MechanoCulture TX software by selecting *Access Test Data* from the hardware menu or by clicking the 

button in the top right corner.

The Access Test Data menu will appear. Select 'Scan for Data' to scan the SD card and display the data files stored on the device. Files can be selected and downloaded to the PC by first selecting a destination folder using the *Browse* button then selecting the data files of interest and downloading them using the *Download Selected* option.

Unwanted data can be cleared off of the onboard memory by simply selecting the desired files and using the *Delete Selected* button.

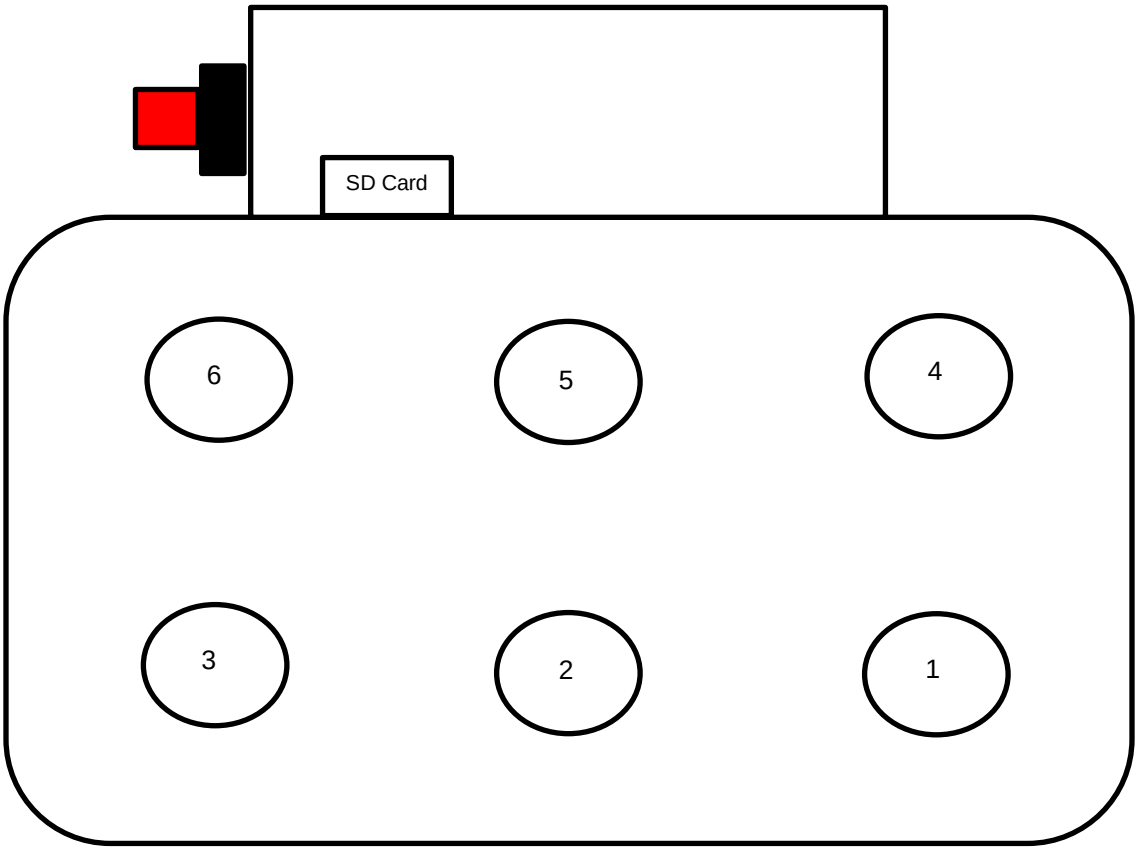


Files that are downloaded will appear as a .csv data file in the specified download folder location.

The 'Advanced' option is reserved for the rare case in which you have removed the device's SD card, moved raw test data from the card to the computer, and would now like to download and process that data from the computer. Select 'Advanced', check the checkbox after reading the message, and select the computer directory storing the raw test data files. These files will then appear in the list.

Csv data file

The .csv (comma separated value) data file contains the collected test data. The output contains information about timing, cycles, as well as position and force information for every well. The numbering for the wells is shown in the following figure of the top view of the device:



Stress information can be calculated from the force data if specimen cross sectional areas are known. Strain information can be calculated from the position data if specimen heights are known.

.Tmt Protocol file

The .tmt template file contains the protocol and settings that were used during that test and can be opened like any other template file.

Csv _Raw data file (contained in TestLogs sub folder)

The raw data file contains data in raw sensor units and is normally not used or viewed with the exception of troubleshooting or debugging.

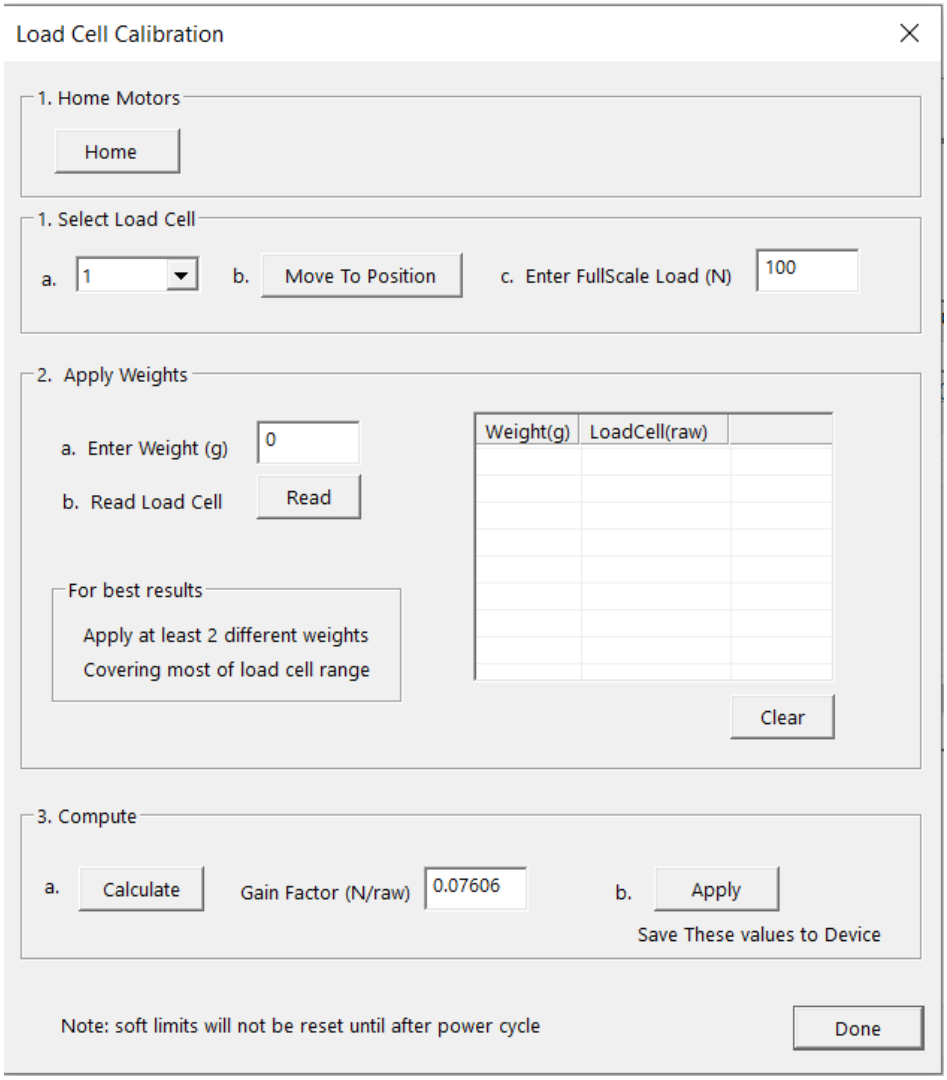
Csv _Full data file (contained in TestLogs sub folder)

The full data file contains additional output columns such as specified pressure and actual pressure and is normally not used or viewed with the exception of troubleshooting or debugging.

6. Additional Settings

Load Cell Calibration

Calibrating the load cells is done at the factory and does not need to be redone unless the load cell has been overloaded or the user suspects one of the load cells may be broken. To access the calibration dialogue window, select the Hardware drop down menu and select Load Cell Calibration. It will open the below window:



Each load cell is calibrated individually, before doing the calibration the system must be removed from the clear well plate and flipped upside down.

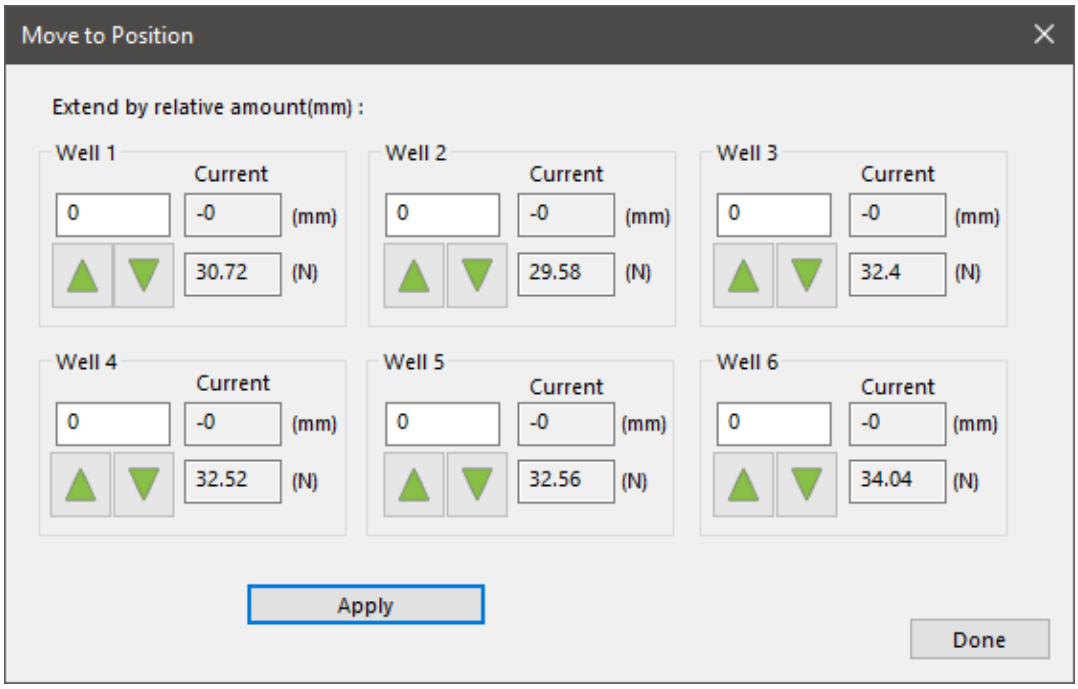
MechanoCulture TX Note

Move to Position Button must only be pushed once or else there is a risk of damaging the loadcell

Move to Position

Each well can be manually moved to a set position using the Move to Position window in the Hardware menu. Select the arrow key under each well to move the compression platen. Alternatively, you can enter the value to move in mm. Click Apply to move the actuators.

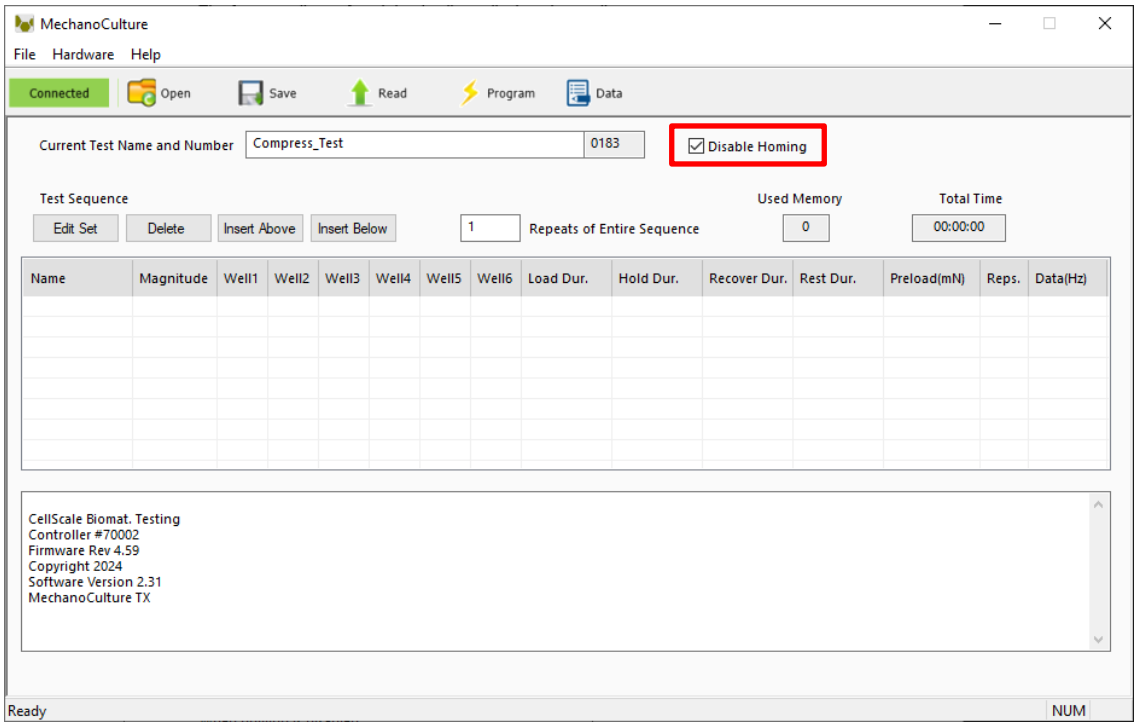
Note the movements are relative to the current position of each actuator. The force readings of each load cell are displayed as well.



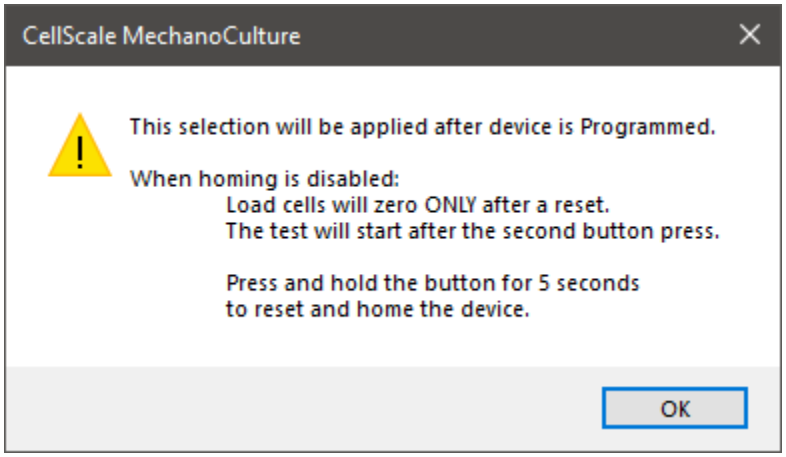
Disable Homing

The homing protocol before each test can be disabled in the software by selecting the check box at the top of the window. Click the Program button to apply it to the device. This is important if you are setting a manual position with the Move to Position and do not want the device to reset at the start of the test sequence.

Note two button presses will still be required to start a test protocol. the first press will begin the test sequence, but it will not execute the homing protocol. The second press will execute the programmed test parameters.



If Disable Homing is selected, manual reset will be required to home the motors and zero the load cells. This can be done by pressing and holding the system button for 5 seconds.



7. Sample Test Sequences

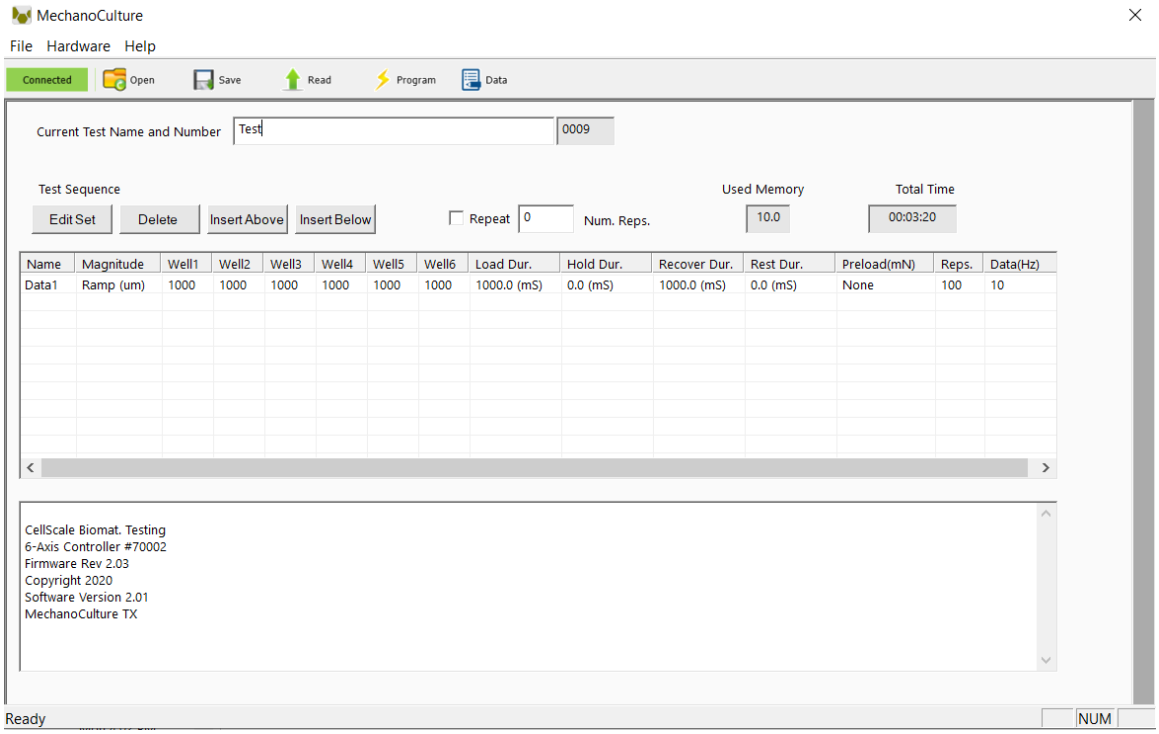
The MechanoCulture TX software allows for complex test sequences to be generated. The examples in this section illustrate how to generate different types of test protocols.

Example 1: Continuous cycling for fixed duration

A long test using one protocol may be desired. To do this, first insert a row by pressing the “Insert Above” button. Then double click on that row to modify it.

The below test protocol will compress 1000um in 1000ms and recover 1000um in 1000ms for 100 cycles then stop with data collected at a frequency of 10Hz

Field	Value
Magnitude	1000um
Stretch Duration	1000 ms
Hold Duration	0.0 s
Recover Duration	1000 ms
Rest Duration	0.0 s
Repetitions	100
Data	10Hz

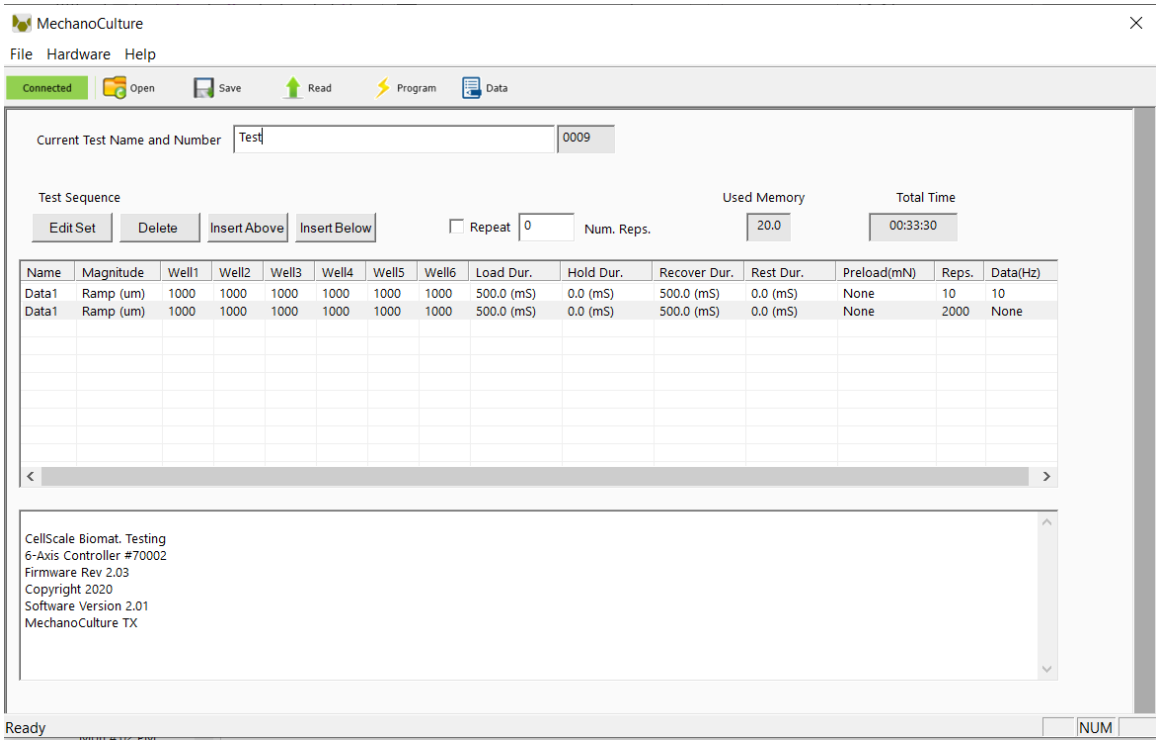


Example 2: Multiple Sets (Smaller Data Files)

A test sequence where the protocol changes during the test may be desired. You may want to test the sample with a smaller compression and then a larger compression.

The following program executes an example test, which includes a 1000um compression for 10 cycles collecting data at 10Hz followed by the same test for 2000 cycles collecting no data.

Field	Precondition Value	Main Value
Magnitude	1000um	1000um
Stretch Duration	500 ms	500 ms
Hold Duration	0 ms	0 ms
Recover Duration	500 ms	500 ms
Rest Duration	0 ms	0 ms
Repetitions	10	2000
Data	10 Hz	None

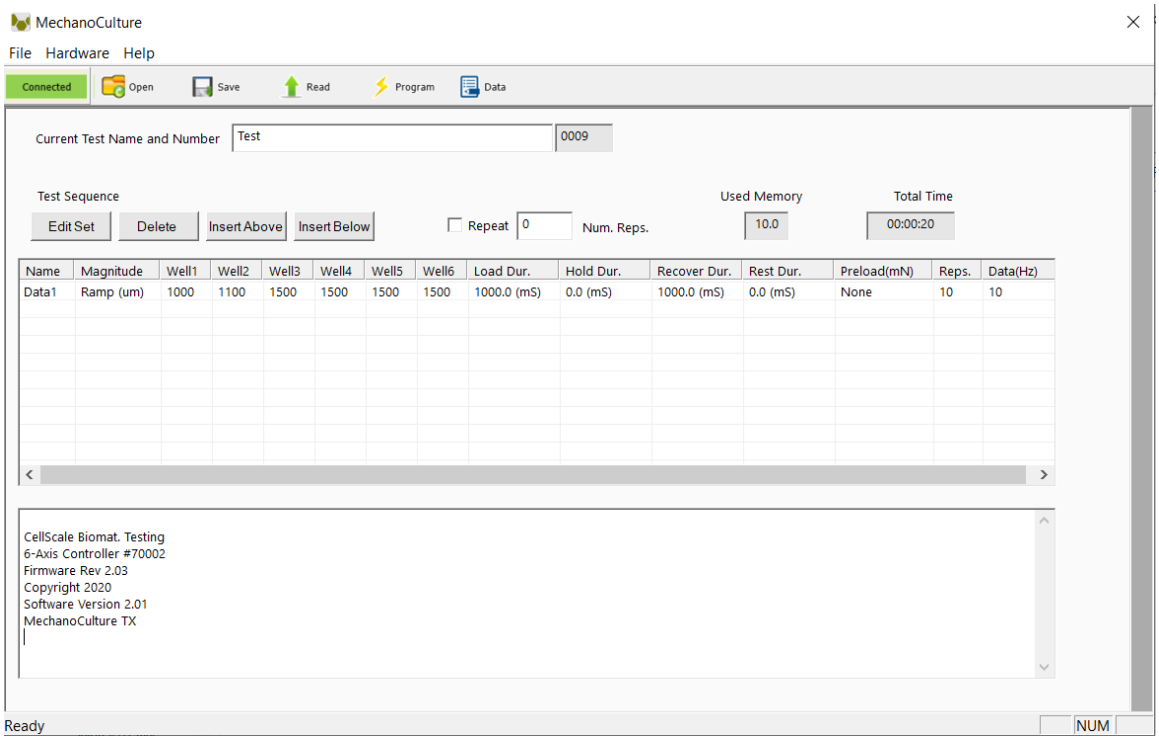


Example 3: Unique Well Compression

A test sequence where each well is programmed individually is possible but each well must be running the same test mode.

The following program executes an example test, which includes a 1000um compression for Well 1, a 1100um compression for Well 2, and a 1500um compression for Wells 3-6.

Field	Main Value
Magnitude	1000um, 1100um, 1500um
Stretch Duration	1000 ms
Hold Duration	0 ms
Recover Duration	1000 ms
Rest Duration	0 ms
Repetitions	10
Data	10 Hz

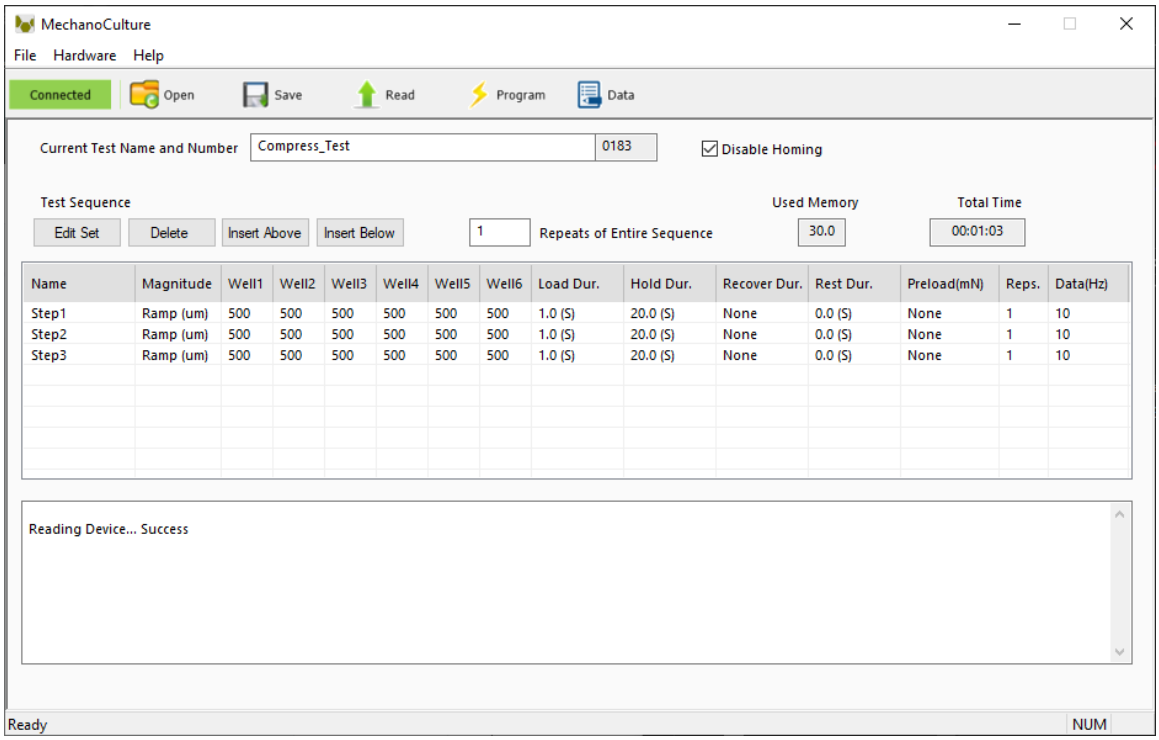


Example 4: Step Compression

A test sequence which compresses the specimens at a set displacements without returning back to zero. If you would like to start at a position that is not zero, disable homing will need to be selected and a manual position (Move to Position) will need to be set priro to the start of the test.

The example below is a 3 step compression protocol. This will compress the sample 500um over 500ms in incremental steps wiith 20 seconds hold each in between.

Field	Step 1	Step 1	Step 1
Magnitude	500um	500um	500um
Stretch Duration	1s	1s	1s
Hold Duration	20s	20s	20s
Recover Duration	0s	0s	0s
Rest Duration	0s	0s	0s
Repetitions	10	10	10
Data	10 Hz	10 Hz	10 Hz



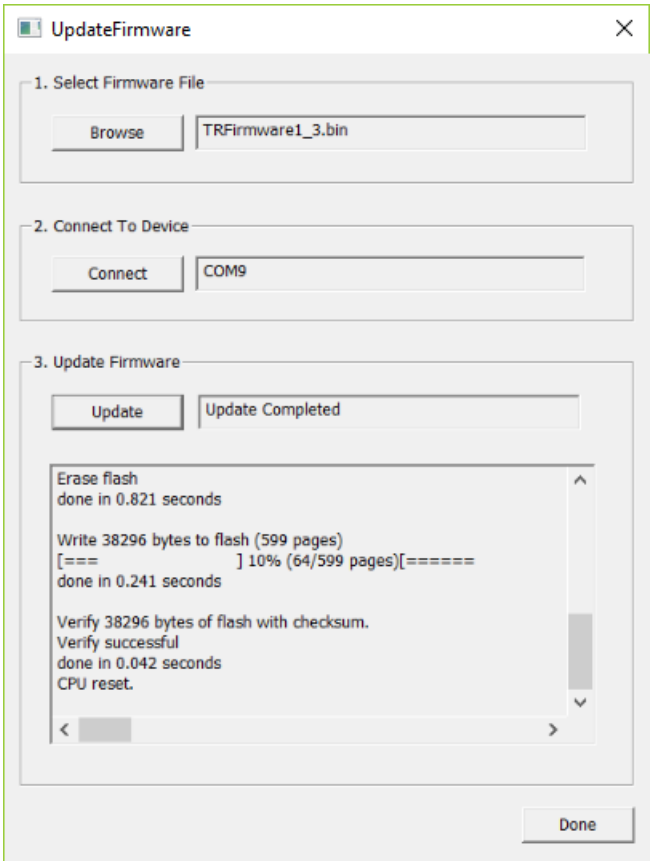
8. Troubleshooting

Error	Remedy
Error 6 readfile error Or other errors when connecting to device	Power cycle software and device
Error reading data from device	Close and Re-open the data collection window

LED Error Code	Meaning and remedy
Red LED 2 Pulse	SD Card not present of unable to access, check that Micro-SD card is inserted properly
Red LED 3 Pulse	No Test Programmed, program a new test protocol in software
Red LED 4 Pulse	No Loadcell calibration data detected, ensure loadcell calibration data is correct.
Orange LED 4 Pulse	Soft limit reached during test (displacement or force), test will continue and finish on all wells that did not reach soft limit
Green LED Slow Flash	Device is paused, press button to resume test
Green LED Solid On	Device is currently operating, either homing protocol or test sequence
Green LED Fast Flash	Device has finished running and is ready to proceed with button push to begin test or homing sequence.

9. Appendix A: Updating the Firmware

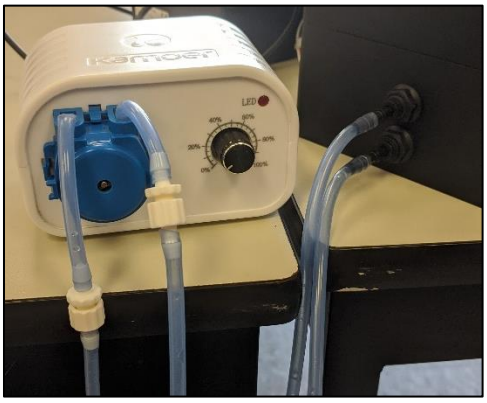
1. With the MechanoCulture TX connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities> UpdateFirmware.exe.
2. Load the firmware file (*.bin) using the “Browse” button.
3. Click the “Connect” button.
4. Execute the update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

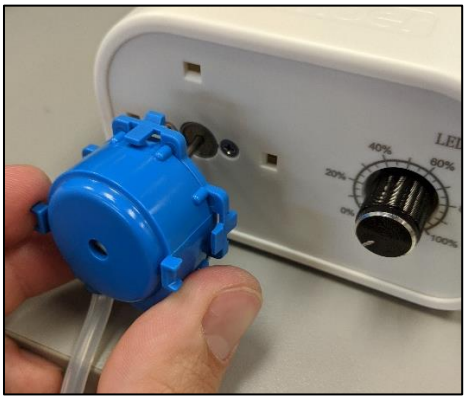


10. Appendix B: Replacing Peristaltic Pump Tubes

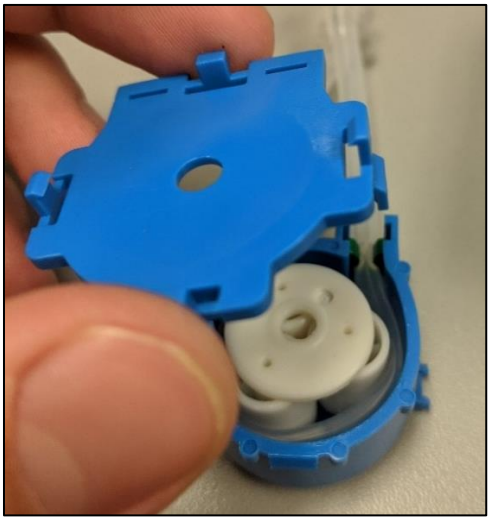
After approximately 50hrs of operation the peristaltic pump tubing must be changed to avoid a rupture of the line and leakage inside the pump. To replace the tubing follow the instructions below:

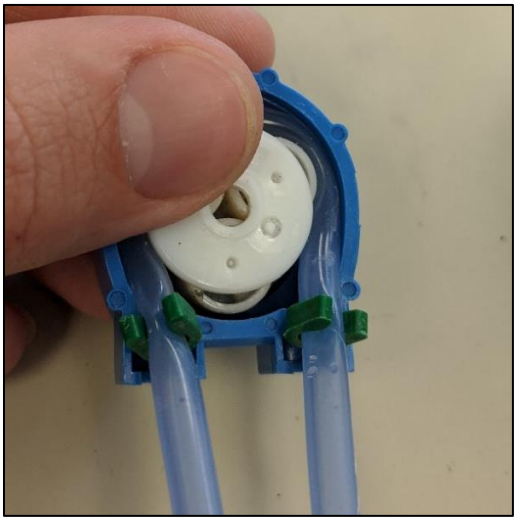
1. Remove the tube from the system and the water reservoir and run the pump dry to remove any excess liquid.
2. Now that the tubes are clear of any liquid, remove the long sections of tubing from the pump head by twisting the Luer lock fittings. Now the pump head can be removed from the pump motor. This is removed by pinching in the 2 side clips and pulling it off as seen in the image.





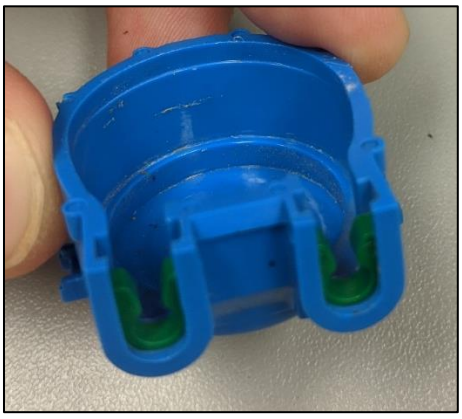
3. Once the pump head is removed take the back cover off to expose the rotating cam and tubing. With the back cover off lift the tubing up and out of the green clips which are used to hold it in place. Once the green clips are removed you can lift the cam and tubing out of the assembly. Be careful as the cam is made of loose parts that can roll away.

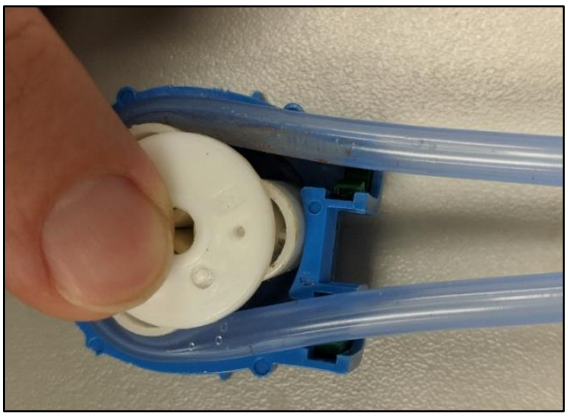




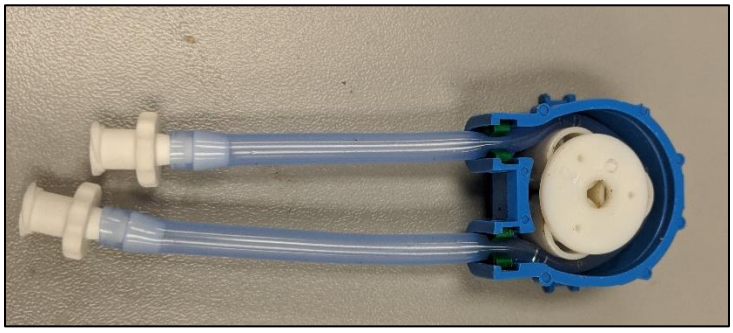
4. With the cam and old tube removed, you can remove the luer lock barbed fittings and place them into the new tube.

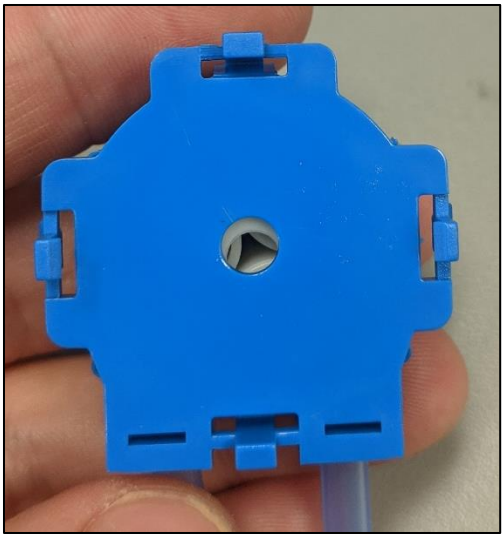
5. Place the green clips back into their slots, wrap the tube around the cams and push it into place in the center of the housing.





6. Slide the tube into each of the green clips and place the back cap back on and snap the pump head back in place with the new tube.



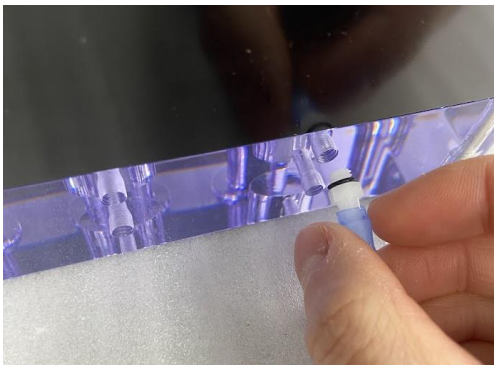


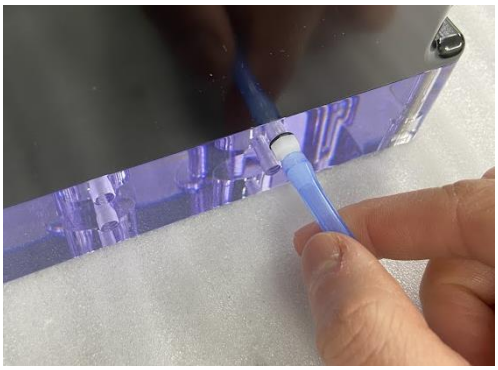

LED
40%
60%
20%
80%
0%
100%

11. Appendix C: Fluid Profusion Setup

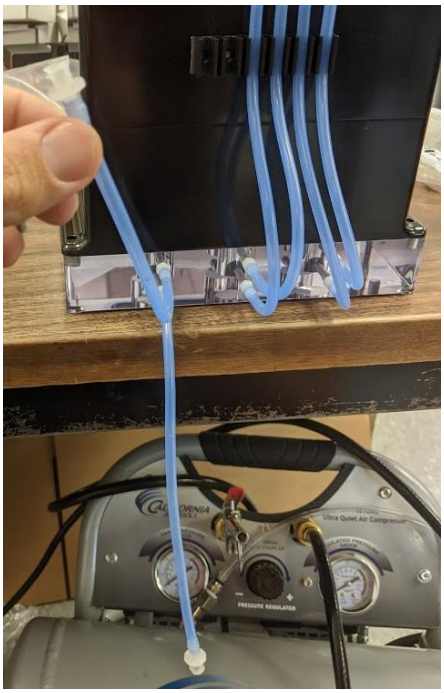
If your system is set up to do fluid profusion your clear polycarbonate well plate will have 2 ports in each well. The top port is for loading new media and the bottom is for removing old media.

To set up the fluid profusion, place an o-ring over each barb fitting and thread each tube into a port. **Only finger tighten the fittings.** Do not over tighten the fittings as it can damage the threads of the well plate.

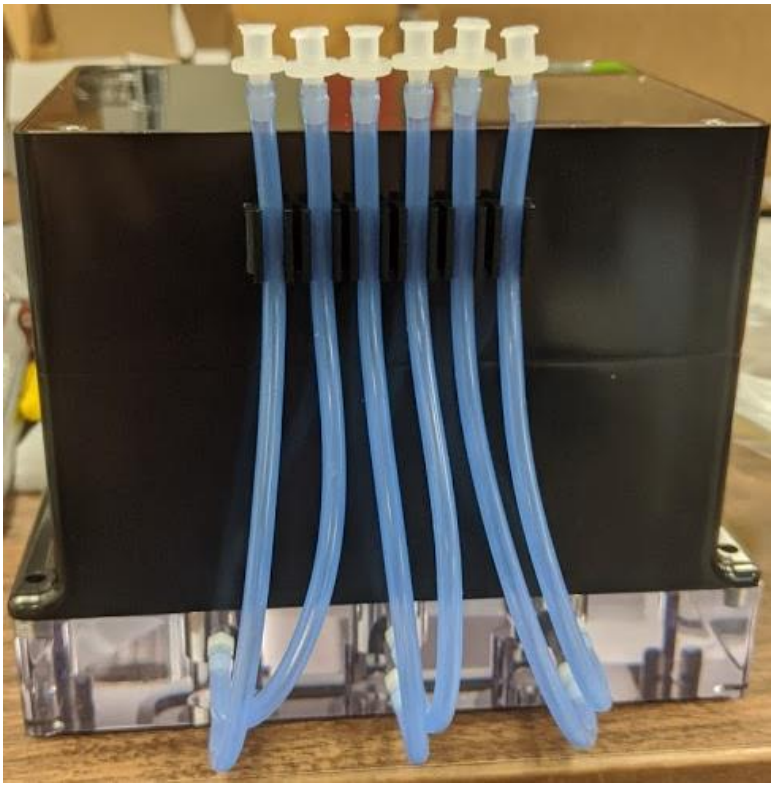




To remove old used media from the well the plug can be removed from the end of the tube and the tube can be removed from its upright position and released into a basin to catch the media as seen below:



To fill the well with new media the plug for the bottom tube must be replaced and the tube must be placed back into the upright position in its holder. Next a syringe can be attached to the luer lock fitting on the top tube and media may be flowed into the well until the desired amount has been inserted. Once the well is filled the plug can be replaced and the tube can be put back into its upright position. These steps can be repeated for all 6 wells to fill the system. If sterility is not crucial, the well plate can be filled before installing the top unit.

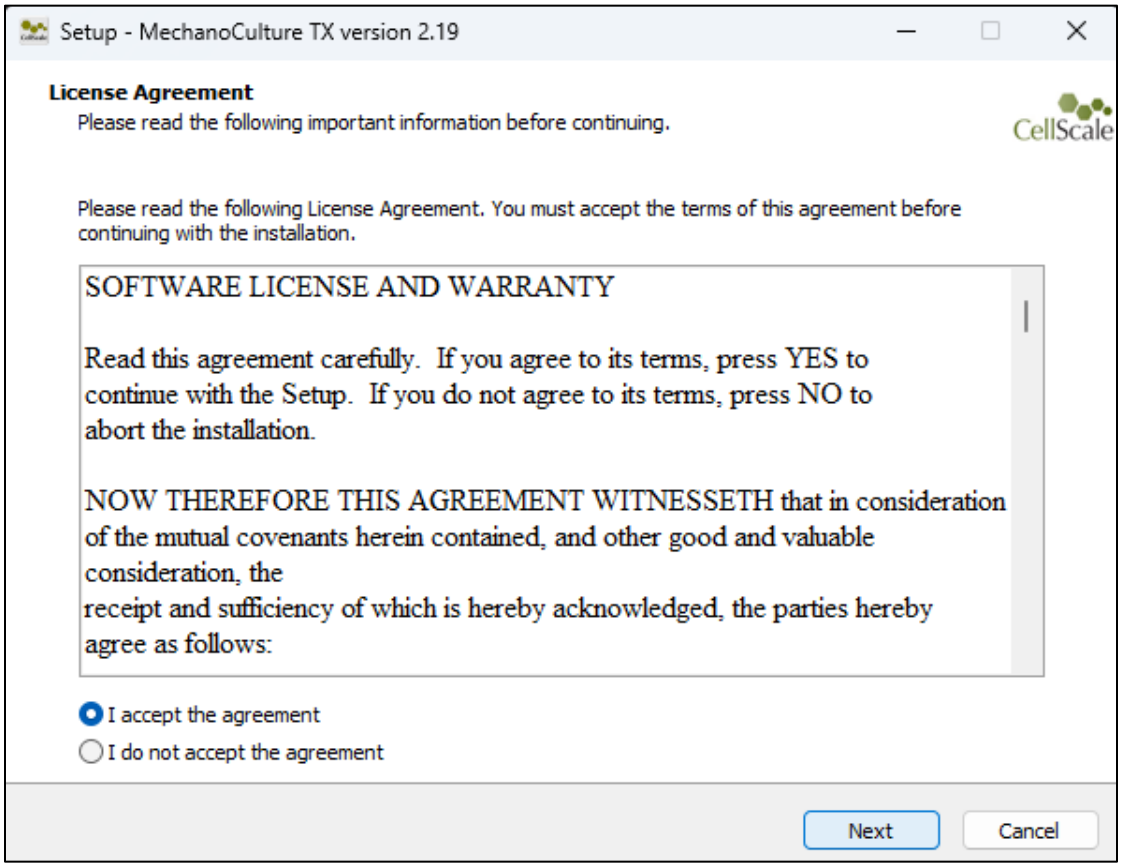


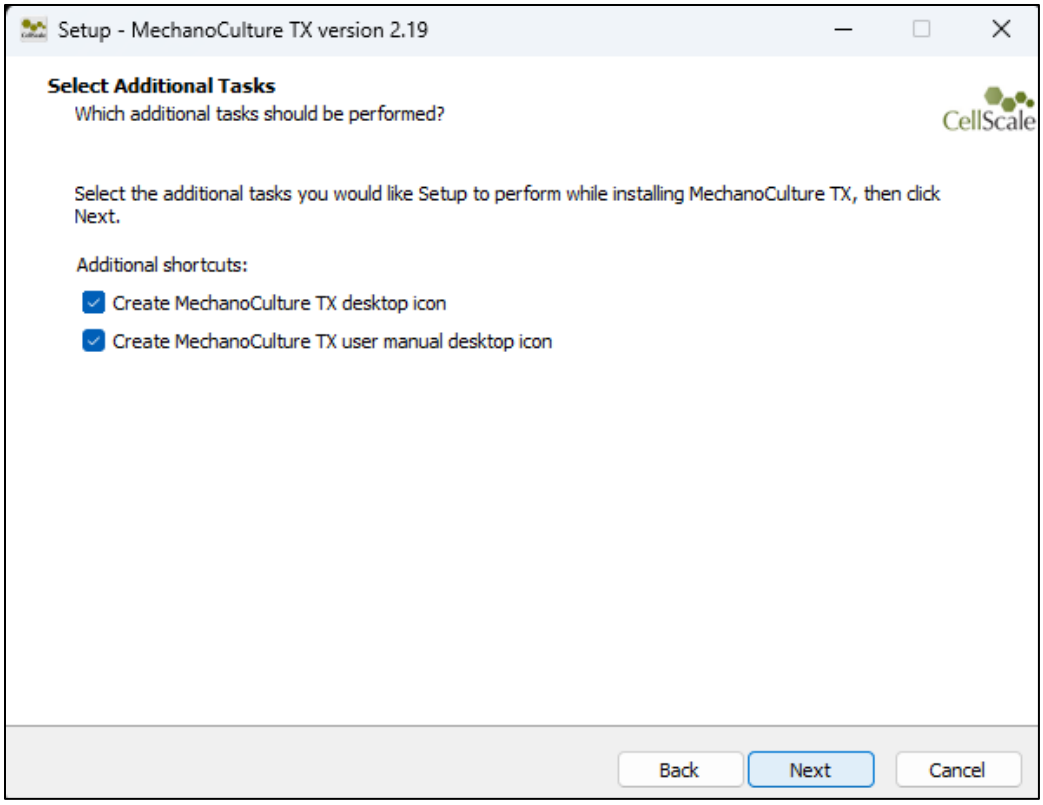
12. Appendix D: Software Installation

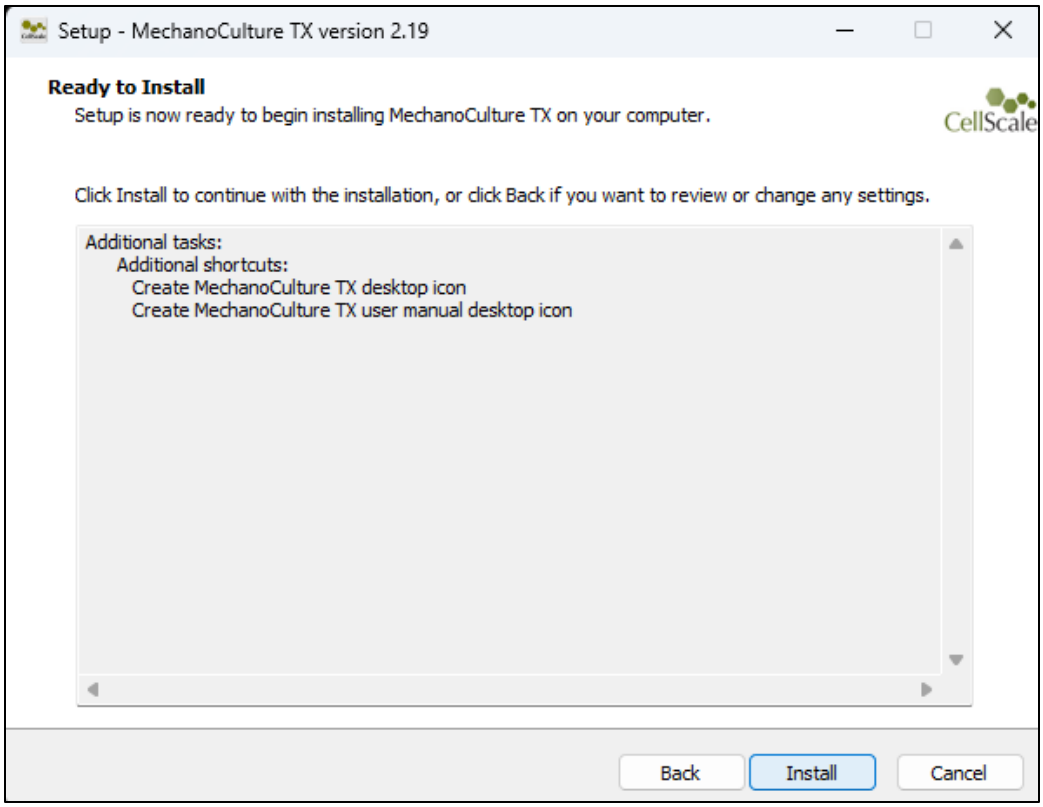
Included in your shipment is a link to install the MechanoCulture TX software. This installation package contains the installer for the software to program your MechanoCulture TX devices.

To install the software:

1. Go to the provided web address and download the compressed installation package.
2. Transfer this file to the computer which will be running the MechanoCulture TX device.
3. After this is complete, go to the uncompressed installation folder and select *MechanoCulture TX Installer.exe*. Details of typical dialogue boxes are shown below.




Setup - MechanoCulture TX version 2.19
Select Additional Tasks
Which additional tasks should be performed?
CellScale
Select the additional tasks you would like Setup to perform while installing MechanoCulture TX, then click Next.
Additional shortcuts:
Create MechanoCulture TX desktop icon
Create MechanoCulture TX user manual desktop icon
Back
Next
Cancel


Setup - MechanoCulture TX version 2.19
Ready to Install
Setup is now ready to begin installing MechanoCulture TX on your computer.
CellScale
Click Install to continue with the installation, or click Back if you want to review or change any settings.
Additional tasks:
Additional shortcuts:
Create MechanoCulture TX desktop icon
Create MechanoCulture TX user manual desktop icon
Back
Install
Cancel