

# Cytostretcher

User Manual

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Cell Stretching Instrument  
for Biomimetic Experiments



# Terms and Conditions

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## Release of Liability

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The Curi Cytostretcher is designed to work exclusively with Curi cell culture chambers. Use of chambers that are not supplied by Curi Bio, Inc. may void the warranty. Use of the Cytostretcher Chambers are covered in section 2 and 3 of this manual, which discusses preparation and treatment of the chambers for use.

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## Conditions of Use

The Curi Cytostretcher is for life science research use only. You are responsible for understanding and safely performing the protocols described within this guide. Curi Bio, Inc. does not guarantee any results you may achieve. These protocols are provided as Curi Bio, Inc. recommendations to you and does not constitute a guarantee of success.

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## Technical Support

Contact [support@curibio.com](mailto:support@curibio.com) for any assistance.

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# Section 1: General Information

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## Thank You

Thank you for purchasing the Curi Cytostretcher instrument. This device is designed to apply cyclical stretch to Curi's proprietary *in vitro* cell culture chambers. The Cytostretcher can be operated on a bench top or inside a cell culture incubator.

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## Handling

Please follow the instructions in this manual carefully. Before using the Curi Cytostretcher, it is recommended that you sterilize the instrument. We recommend wiping the unit down with 70% ethanol and ultrapure water. Avoid getting liquid into the cable ports. Ensure that the instrument is fully dried before connecting any cables. Note that the motor and the electronics are sealed from liquid intrusion but using excessive amounts of liquid, spraying, or immersing parts of the Cytostretcher can damage the instrument.

There are no user serviceable parts inside the Cytostretcher instrument. Opening the Cytostretcher case will immediately void the warranty as it could result in damages to the internal components and will destroy the factory environmental seal. For service-related inquiries, please contact us via email at [support@curibio.com](mailto:support@curibio.com) or call +1-800-913-4403 x704.

**⚠ Note:** It is recommended that you sterilize the cell culture chambers before use. This can be accomplished by first autoclaving the chambers followed by rinsing with ultrapure and sterile water, then drying in a sterile cell culture hood or under vacuum. While the chamber's cell culture surface is durable, Curi advises against making direct contact with the surface. For best results, avoid touching the surface with items such as pipette tips, tweezers, etc.

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## Instrument Overview

The Cytostretcher instrument is comprised of two primary parts: the Main Body and the External Controller. The Cytostretcher Main Body is designed to apply mechanical stimuli inside a cell culture incubator or bench-top space while the External Controller magnetically attaches to the outside of the incubator to allow the user to conveniently operate the instrument while saving valuable incubator space.

**⚠ Note:** In some cases, the Cytostretcher may generate excess heat that cannot be efficiently dissipated based on the make and the model of the cell culture incubators. This heat issue may be exacerbated by high-frequency stretch protocols. In these cases, the Cytostretcher Cooling Unit can be used to maintain the temperature of the Cytostretcher chamber environment at a set value. At the factory, the Cooling Unit is programmed to maintain a temperature of 37°C inside the cell chamber.

This section summarizes the components and functions of each part of

the Cytostretcher instrument.

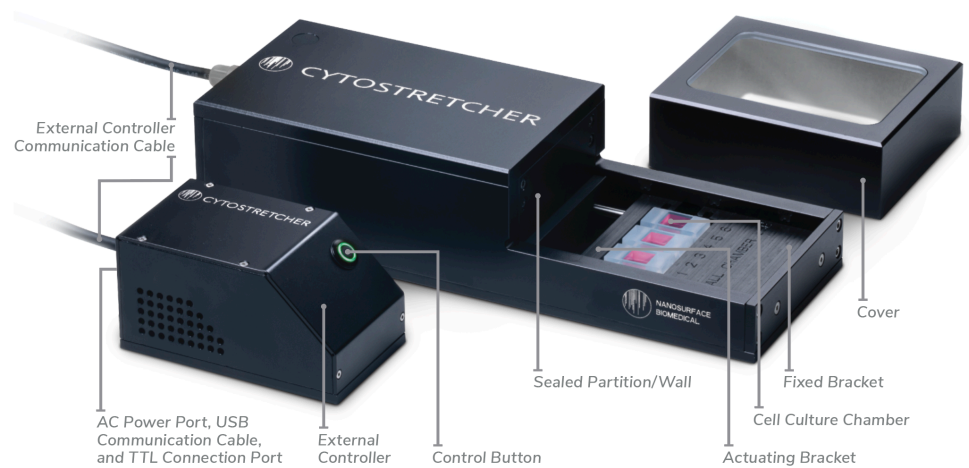


Figure 1: Components and functions of each part of the Cytostretcher instrument.

## Cytostretcher Main Body

**Sealed Partition/Wall:** This wall seals the internal electronic components from the humid environment of the incubator. Make sure not to damage the seals at the edges of this partition.

**Fixed Bracket:** Holds one side of the cell culture chamber. Remains fixed during the stretch cycle.

**⚠ Note:** There are two different types of fixed brackets for the Cytostretcher. The 'Large Bracket' is used for 5 mm X 5 mm and 12 mm X 12 mm chamber size formats. The 'Small Bracket' is used for 50 mm X 50 mm and the high-throughput chamber formats. For more details regarding the chamber formats and the fixed brackets, please refer to the "Chamber Composition and Structure" and "Instrument Setup" Sections in this manual.

**Actuating Bracket:** Holds the other side of the cell culture chamber and moves back and forth to apply the programmed stimuli.

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

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

**Cover:** The cover protects the cell culture chamber's environment from potential contamination while allowing for gas exchange and air flow. It is recommended that the cover is put in place while the unit is operating.

**External Controller Communication Cable:** This cable connects the Cytostretcher Main Body to the External Controller.

## Cytostretcher External Controller

**External Controller:** Contains the control electronics for the stretcher. The unit must be located outside of the cell culture incubator. The bottom of the controlling unit is magnetic, allowing it to mount onto the outside wall of most incubators and providing easy access and control to the Cytostretcher instrument.

**Control Button:** Pushing the button pauses and resumes the stretch cycle. Also, holding the button can be used to turn the Cytostretcher on and off.

**AC Power Port/USB Communication Cable:** Both of these ports are located on External Controller. The USB port connects the controlling unit to the computer to interact with the NaOMI software and upload new stretch protocols.

**TTL Connection Port:** This port allows for external/third party instruments to interact with Cytostretcher as needed by the user.



Figure 2: The Cytostretcher sitting on top of the Cooling Plate (left) and the Cytostretcher External Controller mounted beside the Cytostretcher Temperature Control Unit (right).

## Cytostretcher Cooling Unit

**Temperature Control Unit:** Contains a temperature-activated pump and water reservoir for cooling the Cytostretcher chamber environment. The Temperature Control Unit connects to the Cooling Plate via two silicone tubing water lines (input and output) and a communication cable. When an overheat is detected, water is pumped through the cooling pad until the preset temperature is achieved. The unit can be left on indefinitely but must be located outside of the cell culture incubator. The bottom of the unit is magnetic, allowing it to mount onto the outside wall of most incubators and providing easy access and control to the Cooling Plate.

**Cooling Plate:** Contains a temperature sensor and connects to the Temperature Control Unit via two lines of silicone tubing and a communication cable. The Cooling Plate is placed directly underneath the Cytostretcher Main Body to dissipate excess heat generated from the instrument.

## Chamber Composition and Structure

The Cytostretcher 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  $\mu$ m).

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  $\pm$  0.015 mm). For the patterned formats, the nanopattern structure consists of submicron grooves and ridges.

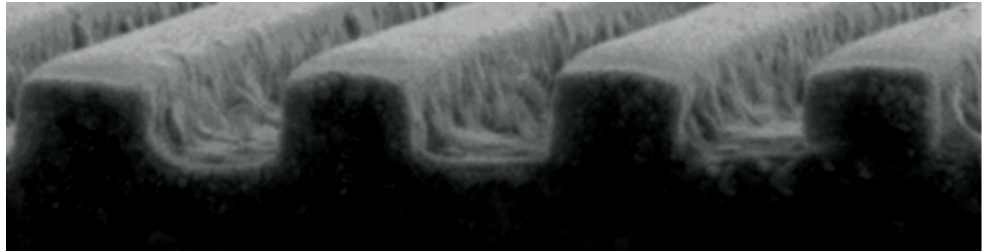


Figure 3: A scanning electron micrograph of the nanopattern surface topography.

Cytostretcher chambers are available in the following five 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
- **Large** – 50 mm x 50 mm Chamber
- **High-throughput** – 6 mm x 6 mm 24-well Chamber

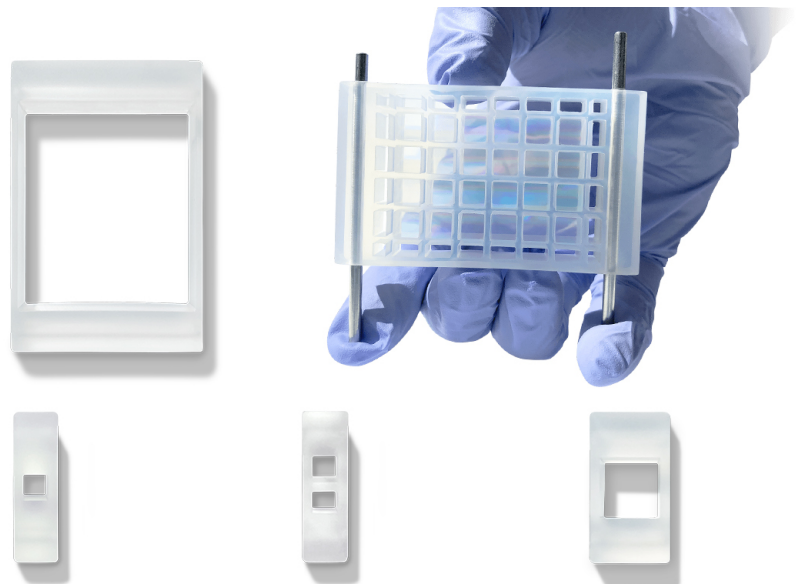


Figure 4: Top row, from left to right: Large and High-throughput chamber formats. Bottom row, from left to right: Small (Single), Small (Double), and Medium chamber formats

## 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](mailto: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 5: NaOMI Software running on laptop.



# Section 2: Installing and Using The Cytostretcher

## Instrument Setup

First, determine the type of fixed bracket suitable for your chamber format. As explained in Section 1, there are 2 fixed brackets included with each Cytostretcher instrument. The large bracket (the default option) is used for the 5 mm X 5 mm as well as the 12 mm X 12 mm chamber size formats whereas the small bracket is used for the 50 mm X 50 mm and High-throughput chamber size formats. If you need to change the brackets before or in between your experiments, follow the step-by-step process listed here:



*Figure 6: Close-up of the Cytostretcher instrument, with arrows pointing out the two front bolts.*

1. Remove the 2 bolts on the panel that holds the bracket, as indicated by the arrows on Figure 6 (you will need a 2.5 mm hex wrench that is included with the instrument).
2. Once removed, attach the new bracket with the same bolts by hand until firm and secure. Avoid applying excessive torque.

Following the attachment of the proper fixed bracket, you can proceed with setting up the Cytostretcher. The Cytostretcher instrument is designed to function inside either a cell culture incubator or on a bench-top space. To properly set up the Cytostretcher, follow the step-by-step process described here.

1. Setting up the Cytostretcher Inside a Cell Culture Incubator:
  - a. Place the Cytostretcher Main Body inside the incubator.

- b. Attach the External Controller to the outside of the cell culture incubator. Note that the bottom of the External Controller is magnetic, so it can easily attach to the outside wall of most incubators.
- c. Run the External Controller communication cable from the External Controller to the Cytostretcher Main Body. Curi recommends running the cable through the small port located on the upper back wall of the incubator. Connect the cable to the Cytostretcher body once you are satisfied with its placement.
- d. Connect the power and USB cables to the External Controller.  
**⚠ Note:** The Cytostretcher main body can be left in the incubator indefinitely. If you would like to remove it at any time, Curi recommends leaving the External Controller communication cable in place.

## 2. Setting up the Cytostretcher on a Bench-top:

- a. Place the Cytostretcher Main Body on a solid, flat bench.
- b. Plug in the Cytostretcher External Controller. Note that the bottom of the controller is magnetic and can be mounted on most metal surfaces. The External Controller also has rubber feet and can be placed adjacent to the Cytostretcher.
- c. Connect the power and USB cables to the External Controller.

**⚠ Note:** As mentioned in Section 1, in some cases when using the Cytostretcher inside a cell culture incubator, the Cooling Unit needs to be setup as well to properly dissipate any excess heat from the Cytostretcher chamber environment. To properly setup the Cooling Unit, please follow the step-by-step process described under "Setting up the cooling unit".

### Setting up the Cooling Unit

1. Attach the Temperature Control Unit to a flat, vertical, and stable surface (as shown in Figure 7). The unit has magnets that allow it to attach to the side of the incubator hood.
2. Ensure that the unit is upright, with the power switch at the top and the silicone tubing port at the bottom.

**⚠ Note:** Do not plug in any cables at this time.

3. Use the included syringe and filling tube (a small section of tubing with a plastic fitting at the end) to fill the Temperature Control Unit with water:
  - a. Remove the black silicone plug at the top right of the Temperature Control Unit (referenced in Figure 7).
  - b. Fill the Syringe with ultrapure water: The capacity of the Temperature Control Unit is 150-180 mL so you may need to repeat this process a few times to fill the unit.

- c. Attach the fitting on the end of the fill tube to one of the ports on the bottom of the Temperature Control Unit (referenced in Figure 7). You must depress the metal clip on the fitting to fully engage the fill tube.
- d. Once attached, empty the contents of the syringe into the Temperature Control Unit.
- e. Ensure that the end of the fill tube is higher than the water reservoir window. Failure to do so will result in leakage of water.
- f. If you need more than one syringe load, remove the syringe and hold the tube above the reservoir. If this is too difficult, simply remove the fill tube from the Temperature Control Unit fitting (it will not leak; the fitting automatically closes). And repeat the same process.
- g. Fill the Temperature Control Unit until the water level reaches the top line of the water level window (referenced in Figure 7).
- h. Remove the syringe and fill tube from the fittings.
- i. Replace the black silicone plug on the top right of the Temperature Control Unit.



*Figure 7: Image of Cooling Unit, referencing specific parts mentioned in the setup process.*

4. Place the Cooling Plate inside the cell culture incubator and directly underneath the Cytostretcher Main Body.
 

**⚠ Note:** There are small indentations on the Cooling Plate that dock with the rubber feet of the Cytostretcher Main Body.
5. Run the Cooling Plate cable bundle through the back port of the incubator hood and plug the water cooling lines into the Temperature Control Unit; it does not matter which line goes into which fitting.

6. Plug in the temperature sensor and power cable to the Temperature Control Unit. Turn on the unit.
7. The set temperature should be 37°C. The indicated temperature may be lower than the set value while the unit equilibrates to the incubator environment.
8. Let the unit run for about 10-20 minutes, and re-check the water level. You may need to top off the water as the system begins pumping out air that remains from assembly. Simply unplug both water lines and add more water with the syringe and filling tube.  
**⚠ Note:** If you do not plan to use your Temperature Control Unit often, please be sure to turn on the instrument and let the water run through your Cooling Plate for at least 1-2 hours once every two weeks to ensure no blockage forms.  
**⚠ Note:** If water is running through the Cooling Plate and the temperature is not decreasing, you may need to replace the fuse. The fuse is located directly below the power socket of the Cooling Unit. To replace, first pry the labeled fuse housing area with a flat head screwdriver. When out, replace the fuse using your hands and put the fuse housing area back into the Cooling Unit. A spare fuse is included in your original shipment.

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## Software Installation

The NanoSurface Operational Mechanics Interface - NaOMI software is designed to exclusively operate with Windows 10 Operating System. To make sure the software is properly installed and ready to use with the Cytostretcher, please follow the step-by-step installation process outlined here. Alternatively, there are NaOMI tutorial videos on our website. Please contact us at [support@curibio.com](mailto:support@curibio.com) to gain access to the tutorials.

1. Download NaOMI
  - a. For the latest software version, please contact us at [support@curibio.com](mailto:support@curibio.com).
  - b. The software will be in a ZIP file and the contents need to be decompressed/extracted.
2. Install NaOMI
  - a. Make sure you are logged in as an administrator on your windows user account
  - b. Double-click on the Setup icon.
  - c. Depending on your windows security settings, you may have a warning that says this software is from an unverified publisher. Simply override this warning to continue installation.
  - d. Proceed with the installation. The installer should leave a shortcut on your desktop.

**⚠ Note:** The USB driver comes in a bundle with the NaOMI setup file. While the driver is automatically installed along with the software on most systems, in some cases (and based the settings of the operation systems) the driver may not be properly installed. In these cases, please follow the "Installing the NaOMI USB driver" installation steps outlined here. Otherwise, please disregard the steps in this note.

#### **Installing the NaOMI USB driver:**

1. The Cytostretcher requires a specific USB driver. You can download the driver from here (linked).
2. Install the driver. The driver will not affect other USB devices attached to your computer.
3. Confirm that the driver is in the "Cytostretcher USB driver" folder.
4. Once the driver is installed, restart your computer.

**⚠ Note:** Curi's NaOMI software requires the use of the "Roboto" font/typeface. Some PCs do not come with this pre-installed. If this font is not installed on your computer, the software window will not display properly. Installation is quick and simple. In case your system does not already have this font installed, please follow the "Installing the NaOMI font Package" instructions here. Otherwise, please disregard the steps in this note.

#### **Installing the NaOMI Font Package:**

1. Locate the "NaOMI Typeface Package" folder in the software installation folder that you downloaded from Curi. This folder has the comprehensive Roboto Font.
2. 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".
3. 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'.

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## **System Operation**

The Cytostretcher 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 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:

**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:

$(\text{Desired Stretch Distance in mm} / \text{Known Stretch Distance on the Table in mm}) \times (\text{Known \% Strain on the Table}) = \text{Desired \% Strain}$

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

$$(1.4 \text{ mm} / 2.0 \text{ mm}) \times 10 = 7.0\% \text{ Strain}$$

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.

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
6 mm Chamber	20%	4.00
	15%	3.00
	10%	2.00
	5%	1.00
	1%	0.20
12 mm Chamber	20%	4.00
	15%	3.00
	10%	2.00
	5%	1.00
	1%	0.20
50 mm Chamber	18%	10.00
	15%	8.40
	10%	5.60
	5%	2.80
	1%	0.56

**⚠ 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. 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.
- b. Connect your computer to the Cytostretcher 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 chamber area.



# Section 3: Culturing Cells in the Cytostretcher Chambers

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## Chamber Sterilization

The Cytostretcher 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 the chambers 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.

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## 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 CH<sub>3</sub> 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 the 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.

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## 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 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 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 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 4: Warranty and Technical Specifications

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## Warranty

The Curi Cytostretcher 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](mailto:sales@curibio.com). If the Cytostretcher 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 to Curi's headquarters in Seattle, WA, USA. Curi will pay for the 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](mailto: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 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.

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## Specifications

**Instrument Footprint:** 280 x 102 x 65 mm

**Instrument Mass:** 2.6 kg

**External Controller Footprint:** 110 x 64 x 60 mm

**External Controller Mass:** 0.5 kg

**Culture Area:** 2,500 mm<sup>2</sup>, 144 mm<sup>2</sup>, 36 mm<sup>2</sup> or 25 mm<sup>2</sup>

**Connectivity:** USB and onboard memory for stored protocols

**Power:** Standard AC adapter. Input 110-240V @ 50/60Hz. Output 24V 0.5A tip positive.

# FAQs

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**What is the substrate thickness on 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.

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

Cytostretcher 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.

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**Are Curi Cytostretcher Chambers tissue culture treated?**

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

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**Can I grow cells directly on Curi Cytostretcher 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.

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**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 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](mailto:support@curibio.com) so that our applications team can provide you with the relevant suggested protocol.

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**Do you have suggested cell seeding protocols for different cell types?**

As with any cultureware, optimal seeding density on Curi Cytostretcher 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](mailto:support@curibio.com).

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**What is the shelf life of Cytostretcher Chambers?**

We recommend using your Cytostretcher Chambers within 2 years of purchase.

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**What is the storage condition for Cytostretcher Chambers?**

We recommend storing Cytostretcher Chambers where they are protected from light and under climate controlled conditions (Ex. 20-25 degrees Celsius, 30-50% humidity).

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**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.

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**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.

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**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.

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**What is the maximum frequency that a Cytostretcher can stretch at?**

The Cytostretcher is capable of applying maximum of 5Hz frequency.

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**More Questions?**

If you have questions or concerns, contact [support@curibio.com](mailto:support@curibio.com) for assistance.