



STREX

Cell Stretching System

Model # STB-190-XY

User Manual

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Section 1: Main Components

Cell Strain Instrument

Y axis motor:

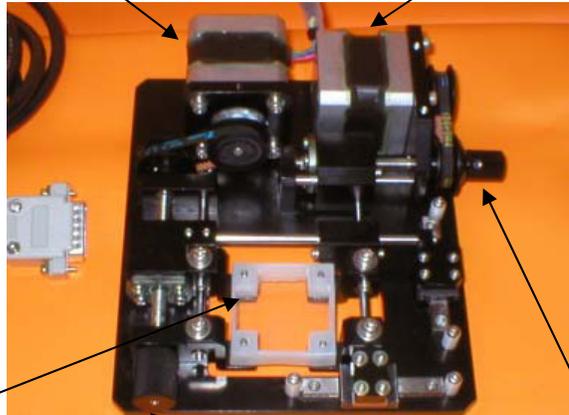
Motor to stretch/compress along Y axis

X axis motor:

Motor to stretch/compress along X axis

Connector Cable:

Cable connecting Control Unit and Strain Unit



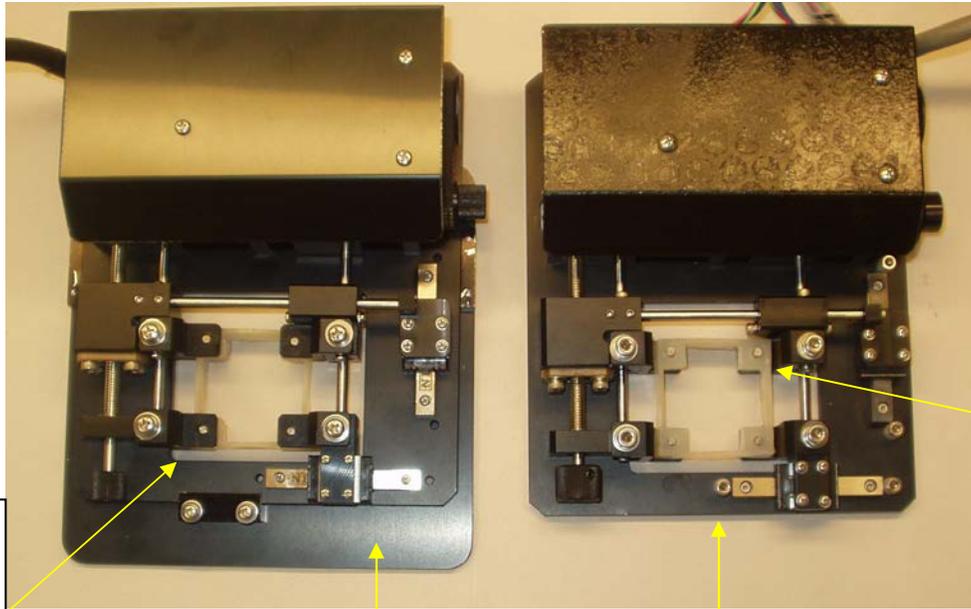
Strain chamber bracket:

Chamber is mounted on brackets by 4 pins.

Chamber length adjusting knobs:

Adjusts distance of chamber brackets to maintain tension on the chamber.

Microscope adapters



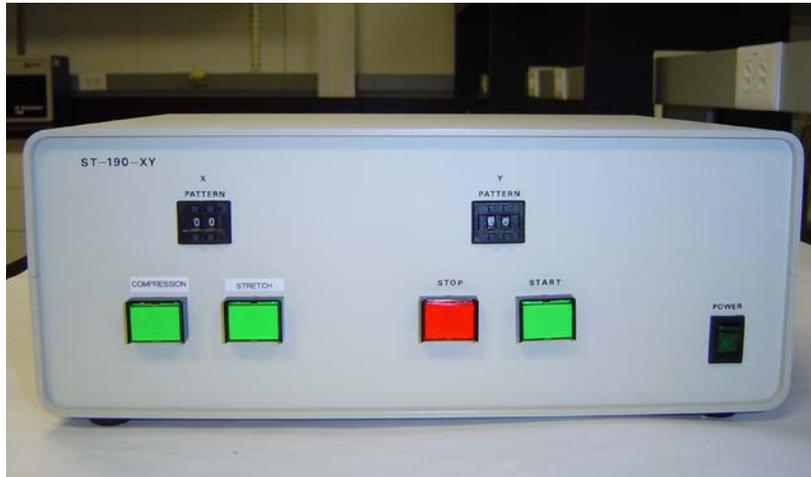
Strain chamber
mounts onto the
pins from below
the stage

Designed for Leica and Zeiss microscopes
with stage adapter

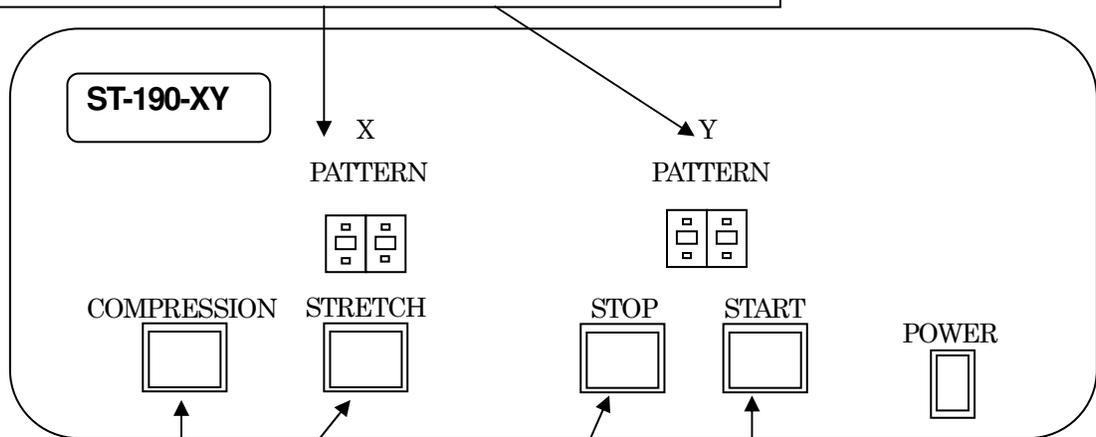
Strain chamber
mounts onto the
pins from the
top

No stage adapter is needed
for Olympus and Nikon
microscopes

Control Unit Front Panel



X axis / Y axis Parameter setting digit:
Left digit: Strain frequency
Right digit: Strain ratio
Use upper and lower buttons to change the parameters



Stretch and Compress Selector:
Push button to select stretch or compress

Stop Button:
The system stops when button is pressed.

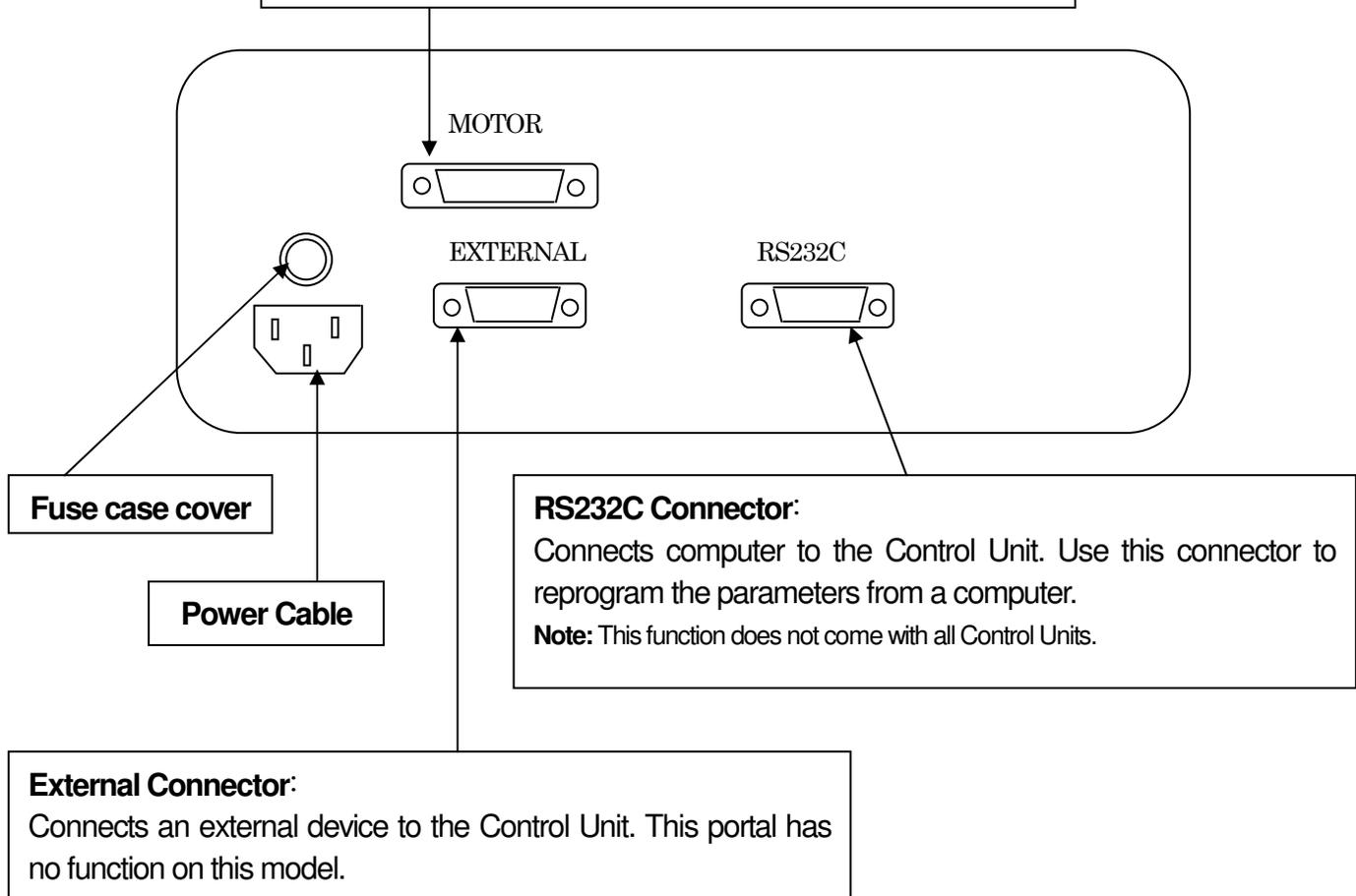
Start Button:
The system starts when button is pressed.

Control Unit Back Panel

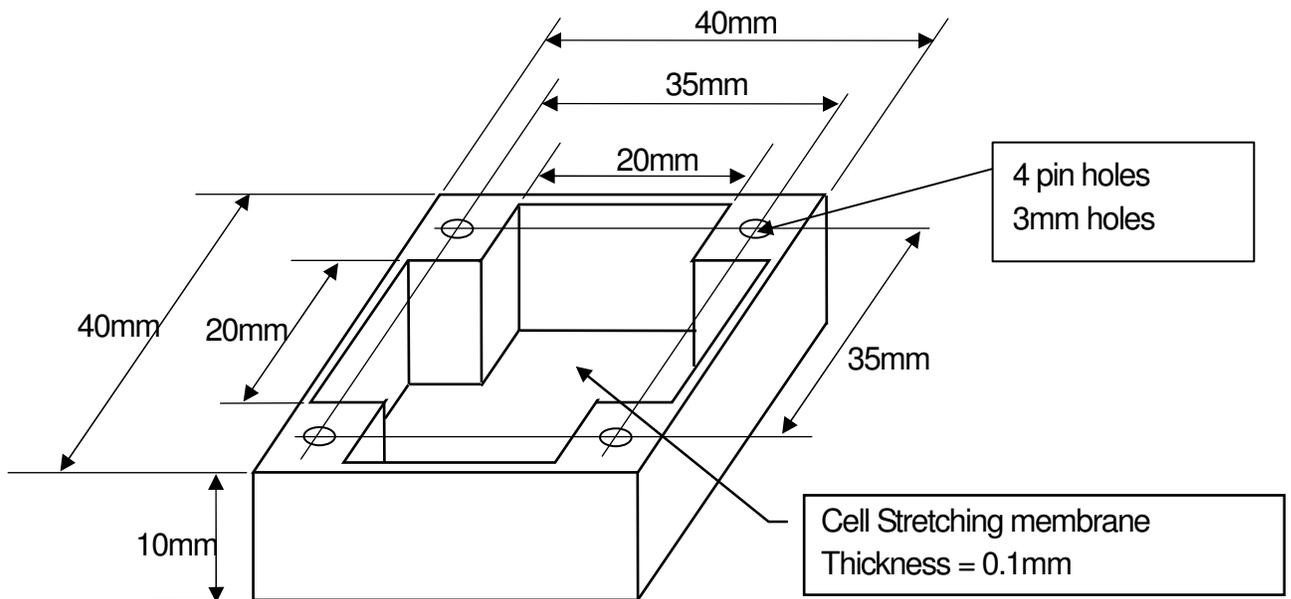


Control Connector:

Outlet to connect cable between Strain Unit and Control Unit.



Silicone Chamber



Note: Adhesiveness and strength of chamber is not guaranteed for more than 3 uses. The chambers are durable enough to withstand approximately 900,000 stretch of 20%.

Section 2: Use of the Cell Strain Instrument

Preparation of the Cell Strain Instrument

Before using the Cell Strain Instrument, sterilize the unit — especially the chamber mounting area — using ethanol-immersed swabs.

System Operation

Operation of the Cell Strain Instrument is very straight forward and intuitive. Below are the basic steps of the process to use this instrument. The step motor that moves the chamber brackets is made to operate 15-20 minutes continuously. It is not recommended to extend the use of the motor beyond 20 continuous minutes because of risk to overheating and burning out the motor.

► Set-up of the Strain and Control Unit

1. Connect the Strain Unit to the Control Unit by the Connector Cable.
2. Connect the Control Unit to the Power Supply (110 volts) by the Power Cable.
3. Turn on the Power Switch to start operation. The Power Switch will light up and the Stop Button will flash.

BEWARE: The step motor cooling fan will be on. Do not obstruct the fan blades with any objects.

4. If the electrical system is operating properly, turn off the Power Switch.
5. Make sure that the microscope is level on the work bench and the Strain Unit when placed on the microscope stage is also level.

► Start Cell Strain

1. Make sure that the chamber is set up on the bracket properly. Set up the Strain Unit by connecting it to the Control Unit.
2. Adjust the stretch or compress frequency and strain ratio using the controller and programs outlined in Section 3.
3. Turning on the Main Power. Press either the STRETCH or COMPRESSION Button. Press the START button to start the stretch or compress cycle. During the action cycle, the START button will be illuminated. To stop movement at any time, press the STOP button.
4. **Do not change the Strain Ratio, Strain Frequency, or the stretch/compression function during the operation of a strain cycle.** Press the Stop Button and wait for the final cycle to complete. Only after the chamber returns to its original start position can the strain parameters be safely changed. Changing parameters during a strain cycle may damage the motors.

5. **The Power Switch must be OFF to freely rotate the Chamber Length Adjustment Knob.** A silicone chamber is attached to the Strain Unit by inserting the 4 pins into the corners of the chamber. Rotate the Adjustment Knob to move the brackets the correct distance apart. A clockwise rotation will create tension on the chamber. The silicone membrane at the bottom of the chamber should be taut.

Note: A 360° turn of the knob changes the chamber length by 5% or 1 mm of the ST-CH-04-XY chamber.

6. After 5 minutes, stop movement and check the condition of the cells. If the cells have not detached, proceed with your experiment. If the cells are detached, the adhesion matrix coating was probably insufficient. Recoat the chambers.
7. The region of the chamber that is subjected to the full amount of both the X and Y strain parameters is the center 20mm x 20mm. Therefore, measurements and assays should be performed using cells in this area.

Culturing Cells in the Silicone Chambers

1. Seed cells at the appropriate concentration in the freshly coated chamber.

Important: Do not over expose the cells to dissociation enzymes. Cells should be treated in the same manner (type and concentration of enzyme, temperature, and time for digestion) for all experiments.

Important: Cells should not be cultured at a high cell density in the chambers. For example, epithelial cells often form a cell-sheet and the cell-cell adhesion seems to be stronger than a cell-surface adhesion. When this happens cells may detach from the chamber. Additionally, cultures that are grown over a week in the chambers may detach.

2. After overnight incubation, inspect cells with the microscope to ensure that they have adhered to the chamber.

Preparation of Silicone Chambers

Before using the chambers, they should be sterilized then coated with a cell adhesion matrix. The procedure below can be adapted for use with other matrices, such as collagen, elastin, pronectin, and laminin.

Sterilize chambers in an autoclave for 20 minutes at 121 °C. The silicone chambers can withstand temperatures up to 180 °C. Use of an autoclave is preferable. However, if an autoclave is not available, the chambers may be sterilized by submerging them in 70% ethanol, rinsing with water, then drying in a sterile environment.

Place the sterile silicone chambers in a Petri dish in preparation for coating.

► Fibronectin Coating

Preparation of fibronectin solution:

1. Dilute human or bovine fibronectin to a final concentration of 50 to 100 ug/ml in Phosphate Buffered Saline (PBS)

Coating with fibronectin solution:

1. Pour 3-6 ml of the fibronectin solution into each strain chamber
2. Incubate at 37°C for more than 30 minutes.
3. Aspirate the fibronectin solution. If coating is successful, water will not be repelled after removing the fibronectin solution.
4. The liquid solution can be used to coat 3 or 4 chambers before discarding.

► Gelatin Coating

Preparation of gelatin solution:

1. Add gelatin powder to PBS at a concentration of 2%
2. Autoclave the mixture to dissolve and sterilize

Coating with gelatin solution:

1. Pour 3-6 ml of the gelatin solution into each strain chamber
2. Incubate at 37°C for more than 30 minutes.
3. Aspirate the gelatin solution. If coating is successful, water will not be repelled after removing the gelatin solution.
4. The liquid solution can be used to coat 3 or 4 chambers before discarding.

► Collagen Coating (Cellmatrix 1-C, P, Type 3 or 4)

Preparation of collagen solution:

1. Combine 1 part collagen to 10 parts HCl, pH 3, in a sterile tube

Coating with collagen solution:

1. Coat chamber with a thin layer
2. Aspirate excess
3. Dry in biological safety cabinet at 25°C or below. The chamber can be stored at the same temperature.
4. Wash the chamber twice with culture medium. If coating is successful, water will not be repelled.

PBS (per liter):

NaCl	8.00 g
KCl	0.20 g
Na ₂ HPO ₄ (anhyd.)	1.15 g
KH ₂ PO ₄ (anhyd.)	0.20 g

Note: Dulbecco's PBS solution for tissue culture applications is commercially available.

The PDMS (silicone) chambers are very hydrophobic with two methyl-base on the surface. Cells adhere to chambers coated with fibronectin or collagen via integrins. This form of cell adhesion is very different compared to attachment of cells to plastic or glass dishes because the surface of plastic or glass is charged, resulting in non-specific cell binding. If the cells are having difficulty attaching to the coated chamber or easily detached upon stretching, coat the silicone chamber overnight with a higher concentration of fibronectin or collagen.

Section 3: Stretch and Compress Parameter List

This instrument does not include standard programs. All programs are custom designed by researcher.

Function of settings

Switch	LEFT digit	RIGHT digit
X PATTERN (X axis parameter)	Frequency settings	Strain Ratio settings
Y PATTERN (Y axis parameter)	Frequency settings	Strain Ratio settings
STRETCH/COMPRESS	Select stretch cycle or compress cycle	

Setting parameters

LEFT Digit: Frequency of Stretch or Compression

Digit	Parameter	Detail of parameters
0	No action	Select only X or Y direction
1	Single cycle: Hold 5 seconds	Stretch or Compress X and Y direction - hold -contract
2	Single cycle: Hold 10 seconds	Stretch or compress X and Y direction - hold- contract
3	Single cycle: Hold 30 seconds	Stretch or compress X and Y direction - hold- contract
4	Single cycle: Hold 60 seconds	Stretch or compress X and Y direction - hold- contract
5	Cyclic cycle:0.5Hz	30 cycle/minute stretch or compress X or/and Y direction
6	Cyclic cycle:0.2Hz	10 cycle/minute stretch or compress X or/and Y direction
7	Cyclic cycle:0.05Hz	3 cycle/minute stretch or compress X or/and Y direction

Note: If either the X or Y axis is set for a single cycle, both the X and Y axis will perform a single cycle regardless of other frequency settings.

RIGHT Digit: Strain Ratio (Degree of stretch or compression)

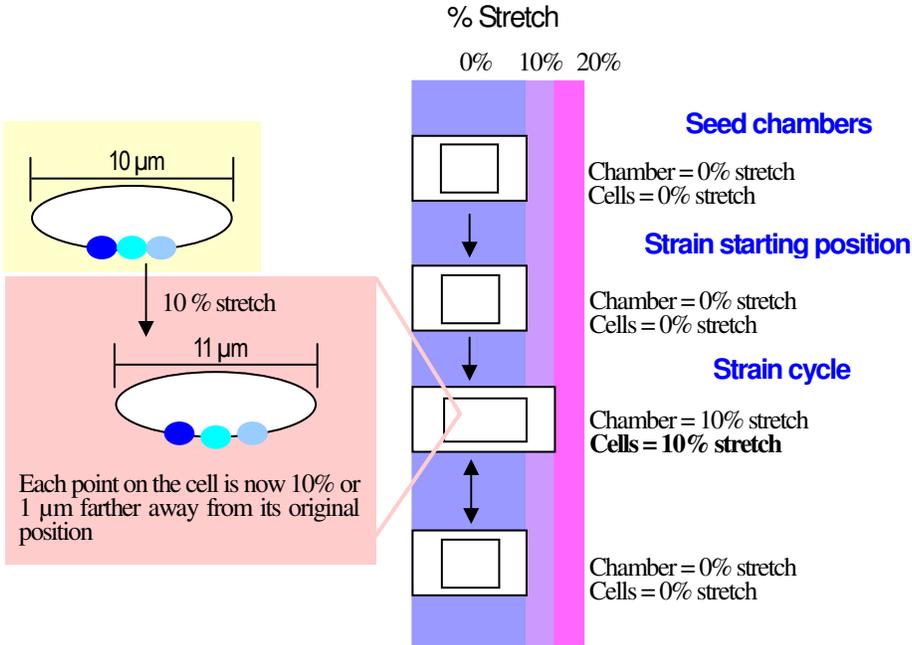
Digit	Rate
0	2%
1	4%
2	6%
3	8%
4	10%
5	15%
6	20%
7	30%

Note: The 30% stretch or compression can only be performed in either the X or Y direction (independently) but not at the same time.

Examples of strain parameters

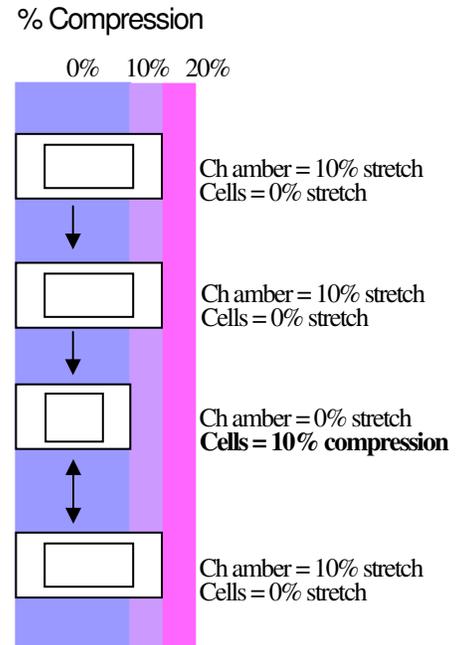
► Stretching Cells

Strain Program: 10% stretch



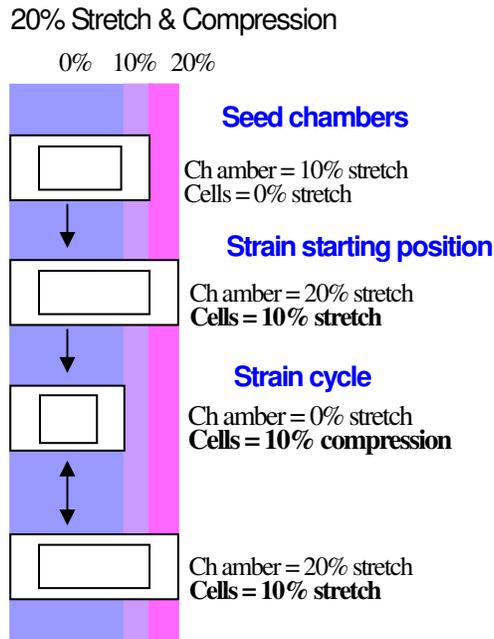
► Compressing Cells

Strain Program: 10% compression



► 10% Stretch & 10% Compression of Cells

Strain Program: 20% stretch or compression



Use the Chamber Length Adjustment Knob to manually stretch the chamber. Use the knob to adjust for the seeding and start positions. Each 360° turn of the knob changes the total chamber length by 5% or 1 mm. ST-CH-04-XY chamber length is 20 mm.

Section 4: FAQ

Q1: What are the characteristics of the silicone chamber?

A1: The strain chamber is made from silicone elastomer consisting of polydiethylsiloxane as its major component. The chamber surface is strongly hydrophobic and cells have difficulty attaching to it; therefore, the chamber surface should be coated with an extra-cellular matrix like fibronectin, collagen, laminin, or gelatin before cultivation.

Q2: Cell attachment on the stretch chamber is not consistent.

A2: There may be wrinkles or bubbles on the bottom surface of the strain chamber when seeding cells. Although the chamber is carefully made not to have wrinkles on it, some products might have little wrinkles due to its thin structure. We recommend the following steps. Place a small volume of ethanol in a Petri dish that is large enough to hold the strain chamber. Gently place the chamber in the culture dish starting at one edge and moving toward the opposite edge of the chamber to remove air bubbles between the dish and chamber. Allow the ethanol to evaporate before spreading your cell suspension in the chamber.

Q3: Cell attachment on the stretch chamber was confirmed by microscopy. But the cells detached from the chamber surface after stretching.

A3: Try seeding your chambers at a lower concentration of cells. Over-confluent cells generally adhere to neighboring cells rather than to the base matrix (dish surface). When an excess amount of cells are put in a culture dish, the cells connect to each other after growth. Such behavior of the over-confluent cells, which are often observed in normal culture dishes, is even worse in the strain chamber.

A second possibility for cell detachment is that the cells were damaged by enzyme treatment such as trypsin before seeding. The damaged cells sometime attach to surfaces by non-specific binding and are not specifically bound to the extra-cellular matrix coating on the chamber; therefore, time, concentration, and temperature for the enzyme treatment should be optimized to reduce cell damage.

A third possibility is insufficient coating of the chamber preventing the cells from attaching to the chamber. Longer coating time is recommended. Some researchers coated the chamber with two or more kinds of the extra-cellular matrix materials to increase binding effectiveness.

Q4: How long can cells be stretched?

A4: The duration depends on cell strain and condition. However, the step motor in an ST-150, ST-195, and ST-190-XY is made to operate for 15-20 continuous minutes. The ST-140 models can run for hours to days if the cooling system is on.

Q5: How can I obtain protein or mRNA samples from the cells attached to the silicon membrane?

A5: (1) Proteins for Western blotting: Wash the cells once with PBS. Add SDS-PAGE sample loading dye directly into the chamber, and collect the cell extract by using a cell scraper.

(2) Proteins for Immunoprecipitation: Wash the cells once with PBS. Add cell extract buffer directly into the chamber, and collect the cell extract by using a cell scraper.

(3) RNA: Wash the cells once with PBS (for RNA preparation). Add RNA extraction buffer directly into the chamber, and collect the cell extract by using a cell scraper.

Q6: I want to use recombinant cells for an experiment.

A6: Direct transfection of cells in the strain chamber may be possible. However, transfection itself may damage the cells, which may make getting clear image data difficult. We recommend performing the transfection in a normal culture dish then transferring the recombinant cells into the strain chamber.

Q7: Cells seem to be crowded in the center of the chamber instead of being uniformly distributed throughout the chamber.

A7: Vibration from the incubator may disrupt the distribution of the cells. We recommend gently rocking the chamber 15 mins after seeding your cells.

Section 5: References

1. Effects of repetitive stretch stimulation on neonatal rat cardiocytes in vitro., K. Kada, K. Yasui, K. Naruse, and J. Toyama, 40: 69-72, Environmental Medicine, 1996.
2. Inhibitory action of repeated stretch stimulation on apoptosis in neonatal rat cardiocytes., K. Yasui, H. Shimano, K. Kada, K. Naruse, and J. Toyama, 40: 175-177, Environmental Medicine, 1996.
3. Mechanosensitive ion channels: Single channels vs. Whole cell activities., M. Sokabe, K. Nunogaki and K. Naruse, 6:139-149, Progress in Cell Research, 1997.
4. Up-regulation of integrin beta3 expression by cyclic stretch in human umbilical endothelial cells., M. Suzuki, K. Naruse, Y. Asano, T. Okamoto, N. Nishikimi, T. Sakurai, Y. Nimura, and M. Sokabe, 239:372-376, Biophys. Biochem. Res.Com., 1997.
5. Mechanotransduction and intracellular signaling mechanisms of stretch-induced remodeling in endothelial cells, Masahiro Sokabe, Keiji Naruse, Shorei Sai, Takako Yamada, Keisuke Kawakami, Masumi Inoue, Kichiro Murase and Motoi Miyazu, S12:191-193, Heart Vessel, 1997.
6. Involvement of SA channels in orienting response of cultured endothelial cells to cyclic stretch., K. Naruse, Y. Yamada, and M. Sokabe, 274:H1532-H1538, Am. J. Physiol., 1998.
7. Up regulation of COX expression by uni-axial cyclic stretch in human lung fibroblast cells, T. Kato, N. Ishiguro, H. Iwata, T. Kojima, T. Ito and K. Naruse, 244:615-619, Biophys. Biochem. Res.Com., 1998.
8. Pp125^{FAK} is required for stretch dependent morphological response of endothelial cells. K. Naruse, T. Yamada, X. Sai, M. Hamaguchi, and M. Sokabe, Oncogene, In press.
9. Orientation Change of Cardiocytes Induced by Cyclic Stretch Stimulation: Time Dependency and Involvement of Protein Kinases, K. Kada, K. Yasui, K. Naruse, and J. Toyama, , J. Mol. Cell. Cardio. In press

Section 6: Safety Instructions and Precautions

Please read this section carefully before using the instrument. Items in this section alert the user to operational dangers that, if not followed, may damage the instrument or, more significantly, result in serious injury or death of the user. To ensure safe operation of the instrument, it is therefore imperative that you follow these instructions carefully.

Cables

To avoid possible short circuit, shock, or fire...

- Only use the power cable provided with the Cell Strain Instrument.
- Do not touch the cable with wet hands.
- Do not use the machine with other voltage than that specified. Do not use 200 volts for an instrument designed for a 100 volts load—overheating, short-circuiting, and/or fire may occur.
- Do not staple around the power cable.
- Do not bend the cable or place heavy objects on it.
- When pulling a connector from an outlet, pull to disconnect gently by holding its plug, not the cable.
- Do not plug many objects into a single electrical outlet since it may cause fire.
- If you are using an extension cord, ensure it can withstand the total current to be used.
- Disconnect power from the unit when it is not in use.
- Connect the instrument to a power-surge protected outlet.

Installation Location and Environment

- Keep the instrument on a stable, leveled floor or a table, secure from vibrations. Be sure you have enough space.
- Do not keep the instrument in a humid or dusty place. Over time, excessive humidity or dust may cause deterioration that can result in an electrical short-circuit and possibly fire.
- Do not use the machine in a place where the temperature is excessively high. Do not place and run the machine near a heater or in a place being exposed to a direct sunlight.
- To avoid possibly explosion, never place and run the instrument nearby the presence of flammable solid substance, liquid, or gas. It may cause explosion or fire.
- Use the machine in well lit conditions.
- Do not use the machine outdoors in direct sunlight or rain, which may cause overheat or short circuit.

Operational Concerns

- Please make sure to read the manual prior to running the unit. Those who are not familiar with the machine should not operate it.
- Do not put your hand closer to mechanical parts or alike while the unit is running.
- Do not put any foreign substances inside the machine. Water, metal, or paper in motor area, may cause fire or electrical shock.
- Do not make any attempt to disassemble nor modify the machine. Do not remove the cover in an attempt to touch the mechanism inside, which may cause you an electrical shock.
- Please refrain from modifying the machine without our permission, you may be shocked or injured. If you do attempt to modify the machine, the warranty on the unit is void and we will not be responsible for any performance deterioration or unit malfunction.
- In the case of any abnormal sound, smell, or smoke, disconnect the power immediately and contact Strex USA.
- Do not run the machine overloaded.
- Be cautious as to your clothing and hair when operating the instrument. Baggy clothing, ties, necklaces, etc., can get tangled in moving parts of the unit. Take appropriate precautions to prevent this occurrence.
- Keep the machine clean and periodically inspect the instrument for excessive wear or damage. Contact Strex USA if you have any concerns.

Safety Precautions

These Safety Precautions are to ensure that you use the product safely and correctly and to prevent harm or injury to users and other people. To prevent injury or harm please read and understand the below text.

 WARNING	Indicates handling prior to reading may cause serious injury or death.
 CAUTION	Indicates handling prior to reading may cause physical harm or damage.

Disclaimer

- We are not responsible for any damage to equipment or facilities during the installation, use, or removal of the product.
- We are not responsible for damages caused by earthquakes, thunder, wind, fire, flood, or a third party to the machine. Negligence, misuse, or abnormal conditions resulting in damage are also not our responsibility.
- We are not responsible for damages caused by malfunctions due to combinations of equipment or software not involving Strex.
- We are not responsible for any incidental damage caused by the use or misuse of this product including loss of business income, interruption of business, loss of stored data, theft of machine, etc.

WARNING

Please do not place water or water-containing vessels on or near the machine:

- Cups, vials, tubes etc. containing water should not be located on or near the device.
- Be careful as to not wet the connection cable or power cable. Failure to do so could lead to fire, electric shock etc. Do not disassemble or reconfigure.
- Do not attempt to disassemble or reconfigure this machine. Doing so may result in fire, electric shock, or equipment malfunction. Please do not use under abnormal conditions.
- If the machine is overheating, emitting a strange odor, etc. disconnect the power cable from the outlet immediately. Failure to do so may result in a fire or electric shock.
- Do not use voltage other than the indicated power supply voltage. Failure to do so may result in fire or electric shock. Be sure to use the supplied power cable.

- Do not exceed the rating of outlets and wiring equipment. If rating is exceeded with the multiple electrical components fire may be caused due to heat generation.
- Do not touch the main unit or the power cable during severe weather events. It may cause electric shock.
- Do not damage the power cable, forcibly bend it, twist it or pull it. Also, please do not place heavy or heated objects on the power cable. The power cable may be damaged, causing fire, electric shock accident, etc.
- Please contact your distributor to replace the power cable.
- Do not handle power cable with wet hands. Be aware of foreign matter entering instrument
- Unplug the machine immediately if foreign matter, such as water or excessive dust, is expected to have entered it to prevent risk of electric shock. If you dropped or damaged the machine.
- Unplug the power cable if the machine has been dropped or damaged. Not doing so may result in electric shock.

CAUTION

Proper Handling of This Equipment

- Do not place the power cable close to a heating source such as a hotplate or open flame. The cable cover may melt, causing fire, electric shock, malfunction, etc.
- When unplugging the power cable from the outlet, please do not pull on the cable part, but remove at the plug. Pulling the cable will damage the cable and cause fire, electric shock, breakdown, etc.
- Regularly check the condition of the plug. If it is damaged or if dust gathers in the plug insulation failure may result, causing fire. Also, if the plug is incompletely inserted, it may cause electric shock or fire. Do not place heavy objects on top of this machine.
- If you place heavy objects on the machine, the items may collapse or fall and cause injury.

Usage Notice

Periodically clean the plug and receptacle once a month and check that it is securely inserted. When you are not using the machine for a long time, please be sure to unplug the power cable from the outlet for safety.

Section 7: Warranty

1. The warranty is for one year, commencing the date the customer receives the product and includes the instrument casing, non-wearable parts, as well as, the motor and bearings. The cell culture chambers are considered consumables, Strex USA is responsible for repair or replacement of chambers, only if they are received and found defective.
2. The warranty does not cover damage to the instrument that is a result of the following circumstances:
 - ① Damage caused by dropping, or other impact.
 - ② Damage caused by inappropriate operation of the instrument.
 - ③ Damage resulting from an attempted repair or modification of the instrument by the user.
 - ④ Damage caused by unavoidable external causes such as earthquakes, lightening, fire, flood, gas leak, power surges, or other acts of providence.
3. Strex USA is free from any responsibility for effects or loss or damages arising from the result of the machine operation.

This warranty assures that Strex USA will repair our product free of charge as stipulated in our warranty policy. Any shipping charges will be the buyer's responsibility.

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The information contained herein such as specification, configuration, and data or alike in part or in whole may be subject to change without notice.