Cytostretcher-LV

User Manual

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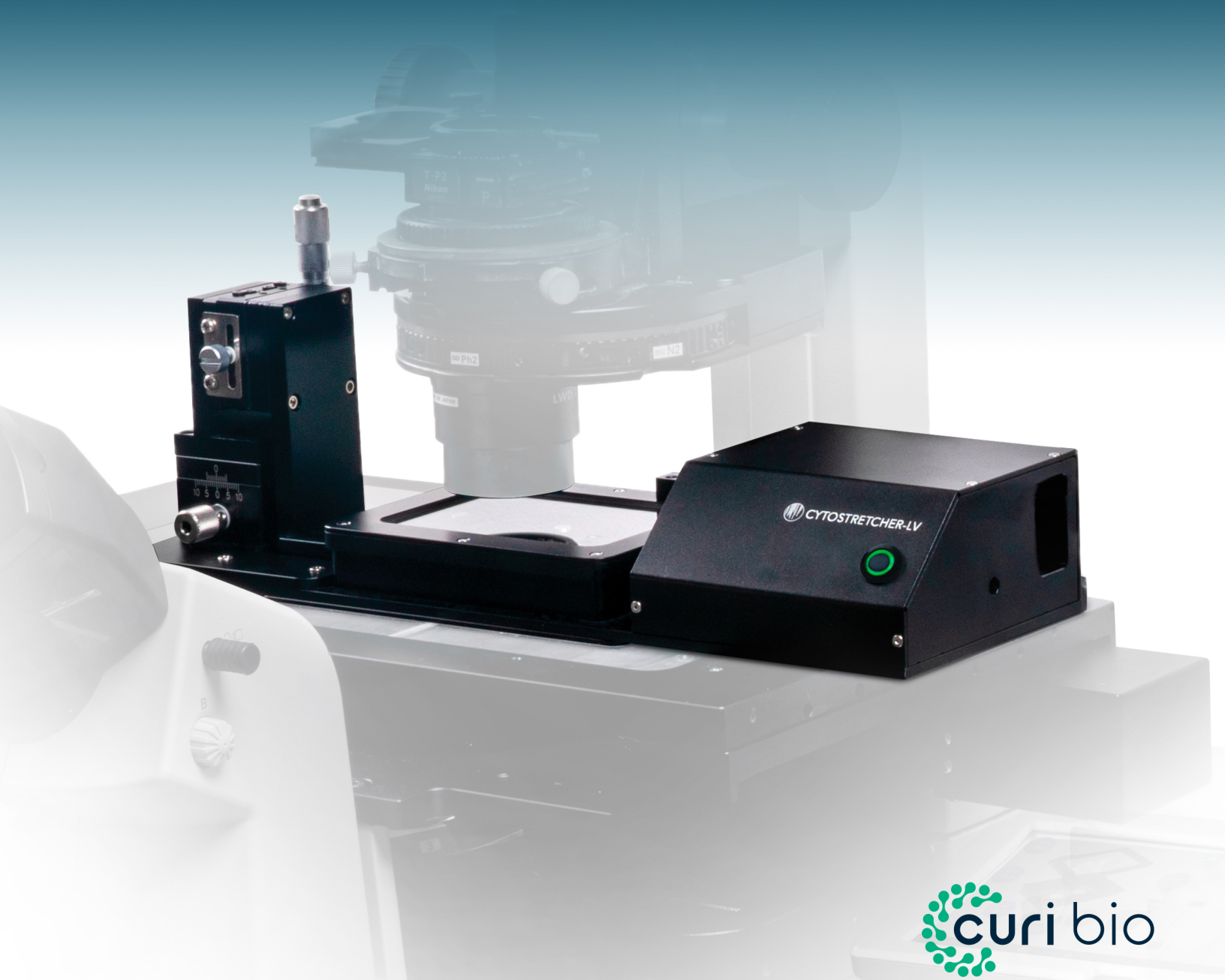
Live-View Compatible Cell Stretching Instrument for Biomimetic Experiments



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Technical Support

Contact support@curibio.com for any assistance.

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Section 1: General Information

Thank You

Thank you for purchasing the Curi Cytostretcher-LV. This device is designed to stretch Curi's proprietary *in vitro* culture chambers on top of an inverted optical microscope.

Introduction

The Cytostretcher-LV is compatible with Curi Bio, Inc.'s Environmental Control Unit (ECU). The ECU is an optional accessory that controls the temperature and CO2 content of the environment inside the imaging chamber. Operation of the ECU is covered in a separate manual. If you need to add the ECU accessory to your Cytostretcher-LV, please email sales@curibio.com or your local distributor.

Please read this manual thoroughly. It contains important information to ensure safe and proper use of the instrument. Critical information that could result in injury to you or in damage to the instrument will be highlighted with a warning triangle. Do not ignore these important warnings!

This manual and the product described herein is subject to change. Changes to the product may not be accurately reflected in this manual. Images of the product may not be identical to the latest shipping product. If there are any questions, please contact us.

⚠ **Note**: This product is intended for research use only.

⚠ ***Warning***: Please follow the instructions in this manual carefully. Failure to do so could result in injury to the user or damage to the instrument.

⚠ ***Warning***: There are no user serviceable parts inside the Cytostretcher-LV. Opening the Cytostretcher-LV case or removing mechanical components could result in damage to the instrument. Opening the Cytostretcher-LV will immediately void the warranty. For service inquiries, please email support@curibio.com or call +1-800-913-4403 Ext. 704.

Section 2: System Overview

Instrument Overview

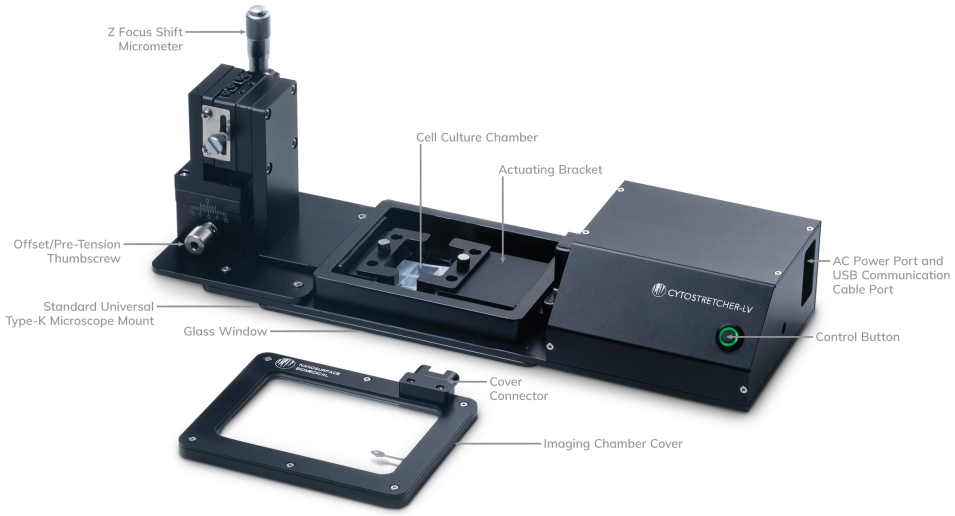

Figure 1: *Components and functions of each part of the Cytostretcher-LV instrument.*

Curi Cytostretcher-LV Main Body

Control Button: Pause and resume stretch. Hold this button to turn the Cytostretcher-LV on and off.

AC Power Port and USB Communication Cable Port:

Offset/Pre-Tension Thumbscrew: Allows you to pre-stretch the membrane.

Actuating Bracket: This component holds part of the chamber and moves back and forth to provide stretch.

⚠ ***CAUTION***: The actuating bracket moves back and forth when the Cytostretcher-LV is operating. Keep objects and fingers clear of this bracket, as it can result in injury to the user or damage to the Cytostretcher-LV.

Cell Culture Chamber: The chamber is placed between the two brackets and on the slots available on each side.

Imaging Chamber Cover: Removable glass window for maintaining chamber CO2 and Temperature control with the optional ECU.

Cover Connector: Magnetic connector to interface with the optional ECU.

Z Focus Shift Micrometer: Adjusts the Z-plane of the chamber as it stretches in order to maintain focus under stretch.

Standard Universal Type-K Microscope Mount: Mounting structure located on the bottom/underside of the instrument. Contact us to inquire about other mount styles.

Glass Window: Located on the underside of the instrument, the Glass Window allows for live-viewing when the instrument is mounted on the microscope stage-top.

⚠ **Note**: If the glass window needs to be removed or replaced, you can do so by removing the four bolts that hold the window frame in place. After removing the bolts, gently lift the glass out with a small tool or pair of tweezers. If the window is beyond cleaning or damaged, please contact support@curibio.com.

Chamber Composition and Structure

The Cytostretcher-LV Chambers are composed of an optically transparent proprietary polymer. The modulus of the polymer is approximately 3 MPa and has a refractive index of 1.51. It has excellent transmissivity above 350 nm to IR (>1.2 μm).

The chambers are available in three topography formats: unpatterned 'flat', patterned parallel to stretch, and patterned orthogonal to stretch. The total thickness of all formats are equivalent to No. 1.5 (0.175 ± 0.015 mm). For the patterned formats, the nanopattern structure consists of submicron grooves and ridges.

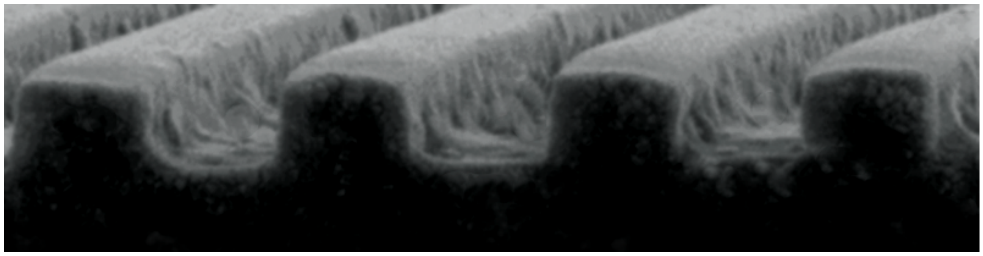

Figure 2: *A scanning electron micrograph of the nanopattern surface topography.*

Cytostretcher-LV chambers are available in the following three size formats:

- **Small (Single)** – 5 mm x 5 mm Single-well Chamber
- **Small (Double)** – 5 mm x 5 mm Two-well Chamber
- **Medium** – 12 mm x 12 mm Chamber

Figure 3: *Culture Chamber size visuals. In order corresponding to the bullet list directly above, left to right.*

Software

The NanoSurface Operational Mechanics Interface (NaOMI) is used to design stretch protocols and operate the Curi Cytostretcher. Please email us at support@curibio.com for the latest version. The NaOMI software is designed to exclusively operate on Windows 10. Operation of the software will be detailed in the next section.

Figure 4: *NaOMI Software running on laptop.*

Section 3: Using the Cytostretcher-LV

Preparing the Cytostretcher-LV

1. Before using the Cytostretcher-LV, it is recommended that you sterilize it. We recommend wiping the unit down with 70% ethanol and ultrapure water. Avoid getting liquid into the power or USB ports. Ensure that the unit is dry before connecting the power or USB cables. Note that the inner motors and electronics are sealed from liquid intrusion but using excessive amounts of liquid or immersing parts of the Cytostretcher-LV will damage the instrument.
2. Sterilize and coat the chamber (as described in the next section).
3. Connect the power cable to the Cytostretcher-LV and plug into the power source. The Cytostretcher-LV will begin calibration and get ready for stretching. Note that many cell culture incubators have ports located on the back wall; many of our customers choose to route their USB and power cables through this port.

Software Installation

The Cytostretcher is controlled by NanoSurface Operational Mechanics Interface (NaOMI). Please email support@curibio.com for the latest version. NaOMI is designed to run exclusively on a Windows 10 PC.

⚠ **Note**: Curi's NaOMI software requires the use of the "Roboto" font/typeface. If your system does not already have this installed, please see "Installing the NaOMI Font Package" at the end of this section for instructions.

Installing the NaOMI USB Driver

1. Download the Software
    a. Curi's NaOMI software is designed to exclusively run on Windows 10. Makes sure you are logged in as an administrator on your windows user account prior to install and use of the software. For the latest software version, please contact support@curibio.com.
    b. The software will be a ZIP file. Unzip this file and you will see two folders: one for the USB driver, and one for the software.
2. Install the USB Driver
    a. The Cytostretcher-LV requires a specific USB driver. The driver is included with the ZIP archive that contains the software. You can also download it here (linked).

b. This driver will not affect other devices attached to your computer.
c. The driver is in the “Cytostretcher USB driver” folder.
d. Once the driver is installed, restart your computer.

3. Install NaOMI (Cytostretcher-LV Software)

a. Double-click the setup icon.
b. Depending on your windows security settings, you may have a warning that this software is from an unverified publisher. Simply override this warning to continue installation.
c. Proceed with the installation. The installer should leave a shortcut on your desktop.

Installing the NaOMI Font Package

⚠ **Note**: The “Roboto” typeface must be installed on your PC for NaOMI to display properly. If it is not installed, the software will not display properly and some functions may be hidden or missing. Many PCs do not come with this pre-installed. Installation is fast and easy, follow these steps:

a. Locate the “NaOMI Typeface Package” folder in the software installation folder that you downloaded from Curi. This folder has the comprehensive Roboto Font.
b. Locate and open the system fonts folder on your PC. You can do this by searching for “Fonts” in the search bar/Cortana bar. Click on the item that says “Fonts–Control Panel”.
c. Select all of the fonts in the NaOMI typeface folder (from step 1) and drag them into the system fonts folder (from step 2). This will install the correct fonts onto your PC. Alternatively, you can just select all the fonts, right click, and select ‘install’. Done!

Focus Tilt Adjustment

Curi recommends adjusting the angle of the Cytostretcher-LV chamber to ensure that the pre-stretch and post-stretch states remain in the same focal plane. This is accomplished by adjusting the four M3-hex head bolts located adjacent to the chamber rods. To ensure cell viability, this can be performed before cells are cultured onto the chamber. Note that different chambers may require individual adjustments.

1. Load the chamber into the Cytostretcher-LV. Make sure you clamp the rods down with the thumbscrews.
2. Turn the Z focus adjust micrometer until the adjustment stage is at its lowest position. You should be able to look at the chamber and see that the left side is lower than the right.
3. Using the NaOMI software, program a stretch routine that has the same amount of stretch that you plan to use. Program a cyclical

stretch with a delay of a few seconds in between stretch and relax. This delay should be long enough to allow you to focus the optical microscope between stretch and relax phases.

4. Use the microscope to focus on the surface of the chamber.
5. Start the stretch routine.
6. When the sample is fully stretched, you will have to adjust the microscope focus knob to keep the sample in focus.
7. Iteratively adjust the Z micrometer in steps while re-checking focus. You may not see a big difference in how much you need to focus even after several Z adjustments.
8. Over several adjustment cycles, you will find that the focus positions become much closer to each other; at this point continue the iterative adjustment with smaller steps until the focus at stretch versus relaxation is the same.

System Operation

The Cytostretcher-LV is controlled by the Curi NaOMI software which allows the user to design a stretch protocol and run the instrument. Once the desired stretch protocol is determined, connect your computer to the instrument via USB connection to upload your protocol to the instrument. Once the protocol has been sent to the instrument, you may remove the USB connection. The instrument does not need to be connected to the computer during the actual stretch protocol.

⚠ ***CAUTION***: Do not load your chamber into the instrument until you are ready to stretch.

The NaOMI software consists of 5 different sections that collectively help design your desired experimental protocol and operate the instrument as explained here:

1. Summary Section: This section is located at the top of the software window and contains a summary of the instrument's status and reports of the protocols. The user can:
    a. 'Open' the previously saved protocols
    b. 'Save' any new protocols to be used later
    c. Connect the software to the Cytostretcher-LV and monitor the state of 'Connection'
    d. Monitor the 'Protocol Status' such as remaining time, cycles, and the frequency of stretch
    e. Keep a record of your Protocol Status section by activating the 'Stretch Log'
2. Edit Section: Protocols are built as a series of steps or "Stretch Segments." Your protocol can consist of a single or many Stretch Segments. Stretch Segments can be easily added, removed,

duplicated, or re-ordered under the 'Edit' section located at the top left half of the panel. By clicking on "Add Segment," a new window named "Stretch Segment Editor" will pop up that allows the user to define different parameters of the Stretch Segment. Each Stretch Segment will then appear as a line under the "Protocol Segments." When working with "Stretch Segment Editor":

a. First, define the amount of stretch ("Stretch distance"). The table below shows you how distance relates to % stretch for commonly used stretch percentages:

Product Type	Desired % Stretch	Stretch Distance (mm)
5 mm Chamber	20%	3.40
	15%	2.55
	10%	1.70
	5%	0.85
	1%	0.17
12 mm Chamber	20%	4.00
	15%	3.00
	10%	2.00
	5%	1.00
	1%	0.20

Calculating Strain: The amount of strain is a function of chamber size and total actuation. The strain changes linearly with actuation. So, to calculate your desired strain, use the following formula:

(Desired Stretch Distance in mm / Known Stretch Distance on the Table in mm) X (Known % Strain on the Table) = Desired % Strain

For example, with applying 1.4 mm of stretch to a 12 mm chamber, you get:

(1.4 mm / 2.0 mm) X 10 = 7.0% Strain

Here, we used 2 mm stretch distance and 10% Strain for 12 mm chambers as a reference point for calculating our desired strain and stretch distance. Please, note that since the % strain vs displacement is linear on the chambers, you can use any of the data points on the table for your calculation.

b. You can define the waveform type to either linear ("ramp") or sinusoidal ("sine") for both stretch and relaxation cycles.

⚠ **Note**: The Stretch and Relaxation sections can be designed independent from each other to add more flexibility for the design of asymmetrical stretch cycles, where the stretch and relaxation may have different parameters.

c. You can then choose to apply a "pre-hold" or "post-hold" to your segment, which are pauses before and after the stretch cycle.

d. You can define the duration of the entire segment.

e. Lastly, you can choose whether or not to add a delay to the end of the segment.

3. Protocol Segments: Located at the top-right of the software window, this section displays each designed Stretch Segments in the protocol as a line, listing their corresponding parameters. Double-clicking the Stretch Segment will open up the “Stretch Segment Editor” window, which enables you to change various aspects of the segment.

4. Protocol Graph: Once a protocol is built, the graph at the bottom-right of the software window will provide a representation of the Stretch vs. Time.

   ⚠ **Note**: The graphical representation of the protocol will show the entire stretch protocol up to 100 cycles. If the programmed protocol exceeds the 100 cycles limit, the graph will only show the first cycle as a representation of the entire protocol. This only effects the visual representation of the protocol and does not effect the programmed parameters in any way.

5. Run Section: This section is located at the bottom-left of the software window and contains the communication options with the External Controller. This section can be used to:

   a. Send a protocol to the device using the ’Send to Device’ option, which ultimately allows for the instrument to run the desired protocol without any connections to the computer. Here, the color of the indicator is used to determine the status of the ’Send to Device’ function. When using this option, the indicator will change from Red to Yellow, showing that the transfer is in progress. The indicator will then turn Green with the completion of the transfer.
   b. ’Start Protocol’ directly from the computer.
   c. ’Pause Protocol’ during the stretch.
   d. ’Stop Protocol’ at any time. Using this function will move the actuating bracket to its starting point.
   e. ’Restart Protocol’.
   f. Apply ’Stretch Offset’ prior to stretch. This function is often used to apply pre-tension to the chamber. The offset will then replace the zero point of stretch and is used as the starting point for any stretching routines, with all stretch distances being relative to the offset point. You can make these adjustments by pressing the arrow buttons or moving the slider.
   g. ’Calibrate’ the Cytostretcher-LV. This button resets the zero point of the instrument to default. Curi recommends calibrating the instrument before each use.

      ⚠ **Note**: Pressing this button will actuate the stretcher. If any cells are in the device, they will be stretched to a potentially dangerous degree.

When you are satisfied with the design of your stretch protocol and before loading the chamber(s):

a. Power on the Cytostretcher-LV.

b. Connect your computer to the Cytostretcher-LV using the USB cable. After the USB is recognized by the computer, click on ”Connection” on the NaOMI software.

c. Once connected, re-set the zero point of the instrument by clicking on “Calibrate”.

d. Set your program and send it to the instrument.

   ⚠ **Note**: Each of these steps may result in an actuation of the device. After doing this, load your chamber.

To mount the chamber, insert the included stainless-steel rods through the round holes in the sides of the chamber. Use these rods to mount the chamber into the slots available in the Cytostretcher-LV chamber area.

Section 4: Culturing Cells in the Cytostretcher-LV Chambers

Chamber Sterilization

The Cytostretcher-LV chambers are made of medical-grade silicone. These silicones are common in many biomedical applications, including human implantation, cell culture and microfluidics.

Before using the chambers, remove the protective PET films below the substrate and on top of the chamber. It is recommended to rinse them with ultrapure water and then sterilize them. The sterilization can be accomplished by autoclaving the chambers or by briefly submerging the chambers in 70% ethanol:water (sonication is recommended).

⚠ **Note**: If you are sterilizing the chambers using 70% ethanol, make sure to rinse them with ultrapure and sterile water afterwards and then dry the chambers in a sterile cell culture hood before use.

Chamber Functionalization

Silicone elastomers are usually hydrophobic. This means that they have lower wettability properties, and pre-treatment with proteins and cell culture may be more difficult. The untreated silicone can still absorb proteins, however it will take longer incubations and more efforts to accomplish this. There are a few methods to address this problem:

1. Plasma Treatment (Recommended): Plasma treatment involves exposing the silicone chambers to a high-energy plasma. This is normally done using a dedicated plasma device, through a UV-Ozone device (or “UVO Cleaner”) or a corona discharge. These devices change the surface chemistry of the silicone from hydrophobic CH3 methyl groups to hydrophilic-OH. There are two caveats with this strategy.
    a. First, a plasma treated silicone surface will revert to its hydrophobic state within a few hours of the treatment; this phenomenon is known as “hydrophobic aging.” The recovery effect occurs on the timescale of hours, and happens faster in the air than it does in water. There are some strategies in literature that discuss ways to prolong the life of a plasma-treated silicone.
    b. Second, overly-aggressive treatment can cause nanoscale cracking and damage to the surface which can affect its function.

    ⚠ **Note**: There are several plasma treatment protocols in literature. Most of these protocols use pressures of 200- 500 mTorr, 10-30 W RF power, and 0.5-5 min treatment time. Because these devices vary in power output and vacuum, and because the degree of attachment

to silicone can vary across cell lines, we suggest testing your plasma treatment strategy before running your experiment.

2. Chemical Modification: There are several chemical coatings that can be used to improve protein coating and cell adhesion in the absence of a plasma machine. One of the methods suggested by Curi Bio, Inc. is:

    a. First, coat the chambers with 100µg/ml of PDL (Poly-D-Lysine) and incubate overnight at 37°C.
    b. Second, remove any excess liquid from the chambers and wash them fully 3 times with 1x PBS.
    c. Last, coat the chambers immediately with your selected protein. Please refer to next section (Suggested Protocols) for more details.

    It has also been reported that when exposed to water, silicone will become more hydrophilic. This change is also helped by elevating the temperature compared to room (25°C). Curi Bio, Inc. has not tested these strategies with the Cytostretcher chambers.

Suggested Protocols

After successful plasma treatment, the surfaces can be coated with extracellular matrix proteins (fibronectin, laminin, etc.) as you would with standard plastic or glass cultureware. Below are some protocols based on coating with standard ECM protein molecules, and they can be adapted to your specific cell type:

1. Collagen

    a. Sterilize the Cytostretcher-LV using the guidelines above.
    b. Do all of your work in a sterile environment, and handle all materials using standard sterile techniques and equipment.
    c. Prepare a sterile solution of 5-10µg/mL collagen in 0.02M acetic acid. An HCL solution of 0.01M can be used as well.
    d. Cover the bottom of the chamber with the gelatin solution. Due to the hydrophobic nature of the solution, you may have to use a significant amount of solution. If the drop is very difficult to spread, use the side of a clean, sterile pipette tip to ‘brush’ the solution across the surface. You may have to do this several times to get full coverage.
    e. Incubate the solution for several hours, or overnight. This can be done at room temperature or at 37°C.
    f. After incubation, remove the solution and immediately seed your cells.
    g. Continue with your standard cell culture protocol.

2. Gelatin

    a. Sterilize the Cytostretcher-LV using the guidelines above.

b. Do all of your work in a sterile environment, and handle all materials using standard sterile techniques and equipment.
c. Prepare a sterile 2% gelatin solution in PBS.
d. Cover the bottom of the chamber with the gelatin solution. Due to the hydrophobic nature of the solution, you may have to use a significant amount of solution. If the drop is very difficult to spread, use the side of a clean, sterile pipette tip to 'brush' the solution across the surface. You may have to do this several times to get full coverage.
e. Incubate the solution for several hours, or overnight. This can be done at room temperature or at 37°C.
f. After incubation, remove the solution and immediately seed your cells.
g. Continue with your standard cell culture protocol.

3. Fibronectin

a. Sterilize the Cytostretcher-LV using the guidelines above.
b. Do all of your work in a sterile environment, and handle all materials using standard sterile techniques and equipment.
c. Dilute your fibronectin stock to a concentration of 5-10µg/mL in sterile DI water or PBS. Note that fibronectin solutions can be very viscous, so you may need to gently pipette the liquids.
d. Cover the bottom of the chamber with the fibronectin solution. Due to the hydrophobic nature of the solution, you may have to use a significant amount of solution. If the drop is very difficult to spread, use the side of a clean, sterile pipette tip to 'brush' the solution across the surface. You may have to do this several times to get full coverage.
e. Incubate the solution for several hours, or overnight. This can be done at room temperature or at 37°C.
f. After incubation, remove the solution and immediately seed your cells.
g. Continue with your standard cell culture protocol.

Section 5: Warranty and Technical Specifications

Warranty

The Cytostretcher-LV possesses a one year warranty, effective from the date of purchase, for any defects or failures in parts or workmanship. Additional warranty coverage may be purchased on a per-year basis by contacting sales@curibio.com. If the Cytostretcher-LV fails or breaks within one year of purchase, Curi will repair it at no charge to the customer. The customer is responsible for the cost of shipping the Cytostretcher-LV to Curi's headquarters in Seattle, WA, USA. Curi will pay for return shipment of the repaired equipment. All warranty returns must be accompanied by an RMA number. For any questions, please contact us at (support@curibio.com or +1 (800) 913-4403).

This warranty does not cover cosmetic damage or wear. Damage due to mishandling, abuse, misuse, or through failure to adhere to the guidelines in this manual will not be covered. Damage to the glass windows of the chamber will not be covered under warranty.

Damage due to using stretching chambers other than those by Curi Bio, Inc. will not be covered under this warranty. This includes custom made chambers or chambers obtained through a third party.

Specifications

Instrument Footprint: 110 x 335 x 122 mm (approximate).

Instrument Mass: 1.97 kg.

Culture Area: 144 mm^2 or 25 mm^2.

Connectivity: USB and onboard memory for stored protocols.

Power: Standard AC adapter. Input 110-240V @ 50/60Hz. Output 12V 0.5A tip positive.

FAQs

What is the substrate thickness on NanoSurface Chambers and is the substrate transparent?

The substrate thickness is specified at 160–190 microns (0.16–0.19 mm). This is the same thickness specification as a No. 1.5 coverglass. The polymer has an index of refraction comparable to that of glass.

Some Curi Cytostretcher-LV Chambers come in flat or nanopatterned topographies. Which one should I use for my application?

Cytostretcher-LV Chambers come in: (1) unpatterned ”flat” and (2) NanoSurface topography. Both formats have a polymer substrate that is softer than conventional glass or plastic cultureware. As a result, the softer substrates are more representative of the mechanical properties of the tissue microenvironment. The NanoSurface substrate possesses submicron grooves with a feature size that is comparable to the collagen microenvironment in the body. As a result, the pattern is also optimized to be in the size of the focal adhesion of cells and facilitate the alignment of adherent cells. Our customers usually use flat substrates either for their improved mechanical properties (softer substrate) or as a control sample for NanoSurface topography to show the differences in the functionality of aligned and non-aligned tissue.

Are Curi Cytostretcher-LV Chambers tissue culture treated?

No, all Curi Cytostretcher-LV chambers need to be sterilized and protein coated following the suggested steps in this manual and prior to cell plating.

Can I grow cells directly on Curi Cytostretcher-LV Chambers or do I need to do ECM protein coating?

We recommend coating the surface with the extracellular matrix protein you typically use in your cell culture workflow (collagen, fibronectin, gelatin, Matrigel, etc.). We also have a suggested concentration in our manual for protein coating of our substrates using some of these proteins. Before starting your experiments, make sure to check our manuals where you can find the details of the protocols for each protein coating.

Is there a suggested cell seeding density for iPSCs?

For most commercially available cell lines, the cell seeding density used on conventional cultureware works with Curi Cytostretcher-LV Chambers as well. If you are using your own cell line, our support team can provide you with suggestions on how to design seeding density optimization experiments.

If you require additional support, please contact support@curibio.com so that our applications team can provide you with the relevant suggested protocol.

Do you have suggested cell seeding protocols for different cell types?	As with any cultureware, optimal seeding density on Curi Cytostretcher-LV Chambers can vary according to cell type and the purpose of the experiment. However, we recommend researchers use the same cell density that they would use for conventional cultureware. For specific applications, please contact us at support@curibio.com.
What is the shelf life of Curi Cytostretcher-LV Chambers?	We recommend using your Cytostretcher-LV Chambers within 2 years of purchase.
What is the storage condition for Curi Cytostretcher-LV Chambers?	We recommend storing Cytostretcher-LV Chambers where they are protected from light and under climate controlled conditions (Ex. 20-25 degrees Celsius, 30-50% humidity).
Can I reuse Cytostretcher Chambers?	We strongly suggest not to reuse the Cytostretcher chambers as the cleaning and reusing process may obscure or damage the substrates.
Do I need to do any surface modifications on Cytostretcher chambers?	The surface of the Cytostretcher chambers is hydrophobic by nature. To allow for faster and better adsorption of the proteins onto the surface, you will need to perform surface modification (physical or chemical) making the chamber substrate hydrophilic prior to use.
Do I need a plasma machine to effectively adhere cells to the surface of Cytostretcher chambers?	Plasma treatment is one of the more effective methods for adequate surface modification. If you do not have access to a plasma machine in your lab, you can also use PDL coating. Please refer to our manual or contact our support team for details of each protocol.
What is the maximum frequency that a Cytostretcher can stretch at?	The Cytostretcher is capable of applying maximum of 5Hz frequency.
More Questions?	If you have questions or concerns, contact support@curibio.com for assistance.