

Mechanical Stimulation System

User Manual version 1.5

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 T6 is a precision instrument specifically designed for deforming flexible membranes and 3D matrices in a sterile fluid environment. This device is capable of uniaxial deformations on flexible cell-seeded substrates or scaffolds. It has an onboard actuator and control board to enable it to run independent of a computer in an environment-controlled incubator.

Electrical Input	24VDC
Current Rating	1.2 Amp
Environmental Conditions	Maximum Operating Temperature 40°C
	0% - 100% Relative Humidity
Installation Category	Category II
Pollution Degree	Degree 2
Data Connections	mini USB for programming

Environmental and Electrical Specifications

System Assembly

The unit does not require initial assembly but requires partial disassembly/reassembly between uses.

Connections to Supply

Connect power cord into properly grounded 100-240VAC power source to ensure safe operation. The mains supply voltage fluctuations should not exceed 10% of the nominal supply voltage.

Safety Warnings

This equipment must be used in accordance with the procedures outlined in this manual to prevent injury and/or damage.

The unit should not be operated without the actuator housing or media chamber lid in place.

Manual Operating Controls

There is one push-button switch on the front of the control unit. A single push will start and stop the test sequence. A double push will cause the actuator to advance to the load position before pausing the test sequence. A push and hold for 5 seconds will advance the actuator and reset the test sequence to the beginning. *The noise heard during at actuator reset move is normal and does not damage the system*.

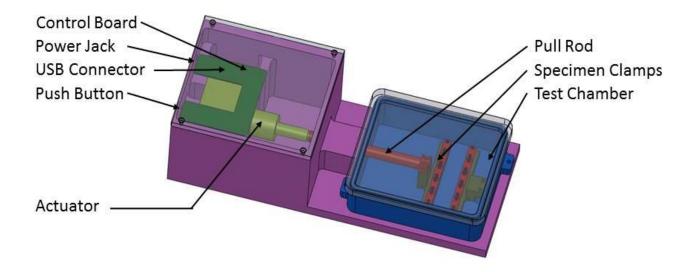
General Maintenance

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

Approvals and Certification

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

2.Components Overview



	Main Body Material: Anodized Aluminum Cleaning: Ethanol on exterior
0	Actuator Housing Lid Material: Anodized Aluminum Cleaning: Ethanol on exterior
	Test Chamber Material: 316 Stainless Steel Cleaning: Ethanol, UV, Autoclave
	Pull Rod Material: 316 Stainless Steel Cleaning: Ethanol, UV, Autoclave
	Specimen Clamps Material: 316 Stainless Steel (incl. fasteners) Cleaning: Ethanol, UV, Autoclave

Chamber Lid Material: Polycarbonate Cleaning: Ethanol, UV, Autoclave
Power Supply
USB Cable
Circuit Board Cleaning: None

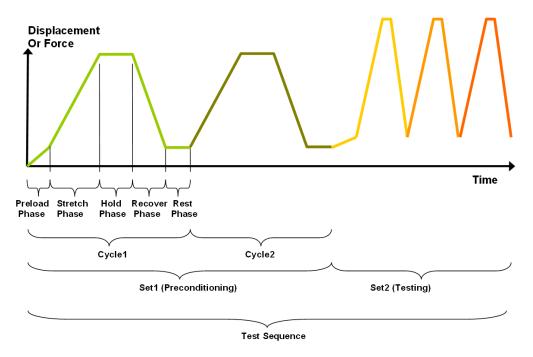
3.Testing Terminology

Multiphase Test Cycles

In order to properly mechanically stimulate/signal cells, it is often necessary to apply more than one load magnitude or frequency during the course of the test.

Phases, Cycles, and Test Sequences

As the following diagram demonstrates, each application and release of the 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

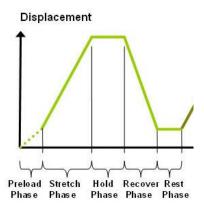
The test phase is the smallest unit of the test specification. There are four phases within a cycle. Each phase serves a specific purpose:

Stretching - During the stretch phase, a deformation is applied to the specimen.

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

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

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



Control Functions

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

The ramp function applies the displacement at a constant velocity.

The **sinusoid function** applies the displacement according to a sinusoid with the desired displacement magnitude and duration. The sinusoid is approximated as a series of linear segments.

4. Software Overview

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

ſ	M	MechanoCulture - Mee	chanoCulture1							×
	Fi	le Hardware Help								
		Connected 🕞 Open 🔜 Save 🛧 Read 🗲 Program 🏟 Jog+ 🔤 Gog-								
		Test Sequence Edit Set Dele	ete Insert 4	bove Insert E	elow	Offset (mm) 6.00	2	Used Memory (%)	T otal Time	
Test		Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions			
Parameters										
Message		CellScale Biomat. Testing							*	
Window		SW# 74051 Rev 1.1 Software Version 1.46	Copyright 2	014						
		MechanoCulture Model T-	6							
									-	
		1								
	Re	ady							NU	ЛМ

Step 1: Initiate a Test Sequence

Test sequences can be initiated in 3 different ways:

- 1. A predefined test sequence can be opened from a MechanoCulture Protocol File (*.tmt). From the File menu, select Open, or press the Open button on the toolbar.
- 2. A currently programmed test sequence on a device can be read. From the Hardware menu, select Read, or press the Read button on the toolbar. To read a MechanoCulture device it must be connected to the computer's USB port with a USB cable and it must be powered on.
- 3. A new test sequence can be created by pressing the Insert Above or Insert Below buttons to add sets to the test sequence. Each set will have its own row in the table and sets will be executed sequentially. A test sequence can have a maximum of 8 sets.

Step 2: Modify Testing Parameters (optional)

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

Note that displacements are specified in mm and are relative to the current position. Time units are applied to the entire set but different sets can have different time units.

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

et Parameter Editor	×
Control Function	Ramp 💌
Stretch Magnitude	5 mm 💌
Time Units	Seconds 🔹
Stretch Duration	10 Do Not Stretch
Hold Duration	0
Recovery Duration	10 Do Not Recover
Rest Duration	0
Repetitions	3
OK	Cancel

Step 3: Program a Device with the Specified Test Sequence

From the Hardware menu, select Program, or press the Program button on the toolbar. To program a MechanoCulture device it must be connected to the computer's USB port with a USB cable and it must be powered on.

MechanoCulture Tip: Saving Test Sequence Protocols

Once you have developed a test sequence, you can save it as a MechanoCulture Protocol file for future use. Select Save from the file menu, or press the Save button on the toolbar. You can then open this test sequence the next time you initiate a test sequence.

5.Designing Test Sequences

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

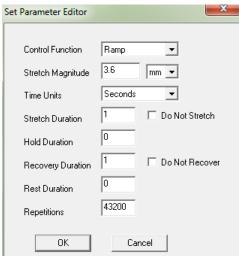
Example 1: Continuous cycling for fixed duration and then stop

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

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

0.5 Hz requires a period of 2 s. In this example this is divided into 1 s of stretch and 1 s of recovery with no hold or rest.

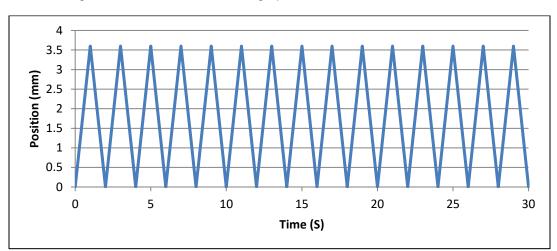
To achieve a 24 hr test duration requires 43200 cycles. (24 hr x 3600 s/hr / (2 s/cycle)).



MechanoCulture Tip: Terminating a test

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

Test Sequence				Offset (mm)	
Edit Set Dele	ete Insert A	bove Insert B	elow	0.00	0
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions
Ramp 3.6 (mm) Ramp 0.0 (mm)	1.0 (S) None	0.0 (S) 0.0 (d)	1.0 (S) None	0.0 (S) 1.0 (d)	43200 21



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

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

1.

2.

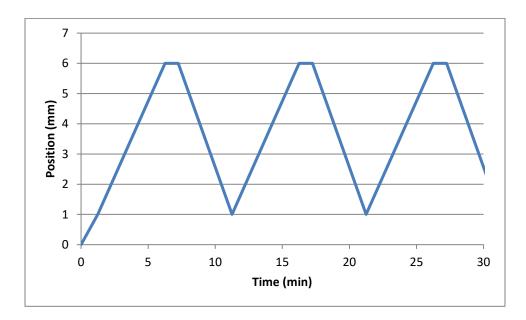
Objective: Create a test sequence that starts with a 1 mm pre-stretch in 75 seconds followed by 100 cycles of an additional 5 mm stretch (5-minute stretch, 1-minute hold and 4-minute recover).

Press Insert Above to create a Set. Then double click on that row to modify it.	Set Parameter Editor	
Check the Do Not Recover box to omit the recovery phase.	Control Function	Ramp
	Stretch Magnitude	1 mm 💌
	Time Units	Seconds 🗨
	Stretch Duration	75 🗖 Do Not Stretch
	Hold Duration	0
	Recovery Duration	0 🔽 Do Not Recover
	Rest Duration	0
	Repetitions	1
	ОК	Cancel
	Set Parameter Editor	
Press Insert Relow to create a second set	Set Parameter Editor	
Press Insert Below to create a second set. Modify this set as follows: Note that the 5 mm	Set Parameter Editor	Ramp 💌
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current		
Modify this set as follows: Note that the 5 mm	Control Function	Ramp V
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the	Control Function Stretch Magnitude	Ramp
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the	Control Function Stretch Magnitude Time Units	Ramp
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the	Control Function Stretch Magnitude Time Units Stretch Duration	Ramp
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the	Control Function Stretch Magnitude Time Units Stretch Duration Hold Duration	Ramp
Modify this set as follows: Note that the 5 mm stretch magnitude is relative to the current position of 1 mm from the previous set (the	Control Function Stretch Magnitude Time Units Stretch Duration Hold Duration Recovery Duration	Ramp 5 5 Minutes 5 Do Not Stretch

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

Test Sequence							Offset (mm)
Edit Set	Delete	e Insert Above Insert Below			0.00			
Magnitude	Stret	ch Dur	Hold	Dur	Recover Dur	Res	t Dur	Repetitions
Ramp 1.0 (mm)	75.0	(S)	0.0 (9	i)	None	0.0	(S)	1
Ramp 5.0 (mm)	5.0 (r	n)	1.0 (m)		4.0 (m)	0.0	(m)	100
Ramp 0.0 (mm)	None	9	0.0 (c)	None	21.0) (d)	1

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



Example 3: Intermittent Stretching

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

1.	Press Insert Above to create a Set. The double click on that row to modify it.	hen	Set Parameter Editor	<u> </u>
	double click on that low to moully it.		Control Function	Ramp
			Stretch Magnitude	2 (mm)
			Time Units	Seconds 💌
			Stretch Duration	0.5 Do Not Stretch
			Hold Duration	0
			Recovery Duration	0.5 Do Not Recover
			Rest Duration	0
			Repetitions	000
			ОК	Cancel
2.	Press Insert Below to create a second set.		Set Parameter Editor	
			Control Function	Ramp
			Stretch Magnitude	0 (mm)
			Time Units	Minutes
			Stretch Duration	0 Do Not Stretch
			Hold Duration	
			Recovery Duration	0 Do Not Recover
			Rest Duration	50
			Repetitions	1
			ОК	Cancel

3. In this case, a test set with lengthy rest duration is not required since the test is designed to repeat indefinitely. The test sequence is shown below:

Test Sequence				Offset (mm)	
Edit Set De	elow	0.00	0		
Magnitude	Stretch Dur	Hold Dur	Recover Dur	Rest Dur	Repetitions
Ramp 2.0 (mm)	0.5 (S)	0.0 (S)	0.5 (S)	0.0 (S)	600
Ramp 0.0 (mm)	None	1.0 (m)	None	50.0 (m)	1

6.Setting Up & Running a Test

Overview

As a user of the MechanoCulture, you are likely to develop your own test preparation and execution protocol, but there are several elements that are common to most users. This manual will not cover cleaning, disinfecting, sterilization, tissue culturing, reagents, and a number of other topics.

The protocol below should be used for running new test sequences. To re-run existing sequences on new specimens, simply power-up the device, ensure the system has been reset, mount specimens, and press the push button to execute the test.

Power-up Device

On power-up, the device may automatically reset the actuator position. *The noise heard during at actuator reset move is normal and does not damage the system*. The only time the actuator will not reset is if the previous protocol was paused prior to power down. In this case, the system can be reset by holding down the device button for 5 seconds. If it is not reset, then a single button press will continue the previously paused protocol.

Connect the Device to the PC via USB

To connect the device, the motor housing cover must first be removed. The mini-USB connector is located on the control board inside the motor housing cover.

Launch the MechanoCulture Software

The software should show a green "connected" box in the upper left corner.

Generate a Test Sequence

Follow the previous instructions to generate a test sequence that will stimulate the specimen in the desired fashion.

Load Specimen(s)

Load specimens and tighten clamp screws to hold them in place. If less than 6 specimens are being tested, position them as close to the middle of the chamber as possible.

It may be helpful to jog or move the specimen clamps to a new starting position. This can be

done using the jog or buttons done using the jog or buttons or the offset function (see Advanced Parameters)

Execute Test

To execute the test sequence, simply press the push button once. To pause the sequence, press the button again at any point. Once the sequence is paused, power can be removed and the then restored without affecting the test protocol. To reset the device, press and hold the button for 5 seconds.

7.Advanced Parameters

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

Hardware Advance	d	×
Series Name	Model Number	Restore Defaults
0	Offset (mm)	
25	Actuator Travel Soft Limit	(mm)
27.9984	Actuator Stroke (mm)	
	Program Do	one

Offset: Offset is a distance that controls the starting stretch magnitude of each test. For example, a stretch of 2mm will stretch from 5mm to 7mm when the offset is 5mm. Click Program and then reset the MechanoCulture using the pushbutton to change the offset. During a reset the actuator fully extends and then retracts a small amount, defined by the offset. The default value for offset is 0mm.

Actuator Travel Soft Limit: The T6 actuator has 1" (or 25.4mm) of travel. The soft limit should be kept slightly less than this to prevent over-travel during manual jogging of the actuator. If desired, this value can be set even lower to prevent accidental damage to specimens. To change the soft travel limit, click program and then reset the MechanoCulture using the pushbutton.

Actuator Stroke: The physical travel capability of the stepper motor to drive the lead screw. For the MechanoCulture T6, this value is 27.9984mm.

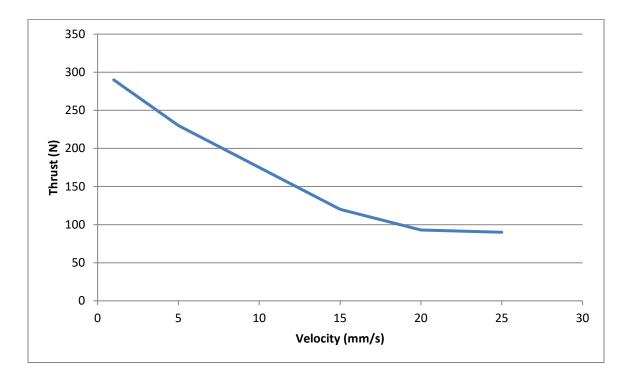
8. Appendix A: Updating the Firmware

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

CellScale Firmware	Update 🛛 🕅
Load Update File	Rev2_0.upd
Not Connected	Status Window
Connect	
Update	

9. Appendix B: Actuator Thrust

The actuator thrust curve for the MechanoCulture T6 is shown below.



10. Appendix C: Software Installation

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

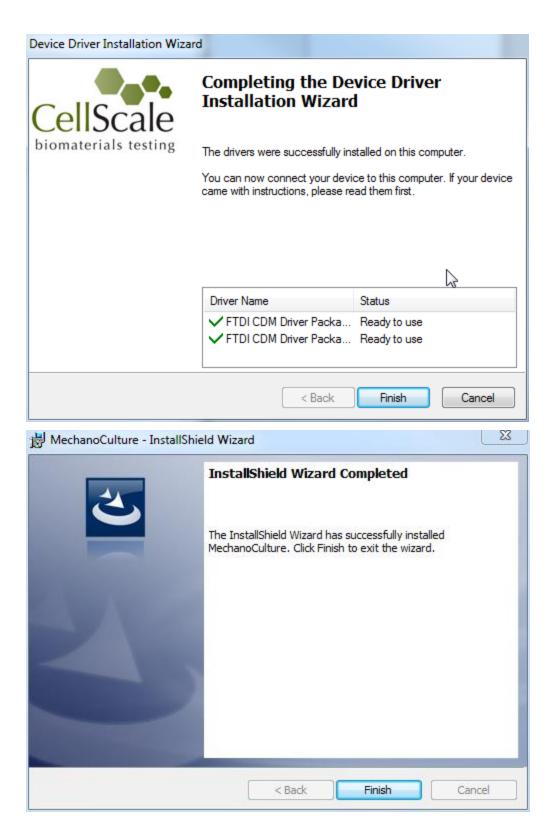
To install the software, go to the provided web address and download the compressed installation package. Transfer this file to the computer which will be programming the MechanoCulture. Right click on the file and select "Extract All..." to unzip. After this is complete, go to the unzipped installation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

岁 Mechano	Culture - InstallShield Wizard	x
	CellScale biomaterials testing www.cellscale.com	
InstallShield —	< Back Next > Cancel	

B MechanoCulture - InstallShie	eld Wizard
2	Welcome to the InstallShield Wizard for MechanoCulture
	The InstallShield(R) Wizard will install MechanoCulture on your computer. To continue, dick Next.
	WARNING: This program is protected by copyright law and international treaties.
	< Back Next > Cancel
。 湯 MechanoCulture - InstallShie	eld Wizard
License Agreement Please read the following licen:	se agreement carefully.
SOFTWARE LICENSE A	AND WARRANTY
-	ally. If you agree to its terms, press YES to f you do not agree to its terms, press NO to
	S AGREEMENT WITNESSETH that in l covenants herein contained, and other good and
• I accept the terms in the licens	
I do not accept the terms in the	e license agreement
InstallShield	
	< Back Next > Cancel

HechanoCulture - InstallShield Wizz	ard			×
Customer Information				
Please enter your information.				
User Name:			_	
User				
Organization:				
CellScale				
InstallShield				
	< Back		Next >	Cancel
HechanoCulture - InstallShield Wiza	ard			×
	ard			×
Ready to Install the Program				×
Ready to Install the Program The wizard is ready to begin installatio	n.		en eliek Reede	2
Ready to Install the Program	n.	setting	gs, click Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any o exit the wizard.	n.	setting	js, dick Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings:	n.	setting	gs, click Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings: Setup Type:	n.	setting	gs, dick Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings:	n.	setting	gs, click Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings: Setup Type:	n.	setting	gs, click Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any o exit the wizard. Current Settings: Setup Type: Typical	n.	setting	js, dick Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\	n.	setting	gs, click Back.	2
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any o exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\ User Information:	n.	setting	gs, dick Back.	2
Ready to Install the Program The wizard is ready to begin installation If you want to review or change any or exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\ User Information: Name: User	n.	setting	gs, click Back.	2
The wizard is ready to begin installation If you want to review or change any of exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\ User Information:	n.	setting	gs, dick Back.	3
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\ User Information: Name: User Company: CellScale	n.	setting	js, click Back.	3
Ready to Install the Program The wizard is ready to begin installatio If you want to review or change any of exit the wizard. Current Settings: Setup Type: Typical Destination Folder: C:\Cellscale\MechanoCulture\ User Information: Name: User	n.	setting	js, click Back.	3

😸 Mechano	Culture - InstallSł	ield Wizard		L	Σ	3
-	MechanoCulture	elected are being instal	led.		2	
12	Please wait while the InstallShield Wizard installs MechanoCulture. This may take several minutes.					
	Status:					
InstallShield –		< Bac	k 🏾 🕅	lext >	Cancel	
Device Driver	Installation Wiza	rd				
	Scale ials testing	Welcome to Installation This wizard helps you computers devices no	Wizard!	tware drivers		



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