CellScale
biomaterials testing

MechanoCulture TR Hydro
Mechanical Stimulation System

User Manual

Version 1.9

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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 TR is a precision instrument specifically designed for hydrostatic compression testing of biomaterials in a sterile fluid environment. This device is capable of 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	1.2 A
Environmental Conditions	Maximum Operating Temperature 40 ˚C 0 – 100% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree 2
Data Connections	USB for programming

System Assembly

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

2. MechanoCulture TR Components

The MechanoCulture TR consists of a cell culture assembly and a pressure regulator control box. As shown in the following table, the cell culture assembly consists of a well plate, O-rings, membrane and top cap. The pressure regulator box contains the pressure regulator and control circuit board.

Component	Description
Well Plate	The well plate contains 9 wells. The well plate is made from polycarbonate.
9 Small O-Rings	Placed in the groove around each well
1 Large O-Ring	Placed in the grove surrounding all the wells to completely seal the sterile culture area from the pressure cavity above that applies the forces to the specimens.
Flexible Membrane	Covers and seals the culture area. When the pressure is applied in the cavity above, the membrane will deform and the specimens will compress.
Fluid port plugs (Optional)	There are inlet and outlet ports that allow media perfusion media if the protocol requires it. Otherwise they must be plugged using the fluid port plugs.
Top Cap	Acts as a pressure manifold which distributes force to each well.
Nuts and Washers	Used to secure the Top Cap to the Well Plate
Pressure Regulator Box	Contains the pressure regulator, control circuit board and power for the system as well as electrical and pneumatic connection ports.
Pressure Supply (not included)	A pressure supply with an air hose is required to use the MechanoCulture TR. For most systems, a supply of between 75-100 psi is adequate. This is dependent on the pressure regulator being used with your specific system.

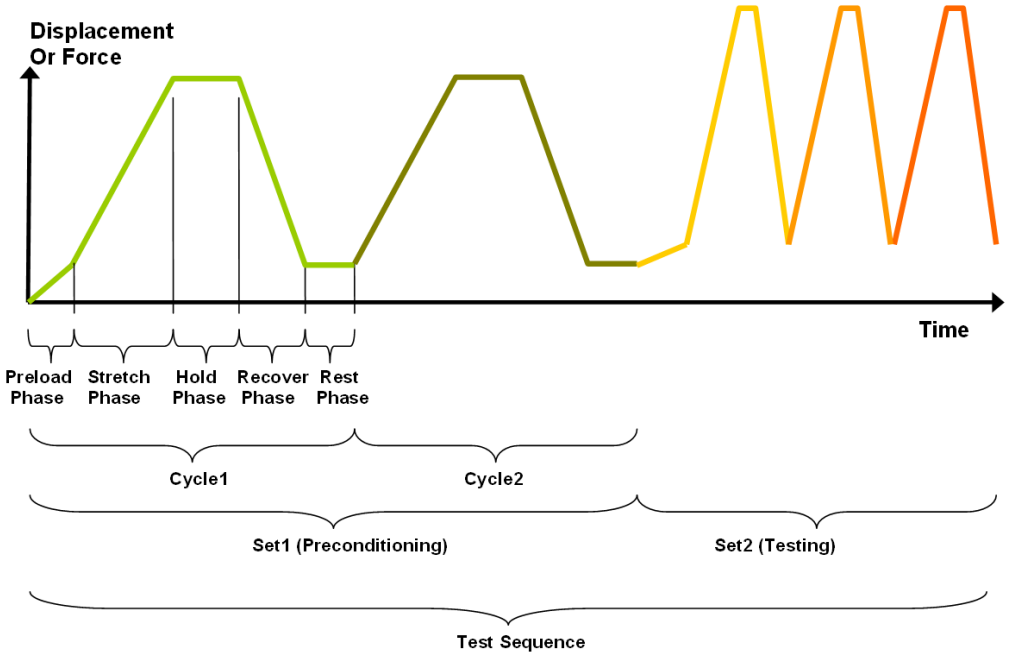
3. Testing Terminology

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

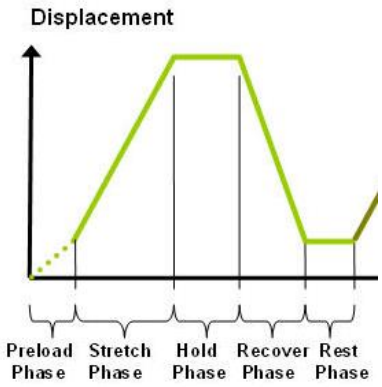
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

Compressing - During the Compress phase, a deformation is applied to the specimen.

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

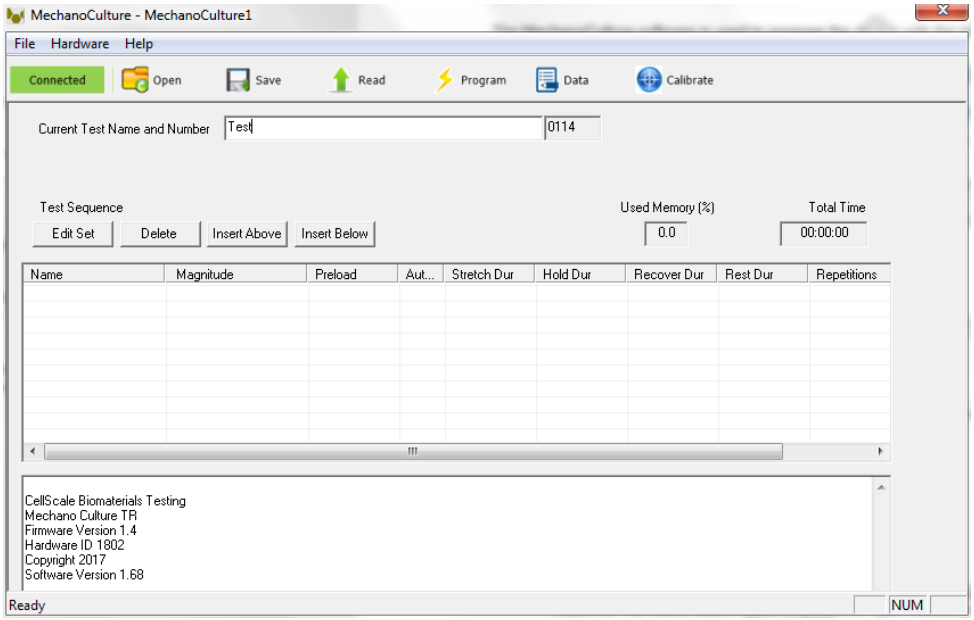
Recovering - The recovery phase is the time during which the deformation imposed during the compress phase is removed.

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 TR 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

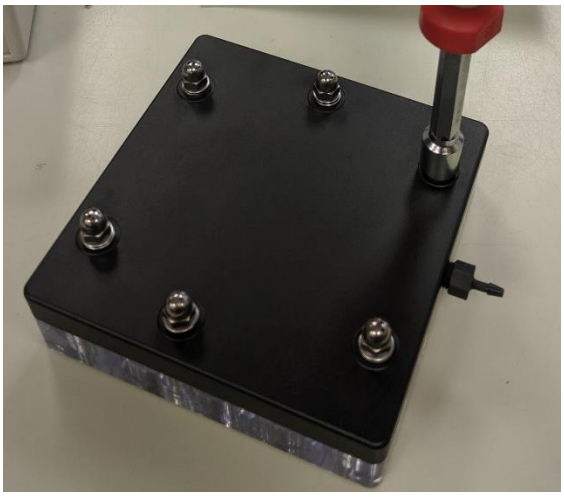
The test parameters section of the main window shows the current testing protocol. Errors and other information are displayed in the message window section.

5. Setting Up and Starting a Test

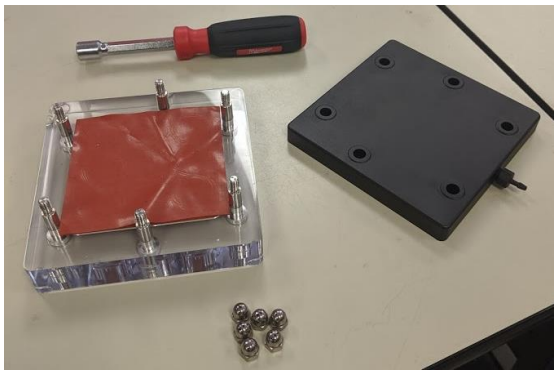
Setting up a test involves loading specimens and generating a test sequence protocol. Data generated during testing can be accessed after the test has completed. Details of this process are described below and in the following video https://cellscale.com/products/mctr/ (see videos section).

Step 1: Specimen Loading

Disassembly

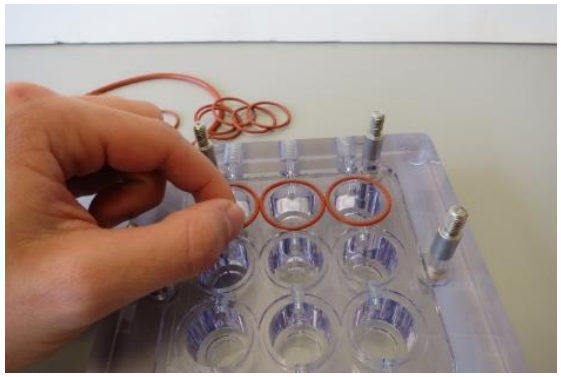


1.Begin disassembly by removing the 6 cap nuts holding the top cover and specimen holder together using the 10mm nut driver provided.



2. Remove the washers and lift the top cover off the specimen holder.

3. Remove the membrane and samples from the specimen holder.



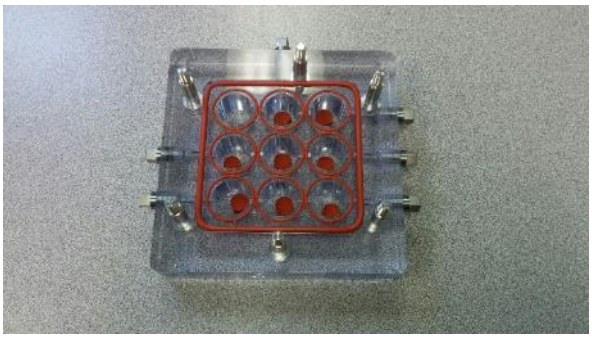
5. Collect the small and large O-rings from the grooves in the specimen holder.

6. Perform any necessary cleaning or sterilization of the MechanoCulture TR components.

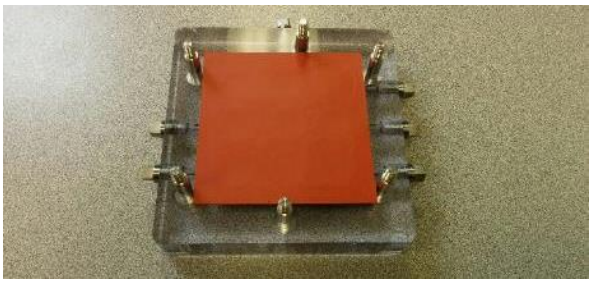
System Assembly

1. Load a specimen into the center of each of all nine specimen wells. After loading, fill all nine wells completely with liquid.

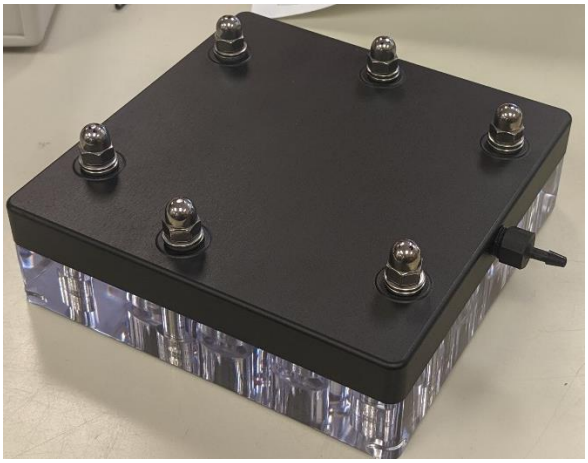
> ***MechanoCulture TR Tip: Liquid Loading***
>
> When filling the sample wells with liquid, please ensure that all wells are completely filled. The system will not function properly in hydrostatic testing mode if an air gap is present.



2. Place nine small O-rings and one large O-ring into the grooves.



3. Cover the fluid filled wells with the provided hydrostatic membrane.



4. Place the top cap onto the well plate assembly. Add a washer and cap nuts onto each of the six compression bolts. Using the 10mm nut driver provided, fully tighten the 6 cap nuts in a crisscross pattern. Each nut must be tightened several times to achieve a good seal.

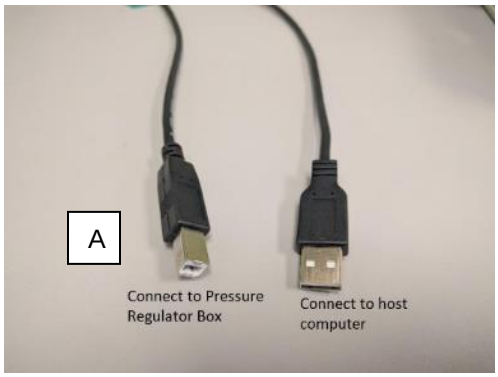
> ***MechanoCulture TR Tip: Proper Cap Nut Tightening***
>
> When tightening the cap nuts on the MechanoCulture TR ensure that they are fully tightened using the provided nut driver in a crisscross pattern. Each nut must be tightened several times to achieve a good seal.
>
> Complete cap nut tightening is essential for proper system sealing

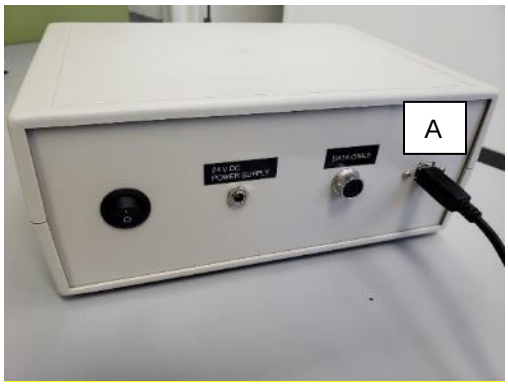
Step 2: System Setup

System setup includes connecting all power and data cables (electrical connections) and connecting the pressurized air hoses and tubing (pneumatic connections). Optionally, fluid connections can also be made to flow media through the chamber wells.

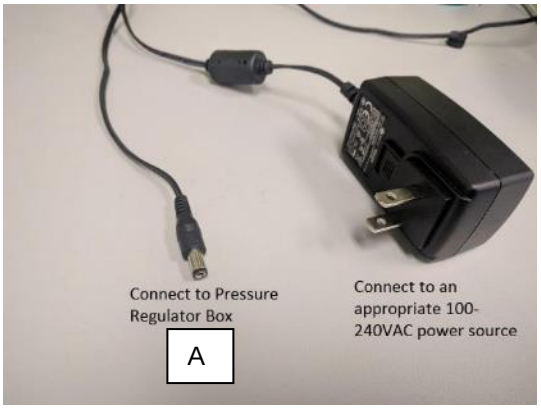
Electrical Connections

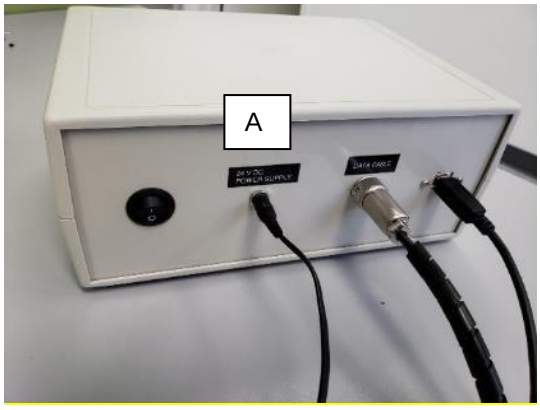
1. Connect the USB cable. This cable connects the control box to the host computer. This cable is necessary for programming testing protocols and accessing collected data. It does not need to be connected while running a test.





2. Connect the 24V DC power supply cable. Plug it into an appropriate 100-240V AC power source and the other end into the pressure regulator box.

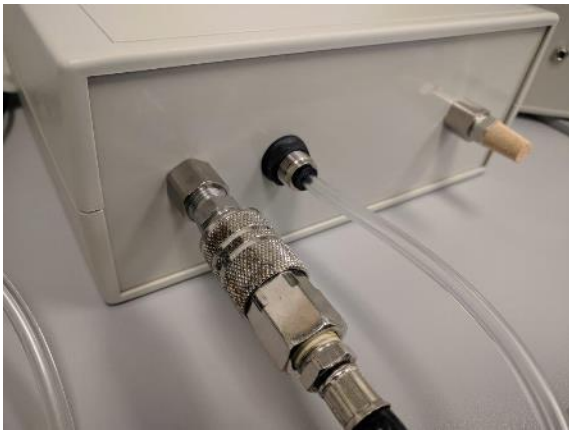




Pneumatic Connections

1. Connect the ¼" O.D. air tubing. This tubing can be cut to size. One end connects to the barbed air connector on the top cap. Connect the other end of the tubing into the pneumatic connector on the control box by inserting it until a click is felt. The tubing can be removed by pressing on the control box connector ring and pulling the tubing out.

2. Connect a pressurized air supply to the control box via the pressure supply port. This could be a 1/4-inch air compressor quick-connect coupler (*Option 1*) or a push to connect fitting for 1/4 inch tubing (*Option 2*) (supplied but not installed), depending on your pressurized air supply.

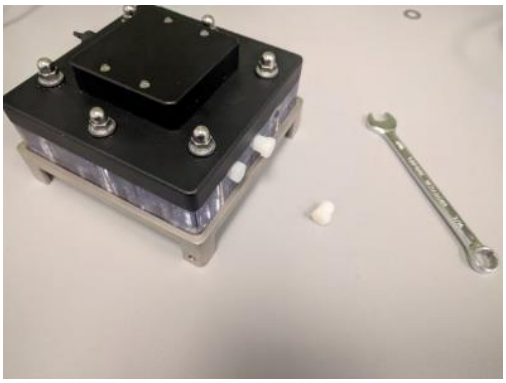


Option 1

Option 2

Fluid Connections

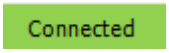
Fluid ports can be attached to a pump system (not supplied) that will circulate or flow media through the specimen wells. Use the barbed fittings if using a pump system. Alternatively, use the fluid port plugs to seal the wells. Fluid port fittings may need to be wrapped in Teflon tape to achieve a good seal.



Or

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 TR Software from the host computer. See Appendix B for more details on Software Installation. Also, please ensure that the power switch on the pressure regulator box is turned on. 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 TR 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 Program button on the toolbar. To program a MechanoCulture TR device it must be connected to the computer's USB port with a USB cable and the switch on the pressure regulator box must be powered on.

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

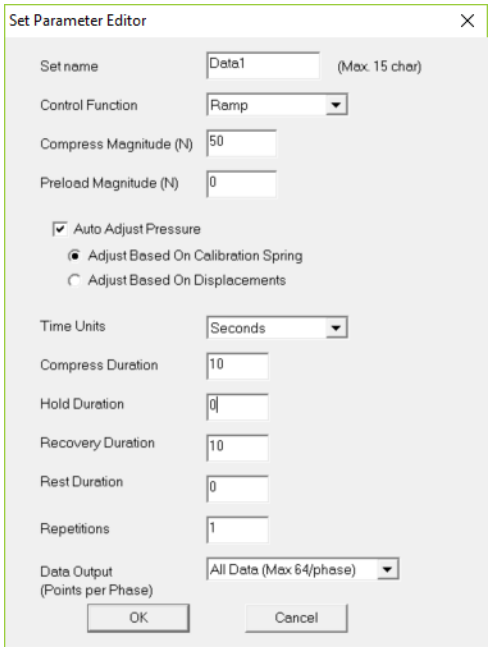
> ***MechanoCulture TR Tip: Saving Test Sequence Protocols***
>
> *Once you have developed a test sequence, you can save it as a MechanoCulture Protocol file for future use. Select Save from the file menu or press the Save button on the toolbar. You can then open this test template the next time you initiate a test sequence.*

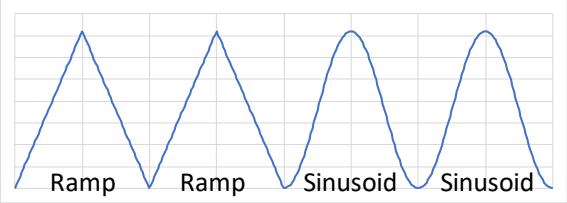
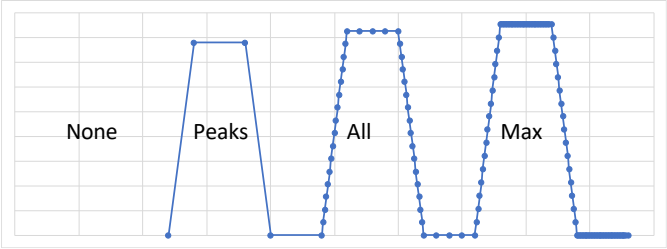
Modify Testing Parameters (optional)

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 control the control function and magnitude of loading as well as the cycle frequency.

The following table describes each of the parameters.



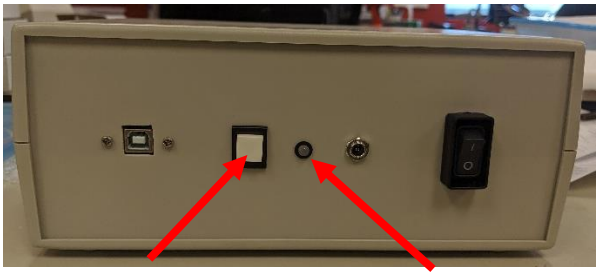
Test Parameter	Description
Set Name	User defined name of each set
Control Function	Forces are applied in a series of linear increments to approximate either a Ramp or Sinusoid function as shown in the following graph: 
Compress Magnitude	The applied force in N. The peak force during testing will be equal to the Compress Magnitude not including the Preload Magnitude. For example, a Compression Magnitude of 50N and a Preload of 5N will still produce total force of 50 N.
Preload Magnitude	A preload is an offset loading value that acts as the baseline for the loading cycle. A preload can be useful to help achieve a well-defined starting position for your tests.
Auto Adjust Pressure	When this option is selected the controller will adjust the applied pressure on every cycle to more accurately achieve the desired magnitudes. Adjustments are based on data from the calibration well or on real time displacements. See Section 7: Understanding Your Data for more details on this system function.
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 be 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 or repetitions of a given test cycle.
Data	The Data menu allows the user to select how much data is recorded by the device. The options are all, max, none or just the peaks of each testing cycle as shown in the graph below. The number of data points collected during the Compress or Recovery phases for All or Max settings depends on the duration of those phases. A minimum of 8 and a maximum of 64 data points will be collected. 

Step 4: Running a Test

Once the device is assembled with specimens and a test sequence is programmed using the MechanoCulture TR 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 start the test sequence. Pushing the button during a test sequence will pause the test. A paused test can be restarted with another single push. A push and hold for 5 seconds will reset the device and terminate any currently running test.



Push Button *LED*

LED Codes

The MechanoCulture TR displays current test state and error codes via the LED located beside the controller push button. The following table describes these codes:

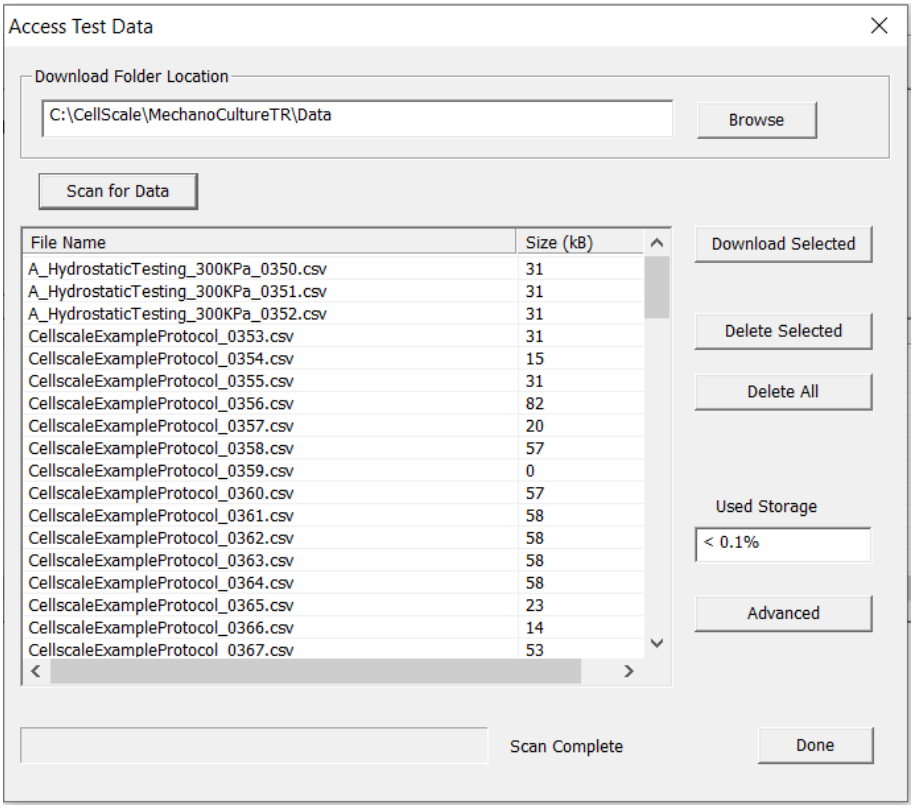
LED Pattern	Status	Troubleshooting
Flashing green	Device is ready	A test protocol can be started with a single button press.
Solid green	Test is running	The device will return to the ready state once the test protocol is complete. The test can be paused with one button press or restarted with a 5s hold.
Solid red	Test is paused	Press the push button to continue testing or hold for 5s to reset.
One red pulse	SD Card error	SD card is not detected, or it is full.
Two red pulses	Run table error	The device has no programmed protocol. Program a protocol using the MechanoCulture TR software and power cycle the device.
Three red pulses	Displacement error	Maximum displacement was exceeded during a test protocol which could cause a membrane rupture. Revise the test protocol to reduce displacement.
Four red pulses	Pressure error	Insufficient pressure provided to the device or a pressure leak been detected.

Step 5: Accessing Data

Data collected during a test protocol is saved directly onto the device and can be accessed from the MechanoCulture TR software by selecting *Access Test Data* from the hardware menu or by clicking the *Data* 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 be placed in a sub-folder with the name of the test. 2 files are downloaded for each test. A .csv data file and a .tmt protocol (template) file. In addition, a TestLogs sub directory is created within each folder. This sub directory contains 2 additional .csv files that may be useful for troubleshooting or debugging.

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 applied pressure information.

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 protocol 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 apart from troubleshooting or debugging.

7. Understanding Your Data

Applied Force vs Specimen Force

Applied forces generated by the applied pressure are split between deforming the flexible membrane and deforming the specimens. Because the amount of force to deform the membrane depends on the membrane displacements which in turn depend on the stiffness of the samples it is not possible to know in advance how much of the applied force will be transmitted to the specimen.

Because of this, the system will initially assume a zero-membrane displacement when calculating the amount of pressure to apply. So, for example, if 50N is the desired force on the specimens, but they deform by 1 mm at that applied pressure, then the membrane forces might be 20N and the specimen forces will only be 30 N.

To account for this difference, either use the Auto Adjust feature (described below), or specify a higher initial magnitude such that the desired applied specimen forces are achieved.

The output data from tests will accurately display the specimen forces but they may not exactly match the desired applied forces.

8. Sample Test Sequences

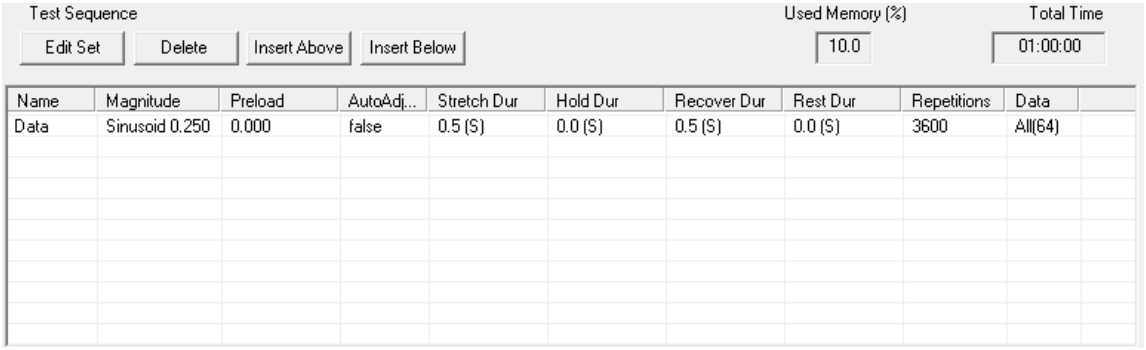
The MechanoCulture TR software allows for complex test sequences to be generated. The examples in this section illustrate how to generate different types of test protocols.

Example 1: Hydrostatic Operation

Hydrostatic testing can be accomplished by first following the setup instructions described in earlier.

The following program executes an example hydrostatic test. The values and steps for this test sequence are described in the following figures.

Field	Value
Wave Form	Sinusoidal
Magnitude	0.25MPa
Preload	0 MPa
Auto Adjust	False
Frequency	1 Hz
Stretch Duration	0.5s
Hold Duration	0.0 s
Recover Duration	0.5 s
Rest Duration	0.0 s
Repetitions	3600
Run Time	60 min
Data	Max



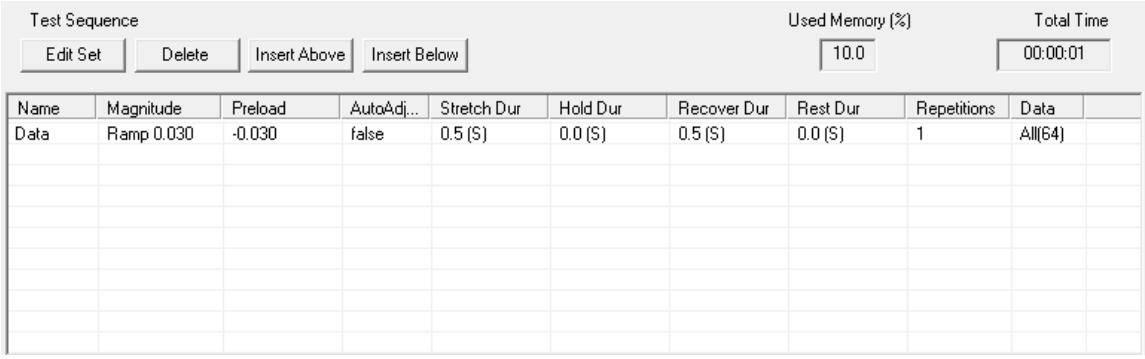
Example 2: Hydrostatic Operation with Pressure and Vacuum

Note: For additional information on select Pressure & Vacuum system operation please see Appendix D.

For select system models, specialized hydrostatic testing which includes a vacuum pressure source can be configured. Vacuum pressures are denoted by a negative pressure and following this notation test settings can be configured as usual.

The following program executes an example hydrostatic test using both a pressure and a vacuum source. The values and steps for this test sequence are described in the following figures.

Field	Value
Wave Form	Ramp
Magnitude	+0.03 MPa
Preload	-0.03 MPa
Auto Adjust	False
Frequency	1 Hz
Stretch Duration	0.5s
Hold Duration	0 s
Recover Duration	0.5 s
Rest Duration	0 s
Repetitions	3600
Run Time	60 min
Data	Max

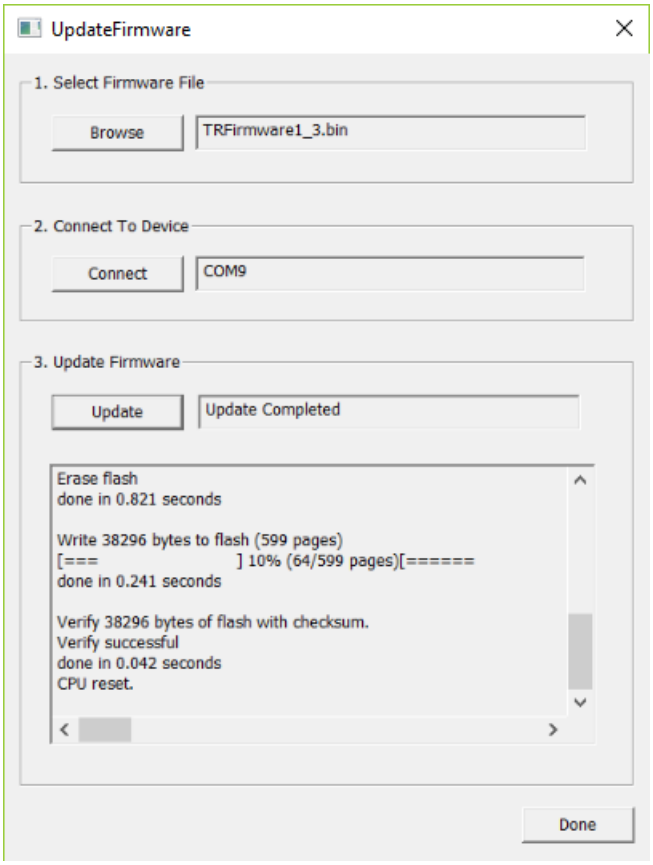


9. Troubleshooting

Error	Remedy
Error 6 readfile error Or other errors when connecting to device	Power cycle software and pressure regulator box
Incorrect Data; Data appears to be significantly off the expected result	1) Make sure the pneumatic connections are fully sealed and not leaking.

10. Appendix A: Updating the Firmware

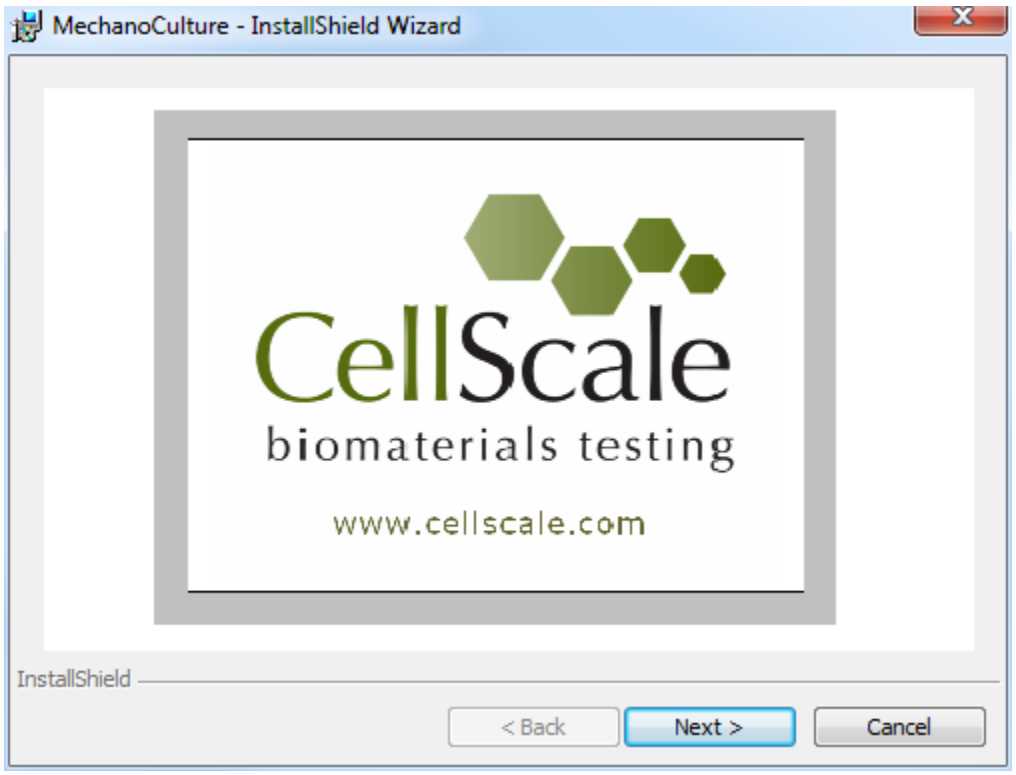
1. With the MechanoCulture TR connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities> UpdateFirmware.exe.
2. Load the firmware file (*.bin) using the “Browse” button.
3. Click the “Connect” button.
4. Execute the update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

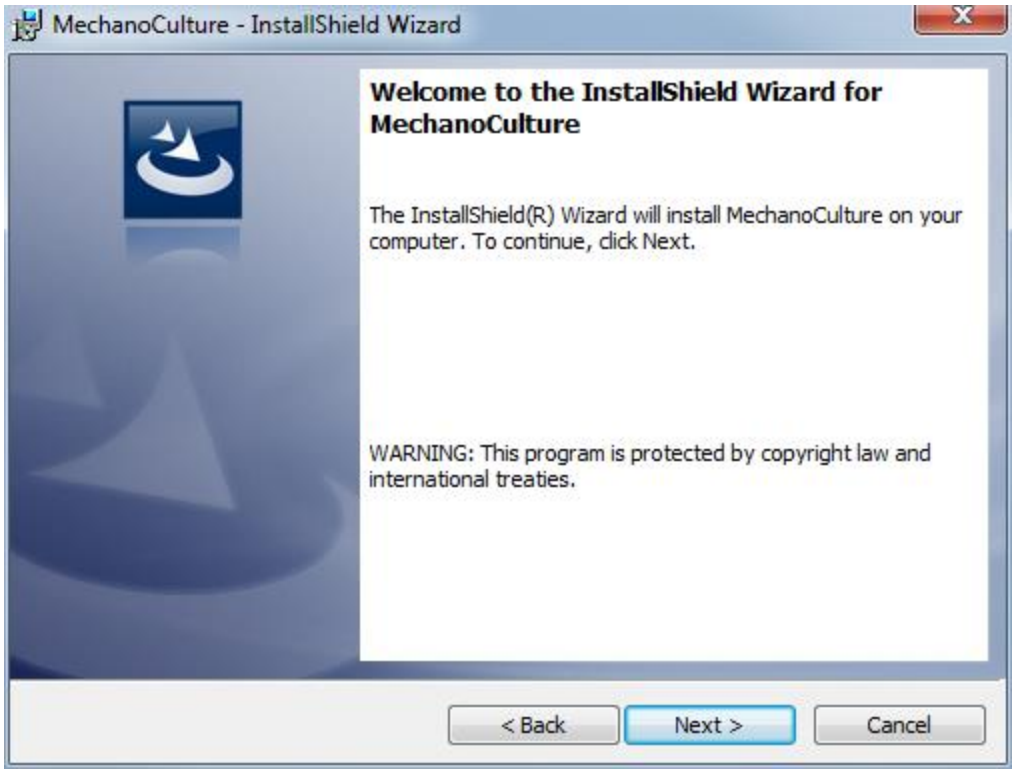


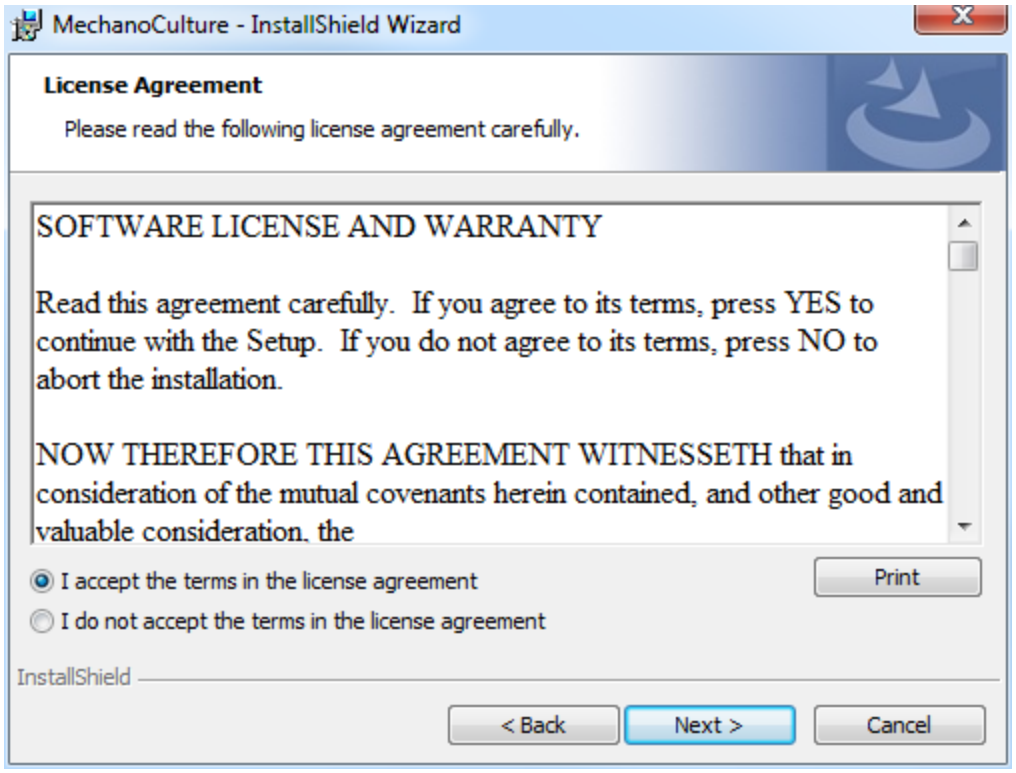
11. Appendix B: Software Installation

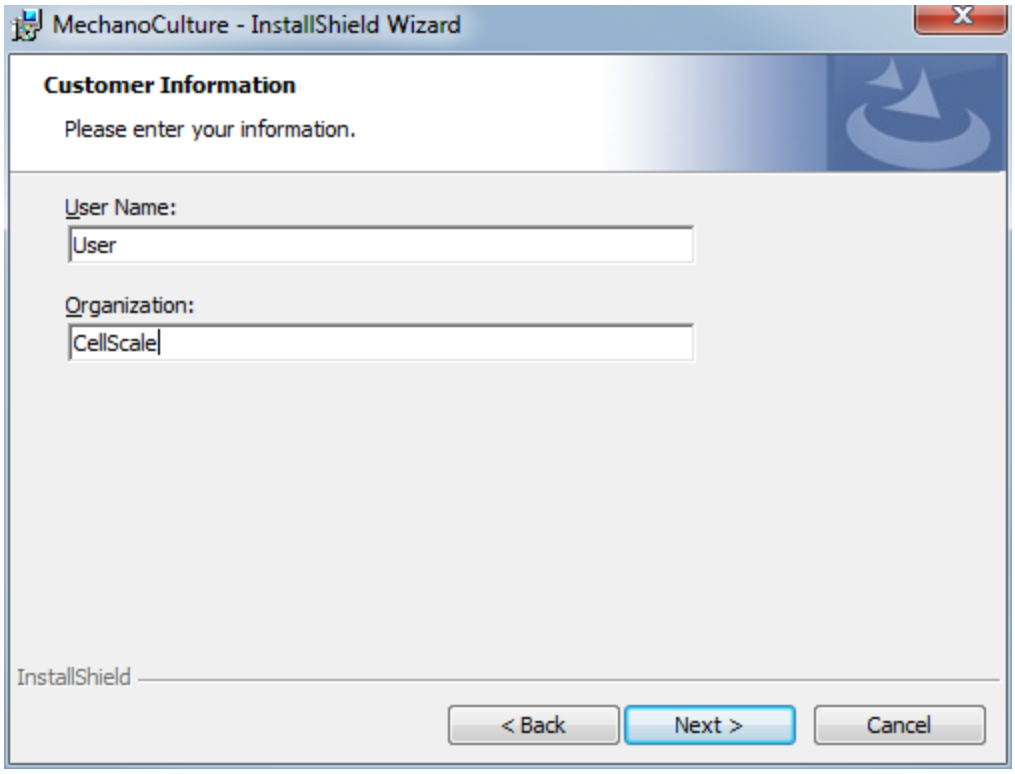
Included in your shipment is a link to install the MechanoCulture TR software. This installation package contains the installer for the software to program your MechanoCulture TR devices.

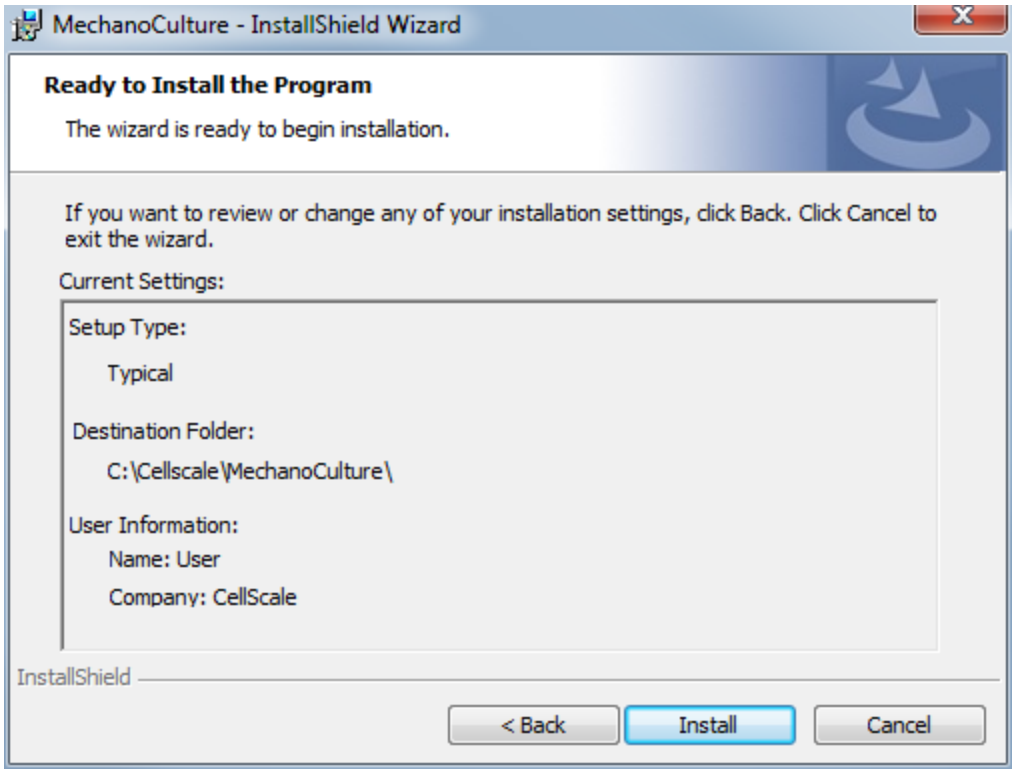
To install the software, go to the provided web address and download the compressed installation package. Transfer this file to the computer which will be programming the MechanoCulture TR. Right click on the file and select “Extract All…” to unzip. After this is complete, go to the unzipped installation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

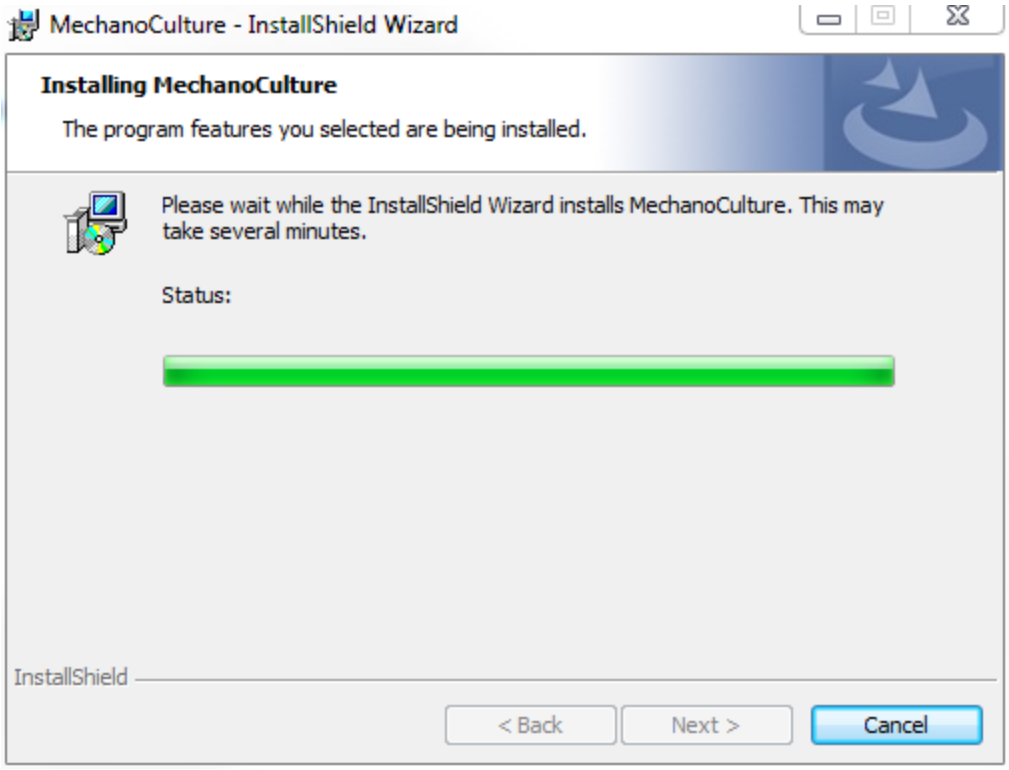


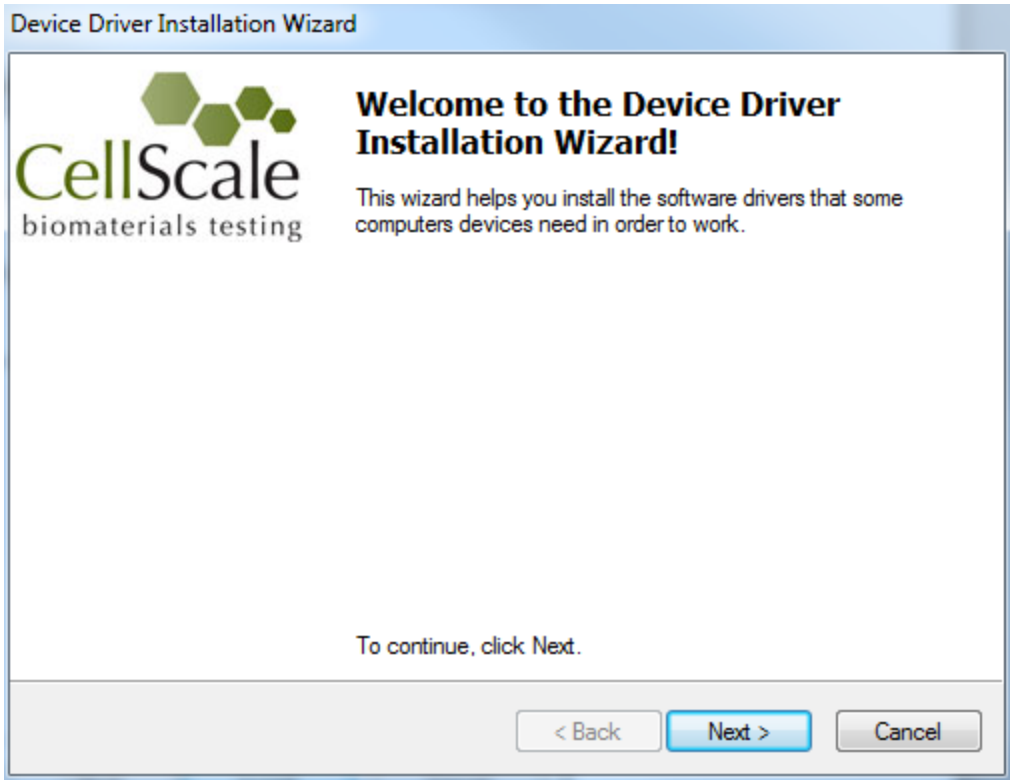

MechanoCulture - InstallShield Wizard
Welcome to the InstallShield Wizard for MechanoCulture
The InstallShield(R) Wizard will install MechanoCulture on your computer. To continue, click Next.
WARNING: This program is protected by copyright law and international treaties.
< Back
Next >
Cancel

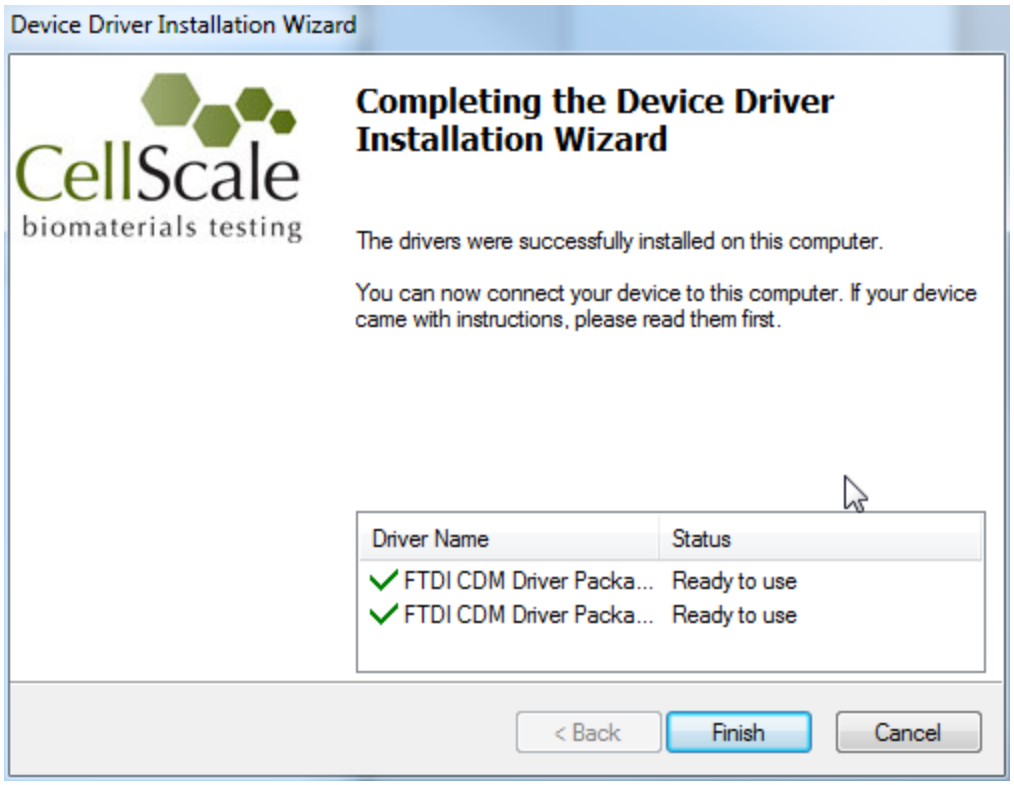

MechanoCulture - InstallShield Wizard
License Agreement
Please read the following license agreement carefully.
SOFTWARE LICENSE AND WARRANTY
Read this agreement carefully. If you agree to its terms, press YES to continue with the Setup. If you do not agree to its terms, press NO to abort the installation.
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

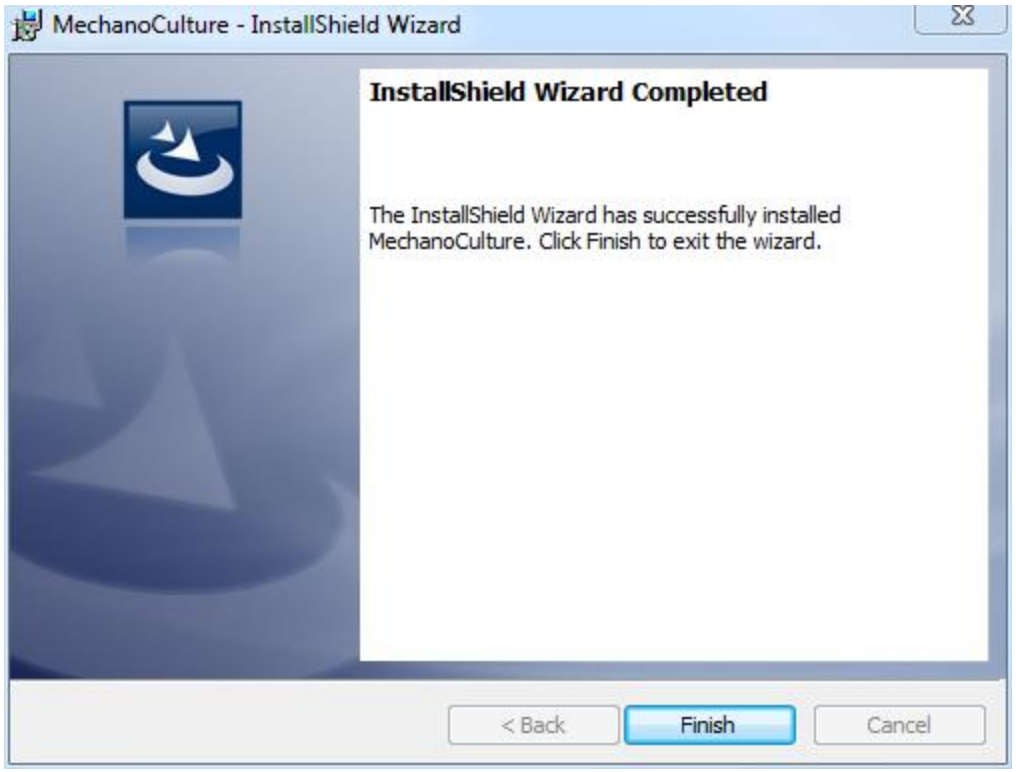

MechanoCulture - InstallShield Wizard
Customer Information
Please enter your information.
User Name:
User
Organization:
CellScale
InstallShield
< Back
Next >
Cancel


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


Device Driver Installation Wizard
CellScale
biomaterials testing
Completing the Device Driver Installation Wizard
The drivers were successfully installed on this computer.
You can now connect your device to this computer. If your device came with instructions, please read them first.
Driver Name
Status
FTDI CDM Driver Packa...
Ready to use
FTDI CDM Driver Packa...
Ready to use
< Back
Finish
Cancel


MechanoCulture - InstallShield Wizard
InstallShield Wizard Completed
The InstallShield Wizard has successfully installed MechanoCulture. Click Finish to exit the wizard.
< Back
Finish
Cancel

12. Appendix C: Pressure/Vacuum System Information

Pneumatic Connections

For select systems, both a Pressure (positive gauge pressure) and Vacuum (negative gauge pressure) inputs are provided, as depicted in the following figure.

For these systems, please ensure to connect both these inputs to their respective supplies even if only one will be used in your test sequence. This is required to completely seal the system to allow fine control of the internal pressure.

Example Operation

For an example of this system in operation, please see Example 2, Section 8 of this document. This describes the system in hydrostatic testing mode, applying both a negative and positive gauge pressure to the samples under test.

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