STREX

STREX Cell Strain Instrument

Cat. # STB-150W

User Manual

StrexCell.com
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Section 1: Main Components

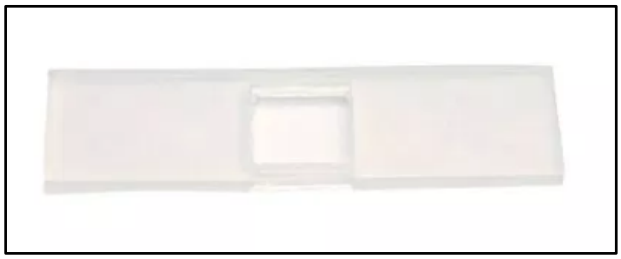
Control Unit
Allows Stretch Distance and Frequency Settings

Motor Cable
Cable connects to Control Unit

Electrical Cable
Connects Control Unit to electric plug for power supply.

Stretching Chamber, Platform and Stretching Arms:
Silicon Chamber where cells are seeded and placed under stretching arms to induce stretch on Platform.

Silicone Strain Chamber (STB-CH-0.02)



Control Unit Front Panel

Main Power Switch

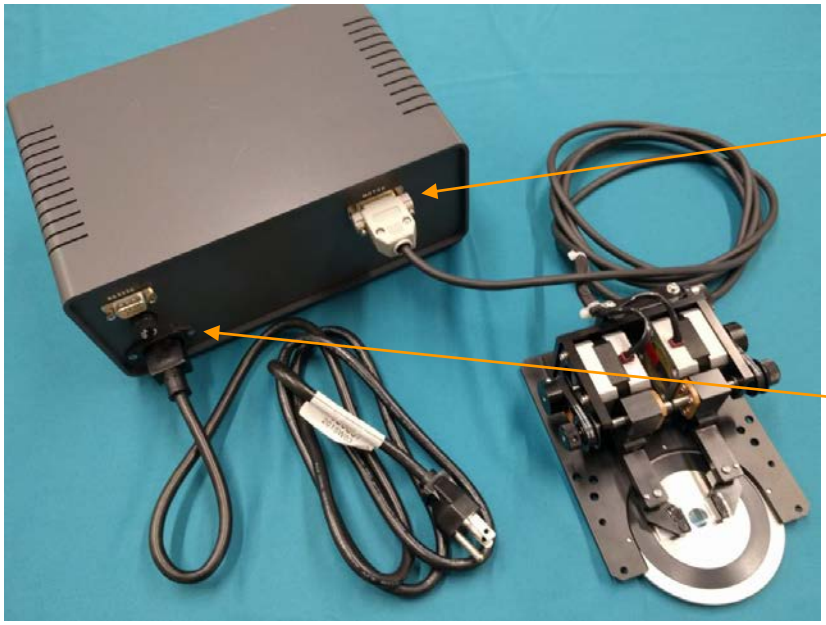
Frequency Selector (left digit)
Use buttons to adjust frequency

Stretch Distance Selector (right digit)
Use buttons to adjust distance

Mode Selector Switch
Switch up for Mode 1 and down for Mode 2

Start and Stop Button
Use to start or stop the stretching action.

Control Unit Back Panel Connections



Motor Cable Outlet
Connects the Stretch Unit to the Control Unit via Motor Cable

Power Cable Outlet

Section 2: Preparation and Use of the Cell Strain Instrument

Preparation of the Cell Strain Instrument

Before using the Stretch Unit, sterilize the unit — especially the chamber mounting area — using ethanol-immersed swabs.

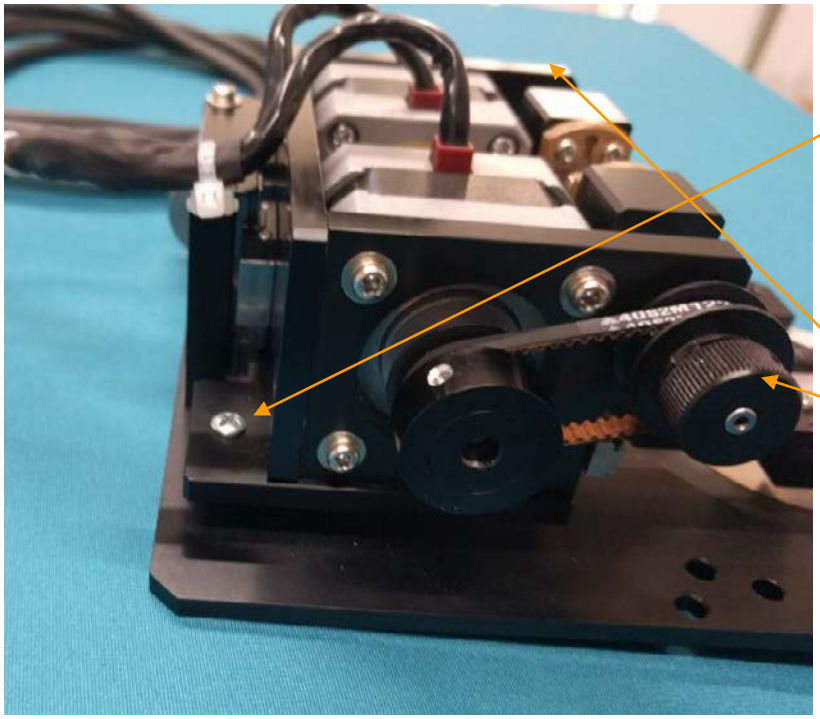
►Set-up of the Stretch Unit and Control Unit

(See Control Unit Back Panel Connections Image on Previous Page)

1. Connect the Stretch Unit to the Control Unit by the Motor Cable. This supplies electricity to the Stretch Unit and enables the two units to communicate.
2. Connect the Control Unit to the power supply 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.
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 Stretch Unit is level on the microscope stage.

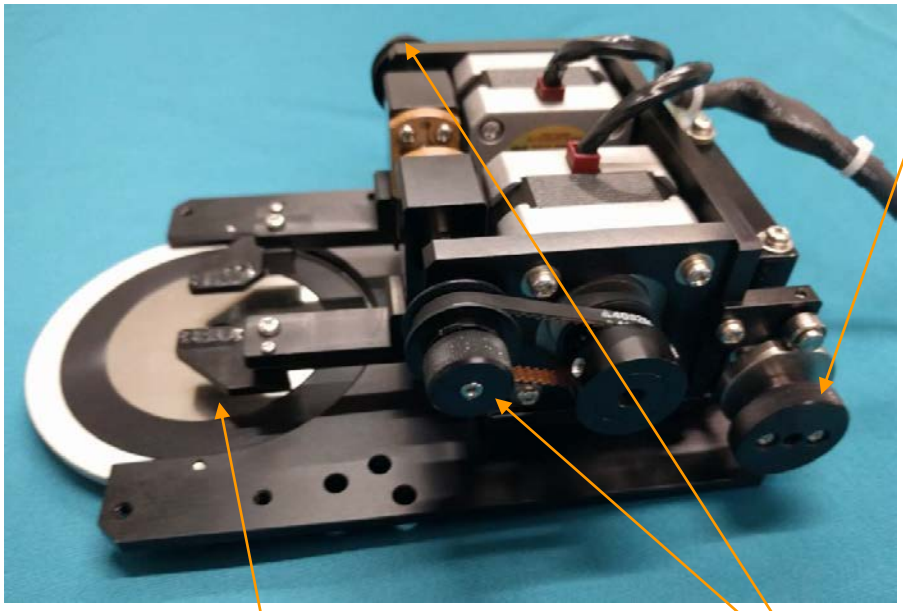
►Releasing and Positioning Motors and Stretching Arms

1. A Retaining Screw is in place to protect the Motors and Stretching Arms from damage during transit. Removal of this screw will allow the Motor and Stretching Arms to move freely up and down.



Retaining Screw
Holds the Motors and Stretching Arms in an elevated position during transit. Removal is required before use.

Stretching Arm Position Knobs (1 pictured)
One on each side can be used to adjust position of each arm independently. Important for Replacement of Cell Stretching Chamber.



Height Adjustment Wheel
Allows height adjustment of Motors and Stretching Arms in order to replace stretching chambers.

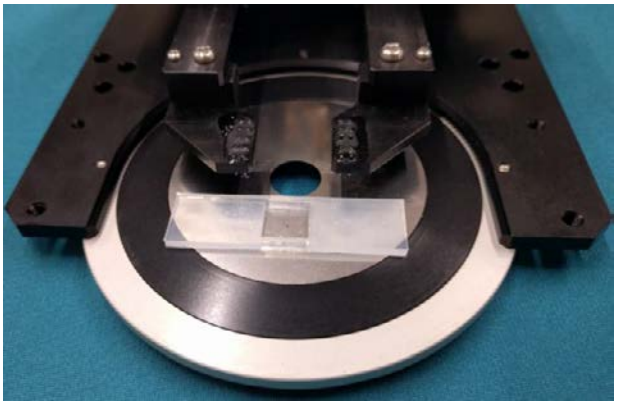
The flat portion of the Wheel is used for the “up” position to raise the stretching arms, as seen here. Rotation of the Wheel will lower the Stretching Arms onto a Stretching Chamber.

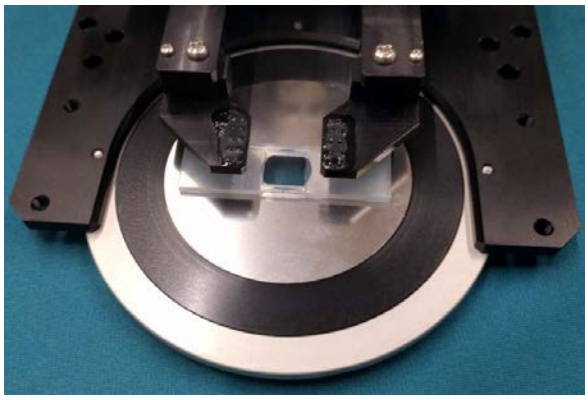
Stretching Arms in “Up” Position
Stretching Arms are raised to replace Cell Stretching Chamber under them.

Stretching Arm Position Knobs

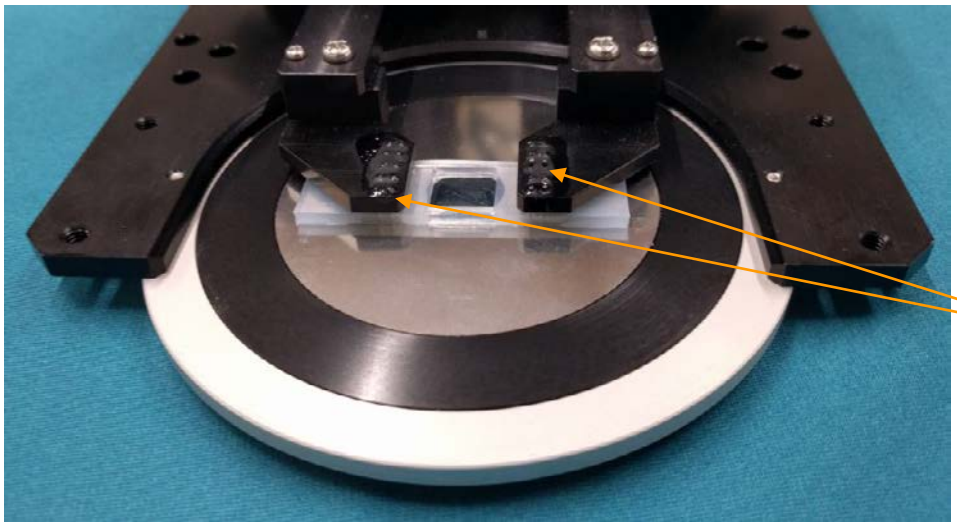
WARNING: Lowering of the Stretching Arms without a Cell Stretching Chamber in place can damage the Platform and/or the pins on the Stretching Arms.

►Replacement of the Cell Stretching Chamber





1. Place a properly prepared Cell Stretching Chamber cultured with cells onto the Platform in front of the Stretching Arms with the Motors and Stretching Arms in the “Up” Position. Slide it carefully to directly under the stretching arms (see above).
2. Ensure the Cell Stretching Chamber is centered above the circular opening on the Platform. Using the Stretching Arm Position Knobs (see page 4 & 5) ensure the Stretching Arms are in a centered position above the stretching chamber (see above).
3. Use the Height Adjustment Wheel (page 5) to lower the Stretching Arms and Motor on to the Cell Stretching Chamber



Note: Gentle downward pressure can be placed on the Stretching Arms above the pins in order to securely attach the Stretching Arms to the Cell Stretching Chamber. Cell Stretching is ready to begin.

Operation of the Cell Stretching Instrument

►Start Cell Stretching

Operation of the Cell Strain Instrument is very straightforward and intuitive. Below are the basic steps to use this instrument. **The stepper motor that moves the chamber brackets is made to operate for 15 minutes continuously. It is not recommended to extend the use of the motor beyond 15 continuous minutes because of possible overheating and burning out of the motor.**

1. Make sure that the electrical/communications for the Stretch and Control Units are properly set up.
2. Load the chamber into the Stretch Unit by Replacement of the Cell Stretching Chamber (see page 6). **The Power Switch must be OFF to freely rotate the Stretching Arm Position Knobs.**
3. Rotate the Stretching Arm Position Knobs to create slight tension on the chamber. The membrane at the bottom of the chamber should be taught.
4. Turn on the Power Switch.
5. Select the desired Stretch Distance and Frequency on the Control Unit. Programs are outlined in Section 3.
6. Press the START button to start the stretch cycle. During the action cycle, the green light on the START button will be illuminated. To STOP movement at any time, press the STOP button.
7. **Do not change the Stretch Distance or Frequency parameters during the operation of a stretch cycle. Press the STOP button and wait for the program to complete the final stretch-contract sequence (returning to the original start position) before initiating the next stretch parameter.** Changing parameters during a stretch cycle may damage the motor.
8. After a few minutes of running a stretch program, stop the cycle and check the condition of the cells. If the cells have not detached from the membrane, proceed with your experiment. If the cells are detached, the chamber coating was probably insufficient. Recoat the chambers per the protocols on page 9 and 10.

Culturing Cells in the Silicone Chambers

1. Seed cells at the appropriate concentration in a freshly coated chamber.
   Important: It is critical to not over expose the cells to dissociation enzymes. Cells should be treated in the same manner (type and concentration of enzyme, temperature, and exposure time) for all experiments.
   Important: Cells should not be seeded at a high 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 an overnight incubation without stretching, inspect the cells with a microscope to ensure they have adhered to the chamber.

Preparation of Silicone Chambers

Before using the chambers, they should be sterilized and coated with a cell adhesion matrix. The coating procedures can be adapted for use with other matrices, such as elastin, pronectin, and laminin.
Sterilize the 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 chambers in a Petri dish in preparation for coating.

The PDMS (silicone) chamber is very hydrophobic with two methyl-bases on the surface; therefore the chamber must be coated with a cell adhesion matrix such as fibronectin or collagen. In the presence of fibronectin or collagen, cells adhere to the matrix by integrins. Integrin attachment is a cell specific interaction unlike cell attachment to plastic or glass dishes, where cells non-specifically attach due to a charged surface.

►Fibronectin Coating

Preparation of fibronectin solution:

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

Coating with fibronectin solution:

1. Pour 3 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 ml of the gelatin solution into each strain chamber
2. Incubate at 37^{o}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.
5. If coating is successful, water will not be repelled.

Important: If cells are having difficulty attaching to the freshly coated chambers or are easily detaching upon stretching, treat the chamber with a higher concentration of the extra-cellular matrix or coat overnight.

Section 3: Strain Parameters

Standard Program

(Custom Programming may be indicated for STB-150W)

RIGHT Digit: Stretch Distance Selector

MODE 1 and 2 can be selected with the Mode Selector Switch (see page 3)

Digit	Distance MODE 1	Distance MODE 2
0	0.1mm	1.5mm
1	0.2mm	2.0mm
2	0.3mm	2.5mm
3	0.4mm	3.0mm
4	0.5mm	3.5mm
5	0.6mm	4.0mm
6	0.7mm	4.5mm
7	0.8mm	5.0mm
8	0.9mm	5.5mm
9	1.0mm	6.0mm

LEFT Digit: Frequency Selector

Digit	Program	Description
0	1 cycle	Stretch >> Pause 0 sec >> Contract
1	1 cycle	Stretch >> Pause 3 sec >> Contract
2	1 cycle	Stretch >> Pause 5 sec >> Contract
3	1cycle	Stretch >> Pause 10 sec >> Contract
4	Continuous stimulation	Repeat 10 times / min
5	Continuous stimulation	Repeat 20 times / min
6	Continuous stimulation	Repeat 30 times / min
7	Continuous stimulation	Repeat 60 times / min
8	One time stimulation	Stretch 0.5 sec >> hold till stop button
9	One time stimulation	Stretch 1/10 distance >> hold till stop button

Note: 60 cycles/minute is the maximum speed at which the instrument can operate.
At this speed 60 cycles/minute, square pattern does not have a hold time and the pattern becomes the same as a sinusoidal pattern.
Inquire with Strex for faster speeds.

Section 4: FAQ

Q1: What are the characteristics of the silicone chamber?

A1: The strain chamber is made from silicone elastomer consisting of polydimethylsiloxane 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. Using a Petri dish that is large enough to hold the chamber, add a small volume of ethanol. Place one end of the chamber in the ethanol and lay the chamber down by slowly moving toward the opposite end of the chamber without trapping air bubbles between the dish and the chamber. The thin layer of ethanol between the dish and the chamber will remove any wrinkles in the chamber membrane. 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 the cells.

A3: Try seeding your chambers at a lower concentration of cells. In typical cell culture dishes, over-confluent cells generally adhere to neighboring cells rather than to the base matrix (dish surface). This behavior is exaggerated when an excess number of cells are seeded into a stretch chamber.

A second possibility for cell detachment is that the cells were damaged by enzyme treatment such as trypsin.

The damaged cells may 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. In this case, longer coating time is recommended.

Some researchers coat 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 a STB-150 and STB-190XY is made to operate for 15 continuous minutes. The STB-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 silicone 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 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 standard culture dish then transferring the recombinant cells into the strain chamber.

Q7: Cells seem to be crowded in the center 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

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Section 6: Safety Precautions & Instructions

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 handing 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.

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.

Power Cable

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. In some cases, a transformer may be used for compatibility. Inappropriate current may result in the machine.
  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, level floor or a table, secure from vibrations. Be sure to have enough space.
- Do not store 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 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 overheating 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 close 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 or modify the machine. Do not remove the cover in an attempt to touch the mechanism inside, which may cause electrical shock.
- Please refrain from modifying the machine without Strex's permission, shock or injury may occur. If attempts to modify the machine are made, the warranty on the unit is void and Strex will not be responsible for any performance deterioration or unit malfunction.
- If an abnormal sound, smell, or smoke is detected disconnect the power immediately and contact Strex.
- Do not run the machine overloaded.
- Be cautious as to your clothing and hair when operating the instrument. Baggy clothing, neckties, 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 if you have any concerns.

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 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, lightning, fire, flood, gas leak, power surges, or other acts of providence.

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