CellScale
biomaterials testing

MechanoCulture J1

Mechanical Stimulation System

User Manual

Version 1.5

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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.

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 J1 is a precision instrument specifically designed for tensile 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	1.5 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, Micro-SD card for data storage

System Assembly

The MechanoCulture J1 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 bottom chamber properly attached.

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, see Appendix B for further instructions.

2. MechanoCulture J1 Components

The MechanoCulture J1 consists of a clear polycarbonate well-plate 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 with 6 individual chambers, O-rings, and cover plates. The control box contains the master controller, the linear actuators, load cells and specimen grips.

Component	Description
Well Plate	The well plate contains 6 wells. The well plate is made from polyether ether ketone (PEEK) and can be sterilized using an autoclave.
Chamber Face Plate O-Ring	Placed in the grove surrounding each well to completely seal the sterile culture area against the front well plate cover.
Specimen Grips	A specimen grip is attached to the end of each load cell and the bottom of the well plate with an O-ring in between to seal the chamber from the bottom. These are used to clamp the specimens in the wells. The top grips are threaded onto a pull rod and locked using a locking nut. How to assemble this device is shown in Section 5.
Control Box	The top control box houses all the linear actuators which drive the system 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, USB to program and read data and a removable MicroSD card
M4 Fasteners	4 x M4x20mm fasteners are used to secure the Control Box to the Well Plate, these are tightened using the long T-handled 3mm hex key.
Peristaltic Pump	This system is liquid cooled using a reservoir of approximately 10L of room temperature water and the supplied 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.

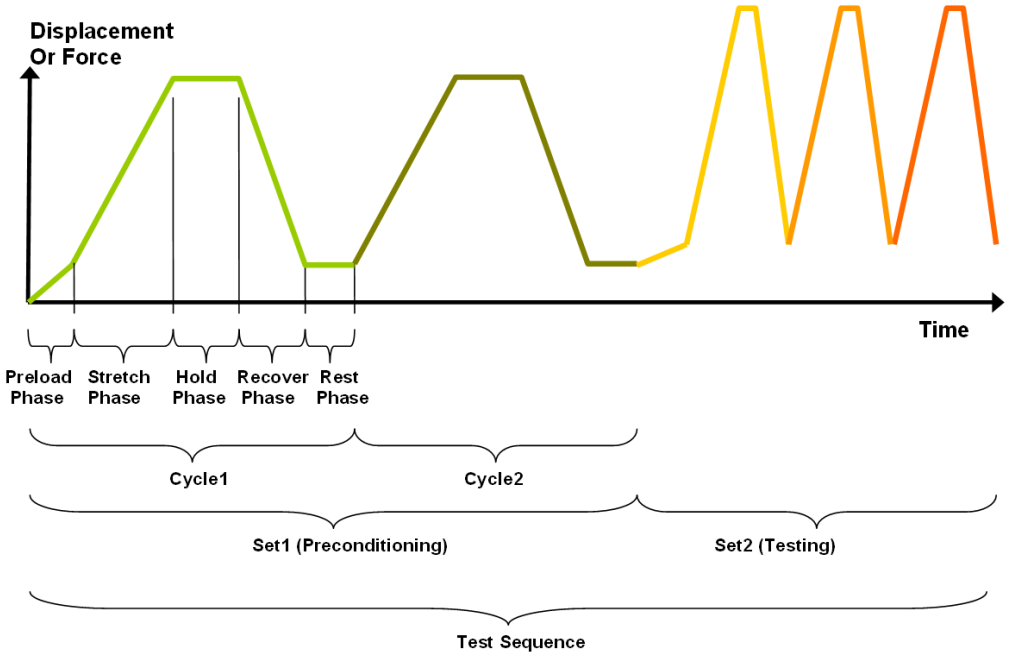
3. Testing Terminology

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

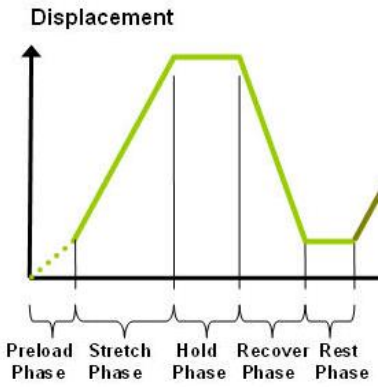
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

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.

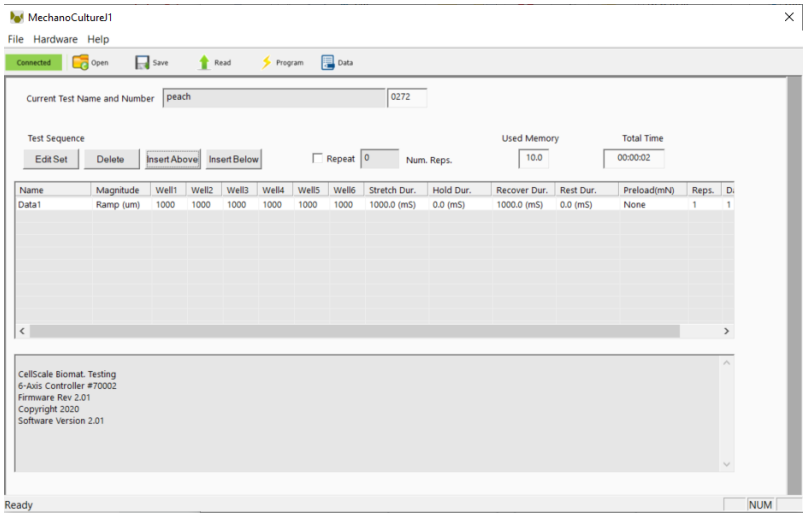


4. Software Overview

The MechanoCulture J1 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

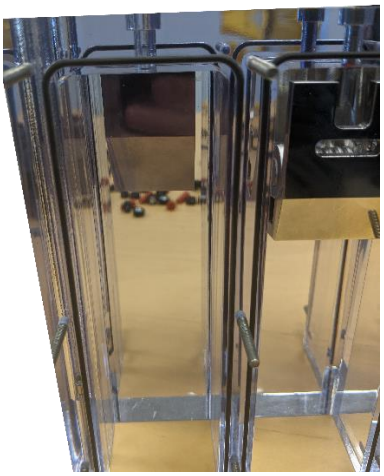


The test parameters section of the main window shows the current testing protocol. Errors and other information are displayed in the Message Window section.

5. Setting Up and Starting a Test

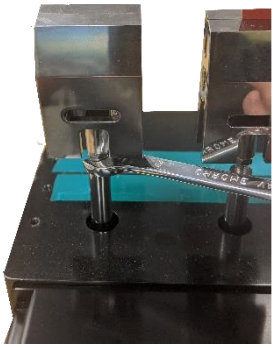
Setting up a test involves loading specimens and generating a test sequence protocol. Data generated during testing can be accessed after the test is completed. Details of this process are described below:

Step 1: System Preparation



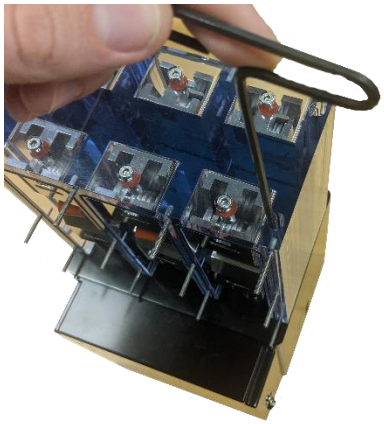
1. Begin by draining the wells if they are filled with fluid. Once it is drained the faceplates can be removed. Next the samples loaded must be removed from one set of grips. Once the samples have been removed, the well plate can be removed by taking off the 4x M4 fasteners.

2. The pull rods, grips, and well plate can be removed and sterilized.



3. Ensure the exterior O-rings are all properly in place. Next the bottom grip can be fastened on the well plate using the M4 fastener and an O-ring.
4. Reinstall the pull rods on each actuator. **Be cautious to not over tighten them**. Next, install the M4 lock nut followed by the top grips on the pull rods. Lock the jam nut against the grips using a 7mm wrench.

   Note: **Do not overtighten** the grips or the pull rods. This can result in damage to the load cells.



5. The well plate can be mounted back on the system using the M4 fasteners.

6. The samples can be inserted into the grips. Open the grips by hand and insert the samples into the grips using the supplied angled forceps.

7. When the samples are loaded, the faceplates can be installed using the M3 thumbnuts. If you are submerging your samples in fluid, the system must be turned right-side up and the plugs must be inserted before filling the wells.

Step 2: System Setup

Once the samples have been loaded into the device the next steps are to connect all power and data cables (electrical connections) and connect the peristaltic pump for the liquid cooling.

Electrical Connections

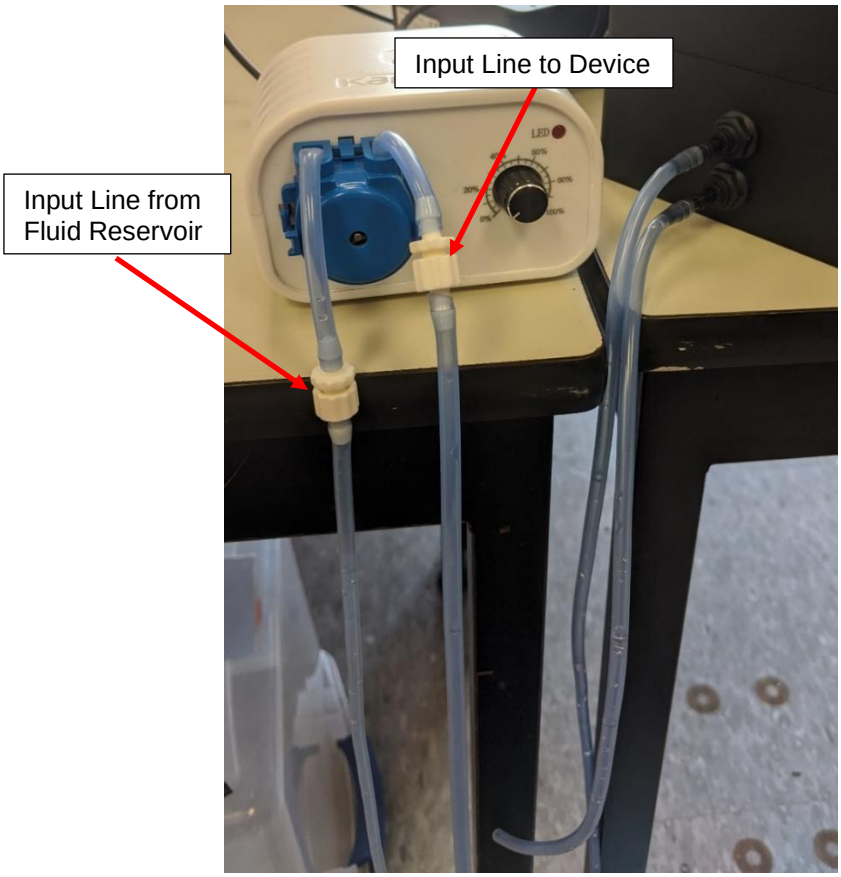
1. Connect the 24V DC power supply cable. Plug it into an appropriate 100-240VAC power source and the other end into the system.

2. 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.

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 J1 Note: Clearing Cooling Lines

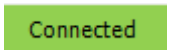
Once you are finished with your test you must clear the cooling tube lines, this is done by running the pump dry. To do this leave the pump running and remove the feed line from the cooling reservoir and leave the system to pump air through the tubing. It is complete once no water is coming out the outlet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

MechanoCulture J1 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 J1 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 

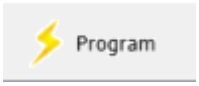
and the hardware and software versions should be listed in the message window.

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 J1 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 J1 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

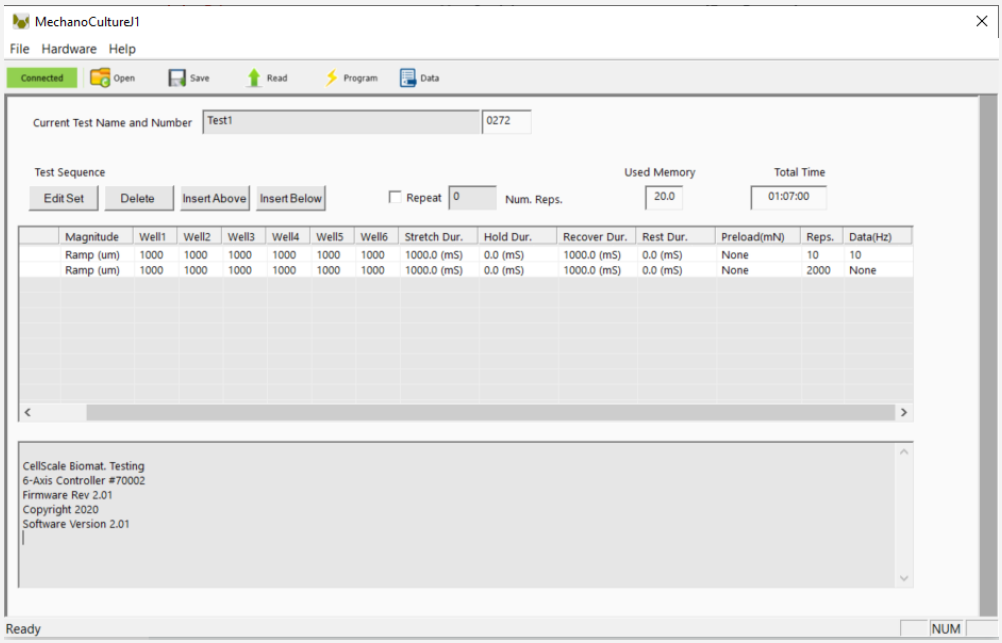
button on the toolbar.

MechanoCulture J1 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.

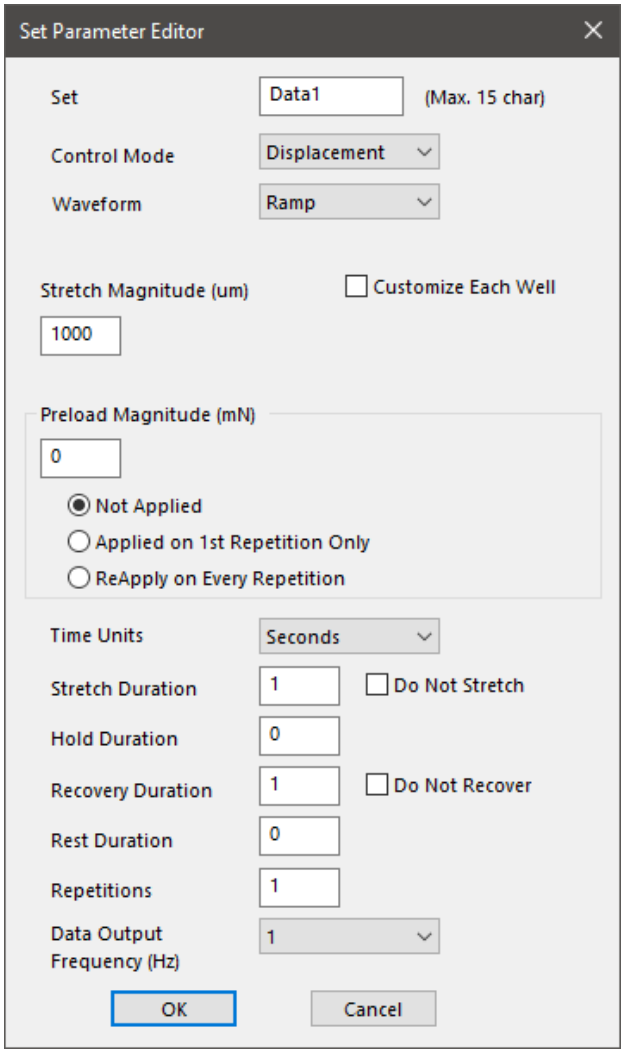
MechanoCulture J1 Note: Long Tests

The MecahnoCulture J1 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:



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 window, seen here, will appear.



The test parameters editor allows you to modify:

- Set Name
- Control Mode
- Waveform
- Stretch 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.
Stretch Magnitude	The displacement magnitude is in um and force magnitude is mN. The maximum displacement magnitude is 20000um. 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.
Stretch Duration	The time over which the stretch phase of a cycle is applied. The total duration of a cycle is the sum of the Stretch, 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.

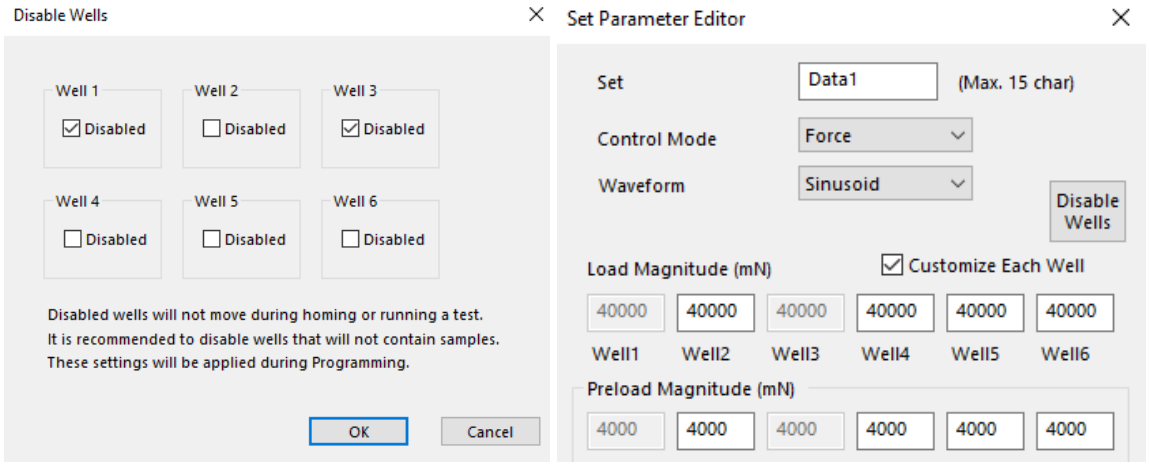
MechanoCulture J1 Note: Do Not Stretch or Recover

For certain specimens or protocols, it may be useful to not stretch 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 clock 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 J1 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.

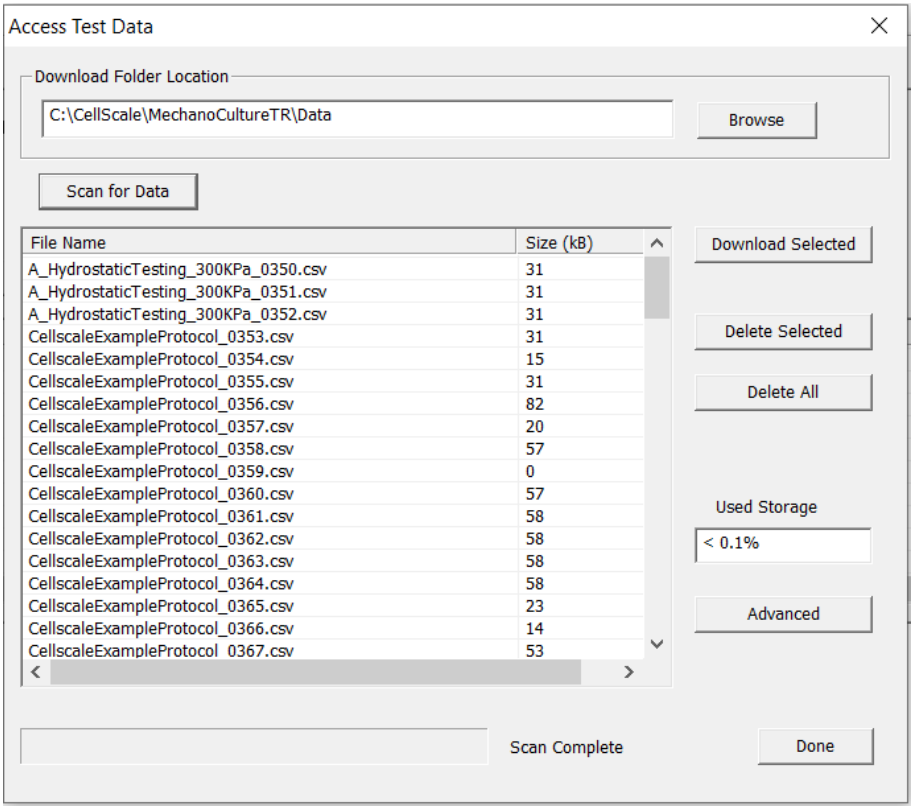
Step 5: Accessing Data

Data collected during a test protocol is saved directly onto the device and can be accessed from the MechanoCulture J1 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.

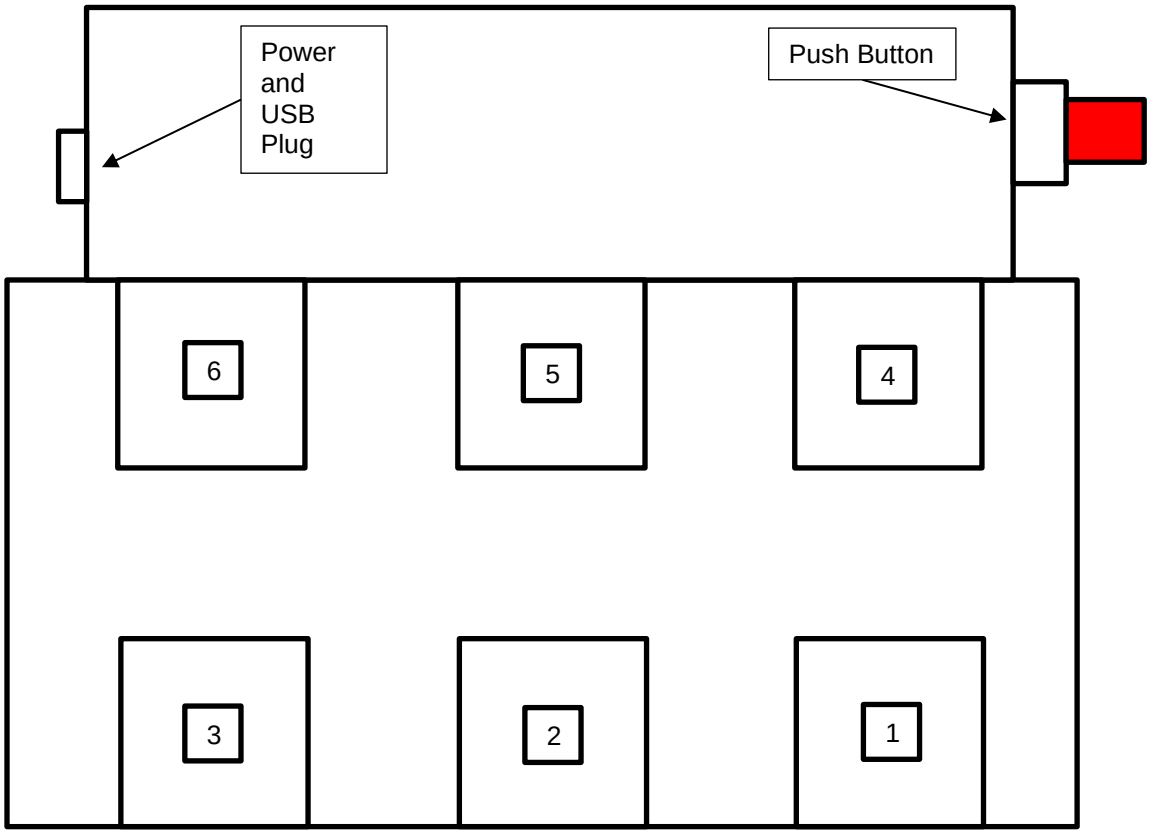


Files that are downloaded will be placed in a sub-folder with the name of the test. 2 files are downloaded for each test. A .csv data file and a .tmt protocol (template) file. In addition, a TestLogs sub directory is created within each folder. This sub directory contains 2 additional .csv files that may be useful for troubleshooting or debugging.

If your test produces extremely large files, the MicroSD card can be removed and plugged directly into the computer and the files can be extracted. The SD card must be re-installed before another test can be run.

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. Well numbering 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 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.

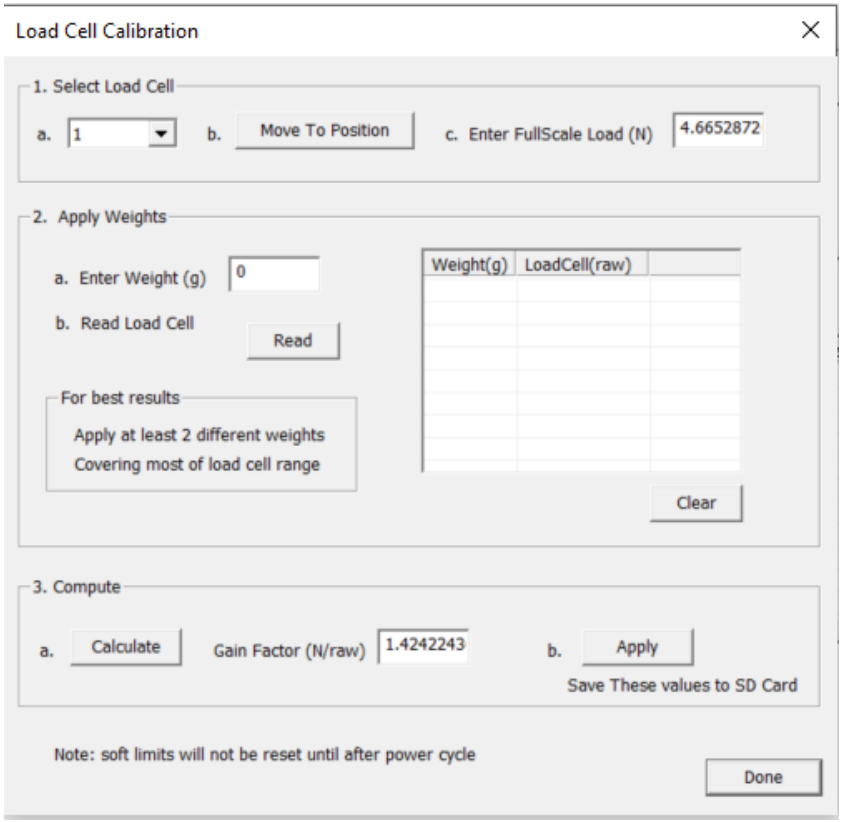
Csv _Full data file (contained in TestLogs sub folder)

The full data file contains additional output columns such as specified pressure and actual pressure and is normally not used or viewed 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:



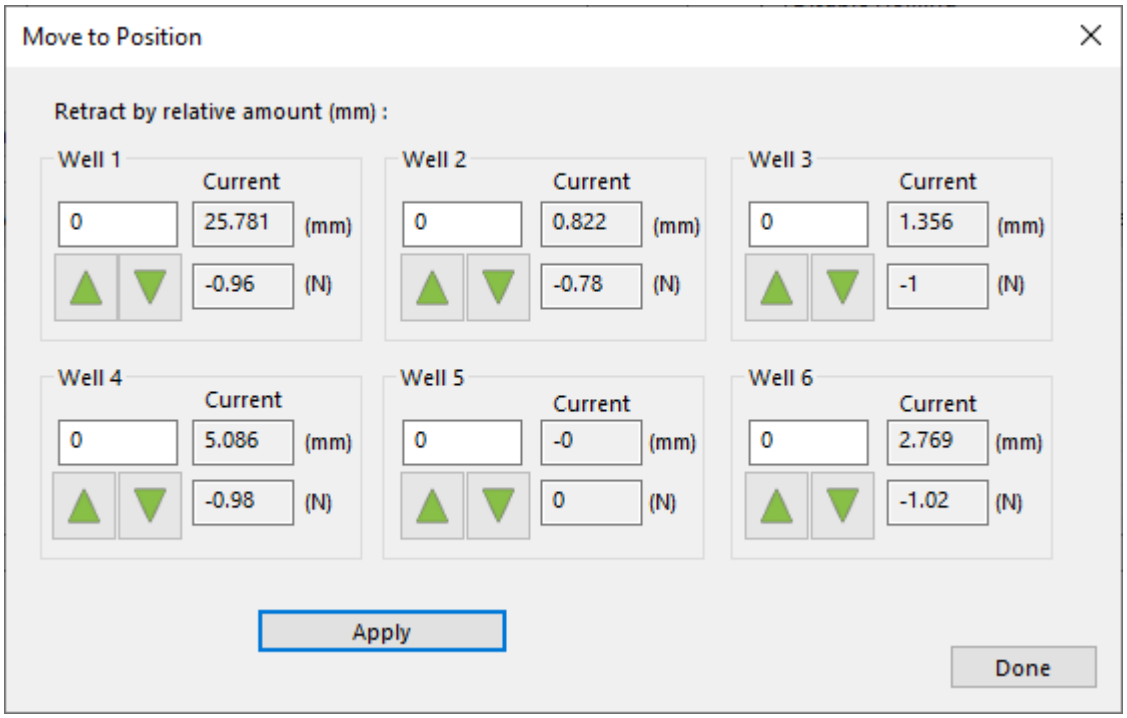
Each load cell is calibrated individually, before doing the calibration the system must be removed from the clear well plate and flipped upside down.

> ***MechanoCulture J1 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.

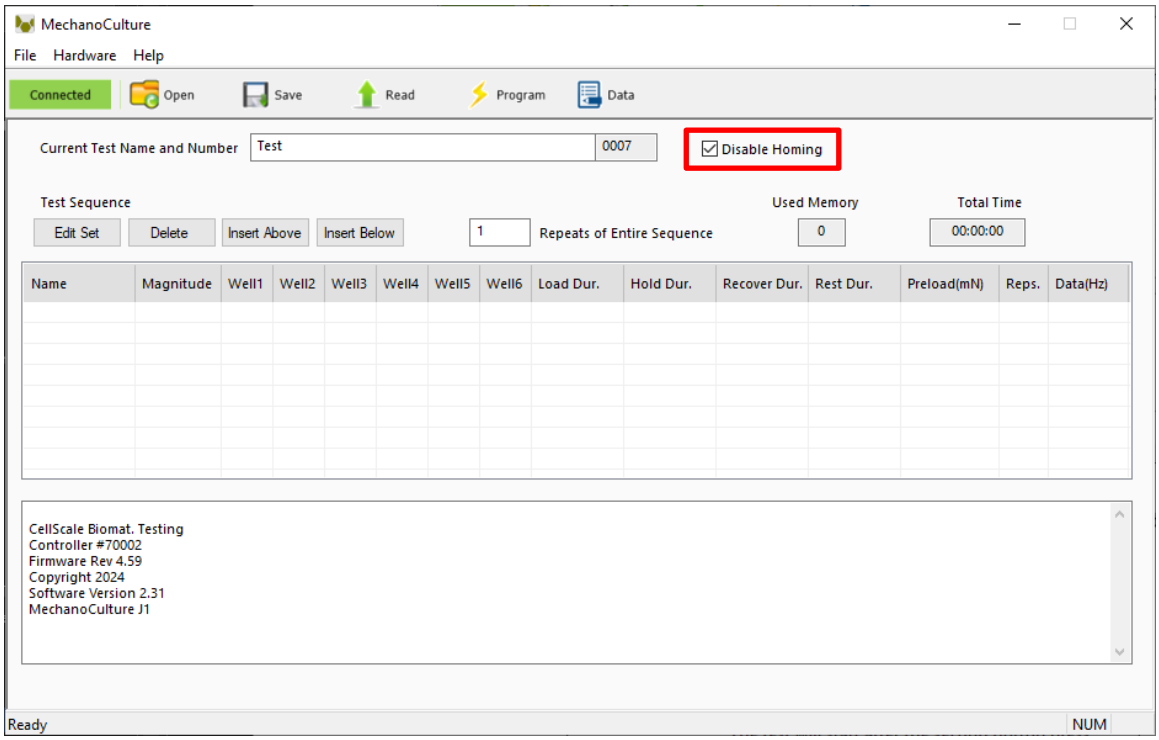
Note the movements are relative to the current position of each actuator. The force readings of each load cell are displayed as well.



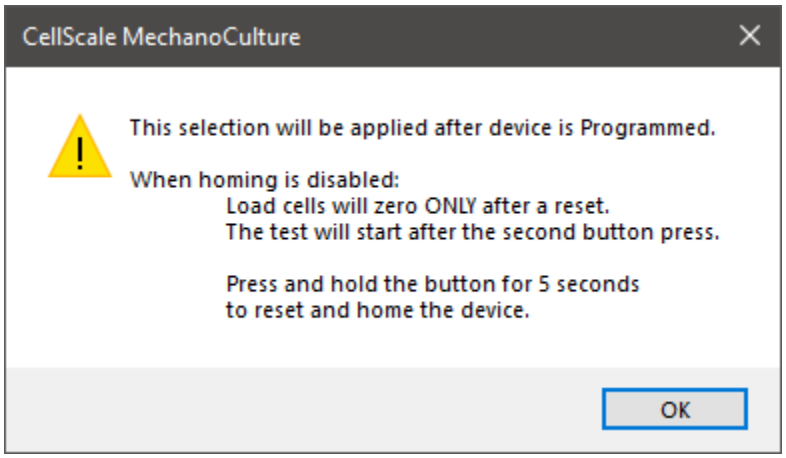
Disable Homing

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

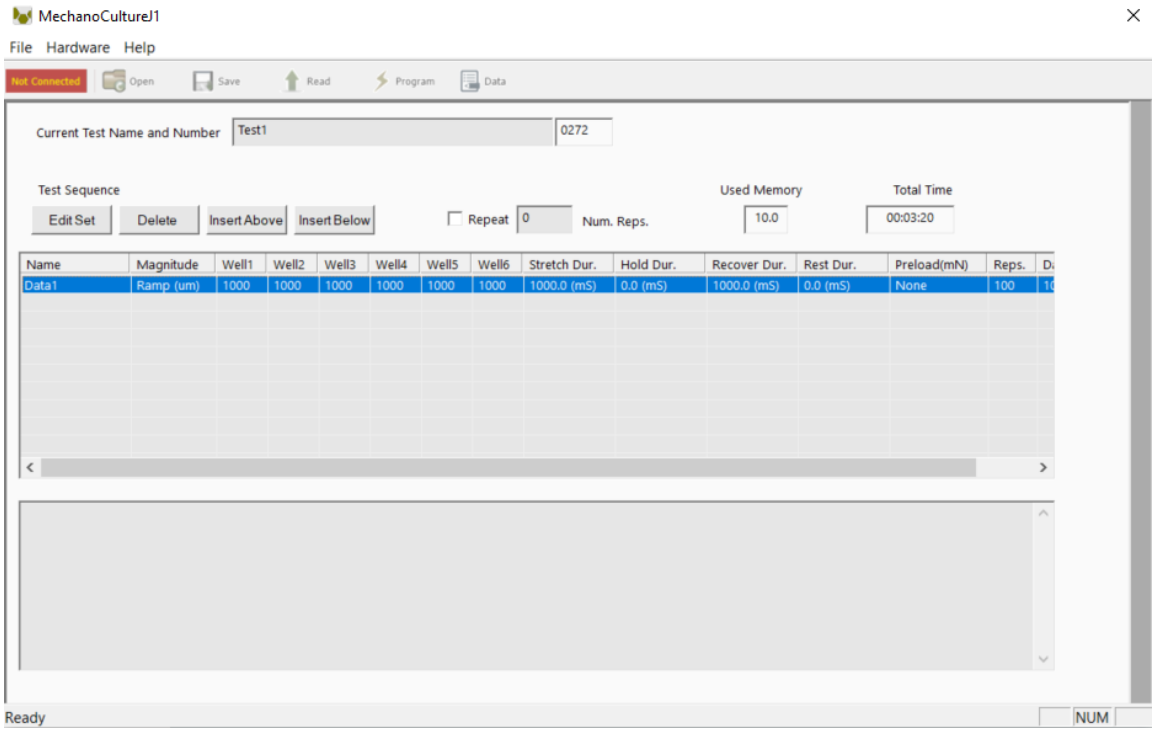
The MechanoCulture J1 software allows for complex test sequences to be generated. The examples in this section illustrate how to generate different types of test protocols.

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 stretch 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

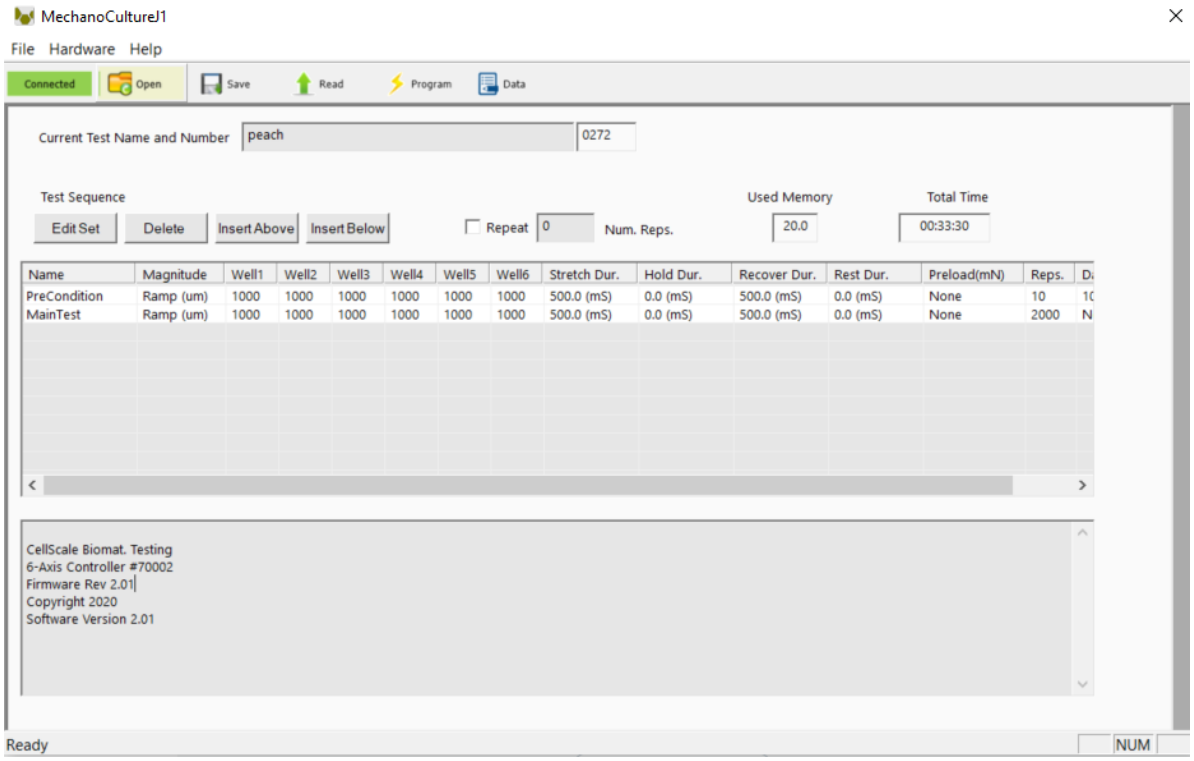


Example 2: Multiple Sets (Smaller Data Files)

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 tension 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

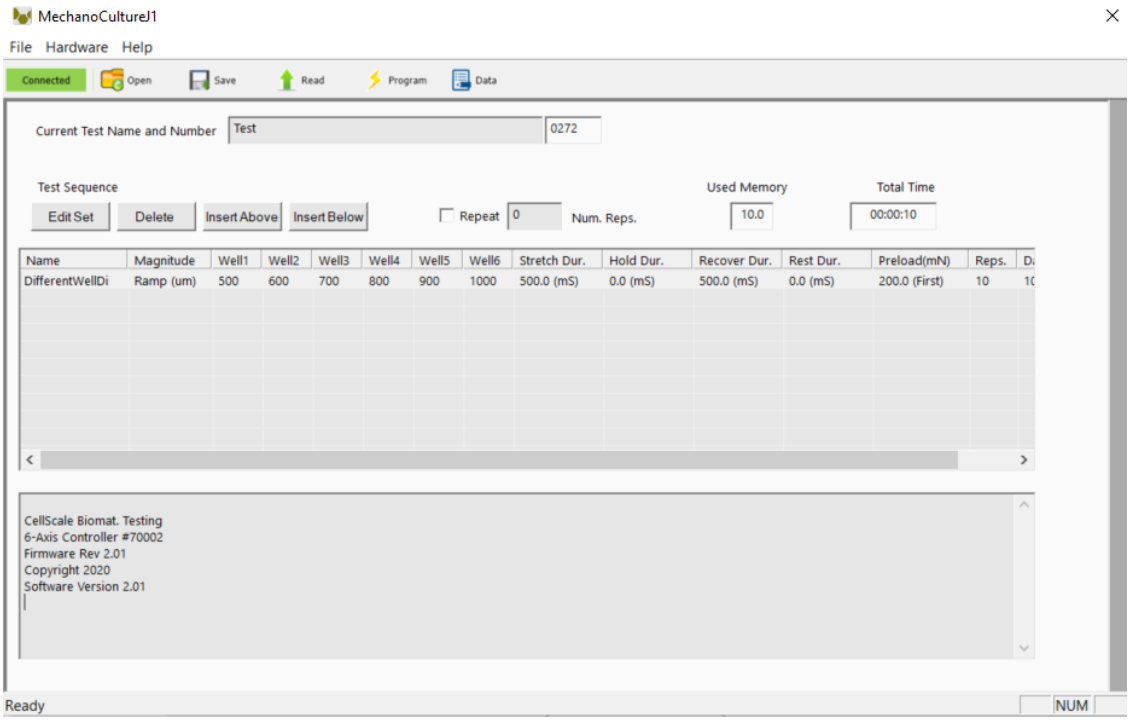


Example 3: Unique Well Displacement

The MechanoCulture J1 is capable of running very customizable tests and this example showcases it. This example is a test where each of the 6 wells is displacing a different stretch magnitude and a preload applied on the first repitition.

The following program executes an example test, which includes a 500um, 600um, 700um, 800um, 900um and 1000um for wells 1, 2, 3, 4, 5, and 6 respectively with a 500mS stretch and 500mS recover for 10 reps with 10Hz data output with a 200mN preload applied on the first repition only.

Field	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
Magnitude	500um	600um	700um	800um	900um	1000um
Preload Magnitude	200mN	200mN	200mN	200mN	200mN	200mN
Stretch Duration	500 ms	500 ms	500 ms	500 ms	500 ms	500 ms
Hold Duration	0 ms	0 ms	0 ms	0 ms	0 ms	0 ms
Recover Duration	500 ms	500 ms	500 ms	500 ms	500 ms	500 ms
Rest Duration	0 ms	0 ms	0 ms	0 ms	0 ms	0 ms
Repetitions	10	10	10	10	10	2000
Data	10 Hz	10 Hz	10 Hz	10 Hz	10 Hz	10Hz

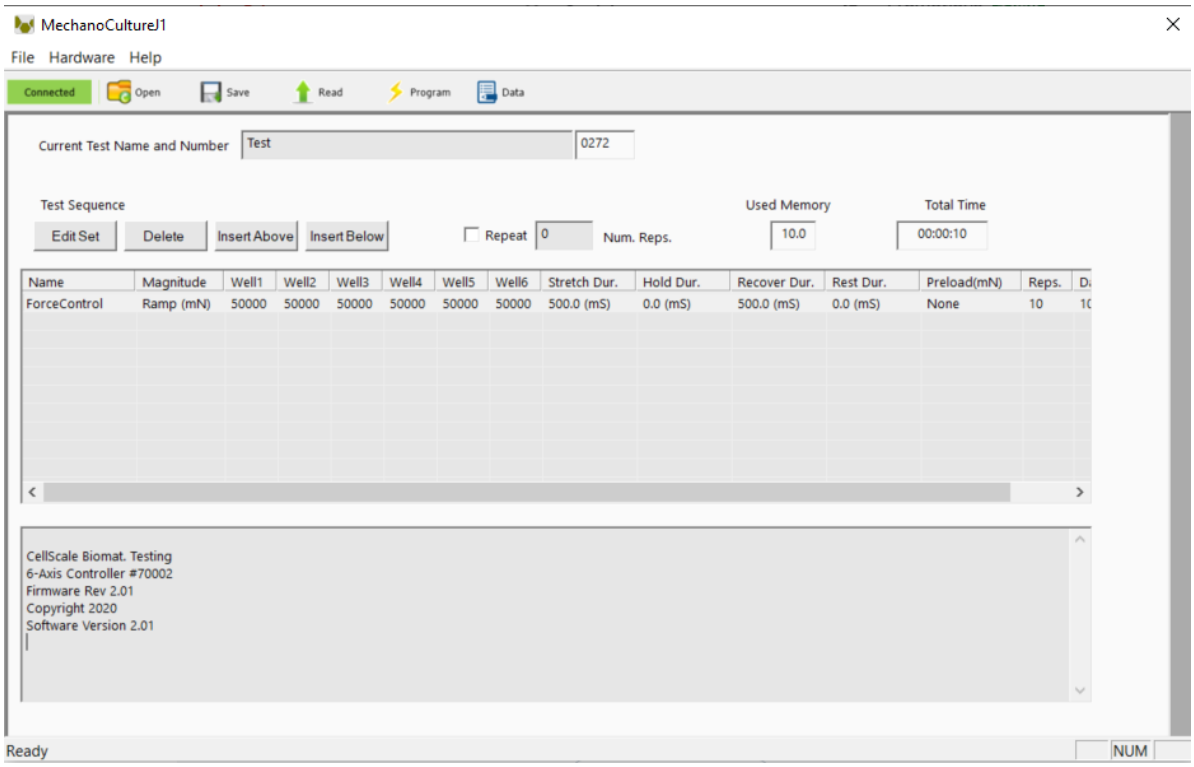


Example 4: Force Control

A test sequence where each sample is diven to the same force regardless of the displacemnt needed to get there is very valuable.

The following program executes an example test, the test is a force controlled test where each sample is tensioned to 50N with a 1000mS stretch and recover for 10 reps with 10Hz data output and no preload applied.

Field	Force Control Test
Magnitude	50000mN
Stretch Duration	1000mS
Hold Duration	0 ms
Recover Duration	1000mS
Rest Duration	0 ms
Repetitions	10
Data	10 Hz



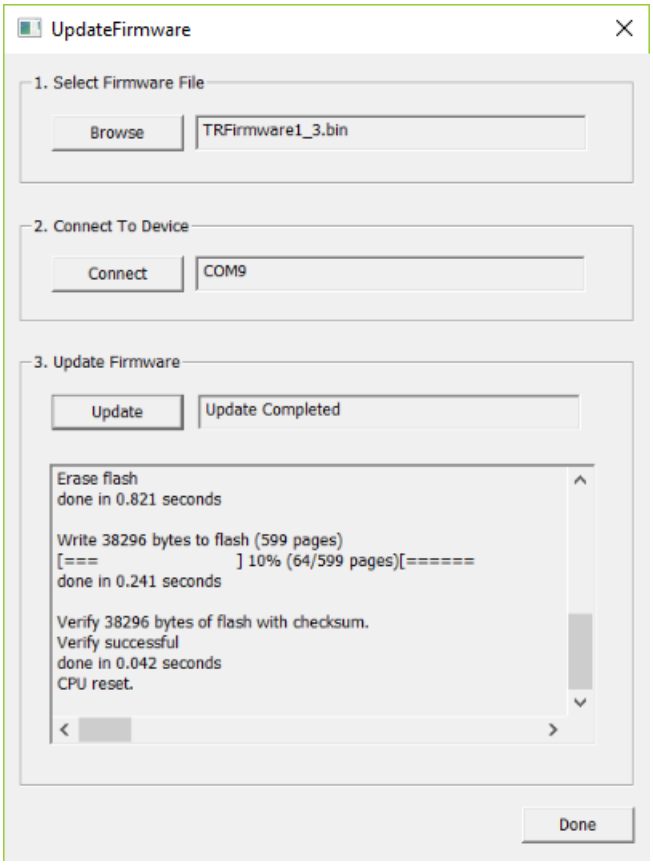
8. Troubleshooting

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

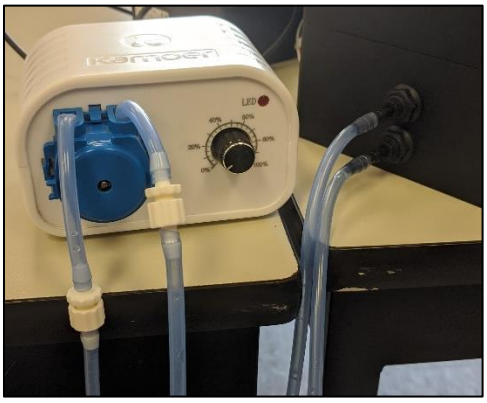
1. With the MechanoCulture J1 connected to the PC and turned on, launch the firmware update software located in the Windows start menu under MechanoCulture>Utilities> UpdateFirmware.exe.
2. Load the firmware file (*.bin) using the “Browse” button.
3. Click the “Connect” button.
4. Execute the update using the “Update” button.

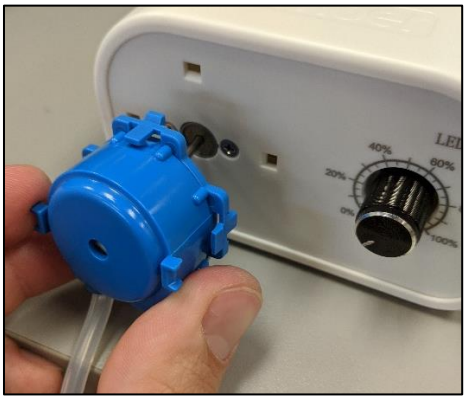


10. Appendix B: Replacing Peristaltic Pump Tubes

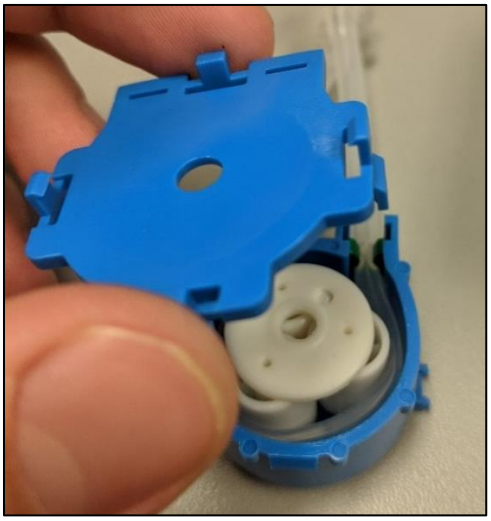
After approximately 50hrs of operation the peristaltic pump tubing must be changed to avoid a rupture of the line and leakage inside the pump. To replace the tubing follow the instructions below:

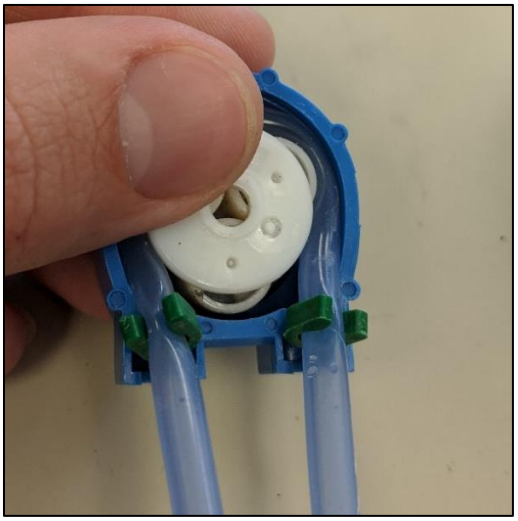
1. Remove the tube from the system and the water reservoir and run the pump dry to remove any excess liquid.
2. Now that the tubes are clear of any liquid, remove the long sections of tubing from the pump head by twisting the Luer lock fittings. Now the pump head can be removed from the pump motor. This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





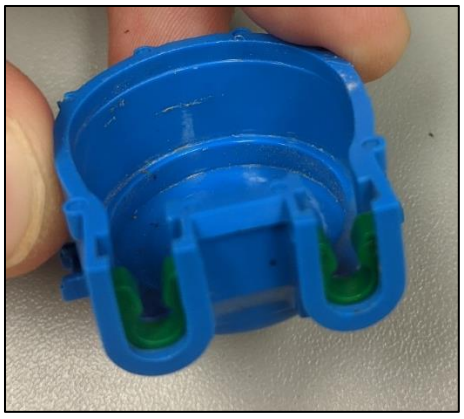
3. Once the pump head is removed take the back cover off to expose the rotating cam and tubing. With the back cover off lift the tubing up and out of the green clips which are used to hold it in place. Once the green clips are removed you can lift the cam and tubing out of the assembly. Be careful as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

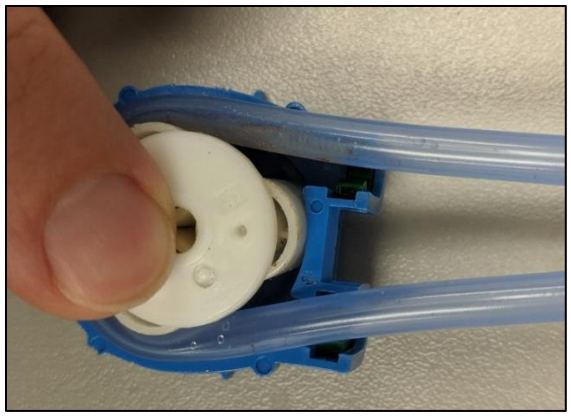




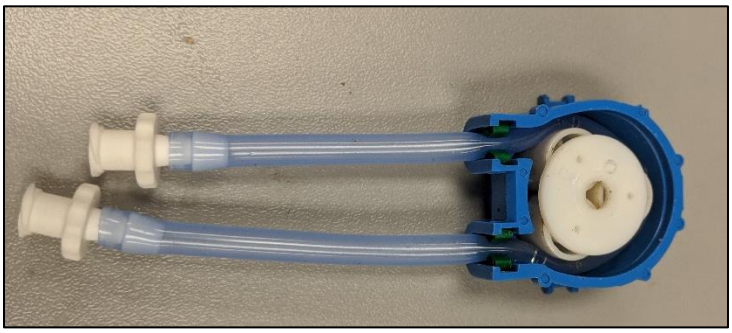
4. With the cam and old tube removed, you can remove the luer lock barbed fittings and place them into the new tube.

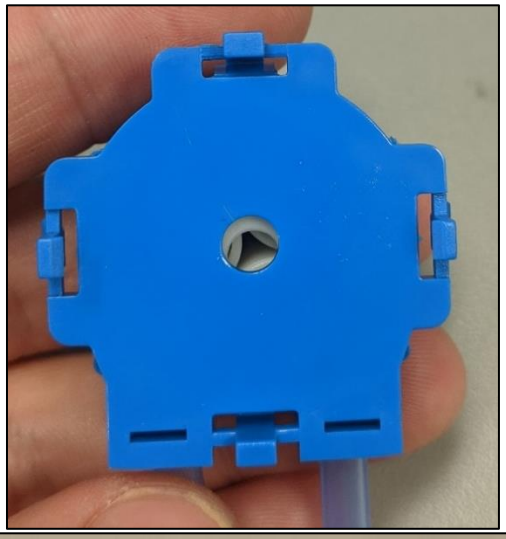
5. Place the green clips back into their slots, wrap the tube around the cams and push it into place in the center of the housing.





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.



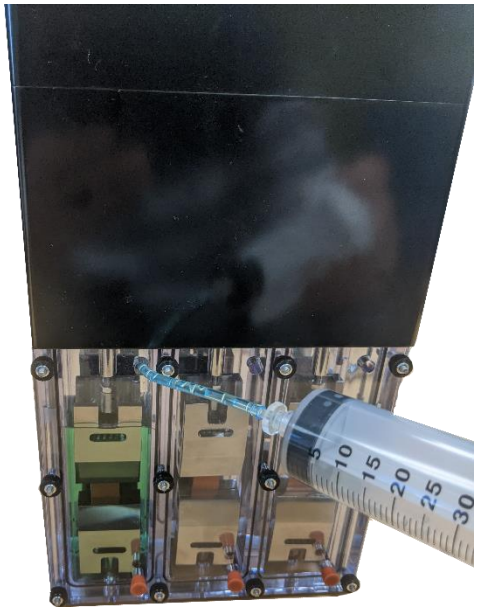


11. Appendix C: Fluid Profusion Setup

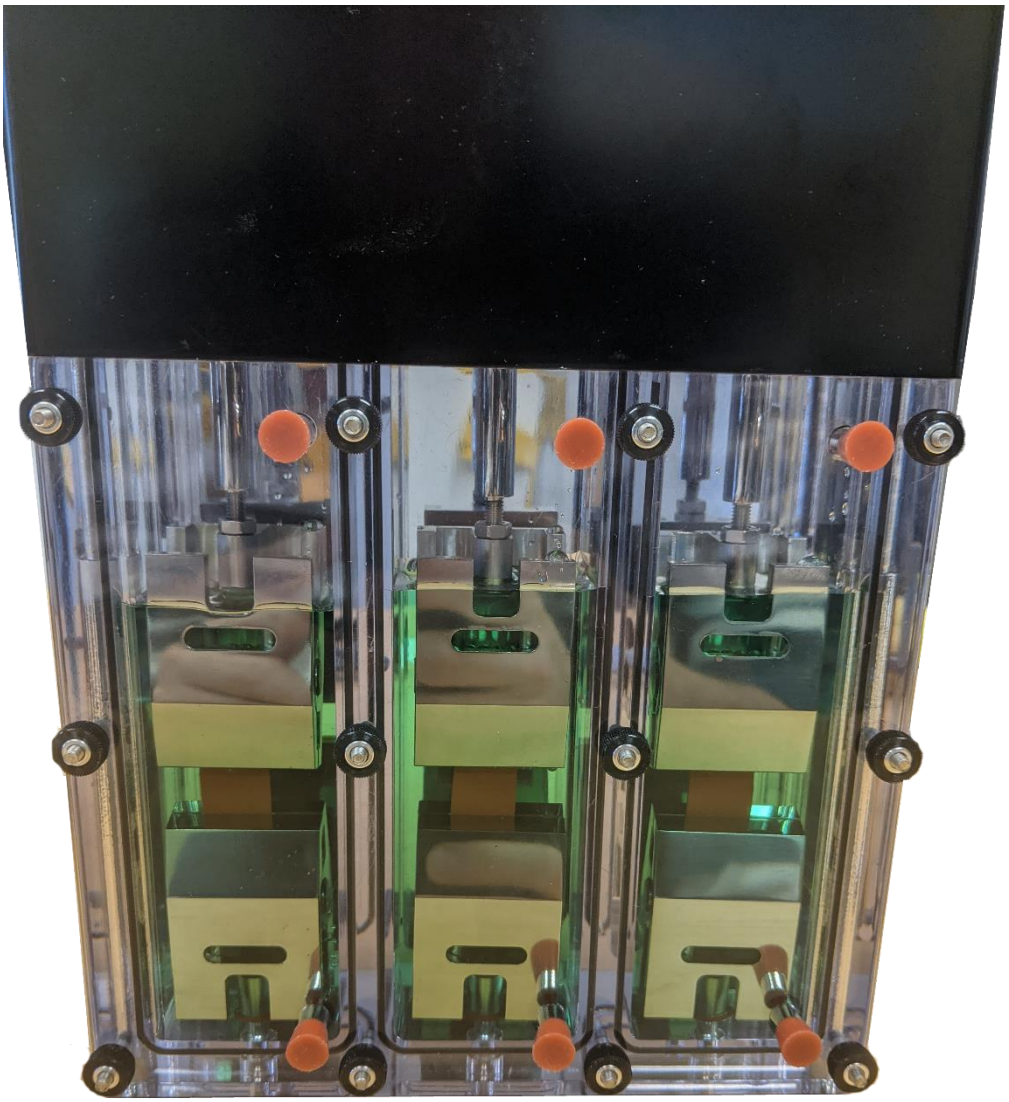
The MechanoCulture J1 system can submerge any samples inside of each well plate. The Large well plate is 40mm wide with a volume of 150mL and the small well plate has chambers 15mm wide with a volume of 30mL. To fill the wells, you will need to follow the below steps:

1. Once the system is fully assembled with the sample mounted properly the well plate can be installed using the 12 M3 thumb screws in order to form an adequate seal. Before filling the chamber make sure the bottom plugs are inserted.

2. A syringe and tubing are required to fill each well one at a time as they are separate from each other. Fill the well simply insert the tubing into the top hole of the well plate and flow the media in.



3. Once each well is filled with the desired amount of media the top plugs can be inserted and the test can be run.



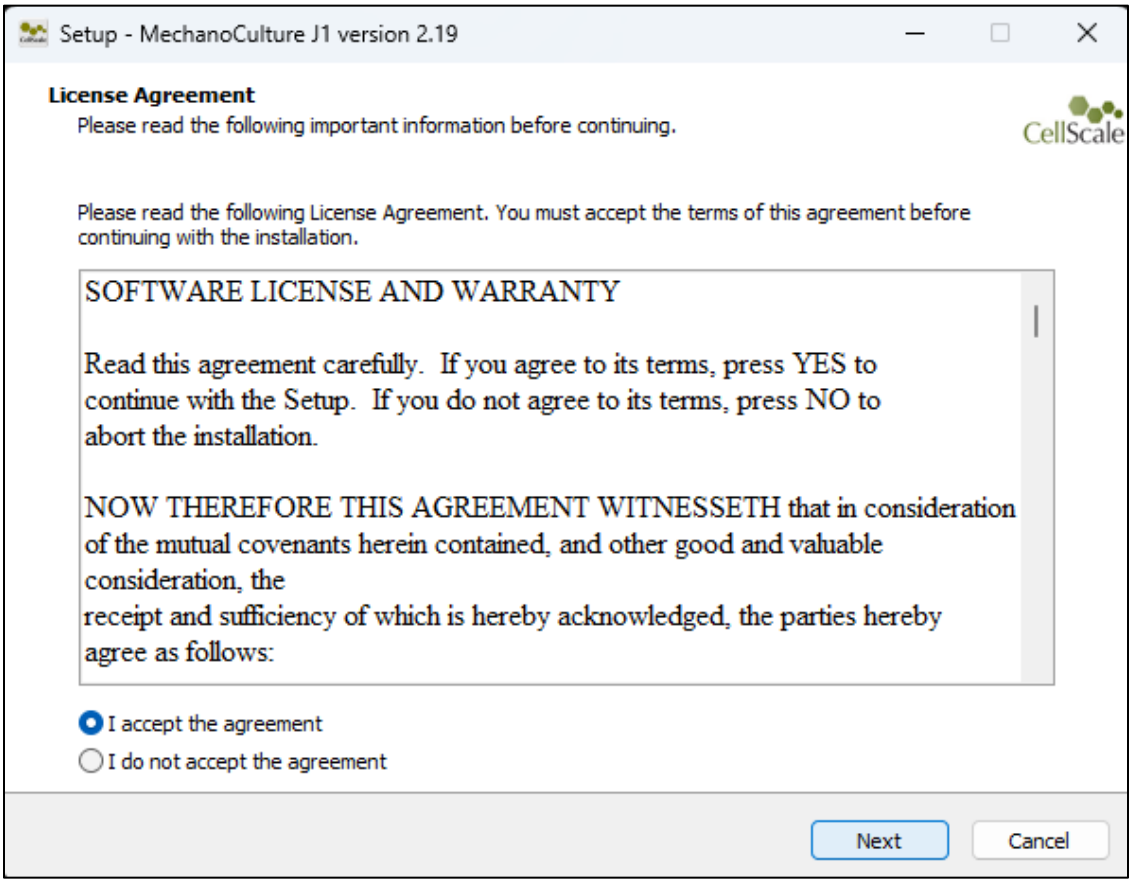
4. Once the test is complete and the media needs to be replaced, bring the MechanoCulture J1 system to where the media is to be disposed of and remove the bottom plugs to drain each well.

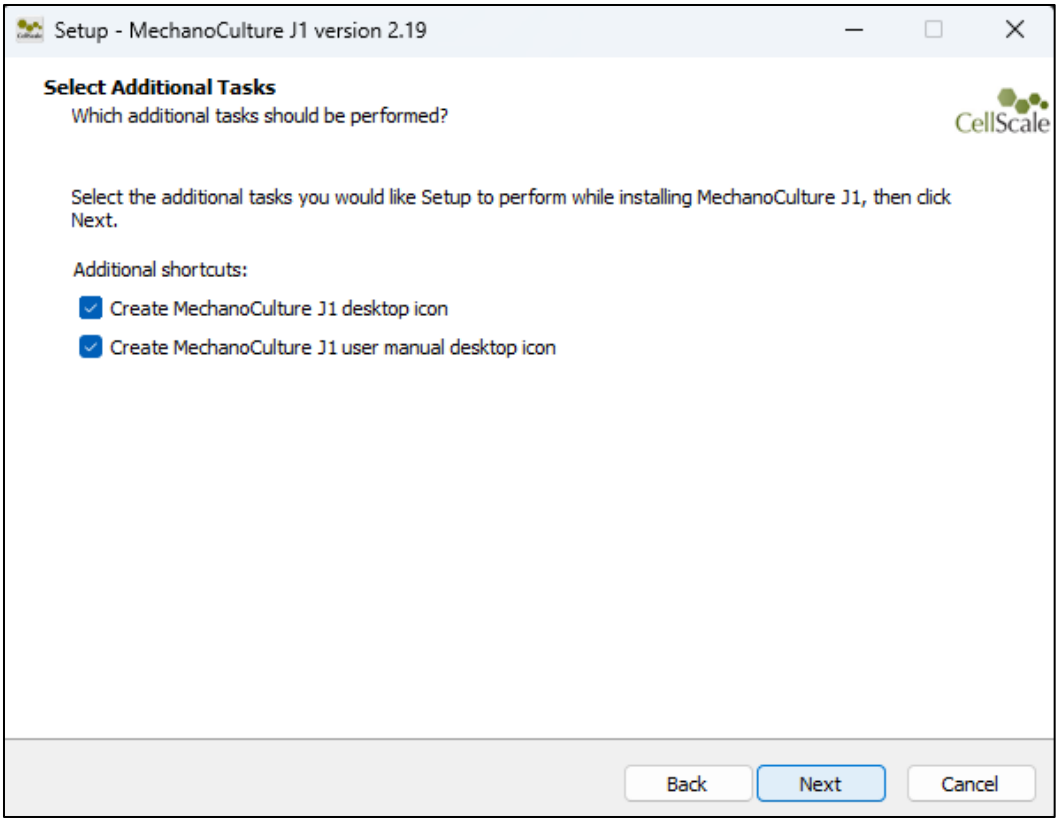
12. Appendix D: Software Installation

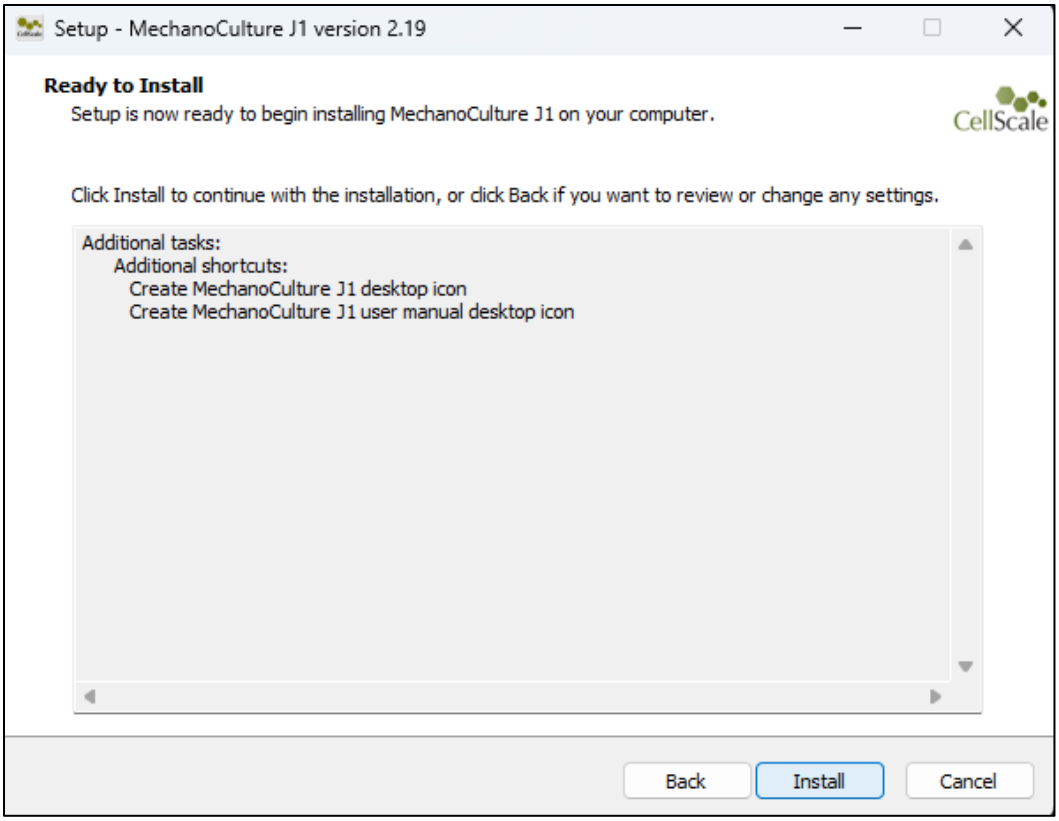
Included in your shipment is a link to install the MechanoCulture J1 software. This installation package contains the installer for the software to program your MechanoCulture J1 devices.

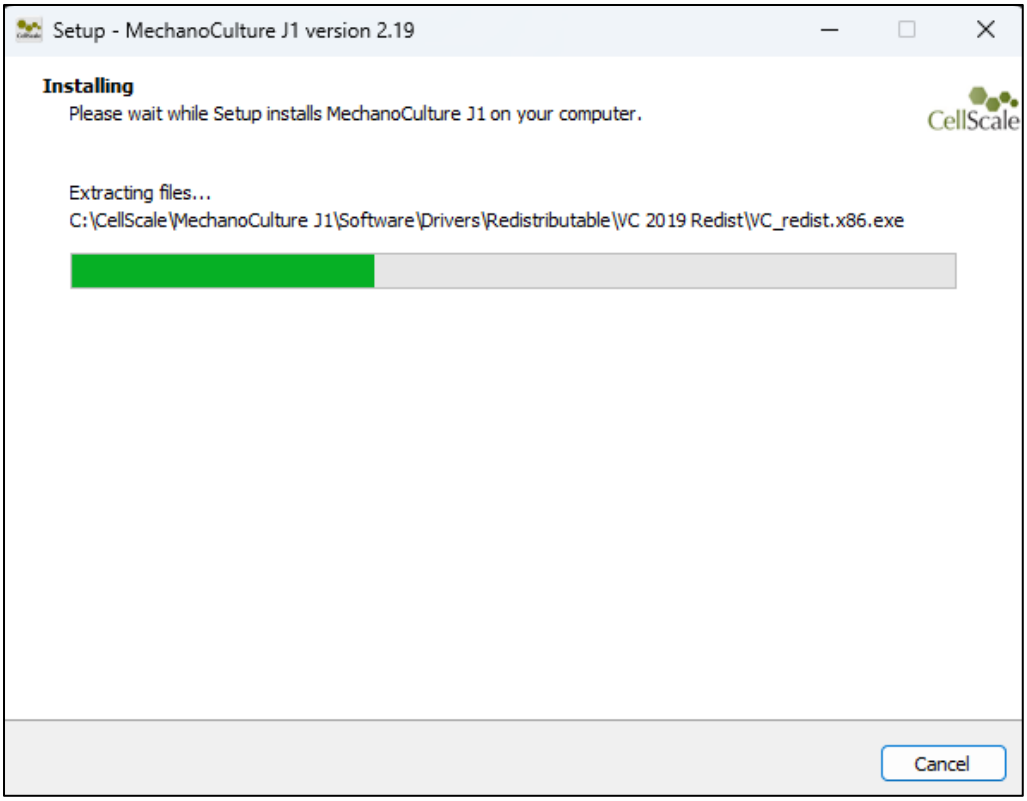
To install the software:

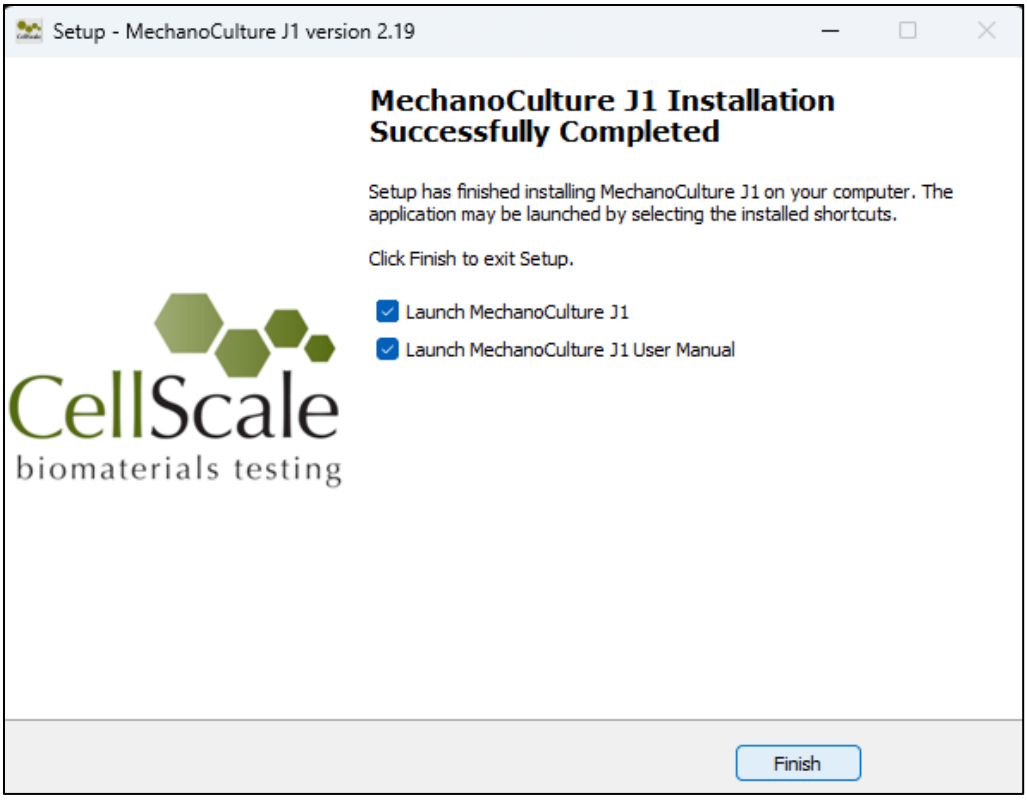
1. Go to the provided web address and download the compressed installation package.
2. Transfer this file to the computer which will be running the MechanoCulture J1 device.
3. After this is complete, go to the uncompressed installation folder and select *MechanoCulture J1 Installer.exe*. Details of typical dialogue boxes are shown below.




Setup - MechanoCulture J1 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 J1, then click Next.
Additional shortcuts:
Create MechanoCulture J1 desktop icon
Create MechanoCulture J1 user manual desktop icon
Back
Next
Cancel


Setup - MechanoCulture J1 version 2.19
Ready to Install
Setup is now ready to begin installing MechanoCulture J1 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 J1 desktop icon
Create MechanoCulture J1 user manual desktop icon
Back
Install
Cancel





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