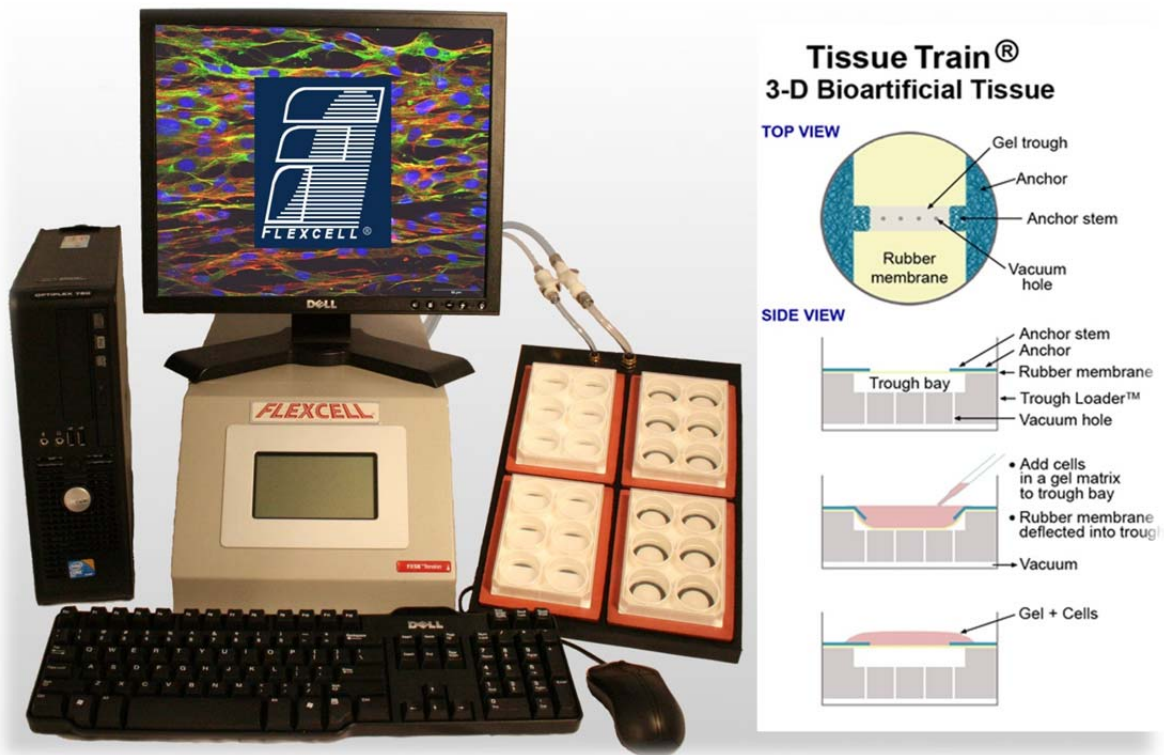




# Flexcell<sup>®</sup> Tissue Train<sup>®</sup> Culture System



12-15-14  
Rev 4.4

*Culturing Cells in a Mechanically Active Environment™*

Flexcell International Corporation • 2730 Tucker Street, Suite 200 • Burlington, NC 27215  
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## ONE YEAR LIMITED PARTS WARRANTY

### Flexcell® Tissue Train® System

1. **FLEXCELL INTERNATIONAL CORPORATION** warrants to the original purchaser/customer all hardware components of the **Flexcell® Tissue Train® System** serial # \_\_\_\_\_ for a period of one year from the date of delivery to the purchaser/customer to be free from manufacturing defects in workmanship or materials with the following exceptions, terms and conditions:
  - a. ITEMS EXCLUDED FROM THE WARRANTY ARE: Dell OptiPlex desktop computer and monitor\*, software, disks, manuals and external peripherals such as printers, mouse or track ball units, imaging devices, vacuum pumps, air tanks, electric voltage converters, compressors, surge suppressers and all other accessory equipment.
  - b. DURING THE WARRANTY PERIOD, the purchaser/customer must notify Flexcell of any warranty claim in writing, by telephone, fax transmission or email identifying each defective part or specifically describe the exact problem no later than the last day the warranty is in effect.
  - c. FLEXCELL AGREES to correct any defect in workmanship or material and supply new or rebuilt parts in exchange for defective parts upon completion and submission by purchaser/customer of a printed "Parts Return Authorization" form furnished by Flexcell. Parts must be properly packed in original container and shipped to our factory service center or distributor with all shipping costs prepaid if the unit is out of warranty coverage. If the original shipping box is not available, Flexcell will send the required protective shipping container. (Flexcell will recommend the insurance value for parts or equipment to be shipped.) Return carrier shipping costs will be paid by Flexcell from the service center. The purchaser/customer is solely responsible for payment of custom fees, taxes, holding fees or value added taxes.
  - d. THIS LIMITED WARRANTY only covers failures due to defects in materials or workmanship which occur during normal use. It does not cover damage which occurs in shipment or failures of original equipment due to products identified as add-ons not manufactured by Flexcell International Corporation or its distributors nor does this limited warranty cover damages or failures which result from accident or disaster such as fire, explosion, flood, wind, lightning, or earthquake or misuse, abuse, neglect, mishandling, misapplication, alteration, faulty installation, modification or service by anyone other than our factory or distributor. Breaking the seal and opening the unit case or violating unit components will void the warranty. This warranty is extended only to the original purchaser/customer unless a transfer of ownership is approved by Flexcell in writing.
  - e. LIMITED LIABILITY. Flexcell or its distributor's only liability shall be to remedy any defect to comply with its warranty and return the repaired equipment to function as designed. Under no circumstances shall Flexcell or its distributors be liable for any special incidental or consequential damages based upon breach of warranty or contract or negligence. Such damages include, but are not limited to: loss of profits, revenue, loss of data, down time, customer's material or time.
  - f. DISCLAIMER OF WARRANTIES: The Limited Warranty expressed in the foregoing language is the only warranty applicable to this product. Any other warranty, expressed or implied warranty or of merchantability or fitness for a particular purpose are hereby disclaimed. No oral or written information or advice provided by Flexcell, through its agents or employees, in the use and functioning of the equipment shall in any way create a warranty or in any way increase the scope of this limited warranty.
  - g. DISCLAIMER: LANGUAGE. This warranty document, accompanying instruction manual and supplemental applicable laws appear in the English language. In the event of any inconsistency in the meaning of the words and terminology and any foreign language translation, the English language shall prevail.
2. **GOVERNING LAW.** The performance of the duties and liabilities of the parties under the terms and conditions of this Limited Warranty shall be governed in all respects by the laws of the State of North Carolina, the United States of America.

APPLICATION OF STATE LAWS: Some states do not allow the exclusion or limitation of consequential damages nor do some states allow limitations on how long an implied warranty lasts, so the above limitations may not apply to you. This warranty gives you specific legal rights and you may also have other rights that vary from state to state.
3. **INTERNATIONAL CUSTOMERS.** The full text of the foregoing limited warranty and all disclaimers is applicable to international customers/purchasers except when the purchase was made from an international distributor or reseller, the warranty will be covered through your distributor or reseller.

If technical advisory support service is not available through your distributor or reseller, for service contact warranty headquarters by phone or fax.

Within the United States only: 1-800-728-3714 (toll free) - Fax: 1-919-732-5196  
International Customer's telephone: 01-919-732-1591 - Fax: 01-919-732-5196  
Email: [info@flexcellint.com](mailto:info@flexcellint.com)

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*\*Customers should contact Dell Inc. for all warranty questions or concerns with regards to the Dell OptiPlex computer and monitor.*



## **Dell COMPUTER WARRANTY INFORMATION**

Dear Customer:

Congratulations on your purchase of the Flexcell® Tissue Train® system. This system comes with a one (1) year warranty. The Dell computer that is included with this equipment will be transferred into the Principal Investigator and University's name at the time of purchase. This computer comes with an excellent warranty through Dell. If you have any problems with this computer, you will contact Dell directly. The details of the warranty are listed below. You will need to have the service tag number and Express Service Code number, which are located on the side of the computer.

- Dell Hardware Limited Warranty Plus Onsite Service Extended Year
- Dell Hardware Limited Warranty Plus Onsite Service Initial Year
- Thank you choosing Dell ProSupport. For tech support, visit <http://support.dell.com/ProSupport> or call 1-866-516-3115
- ProSupport for IT: Next Business Day Parts and Labor Onsite Response 2 Year Extended
- ProSupport for IT: Next Business Day Parts and Labor Onsite Response Initial Year
- ProSupport for IT: 7x24 Technical Support for certified IT Staff, 2 Year Extended
- ProSupport for IT: 7x24 Technical Support for certified IT Staff, Initial year



## **FEDERAL COMMUNICATIONS COMMISSION** **RADIO FREQUENCY INTERFERENCE STATEMENT**

This computing equipment has been type-tested and found to comply with the limits for a Class B digital device in accordance with the specifications for Subpart J of Part 15 of FCC Rules. These limits are designed to provide reasonable protection against harmful radio and TV interference in a residential installation. There is no guarantee that interference will not occur in a particular installation.

The following instructions and precautions must be observed by the user when installing and operating this device:

1. The equipment must be operated in strict accordance with the manufacturer's instructions.
2. The unit(s) must be plugged into a properly-grounded power outlet and used with the power cord supplied with the unit(s), unmodified.
3. The unit must be operated with the factory-installed cover in place.
4. No modifications of the equipment can be made which may result in the equipment failing to meet the limits of the FCC Rules.
5. The equipment must be maintained in a satisfactory state of repair.
6. Shielded cables must be used with this equipment to ensure compliance with the FCC Rules.

If the unit interferes with radio or television reception (which can be tested by turning the unit off and on) the user is encouraged to try the following procedure to correct the interference:

1. Reorient the antenna of the receiver.
2. Relocate the equipment to increase the distance between the unit and the radio/TV.
3. Connect the unit to an outlet on a different circuit from the receiver.
4. Consult with the dealer or an experienced radio/TV technician for advice on ways to reduce interference.



## **TISSUE TRAIN® QUICK START INSTRUCTIONS**

1. Choose a location for the Tissue Train® system that is near the incubator.
2. Place the computer, monitor, keyboard, mouse and FX5K™ Tension FlexLink® on a sturdy, flat surface.
3. Connect the keyboard, mouse and monitor to the computer according to the manufacturer's instructions.
4. Connect the FlexLink® to the Ethernet port on the back of the computer using the Ethernet cable supplied (see Figs. 6 and 7, pg. 9).
5. Connect the computer, monitor and FlexLink® to a power source using a surge protector.
6. Connect the system vacuum to the **SYSTEM** port on the back of the FlexLink® and check the system vacuum supply (see manual section 2.6 for pump specifications recommendations).
7. Cut two lengths of tubing from those supplied: cut a 10' (3 m) length of the ¼" (6.4 mm) and cut a 10' length of the ⅜" (9.5 mm) tubing. Connect each length of tubing to the rear connection of the FlexLink® in the **FLEX IN** and **FLEX OUT** ports, accordingly. Attach provided quick disconnects to the other end of each piece of tubing.
8. Place the Water Traps (see Fig. 5, pg. 9) **inline** with the **FLEX IN** and **FLEX OUT** tubing:
  - a. Make cuts in the tubing for the Water Traps to be positioned as near as possible to the baseplate, outside of the incubator, so that the water will be trapped away from the FlexLink®.
  - b. Place the Water Traps at a position below the FlexLink® unit.
9. Place the baseplate(s) in the incubator:
  - a. Insert the ¼" (6.4 mm) and ⅜" (9.5 mm) tubing into the access opening of the incubator.
  - b. Connect the quick disconnects for the ¼" (6.4 mm) and ⅜" (9.5 mm) tubing to the baseplate(s).
  - c. Place four Trough Loaders™ into the four baseplate wells (optional).
  - d. Place four gaskets on four Tissue Train® plates and place a Tissue Train® plate and gasket in each baseplate well.
10. Turn the FlexLink® and computer system ON. The Windows® desktop should appear on the monitor. Double click on the FX-5000™ icon on the left-hand side of the screen to start the software.



11. In the **Configure** menu, select **User Editor** and register as a user.
12. In the **Regimen** menu, select **Create new** and create a regimen by entering values into the appropriate spaces or click on the **Run Wizard** button in the bottom left-hand corner for a step-by-step procedure. When completed, click on **Save Regimen**. Exit the regimen editor.
13. In **Controllers** menu, select the appropriate FlexLink® number to which the regimen will be downloaded.
14. In the **Regimen** menu, select **Assign**. Choose the proper platform, user, and regimen (**see section 3.7 for assignment instructions**) from the drop-down menus. Confirm with a **Yes** to download the regimen.
15. Once the program is downloaded, click on **Simulate** in the main window display. Once satisfied that the regimen is correct, click on **Stop**.
16. Turn ON the vacuum system. Click on **Start** to run the regimen. The plates should begin flexing. Make a visual check to verify membrane has been pulled downward into the troughs.
17. To pause or stop the regimen, click on **Pause** or **Stop** at any time. Otherwise, the program will stop when the regimen is completed.
18. **Check the Water Traps prior to each experiment** to see if water is accumulating. Empty the Water Traps before they are more than half full.



## PART A

# CREATING 3D CELL- SEEDED HYDROGELS



## 1. INTRODUCTION

Flexcell®'s Tissue Train® culture system utilizes a novel method to culture and mechanically load cells in a three-dimensional (3D) hydrogel or cell-assembled matrix. Mechanical load may be applied using the Flexcell® Tension System with a user defined program. Cell-gel constructs or cell-assembled matrices may be mechanically loaded uniaxially or equibiaxially using special Arctangle® or cylindrical Loading Stations™, respectively. A program defining the % elongation, frequency and duration of mechanical load can be regulated by the user in the FX-5000™ FlexSoft® software.

Flexcell's Tissue Train® Culture System can be used as a stand-alone device to create

three-dimensional geometries (i.e., linear, trapezoidal, or circular) for cell culture in a matrix gel or allow the cells to build a self-assembled matrix that connects to the anchors in the Tissue Train® culture plate. The investigator can also apply regulated strain to the growing tissues. A user can define a frequency, elongation and duration of strain in a regimen that simulates the strain environment of a tissue in the body. The strain regimen is controlled by the Flexcell® Tension System (see Part B, pg. 7) that can apply static or cyclic equibiaxial or uniaxial strain. The following is a method by which to culture cells in a 3D matrix.

## 2. METHOD TO CREATE 3D CELL-SEEDED HYDROGEL

### 2.1 PREPARATION OF CELLS IN 3D LINEAR OR TRAPEZOIDAL GELS IN A TISSUE TRAIN® CULTURE PLATE

of a 6-well Trapezoidal Tissue Train® culture plate.

1. Prepare cells according to the laboratory's established protocol for primary cultures or continuous cell lines in the medium of choice.
2. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase or other means. Add serum containing media to the cells to neutralize the trypsin or collagenase.
3. Count cells and determine the number of cells needed:
  - Linear gel: approximately 50,000-200,000 cells in 150-200 µL for each well of a 6-well Tissue Train® culture plate.
  - Trapezoidal gel: approximately 700,000 cells in 700 µl for each well

**NOTE:** Cell seeding density will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.

4. Wash cells 2 times with medium to remove trypsin or collagenase.
5. Before adding cells, the matrix protein gel solution should be neutralized to pH 7.0 using 1 M sodium hydroxide.
6. Cells may be reconstituted in one volume of media containing 10% fetal calf serum, 70% matrix protein gel fluid and 20% 5X MEM. The objective is to achieve an overall gel-MEM concentration of 1X. The suggested formula for the cell/matrix protein gel combination is as follows:

- 70% by volume matrix protein gel
  - 20% by volume of 5X MEM to yield an overall 1X concentration by total volume
  - 10% fetal calf serum
  - Cells
7. The Tissue Train® culture plate in either the linear or trapezoidal anchor configuration can be used for creating the 3D constructs. For linear-shaped constructs, use a linear Trough Loader™ with a standard Tissue Train® culture plate. For trapezoidal-shaped constructs, use a Trapezoidal Trough Loader™ with a Trapezoidal Tissue Train® culture plate.
  8. Place the appropriate shaped Trough Loader™ in a Loading Station™ beneath the flexible membrane of the Tissue Train® culture plate so that the anchor stems are aligned with the long axis of the Trough Loader™ (Fig. 1). Apply a thin layer of lubricant to the top surface of the Trough Loader™ prior to placement of the Tissue Train® culture plate on top. The lubricant will facilitate uniform and unrestricted conformation of the membrane in the trough.
  9. Place the Tissue Train® culture plate on a BioFlex® baseplate with gaskets and connect to the Flexcell® FX5K Tension FlexLink® or other regulated vacuum source

Vacuum should be applied to the baseplate in a steady “hold” mode so that the flexible membrane is deformed and held in the space in the Trough Loader™. To supply the proper vacuum level with the FX-5000™ system, it is recommended that a maximum of 20% elongation be used with the Tissue Train® Loading Station™ (24 mm) platform setting. This is the equivalent of -90 kPa. Be sure that you

allow enough vacuum tubing for your baseplate to reach from your incubator to your tissue culture hood.

**NOTE:** When using the Flexcell® Tissue Train System use the minimal amount of tubing required, as longer tubing decreases cyclic strain performance. It may be more practical to use two sets of tubing to supply vacuum to the Tissue Train® baseplate. One set should be used for 3D gel formation and should be long enough to span the distance from the vacuum source and both the cell culture hood and the incubator. The second vacuum line set should be dedicated to the cyclic strain process and should be routed through the incubator portal. Such an arrangement optimizes the cyclic strain process and minimizes potential sources of contamination.

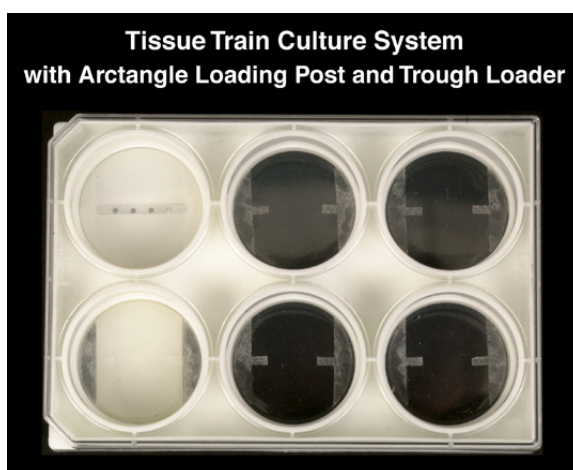


Figure 1. Tissue Train® culture plate for 3D culture of cell-matrix constructs. The top left well has a Trough Loader™ beneath the flexible membrane. The four adjacent wells show the anchors for attachment of cells and gel for a uniaxial construct. The bottom left well shows an Arcangle® loading post to deliver uniaxial tension.

10. Pipette the cell and matrix protein gel solution into the “trough” in each Tissue Train® well (Fig. 2). First pipette a small drop of gel at each end of the trough, under the anchor stems. Then press the anchor stems into the trough and pipette over top of them. Finally, fill the middle of the trough with gel, moving the pipette back and forth to create a uniform strip of gel in the well (see video of Tissue Train®

Bioartificial Tissue Fabrication with Uniaxial Strain on Flexcell®'s web site, [www.flexcellint.com](http://www.flexcellint.com).)

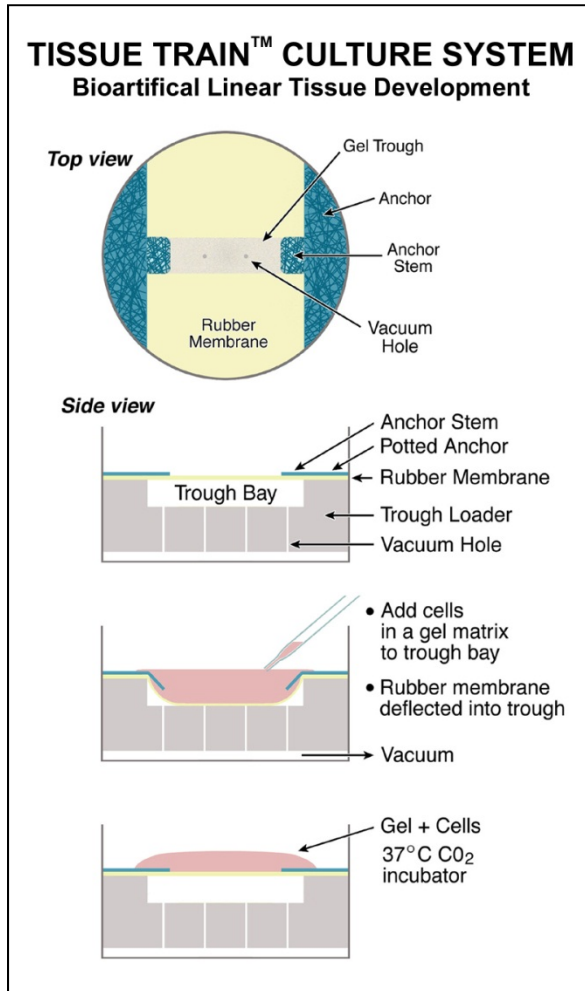


Figure 2. The top view shows a cell-gel construct in a Tissue Train® well attached to the anchor stem. The vacuum holes deform the rubber membrane downward to create a space for the gel. The side view shows the membrane conforming to the trough and the loading of a gel matrix in the trough.

- Place the baseplate with culture plates in a 37 °C incubator and allow the solution to gel. After the gel has set, release the vacuum and add 3 mL of serum-containing media to each well. The gels should appear as a band of gel attached at each anchor end in the Tissue Train® well (Fig. 3). Remove culture plates from the BioFlex® baseplate, if needed.

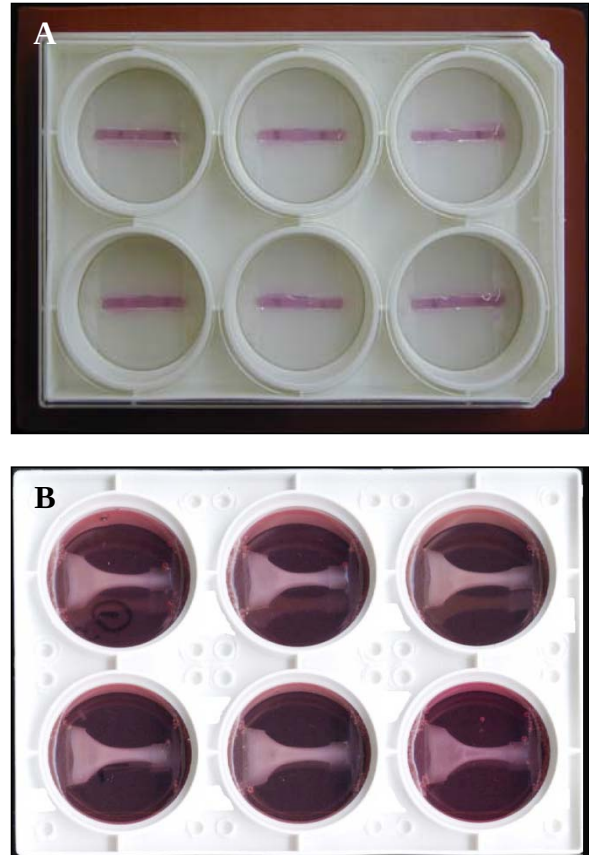


Figure 3. Tissue constructs in A) a linear Tissue Train® plate atop Trough Loaders™ and B) a Trapezoidal Tissue Train® culture plate.

- Culture constructs according to the laboratories established protocol. Cultures may be observed or assayed for various functions. The user can monitor cell shape, organization, migration, division, gene expression, protein expression /secretion, mediator secretion DNA and protein synthesis in the gels, to name a few. Cultures may also be mechanically loaded (see Part B) at any time after gelation or after cells have self-assembled their own matrix. The elongation, frequency and duration of the applied strain to the construct must be determined by the investigator for his/her specific use.

Typically, spherical-shaped cells can be observed in the gel using an inverted phase contrast microscope directly after plating. By day one, the cells will begin to attach

to and spread into the matrix. Cells will then form attachments to each other and intercommunicate. By days 3-5, cells will reorganize and contract the matrix (Fig. 4).

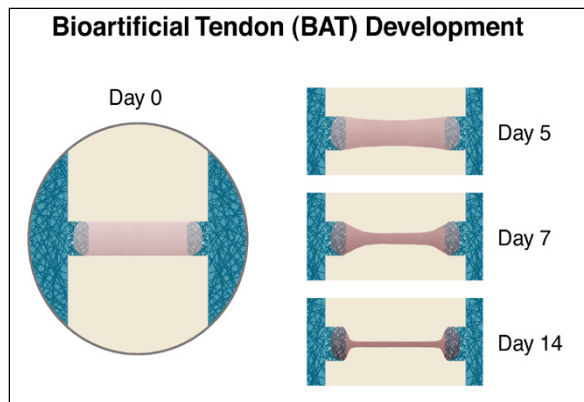


Figure 4. Contraction of a bioartificial tissue construct over time.

## 2.2 PREPARATION OF CELLS IN 3D CIRCULAR GELS IN A TISSUE TRAIN® CULTURE PLATE

1. Prepare cells according to the laboratory's established protocol for primary cultures or continuous cell lines in the medium of choice.
2. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase or other means. Add serum containing media to the cells to neutralize the trypsin or collagenase.
3. Count cells and determine the number of cells needed:
  - Circular gel: approximately 1-2 million cells in 1 mL for each well of a 6-well Tissue Train® Circular Foam culture plate.

**NOTE:** Cell seeding density will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.

4. Wash cells 2 times with medium to remove trypsin or collagenase.
5. Before adding cells, the matrix protein gel solution should be neutralized to pH 7.0 using 1 M sodium hydroxide.
6. Cells may be reconstituted in one volume of media containing 10% fetal calf serum, 70% matrix protein gel fluid and 20% 5X MEM. The objective is to achieve an overall gel-MEM concentration of 1X. The suggested formula for the cell/matrix protein gel combination is as follows:
  - 70% by volume matrix protein gel
  - 20% by volume of 5X MEM to yield an overall 1X concentration by total volume
  - 10% fetal calf serum
  - Cells
7. For circular-shaped constructs, a Trough Loader™ and vacuum source are not required to create the 3D gels. The foam annulus in the Tissue Train® Circular Foam culture plate serves as both the anchor and forms a well into which the cells and gel are cast.
8. Pipette the cell and matrix protein gel solution into the space in the center of the foam anchor and around the inner perimeter of the annulus anchor (approximately 1.5 mm wide). Use the pipette tip to compress the edge of the foam so that the cell and matrix protein gel solution is drawn into it. This step ensures that the matrix and cells will integrate with the structure of the foam and form a secure bond to support the artificial tissue disk during mechanical loading.
9. Place the baseplate with culture plates in a 37 °C incubator and allow the solution to gel. After the gel has set, release the vacuum and add 3 mL of serum-



containing media to each well. Remove culture plates from the BioFlex® baseplate, if needed.

10. Culture constructs according to the laboratories established protocol. Cultures may be observed or assayed for various functions. The user can monitor cell shape, organization, migration, division, gene expression, protein expression /secretion, mediator secretion DNA and protein synthesis in the gels, to name a few. Cultures may also be mechanically loaded (see Part B) at any time after gelation or after cells have self-assembled their own

matrix. The elongation, frequency and duration of the applied strain to the construct must be determined by the investigator for his/her specific use.

Typically, spherical-shaped cells can be observed in the gel using an inverted phase contrast microscope directly after plating. By day one, the cells will begin to attach to and spread into the matrix. Cells will then form attachments to each other and intercommunicate. By days 3-5, cells will reorganize and contract the matrix.

### 3. APPLICATION OF REGULATED STRAIN TO CELLS IN A 3D GEL USING THE TISSUE TRAIN® CULTURE SYSTEM

Cell-matrix constructs can be mechanically loaded in the 3D matrix by using a Flexcell® FX5K Tension FlexLink™ and the FX-5000 FlexSoft Software (see Part B, pg. 7, or the Flexcell® FX-5000™ Tension System). This tension system can be used to apply a regimen of controlled elongation (strain), frequency or duration with added rest periods.

The regimen parameters must be tested beforehand to determine if the cell-constructs can withstand the applied strain regimen. Usually, the cell matrix constructs can be stretched at 1-3% elongation for several minutes to several hours per day without matrix failure. In the case of cardiac myocytes, the myocyte-matrix construct can maintain its beat frequency for a week or more in the matrix.

Uniaxial strain can be applied to linear and trapezoidal-shaped constructs. For application of uniaxial strain, use Arctangle® Loading Stations™ with the Tissue Train® plates.

Equibiaxial strain can be applied to circular-shaped constructs. For application of equibiaxial strain, use 25 mm cylindrical Loading Stations™ with the Tissue Train® plates. Regimens can be programmed with strains ranging from 0-20% and frequencies from 0.01-5.0 Hz. When downloading regimens, choose the Tissue Train Loading Station (24mm) platform setting for applying uniaxial strain to linear and trapezoidal-shaped constructs and choose the BioFlex Loading Stations (25mm) platform setting for applying equibiaxial strain to circular-shaped constructs.

**NOTE:** Higher frequencies will limit the system's ability to maintain higher strain ranges.



## PART B

# SET-UP OF THE TISSUE TRAIN® SYSTEM TENSION EQUIPMENT



## 1. FLEXCELL® TISSUE TRAIN® SYSTEM COMPONENTS

1. Dell OptiPlex desktop computer.

*Do not delete any software or change any settings on this computer. It has been pre-configured to operate the FlexSoft® FX-5000™ software.*

### Preinstalled software:

- FlexSoft® FX-5000™
  - Microsoft Windows® 7
  - Microsoft Office 2010
  - Adobe Acrobat
2. FX5K™ Tension FlexLink®
  3. USB keyboard
  4. USB 2-button entry mouse
  5. 17" Flat panel monitor
  6. Accessory pack of nuts and ferrules
  7. Flexcell® BioFlex® baseplate
  8. Four gaskets
  9. Four Arcangle® Loading Stations™
  10. Four Trough Loaders™
  11. Silicone lubricant
  12. Plexiglas® cover
  13. System Drying Filter device
  14. 25 ft small blue tubing (¼" (6.4 mm) O.D.) for **FLEX IN** connection
  15. 25 ft clear tubing (⅜" (9.5 mm) O.D.) for **FLEX OUT** connection
  16. 25 ft large blue tubing (⅜" (9.5 mm) O.D.) for vacuum source connection
  17. Flexcell® Tissue Train® Culture System user's manual and other ancillary manuals for computer and monitor
  18. Surge protected outlet strip
  19. Water Trap (x2)
  20. Vacuum source (optional)

## 2. SETUP OF THE FLEXCELL® TISSUE TRAIN® SYSTEM

**Do not power up the Flexcell® Tissue Train® system until all connections among components have been properly made and checked! Never power up the computer or the FX5K™ Tension FlexLink® with the cover removed - an electrical shock hazard exists. Operation of the computer without the cover is in violation of FCC regulations and will void the warranty!**

### 2.1 PACKING LIST

Remove the packing list and check off the instrument components as they are removed from the shipping containers. If any parts are missing, contact Flexcell® International Corp. immediately.

### 2.2 LOCATION OF THE TISSUE TRAIN® SYSTEM

Choose a location for the Tissue Train® system (Fig. 5) that is adjacent and as close as possible to the CO<sub>2</sub> incubator and vacuum source. A shorter distance in vacuum lines decreases the air flow resistance for elongation and release of the flexible bottomed culture plates, allowing for a greater range of flexing at higher frequencies. The length of the vacuum line should be a minimum of 4' (1.2 m). All tubing and the Ethernet communications cable connect to the rear of the **FX5K™ Tension FlexLink®** (Fig. 6). All cables (mouse, monitor, keyboard, and Ethernet communications cables) will connect to the rear of the **computer** (Fig. 7). The length of



the clear and blue tubing from the FlexLink® to the baseplate should be 10' (3 m) long to assure proper system performance. This length can vary if needed, but no more than 5' (1.5 m) longer or shorter. Cut the tubing **after** the system is fully set up to ensure of the proper length.

The Tissue Train® system will require a space 28.75" (73.03 cm) wide by 22.44" (57.00 cm) deep if the computer, FX5K™ Tension FlexLink® and monitor are placed side by side with the keyboard and mouse in front. The total weight of the system is 48.37 lbs (21.96 kg).

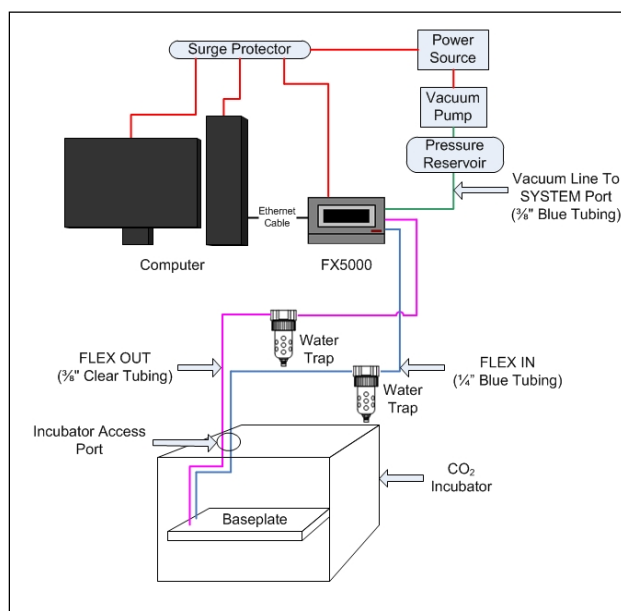


Figure 5. Flexcell® Tissue Train® System

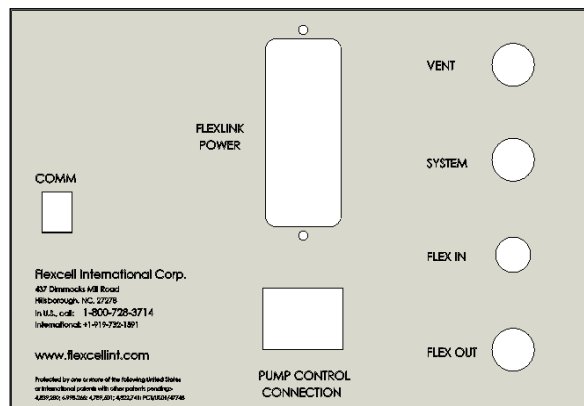


Figure 6. FX5K™ Tension FlexLink® rear view

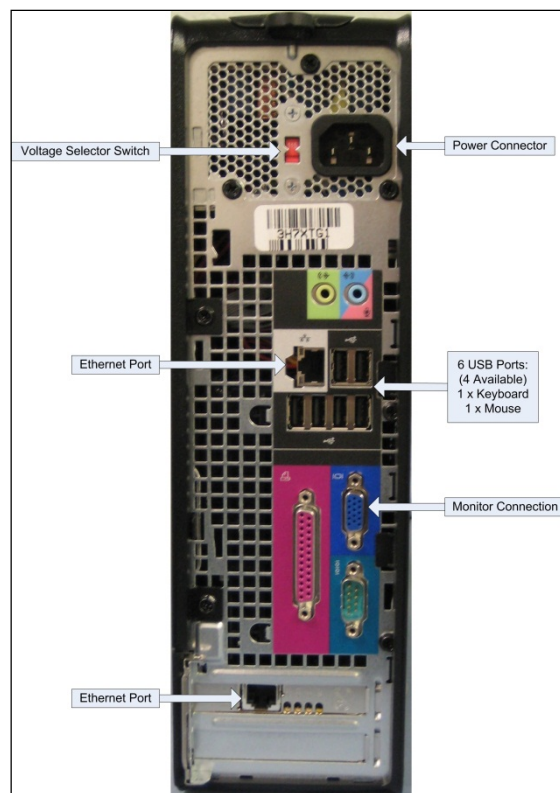


Figure 7. Dell OptiPlex rear view

## 2.3 SETUP OF THE COMPUTER AND FX5K™ TENSION FLEXLINK®

Setup the Dell OptiPlex desktop computer and peripherals according to the manufacturer's instructions. Refer to Figures 5-9 for proper setup of the Tissue Train® system instruments. Place the FX5K™ Tension FlexLink® next to the computer and connect the FlexLink® to the computer via the Ethernet cable provided.

If additional FlexLinks® have been purchased, they are to be placed side by side. For multiple FlexLinks® to be controlled by one computer, connect the Ethernet cable from each FlexLink® to an Ethernet Hub. Then, connect the hub to the Ethernet port on the back of the computer.

Connect the system tubing as follows:

1. Connect the  $\frac{3}{8}$ " (9.5 mm) O.D. blue tubing from the vacuum source to the **SYSTEM** port on the back of the FlexLink® (Fig. 5 & 6).
2. Attach the free end of the clear  $\frac{3}{8}$ " (9.5 mm) tubing to the **FLEX OUT** port on the back of the FlexLink® (Fig. 6).
3. Attach the free end of the blue  $\frac{1}{4}$ " (6.4 mm) tubing to the **FLEX IN** port on the back of the FlexLink® (Fig. 6). The **VENT** port should be left open (Fig. 6).
4. Connect a Water Traps inline with the clear  $\frac{3}{8}$ " tubing and the blue  $\frac{1}{4}$ " tubing. See the Addendum (pg. 24, "B") for further details concerning the placement of the Water Traps.

**NOTE:** When connecting the tubing to the back of the FlexLink®, slip the brass nut with the nylon ferrule over the tubing, then push the tubing onto the fitting as far as possible and thread the nut onto the fitting. Tighten each nut **by hand** until the tubing is crimped to minimize vacuum leakage.

Connect the power cables (115 VAC or 230 VAC) to the rear power inlet (**FlexLink Power**) of the FlexLink® (Fig. 6). If the available power supply is 115 VAC or 230 VAC, be sure the voltage selection switch ON the computer (Fig. 7) is set to 115 V or 230 V, respectively. When the instruments are completely set up, the power cords should be connected to a **surge protected** power strip and then to a 115 VAC or 230 VAC, 60 Hz power outlet.

## 2.4 INCUBATOR ACCESS PORTS

Consult the operations manual of the CO<sub>2</sub> incubator for the location of the incubator access ports. Most incubators have an access port at the top or the rear of the incubator. If this opening is not immediately apparent, consult the incubator manufacturer about the location of an access port. To prevent voiding the incubator warranty or

encountering an electrical hazard, a trained incubator representative should open the incubator access ports.

Once the access port has been located, insert the **FLEX IN** and **FLEX OUT** tubing through the access port into the incubator. Attach the  $\frac{1}{4}$ " (6.4 mm) blue tubing to the  $\frac{1}{4}$ " (6.4 mm) quick disconnect on the baseplate. Attach the  $\frac{3}{8}$ " (9.5 mm) clear tubing to the  $\frac{3}{8}$ " (9.5 mm) quick disconnect on the baseplate (Fig. 8). If two baseplates are to be used simultaneously, connect the baseplates using the configuration as shown in Figure 9.

**NOTE:** Please see Appendix A (pg. 35) for the maximum allowable % elongations when using two baseplates simultaneously.

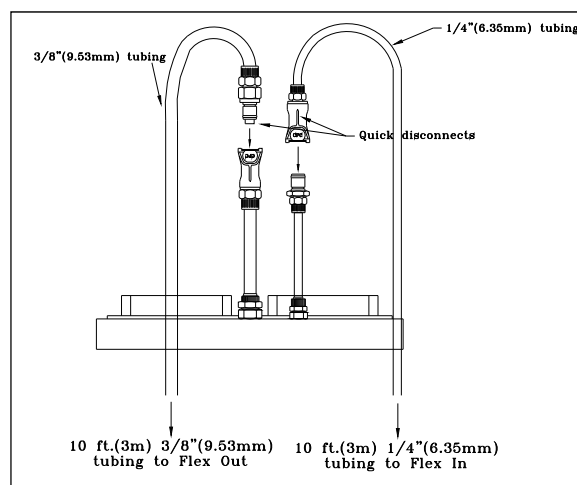


Figure 8. Single baseplate connection

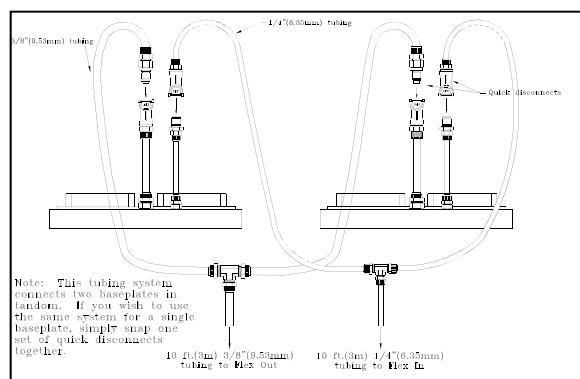


Figure 9. "T" connection for two baseplates



## 2.5 SETUP OF BIOFLEX® BASEPLATE(S) WITH LOADING STATIONS™ AND CULTURE PLATES

Place the BioFlex® baseplate onto one of the incubator shelves. Be sure the shelf is level front to back and side to side in the incubator.

1. Attach the **FLEX IN** and **FLEX OUT** quick disconnect fittings to the respective fittings on the baseplate.
2. If using Loading Stations™, place the four sets of Loading Stations™ into the baseplate wells and apply silicone grease to the tops of the posts (see the *Culture Plate and Loading Stations™* user's manual for detailed instructions).
3. Place the 4 red gaskets onto the bases of the four Tissue Train® culture plates. Be sure that the gaskets are fully pressed onto the culture plates and that no parts are folded under.
4. Place the culture plates and gaskets into the baseplate wells. Check to be sure that the gaskets are not folded up at any point of contact with the baseplate. If the four gaskets feel loose within the plane of the baseplate (can be easily moved right or left, forward or backward without lifting the gasket out of the well), they should seal correctly.
5. Place the Plexiglas® sheet with weights on top (5-10 lbs) over the culture plates to help seal the baseplate.

**NOTE:** After a regimen is run for an hour or so, there should be little or no moisture visible in the lines or Water Traps. Condensation early on in the experiment indicates an air leak. If condensation is present, recheck the seals around each of the culture plates.

## 2.6 CONNECTING THE SYSTEM VACUUM LINES

Connect the blue 3/8" (9.5 mm) vacuum inlet tubing to a vacuum source. A vacuum pump capable of producing a maximum vacuum of -100 kPa and a flow rate of, at least, 5.7 ft<sup>3</sup>/min (161 L/min) is necessary for optimal performance.

Keep the tubing length between the vacuum source and FlexLink® as short as possible. Also, keep in mind that some house vacuum systems will vary in flow rate and/or pressure level. To eliminate or reduce this effect, use of a pressure reservoir between the vacuum source and the FX5K™ Tension FlexLink® is recommended.

Flexcell® International Corp. recommends the Leybold Trivac D8B Pump vacuum pump (includes the D8B pump system, power cable, gas ballast valve and AR 4-8 return filter) as a vacuum source as well as using a pressure reservoir. The vacuum pump and pressure reservoir can be purchased directly from Flexcell® International Corp.

**NOTE:** Even with an optimal vacuum source, there will still be some **system limitations** due to airflow. Combinations of high frequencies and % elongations may not be achievable due to the high vacuum level in the short period of time requested. An example would be a regimen programmed to run a sine wave from 0–20% elongation at 1.0 Hz, using the Arctangle® Loading Stations™. The system is not able to achieve the vacuum required to reach 20% in 0.5 seconds because of airflow limitations. The maximum % elongation may only reach 15%. The exact output is dependent on many system variables. See the Addendum on page 24 for suggestions on maximizing system performance.

To use the auto control pump feature, plug the vacuum pump power cable into the



**PUMP CONTROL CONNECTION** outlet on the rear of the FX5K™ Tension FlexLink® (Fig. 6 and 10).

**NOTE:** When using the auto pump control feature, the power cable from the FX5K™ Tension FlexLink® must be connected directly into the mains power. Failure to do so will cause damage to the FX5K™ Tension FlexLink®.

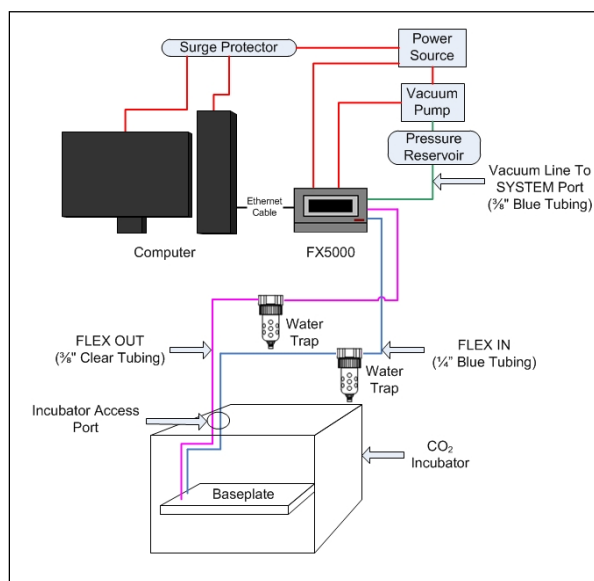


Figure 10. Flexcell® Tissue Train® System power set-up with auto control pump feature

See page 21 for additional comments on setting up the vacuum power to use the auto control feature.

## 2.7 FLEXSOFT® FX-5000™ SOFTWARE AND SYSTEM POWER-UP

The FlexSoft® FX-5000™ software (Flexcell® Copyright Protected Software, Property of Flexcell® International Corporation, 2730 Tucker Street, Suite 200, Burlington, NC 27215) has been pre-installed on the hard drive.

Keep the CD package included with your system and store it in a safe place. Be sure to shutdown Windows® properly by using

the **Shut Down** command activated by the **Start** button. Failure to follow the above directions can cause software and hardware malfunctions.

The computer has Windows® 7 installed on the hard drive. All backup software is provided with the system. Check all connections and connect the surge protector into an appropriate power outlet (115 VAC or 230 VAC). Turn ON the surge protector strip and check that the indicator light on the strip is lit. If the power light does not come on, the surge protector has failed. Replace the surge protector and attempt to determine what caused the surge protector to fail. Turn ON the power switches at the computer, monitor and FX5K™ Tension FlexLink®. The system should then boot and load Windows®. Select and double click on the FX-5000™ icon.

**NOTE:** Flexcell® recommends that the power saving or screen saver features in the Windows software are disabled. Allowing the hard drive to power down while an experiment is running may create the need to reboot the computer before data can be viewed, or before a regimen can be downloaded again. It is recommended that the monitor be put in “sleep” mode when not in use and that the computer be allowed to run at full power during an experiment.

## 2.8 LCD DISPLAY ON THE FX5K™ TENSION FLEXLINK®

The front of the FX5K™ Tension FlexLink® box has an LCD graphic display. When the FX5K™ Tension FlexLink® is turned ON, the LCD will display the FlexLink® number (i.e., #1, #2, #3, or #4) and the regimen status (i.e., Idle, Ready, or Left (the time remaining, in minutes, of a running regimen)). When a regimen has been downloaded and/or is running, the LCD will also display the user name, platform, and regimen name. See Table 1, below, for



the display messages with various FlexLink® statuses.

*Table 1. Messages displayed on the FX5K™ Tension FlexLink LCD.*

| FlexLink Status                       | LCD message                                                                                                                |
|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| Power ON                              | #1 Regimen: Idle<br><br>Flexcell International Corp.<br>www.flexcellint.com                                                |
| Regimen downloaded                    | #1 Regimen: Ready<br>User name<br>Platform<br>Regimen Name<br><br>Flexcell International Corp.<br>www.flexcellint.com      |
| Regimen is running or being simulated | #1 Regimen: Left 5 min<br>User name<br>Platform<br>Regimen Name<br><br>Flexcell International Corp.<br>www.flexcellint.com |
| Regimen has stopped                   | #1 Regimen: Idle<br>User name<br>Platform<br>Regimen Name<br><br>Flexcell International Corp.<br>www.flexcellint.com       |

## 2.9 USING ADDITIONAL FLEXLINKS®

Using additional FX5K™ Tension FlexLinks® will allow two or more baseplates (platforms) with different regimens to run simultaneously. All tubing connections made to the first FlexLink® should be made exactly the same with any additional FlexLinks®. Each FlexLink® will have its own separate **FLEX IN** and **FLEX OUT** tubing for each baseplate. However, the **SYSTEM** tubing can be shared between FlexLinks® with "T" fittings as long as the vacuum source is capable of running two or more FlexLinks®

with the programmed regimens. Regimens with a combination of high frequencies and % elongation levels will require a more powerful vacuum source. The capability of the vacuum source with more than one FlexLink® will need to be tested experimentally with the users' particular setup. FlexLinks® must be placed side-by-side during use. If the user desires to use the vacuum pump auto control feature with each FlexLink®, then separate vacuum pumps will need to be used with each FlexLink®.

To connect an additional FlexLink®(s) to the computer, connect the Ethernet cable from each FlexLink® to an Ethernet hub. Then, connect the hub to the Ethernet port on the back of the computer using the Ethernet communications cable provided. The LCD on the front of the FlexLink® will display the number (#1-4) that the FX5K™ Tension FlexLink® has been assigned at the time of production.

**NOTE:** The numbering of each FX5K™ Tension FlexLink® will not depend on what order it appears in the connection chain, but according to the configuration of each FlexLink® as "FlexLink #1, FlexLink #2...etc" during production.

When powering up the FlexLink®(s) and computer, turn the FlexLink®(s) ON before the computer. To use a particular FlexLink®, select the appropriate FlexLink® number (#1, 2, 3, or 4) from the *Controllers* menu. Assign, download, and run regimens just as would be done when using one FlexLink®.



### 3. OPERATION INSTRUCTIONS

#### 3.1 STARTING THE FX-5000™ PROGRAM

The Flexcell® system should now be set up and ready for operation. Be sure that the system vacuum supply is adequate to operate the instrument(s) (see section 2.6).

Turn ON the power switch on the computer, monitor and FX5K™ Tension FlexLink®. The system should then boot and load Windows®. Select and double click on the FX-5000™ icon (Fig. 11) on the desktop to start the FlexSoft® FX-5000™ software. A main display window will appear (see Fig. 13).



Figure 11. FX-5000™ icon

#### 3.2 USING THE FX-5000™ TUTORIAL

In the **Help** menu, click on **View Tutorial** to view the FX-5000™ tutorial at any time (Fig. 12). The tutorial will appear on the left side of the screen. The tutorial explains the main steps involved in configuring users, creating regimens, and starting regimens. The tutorial will start automatically, as a default setting, when the software is opened. To turn OFF this feature, uncheck the box marked “Show Tutorial at Startup”.

#### 3.3 MAIN DISPLAY WINDOW

The main display window (Fig. 13) for each FX5K™ Tension FlexLink® has several features that allow more precise monitoring of the regimen. To access this window, select the appropriate FlexLink® (**FlexLink#n**) from the **Controllers** menu, where “n” is the FlexLink® number to which the regimen will

be downloaded. The FlexLink® number can be found on the LCD screen on the front of the FlexLink® controller box.

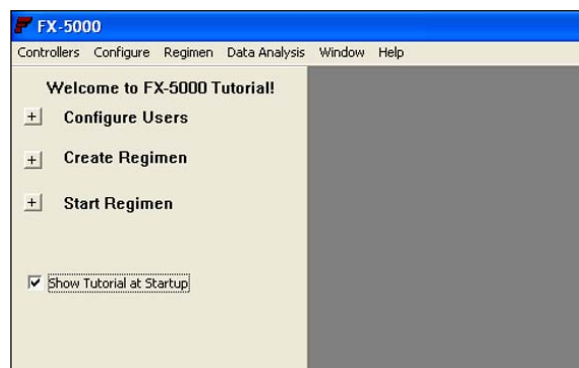


Figure 12. FX-5000™ tutorial

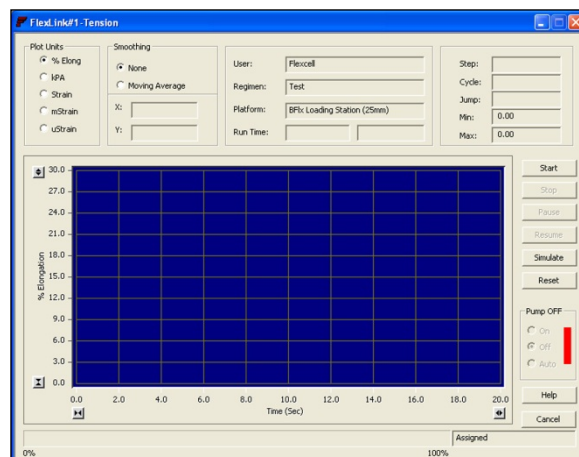


Figure 13. Main display window

**All** of the remaining information and command button states in the main display window reflect the current state of the system for the currently selected FX5K™ Tension FlexLink®.

It is possible to simultaneously assign and run a different regimen for each of the attached FlexLinks®.

In the upper left hand corner of the window is a section labeled **Plot Units**. This function allows users to change the units of the y-axis: % elongation, kPa, strain,



millistrain (mstrain), or microstrain (ustrain). To select a particular unit, click on the appropriate button beside the unit label.

The next block to the right is a section labeled **Smoothing**. One expects data patterns to represent normal distributions of a function describing a straight line, sinusoidal curve, etc. Therefore, the plotted points of a mathematical function should be a predictable data set that is “smooth” (i.e., a curve comprised of points whose coordinates are predicted by the function). For a straight line, the equation would be  $y = mx + b$ ; for a power function, the equation may be  $y = x^2$  or some derivative thereof. In general, data patterns may be confounded with noise. For the case of the pressure plot as a function of voltage from the Tissue Train® System pressure transducer, the rough data yield a plot with a coefficient of variation of only 0.01%. Therefore, the signal to noise ratio for the combined Flexcell® system of circuits, hardware and transducers is high, lending confidence in the displayed plot. Nevertheless, the user can select a data smoothing option that is a simple weighted average of the points. The Smoothing section contains the option of either applying no smoothing (**None**) to the data points returned from the FlexLink® or of applying a three-point moving average filter (**Moving Average**) to the incoming data. Choose the desired affect by clicking on the appropriate button.

Below the box for Smoothing is a section used to show the current **X** and **Y coordinates** for the waveform. These are displayed when the left mouse button is clicked and held down in the graph area. When this is done, a pair of crosshairs appears and the coordinates at the intersection of the crosshairs are shown in the X and Y spaces. The crosshairs and

values will track the mouse as long as the left mouse button is held down.

The next block to the right is a section used to display the current **User**, **Regimen**, **Platform**, and **Run time**. Run time has two blocks. The one on the left displays the elapsed regimen time, and the one on the right displays the remaining regimen time, using the following format: dd:hh:mm:ss (days:hours:minutes:seconds).

The block at the top right contains the current regimen **Step**, **Cycle**, **Jump** (when a regimen step is using the Jump Back to option), minimum level (**Min**), and maximum level (**Max**). The minimum and maximum levels will be listed according to the units specified under the Plot Units section as the minimum and maximum points at which the membrane stretches with each cycle.

The major portion of the main display window is taken up with a **plot** designed to show the current state of a simulated or running regimen. This plot is generated from the data returned by the FlexLink® during regimen activity. When using a platform configuration that uses vacuum to generate a downward stretching of the membrane (elongation), the vertical scale represents increasing elongation, vacuum or strain. When the selected unit of display is kPa, the vertical scale will start at 0.0 and move upwards as an increasing negative pressure level, indicating that a vacuum is being applied to the membrane to stretch it downwards.

The scale on the x- and y-axes can be changed by clicking on one of the two small double-arrow buttons at either end of the axis. The button with arrows pointing away from each other increases the axis

scale, while the button with arrows pointing toward each other decreases the scale.

Below the plot is a **timeline** that displays the percentage of the regimen that has been completed.

To the right of the plot is a set of command buttons which allow the user to:

- 1) **Start** a regimen.
- 2) **Stop** a regimen.
- 3) **Pause** a regimen.
- 4) **Resume** a paused regimen.
- 5) **Simulate** a regimen.
- 6) **Reset** the controller.
- 7) Control the vacuum **pump**.
- 8) Access **Help**.
- 9) **Cancel** the program.

### 3.4 CONFIGURING USERS

Prior to creating and downloading a regimen, a user needs to be registered. Once this task is completed, regimens can be personalized under the registered user name.

In the **Configure** menu, select **User Editor**. A new window will appear entitled **Users** (Fig. 14).

To add a user, type in a user name (must be at least 4 characters) in the provided space and click on **Add User**. The new name should appear in the **Existing Users** box.

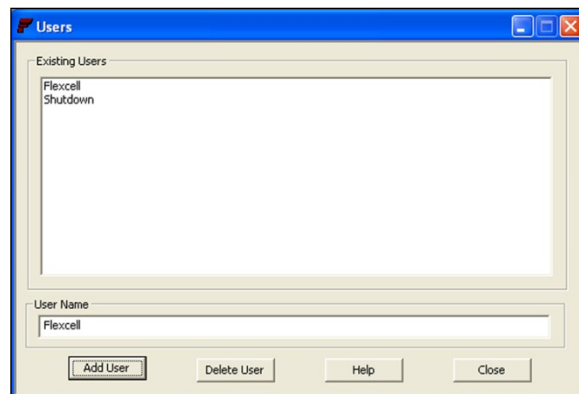


Figure 14. FX-5000™ user editor

To delete a user, click on the appropriate user name in the **Existing Users** box so that it is highlighted in blue and then click on **Delete User**. The program will ask for a confirmation and issue a warning, click **Yes** to confirm deletion. The user and any of the user's defined regimens will be deleted.

To exit the Users window, click on **Close**.

### 3.5 CREATING REGIMENS

**NOTE:** Please see Appendix A (pg. 35) for the maximum allowable % elongations when using two baseplates simultaneously.

Before beginning, a regimen must be created for downloading. In the **Regimen** menu, select **Create new**. A new window will appear entitled **Regimens** with a list of the existing users and their regimens (Fig. 15). In the **Existing Users** box, click on the user name under which the regimen is to be created. A list of current regimens created under this user will appear in the **Regimens for Selected User** box, unless no regimens have been saved under this user, in which case, this box will be empty.

To create a new regimen, click on **Run Wizard** in the bottom left hand corner. This option will bring up a step by step process of creating a new regimen. Alternatively, follow the sequence of steps below:



- 1) Type a regimen name in the box labeled **Regimen Name**.

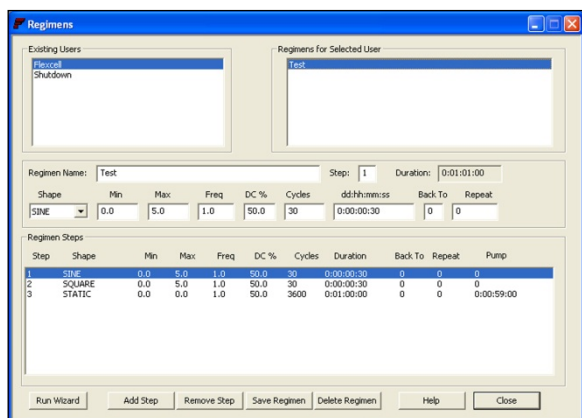


Figure 15. FX-5000™ regimen editor

- 2) Choose the desired waveform shape under **Shape** by clicking on the downward arrow and selecting an entry from the drop-down list.
- 3) Enter the desired minimum and maximum % elongation for the plate membranes (0-30%) in the boxes under **Min** and **Max**.
- 4) Enter the frequency for the waveform (0.01-5 Hz) in the box under **Freq**.
- 5) If a static regimen is required, enter the desired % elongation at which the membrane is to be held in the **Max** box. Enter 0 in the **Min** box, and 1.0 in the **Freq** box (**Min** and **Freq** are irrelevant for static regimens).
- 6) If a duty cycle for the waveform is to be defined, enter the desired value in the box under **DC %**. The duty cycle is the percent of time that the waveform shape remains on at the high (square wave) or rising (triangle) part of the waveform. Duty cycle only affects the triangle and square waves.

7) Enter the number of times for the regimen to repeat (0-65,535) in the box under **Cycles**. When moving out this box, the box underneath **dd:hh:mm:ss** will update to the calculated regimen time according to the set parameters. The format for this entry is days:hours:minutes:seconds. However, if the time is entered first, the program will calculate the number of cycles.

- 8) To make a regimen step (except for the first step) return (jump back) to a previous regimen step, enter the return step number in the box under **Back To**. Then, type the number of times to repeat this process in the box under **Repeat**.

For instance, if the user was entering Step 4 and wanted to return to Step 2 and repeat Steps 2 through 4 three times, enter 2 under **Back To** and 3 under **Repeat** when inputting Step 4.

- 9) When the regimen step is complete, click on **Add Step** at the bottom. Every time a new/edited step is saved, the overall duration of the regimen will be calculated and displayed in the box labeled **Duration** next to the **Step** number box. If the total duration exceeds the maximum allowable duration, this value will be **MAXIMUM**, and the regimen cannot be saved. Adjust the time and/or cycle number to decrease the total duration time.
- 10) To add other steps to a regimen, click in the box labeled **Step** and type in the next step number.

For example, the first step will automatically be Step 1. To add another step, delete the 1 and replace it with a 2.

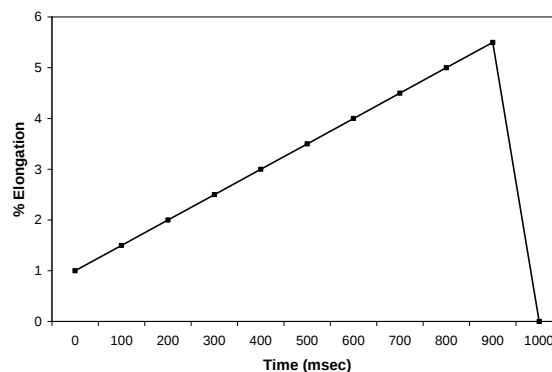


After this is completed, change the parameters as needed for the new step according to steps 2 to 9 above.

- 11) To delete a step that has already been added, click on the step in the window labeled **Regimen Steps**, which contains the list of steps. This will cause the step to be highlighted. Then click on **Remove Step** at the bottom of the window. Confirm the deletion by clicking on **Yes** when prompted.
  - 12) After all steps and parameters have been entered, click on **Save Regimen**.
  - 13) To delete a regimen, simply double-click on the desired regimen name in the **Regimens for Selected User** box, and then click on **Delete Regimen**. Confirm the deletion by clicking on **Yes**.
- NOTE:** *If the regimen to be deleted is currently assigned, the regimen must be removed from assignment before deleting.*
- 14) When finished with regimen editing, click on **Close**.

### 3.6 CREATING A REGIMEN WITH A CUSTOM WAVEFORM SHAPE

In the top row of an Excel spreadsheet, enter the % elongation values for the desired waveform shape for one cycle. Enter one value per cell. Values should be chosen along a curve at even time intervals, i.e. every 100 msec (milliseconds). Save this spreadsheet as a .csv file (comma delimited file format) in the C:/FlexSyst directory.



Example Custom waveform shape: Ramp waveform shape

Example Excel file: Ramp waveform shape

|   |    |   |    |   |    |   |    |   |    |   |
|---|----|---|----|---|----|---|----|---|----|---|
| 1 | 1. | 2 | 2. | 3 | 3. | 4 | 4. | 5 | 5. | 0 |
|   | 5  |   | 5  |   | 5  |   | 5  |   | 5  |   |

Example CSV file viewed in Notepad:

Ramp waveform shape

1,1.5,2,2.5,3,3.5,4,4.5,5,5.5,0

In the **Regimen** menu of the FX-5000™ software, select **Create new**. A new window will appear entitled **Regimens** with a list of the existing users and their regimens. In the **Existing Users** box, click on the user name under which the regimen is to be created. A list of current regimens created under this user will appear in the **Regimens for Selected User** box, unless no regimens have been saved under this user in which case, this box will be empty.

To create a new regimen, click on **Run Wizard** in the bottom left hand corner. This option will bring up a wizard to create a new regimen. Or, follow the sequence of steps below:

- 1) Choose **Custom** from the list of waveform shapes under **Shape** by clicking on the downward arrow and selecting this entry from the drop-down list.

- 2) Choose the appropriate file name under **File** by clicking on the downward arrow and selecting an entry from the drop-down list
- 3) Type a regimen name in the box labeled **Regimen Name**.
- 4) Enter the time interval in milliseconds between two % elongation values in the box under **Time**.
- 5) Enter the number of times for the regimen to repeat (0-65,535) in the box under **Cycles**.
- 6) When the regimen step is complete, click on **Add Step** at the bottom. Every time a new/edited step is saved, the overall duration of the regimen will be calculated and displayed in the box labeled **Duration** next to the **Step** number box. If the total duration exceeds the maximum allowable duration, this value will be MAXIMUM, and the regimen cannot be saved. Adjust the time and/or cycle number to decrease the total duration time.
- 7) Click on **Save Regimen**.
- 8) When finished with regimen editing, click on **Close**.

### 3.7 ASSIGNING REGIMENS

In the **Controllers** menu select the appropriate **FlexLink#n**, where “n” is the FlexLink® number to which the regimen will be downloaded.

In the **Regimen** menu, select **Assign**, then choose the **Platform**, **User**, and **Regimen** from each drop down menu (Fig. 16). A message box will appear to confirm regimen assignment. Click **Yes**.

**NOTE:** Be sure to choose the platform that properly corresponds to the setup being used, otherwise the % elongation values will be incorrect.

For example:

If using the Arctangle® Loading Stations™ with linear or trapezoidal Tissue Train® plates, click on **Tissue Train Plate (24mm Arctangle LS)** for the choice of platform.

If using the 25 mm BioFlex® Loading Stations™ with Tissue Train® Circular Foam plates, click on **BFlx Loading Station (25mm)** for the choice of platform.

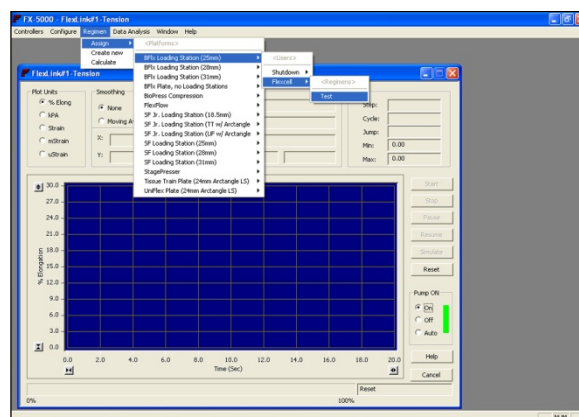


Figure 16. Assigning regimens for downloading to the FX5K™ Tension FlexLink®.

If the download progresses, no further messages will appear, and the **Start** and **Simulate** buttons will be enabled. If an error occurred in downloading the regimen to the FlexLink®, a message will appear alerting the user of a problem, and the regimen will not be able to run. In which case, click **Reset** in the FlexLink® window, and then assign the desired regimen again.

### 3.8 STARTING REGIMENS

To run an assigned regimen, click on **Start** in the main display window (Fig. 13) after the regimen has finished downloading. Be sure that the vacuum pump is ON and all



other connections are made. The membranes should begin flexing and the assigned regimen will be in progress.

The progress of the regimen for the currently selected FlexLink® will be monitored continuously. The displays for Run Time, Step #, Cycle #, Jump #, and Min and Max % will update, as will the **actual waveform data** returned by the FlexLink®. Additionally, a progress bar below the graph will display the percentage of the regimen that has been completed.

### 3.9 SIMULATING REGIMENS

The **Simulate** button is used to simulate a regimen graphically without actually running it. Simulating a regimen will give the user an idea of the waveform output before actually running the regimen. To start a simulation, click on **Simulate** in the main display window. A waveform will begin moving across the graphic display. This waveform corresponds to the ideal waveform as generated by the FX-5000™ software, and is the actual waveform pattern sent to the FlexLink® during the download process. For certain waveforms (e.g. square waves), the idealized pattern may appear to have some unusual elements (e.g. rounded corners). The purpose of these unusual elements is to accommodate for the lag time during sudden changes in pressure (e.g. rising/falling edge of a square wave). Without these unusual elements, the valve controllers may oscillate for a period of time (e.g. dampened sine wave during beginning of plateau in a square waveform).

#### 3.10 PAUSING REGIMENS

To pause a simulated or running regimen, click on **Pause** in the main display window. Click on **Yes** when the program asks for confirmation. Pausing will cause the current

simulation or actual running of the regimen to stop at the next cycle of the current regimen step. Pausing is different from stopping, in that pausing leaves the regimen at its current position, whereas stopping causes the regimen position to be set to the beginning of the first regimen step.

#### 3.11 RESUMING REGIMENS

To resume a paused regimen, click on **Resume** in the main display window. This function is available only when a simulated or running regimen has been paused. The regimen will restart its prior activity (simulation or actual run) from its last point.

#### 3.12 STOPPING REGIMENS

To stop a simulated or running regimen, click on **Stop** in the main display window. Click on **Yes** when the program asks for confirmation. Stopping causes the current activity to cease immediately, and sets the regimen position back to the beginning of the first regimen step. Stopping is different from pausing, in that pausing leaves the regimen at its current position so that it can be resumed from where it stopped.

#### 3.13 DETERMINE THE PROGRESS OF THE REGIMEN

The progress of the regimen for the currently selected FlexLink® will be monitored continuously. The displays for **Run Time**, **Step** number, **Cycle** number, **Jump** to number, and **Min** and **Max** % elongations will be updated in the upper right hand corner of the main display window. The actual waveform data returned by the controller will also be continuously updated on the graph. A timeline progress bar below the plot



illustrates the percent of the regimen that has been completed.

### 3.14 RESETTING THE FLEXLINK®

Resetting a simulated or running regimen causes the current activity to stop at the next cycle of the current regimen step, and disassociate the regimen from the FlexLink® controller. To reset, click on **Reset** in the main display window. Click on **Yes** when the program asks for confirmation. The system will now be in state similar to that which exists when the FX5K™ Tension FlexLink® is first turned ON.

### 3.15 CONTROLLING THE VACUUM PUMP

On the right side of the FlexLink® window, the box labeled **Pump** allows the user to control power to the vacuum pump.

**NOTE:** *The vacuum pump must be plugged into the back of the FX5K™ Tension FlexLink® in the appropriate 110/220V plug prior to system power-up for this option to work, and the ON/OFF switch on the vacuum pump must be in the ON position.*

For manual control of the pump through the FlexLink®, use the **On** and **Off** options. If **On** is selected, the FlexLink® will turn the pump ON. If **Off** is selected, the FlexLink® will turn the pump OFF. For automatic control of power to the pump while regimens are running, select **Auto** prior to downloading the regimen. Once **Auto** is selected, the FlexLink® will turn the pump ON. The software will turn the pump OFF when a regimen is complete. This option also allows the pump to be turned OFF during a regimen step when a static waveform regimen is selected that has a 0% maximum elongation longer than 60 minutes. The pump will power up 10 minutes before the next regimen step begins. Users will be prompted

to select this option or not when creating a regimen. The status of the pump is indicated by a light box to the right side of the On/Off/Auto options. A green light indicates that the pump is ON, and a red light indicates that the pump is OFF.

### 3.16 DATA ANALYSIS

The FX-5000™ software has the capability of storing data (a set of waveform points) from a regimen run to a file on the hard drive. The filename consists of the FlexLink® number, a “V” or “C” code letter (V for vacuum, or C for compression), the regimen name and a sequence number. If a tension regimen is run three times on FlexLink#1, then there will be three data files on the hard drive with numbers ‘000’, ‘001’ and ‘002’ (i.e., 1\_V\_regimename\_001.dat, 1\_V\_regimename\_002.dat, etc.). The file size depends on the regimen duration. The current settings take pressure data every 2 milliseconds (i.e., 500 points/sec or 1,800,000 points/hour). Therefore, a one hour regimen will have approximately a 4.0 Mb data file. To turn this feature OFF, please contact Flexcell®.

To see the stored data, in the **Data Analysis** menu, select **Data Viewer**. Open the appropriate .dat file from the C:/FlexSyst directory. Two new windows will appear (Fig. 17). In the window labeled **filename:1**, the data presented defaults to the raw data. In the window labeled **filename:2**, the data presented defaults to a moving average of the raw data with data averaged every 10 seconds.

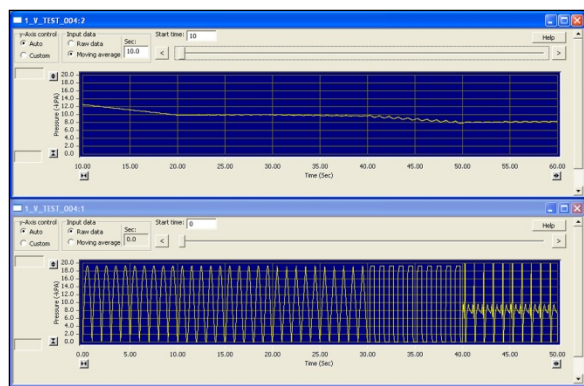


Figure 17. FX-5000™ data display

The **y-Axis control** box allows for automatic or manual control of the scale on the y-axis. If **Auto** is selected, the y-axis scale can be changed by clicking on one of the two small double-arrow buttons at either end of the axis. The button with arrows pointing away from each other increases the axis scale, while the button with arrows pointing toward each other decreases the scale. The minimum value is -2 kPa and the maximum is -100 kPa. If **Manual** is selected, the user can enter the upper and lower limits of the y-axis scale in the white boxes to the left side of the axis. The small double-arrows will not function if **Manual** is selected.

The **x-axis scale** allows for a time interval to be set between 0.1 and 1000 seconds. Click on one of the two small double-arrow buttons at either end of the axis to change this scale. The button with arrows pointing away from each other increases the axis scale, while the button with arrows pointing toward each other decreases the scale.

The **Input data** box allows the user to switch the data in the graph from raw data to averaged data. If the **Raw data** option is selected, the graph will plot the pressure in kPa over time, in seconds, for the selected regimen. If the **Moving average** option is selected, the graph will plot the average pressure over the designated time period (in

seconds, range of 0.1-1000 seconds) shown in the **Time (s)** box.

The **Start time** box can be set so that the data viewed in the plot begin at the specified time entered here. Use the timeline at the top of the graph to navigate through the regimen. Users can move the slider function within the timeline to manually progress through the regimen plot. If the user clicks on the arrows at either end of the timeline, the plot will progress to the next time period as set by the x-axis scale. For instance, if the scale is set to show 20 seconds of data and the user clicks on the right arrow button, the plot will progress to the next 20 seconds of data.

### 3.17 SHUTTING DOWN THE SYSTEM

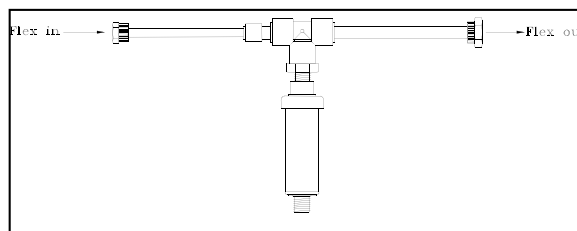
A system **Drying Filter** (Fig. 18) has been supplied with the FX-5000™. This device must be used whenever the system has been operated for more than one day and will not be operated again for more than one day. If the system will be operated again immediately after a one-day run or if the regimen runs for a longer period of time, the Drying Filter can be used at the conclusion of the usage period. The device and shutdown program will help to remove any moisture entrained into the valves during normal operation. This will greatly prolong the life of the valves and reduce expensive repairs.

To use the Drying Filter, first remove the orange plug in the bottom of the filter. Attach the fittings of the Drying Filter to the proper ports on the rear of the FlexLink® by replacing the lines running to the baseplate (**FLEX IN** and **FLEX OUT**). Assign the **Drying** regimen under the **Shutdown** user, using the **BioFlex Plate, no Loading Stations** platform.



If the **Drying** regimen is not in the system or is erased, create a regimen with the following specifications: sine wave, 0-28%, 0.5 Hz, 1000 cycles; all other parameters are standard. The shutdown program will run for about 30 minutes. Dry room air will help to reduce moisture entrained into the lines and valves from the incubator atmosphere. Once the beads in the Drying Filter become pink, the filter is saturated and needs to be replaced.

The baseplate should be removed from the incubator and stored flat to prevent warping. Do not hang the red gaskets on pegs to dry. They will stretch over time.



*Figure 18. System Drying Filter*

### 3.18 EXITING THE FX-5000™ PROGRAM

To exit the FX-5000™ software, in the **Controllers** menu, select **Exit**.



## 4. ADDENDUMS TO THE TISSUE TRAIN® SYSTEM

### FOR OPTIMAL PERFORMANCE OF THE FX5K™ TENSION FLEXLINK®:

- A. Place the FlexLink® higher than the baseplate. If the FlexLink® can safely sit on top of the incubator, put it there. This will reduce the amount of moisture reaching the valves in the system.
- B. It is necessary to place the included Water Traps inline between the FlexLink® and the baseplate. Be sure to place the Water Traps **below** the FX5K™ Tension FlexLink®. Humid air from the incubator tends to condense within the tubing lines. This moisture may in turn lead to problems with long term regimens. This fluid collection can be prevented from entering the FlexLink® by the insertion of the Water Traps.

The Water Traps need to be placed so that they are maintained in a **vertical position**. They should be checked frequently (twice a day initially while a regimen is running) and should be emptied when the Water Traps become half full. On the first trial, monitor the Water Traps every 15 minutes for the first two hours to be sure that a significant amount of water is not accumulating. If so, there may be a leak in the platform and/or gasket/Tissue Train® plate assembly. See “System Troubleshooting, Problem 1” (pg. 25) to correct this problem. The valves and transducers may be damaged if moisture is allowed to accumulate.

- C. Use the system Drying Filter and the shutdown program whenever the unit has

been operating continually for more than one day and will not be operated again for more than one day. See section 3.17 for instructions.

- D. Place the enclosed Plexiglas® sheet over the culture plates and distribute a weight of about 5 -10 lbs (2.25 - 4.5 kg) evenly across the sheet as necessary to prevent vacuum leaks between the Tissue Train® plate, gasket and baseplate. Lead donuts are recommended. As the gaskets are worn in this will no longer be necessary.
- E. Make sure that the brass fittings on the back of the unit are tight and that the tubing is secure.
- F. Since the FX5K™ Tension FlexLink® is calibrated with 4 Tissue Train® plates on the baseplate, Flexcell® recommends having 4 Tissue Train® plates on the baseplate during strain, even if only one plate has been seeded with cells.
- G. Achieving the highest possible combinations of % elongation and frequency:
- Shorten the **FLEX IN**, **FLEX OUT**, and **SYSTEM** tubing to their minimum possible lengths. See section 2.2 for minimum lengths.
  - Use a more powerful vacuum source if recommended specs are not being reached with the current vacuum source (see section 2.6).



## 5. SYSTEM TROUBLESHOOTING

**NOTE:** *If the Tissue Train® system is not functioning properly and the solution is not clearly outlined or defined below, please contact Flexcell® before proceeding with any repair work.*

### **Problem 1: Gaskets on baseplate are not sealing**

#### **Symptoms:**

- a. System is not performing to specified parameters (i.e., not reaching maximum % elongation value entered in regimen)**
- b. Water Traps are filling with water quickly during use with incubator**

Test to determine if this is the problem:

If experiencing symptom **a**, there is likely a leak or crimped tubing somewhere in the system. First, check all tubing to be sure that it is straight and not bent to restrict air flow. To find the location of any vacuum leaks, do the following:

*First, check all vacuum connections at the back of the FlexLink®. If these are tight and without noticeable leaks, then run a regimen with the baseplate and take note of the max and min % elongations. Disconnect the quick disconnects from the baseplate and connect them together. Take note again of the max and min % elongations. These values should change slightly because of the decrease in system volume, however, not by a significant amount (2% elongation or greater). The wave may also be somewhat choppy because of the low system volume. If the outputs were originally only slightly lower or higher than the inputs, the problem is likely not a leak but calibration. Call Flexcell® for further instructions. If the output max % elongation increases significantly when the quick*

*disconnects are connected together, the leak is most likely in the baseplate or gaskets.*

#### **Solution 1:**

Remove gaskets and plates from baseplate. Check to see that each gasket is firmly seated in each plate without any folds in the thin bottom lip of the gasket and with each inside corner butted up against each outside corner of the Tissue Train® plate. Place each plate and gasket carefully into each baseplate well. Check to be sure that the gaskets are flatly seated in the wells; i.e., none of the sides touching the baseplate to the point that the gasket is not able to fully enter the well. Move each gasket forward and back and side to side within the well to be sure that they are somewhat loose in the plane of the baseplate. This will allow the vacuum pressure to pull the gaskets and plates firmly down to seal flat against the baseplate surface. Run a regimen. The plates and gaskets may require some initial hand pressure to start the seal. Place the Plexiglas cover and a couple of lead weights (approx. 10 lbs total; variable) on top of the plates. If there is still a noticeable leak, check all sides of each Tissue Train® plate to see if the membrane is protruding from any side. If there is any noticeable membrane protrusion, remove this with a razor blade.

### **Problem 2: Regimen wave is flatlining**

#### **Solution 2:**

Remove the **SYSTEM** vacuum line at the back of the FlexLink® and check to be sure that there is vacuum coming through the line. Check the **VENT** port at the back of the FlexLink® when the system is running to see if air is moving in or out. If



not, it is possible that the valves are locked due to residue buildup. Try applying positive pressure to the **VENT** port to free the valves.

If the regimen wave is flatlining but the plates are still cycling air pressure, try the following:

Disconnect the blue 1/4" (6.4mm) *FLEX IN* tubing from the back of the unit and place a finger over the end of the tubing while a regimen is running. If air is cycling at the end of the tubing then the problem is with the FX5K™ Tension FlexLink®. Contact Flexcell® International for further instructions.

**Problem 3: Regimen wave is fluttering or not smooth**

**Solution 3:**

This problem could be due to water in the valves or the system being out of calibration. Run the drying regimen with the Drying Filter (as described in the manual) to dry the system and valves. If this does not resolve the problem try running the drying regimen once more. If the problem continues, contact Flexcell® for further instructions.

**Problem 4: Programming a long regimen (1 day or more) but the software will not allow the regimen to be saved.**

**Solution 4:**

The software has a limit on the number of cycles that can be entered into any given step (65,535). If a regimen is programmed with a step that requires more than 65,535 cycles, the software will give an "invalid entry" error. To run the regimen for the time required additional steps will need to be programmed. This will not create any breaks in the regimen. Simply divide the total time in half (or as many times as is necessary) and make additional steps with the same parameters. See section 3.5 of the manual for

instructions on programming additional steps.

**Problem 5: Plate wells are drying up during the experiment**

**Solution 5:**

When vacuum is pulled to expand the silicone membranes at the bottom of the Tissue Train® wells, the volume of the well increases, pulling air into the well from the atmosphere. When the membranes contract, the air is pushed back out. Because of this cyclic airflow during any experiment, the fluid evaporation rate in the Tissue Train® wells will increase. The best solution to this problem is to use more culture medium.

**Problem 6: "An invalid argument was encountered" error is shown when attempting to assign a regimen.**

**Solution 6:**

This problem is caused when a user name is entered into the software without any regimens listed under his/her name. If the assign function sees that a user has nothing to assign an error is displayed. Simply delete the user(s) without a regimen or make and save a regimen under the user(s) name(s).

**Problem 7: Water is accumulating quickly in the Water Traps**

**Solution 7:**

This problem is likely due to a poor gasket seal on the BioFlex® baseplate. See the solution to Problem 1 to correct this. Be sure to empty the Water Traps frequently if this problem occurs. Water accumulation in the FlexLink® can damage the valves.

**Problem 8: One or more of the FX5K™ Tension FlexLinks® connected to the system are not being recognized by the software. This is usually represented by**



**the error message “Cannot Connect to Controller” when attempting to connect a FlexLink® to assign a regimen.**

**Solution 8:**

This problem is usually due to a lack of communication between the computer and one or more FX5K™ Tension FlexLinks®. Check the FX5K™ Tension FlexLink® Ethernet port connection on the computer

and the communications connection on the back of the FlexLink® to be sure that these are secure. Check the FlexLink® to see if the power is ON (the FlexLink screen should power on). If both of these conditions are true, close and restart the software. Try downloading the regimen again. If the problem is not resolved, call Flexcell® for further instructions.



## 6. APPLICATION NOTES

### BASEPLATE AND GASKET CLEANING AND DISINFECTING

The black urethane baseplate should not be autoclaved or washed in a dishwasher because the high temperature will cause warping. The red rubber gasket should also not be autoclaved or washed in a dishwasher. Both baseplate and gasket should be cleaned with regular laboratory detergent, rinsed with warm tap water, followed by distilled or deionized water and allowed to dry. The baseplate should be dried and stored on a flat surface. Do not hang the red gasket on pegs to dry as the gasket can be stretched. Protect both pieces from UV light exposure.

To disinfect, use a mild bleach (commercial bleach solution such as "Clorox" or other laundry bleach solution is normally 5.25% sodium hypochlorite) solution (1:500, bleach to water) or a 1% benzalkonium chloride solution, which can be sprayed, poured or wiped on both pieces and allowed to stand on surfaces for at least 5 min. Rinse with distilled or deionized water. The above solutions are effective against yeast and fungi. A 70% ethanol solution also can be applied and pieces allowed to air dry, do not wipe dry. Tubing on the baseplate may be flushed with 70% ethanol, but be sure tubing is completely dry before connecting to the FX5K™ Tension FlexLink®. Contact time (time surface remains wet) of the above solutions is critical to the effectiveness of disinfecting. Times given are minimal and longer contact times are recommended in cases where contamination is severe.

The above decontamination procedure should be performed before and after each use of the

baseplate and gasket or whenever media is spilled onto these surfaces. Be sure that the openings and the channels in the baseplate are dry and clear of any obstructions before using.

Do not store baseplate or gasket in incubator as the warm, humid conditions will promote growth of microorganisms. After cleaning and disinfecting, seal baseplate and gasket in a plastic bag. Store baseplate flat and be sure that the entire bottom of baseplate is supported to prevent warping. Store gasket away from UV light exposure.

### RUNNING LOW FREQUENCY REGIMENS WITH BIOFLEX® CULTURE PLATES

If running a low frequency or constant stretch regimen with the BioFlex® culture plate without Loading Stations™, keep in mind that the 2-2.5 mL of medium in the well may not cover the entire surface area of the BioFlex® well under stretch. When the membranes are stretched and held for long periods of time, the cells around the outer edge of the well will be exposed to open air and will be in danger of drying out. It is therefore necessary to use additional medium (3-5 mL) when running low frequency and high strain regimens on the BioFlex® plate without the Loading Stations™.

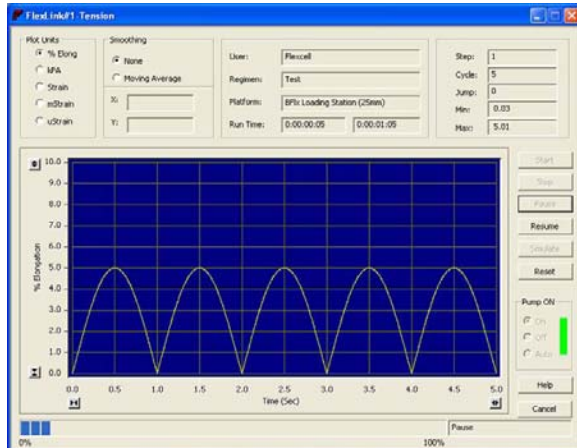
Running Low Frequency Regimens With BioFlex Culture Plates, Flexcell International Corporation, July, 1997, 1p.



## WAVEFORM SHAPE DEFINITIONS

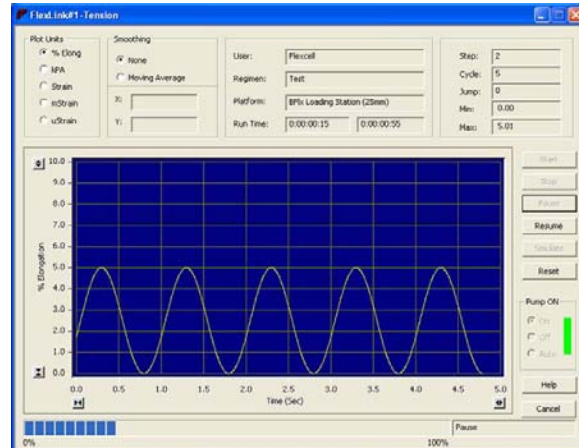
### ½ Sine Wave

This waveform provides a smooth, physiological variation in pressure at the max% with a quick transition at the min%.



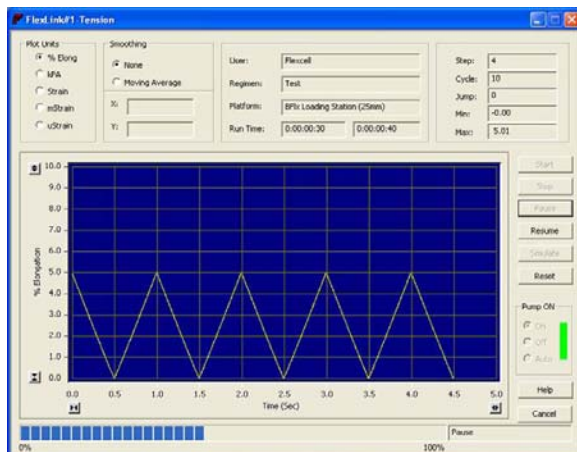
### Sine Wave

This waveform provides a smooth, physiological variation in pressure from min% to max%.



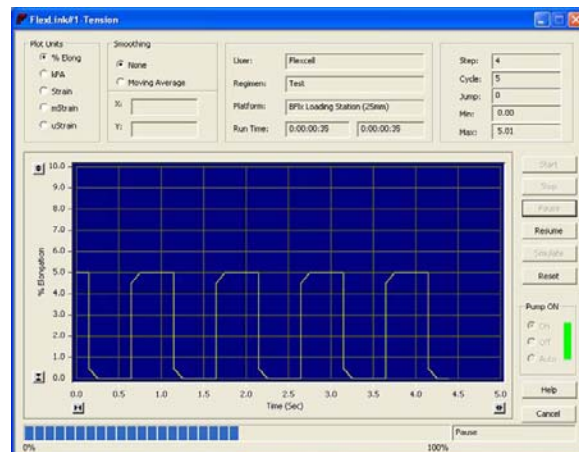
### Triangle Wave

This waveform provides a linear-ramped rise to the max% and a linear-ramped fall back to the min%.



### Square Wave

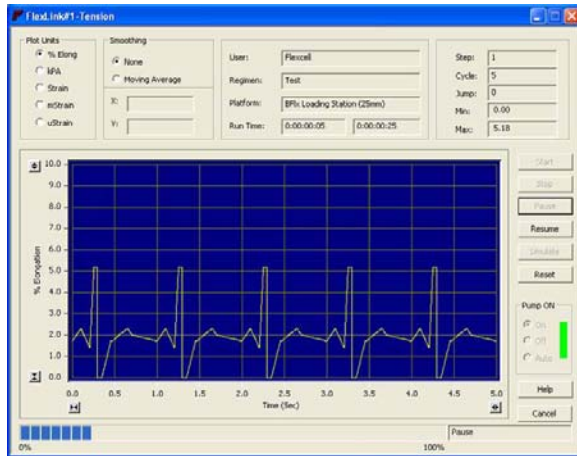
This waveform provides the fastest possible rise to max%, followed by the fastest possible fall to min%.





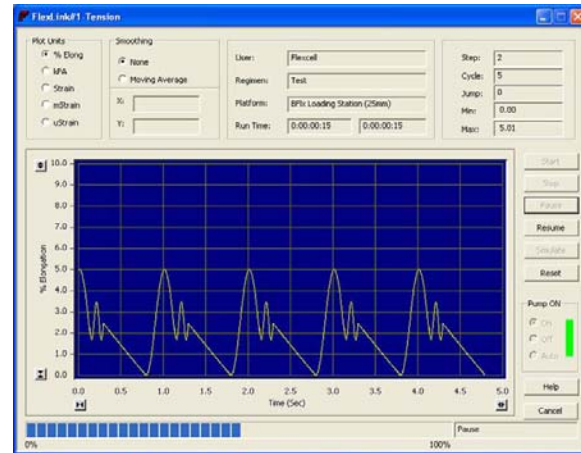
## Heart (E) Wave

This waveform applies a pressure pattern that mimics the shape as seen on an electrocardiogram



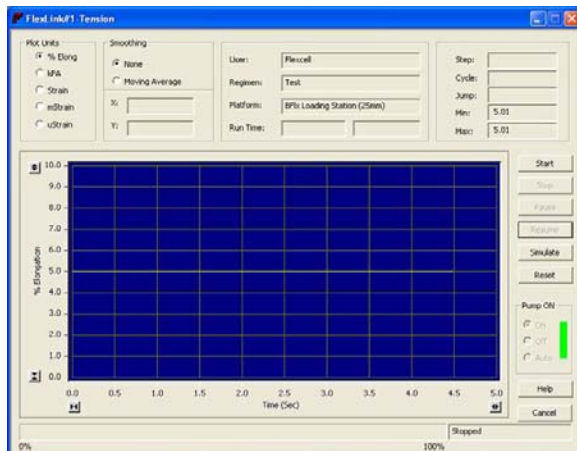
## Heart (P) Wave

This waveform applies a pressure pattern that mimics the actual pressure wave generated by a heart beat.



## Static Wave

This waveform applies a constant state of pressure.





## PART C

# FLEXCELL® FX-5000 TENSION SYSTEM SPECIFICATIONS



## FX-5000™ TENSION SYSTEM FEATURES

- Uses vacuum to deform a flexible-bottom culture plate yielding up to 33% substrate elongation
- Minimum strain resolution capability is 0.1% elongation
- Applies a defined, controlled, static, or cyclic deformation to growing cells *in vitro*
- Housed valving mechanism automatically regulates and maintains pressure to provide the specified strain regimen
- Capable of driving up to 4 independent FlexLink® remote strain and/or compression controllers
- Capable of delivering frequency ranges from 0.01→5 Hz
- Works with BioFlex®, Tissue Train® and UniFlex® culture plates
- Ability to program multiple frequency, amplitude and wave changes in one regimen
- Waveforms available: Static wave, Sinusoidal wave, Heart wave (Electronic and Pressure), Triangular wave, Square wave and custom waveforms
- Provides equibiaxial strain or unconstrained distension to the BioFlex® culture plate membrane
- *Optional:* Tissue Train® and UniFlex® plates can be used in conjunction with Arctangle® Loading Stations™ to achieve uniaxial strain
- *Optional:* StageFlexer® and StageFlexer Jr.® devices allow strain application to cells while viewing in real time under an upright microscope
- *Optional:* FlexStop™ provides a way to prevent pressure-induced flexing of any number of BioFlex® wells

## FX-5000™ TENSION COMPONENTS AND SPECIFICATIONS

- Dell OptiPlex Desktop Computer
- USB Keyboard
- USB Mouse
- 17" Flat panel Monitor
- FlexSoft® FX-5000™ V1.0 (Flexcell® FX-5000™ Software) and Microsoft Windows 7
- FX5K™ Tension FlexLink® controller
- Accessory Pack - nuts, ferrules
- Flexcell® vacuum pressure baseplate, gaskets, and acrylic window for BioFlex® culture plates
- BioFlex® 25mm diameter Loading Stations™ and Lubricant
- Water Traps
- System Drying Filter
- 25 ft small blue tubing (1/4" (6.4 mm) O.D.) for **FLEX IN** connection
- 25 ft clear tubing (3/8" (9.5 mm) O.D.) for **FLEX OUT** connection
- 25 ft large blue tubing (3/8" (9.5 mm) O.D.) for vacuum source connection
- Flexcell® FX-5000™ Tension System User's manual, Culture Plate & Loading Stations™ manual, Vacuum Source Connection Manual, Water Traps and Drying Filter Tech Report and other ancillary manuals for computer and monitor
- Surge protected power outlet strip

## TISSUE TRAIN® ACCESSORY KIT (OPTIONAL)

*(Note: If purchased as a system, the Tissue Train® Accessory Kit is substituted for the Flexcell® vacuum pressure baseplate, gaskets, and acrylic window for BioFlex® culture plates and BioFlex® Loading Stations™)*

- Flexcell® vacuum pressure baseplate, gaskets, and acrylic window for Tissue Train® culture plates
- Arctangle® Loading Stations™ and Lubricant
- Trough Loader™ Loading Stations™

## FX-5000™ TENSION COMPUTER SPECIFICATIONS

- Dell OptiPlex Small Form Factor
- Intel Core i5 processor (3.2 GHz)
- 250 GB SATA 6.0 Gb/s hard drive
- DVD +/- RW Drive



- 2 GB of DDR3 RAM
- Integrated video, Intel® HD2500

**Operating System:**

- Windows 7 Professional

**FX5K™ TENSION FLEXLINK® COMPONENT SPECIFICATIONS**

- Custom design vacuum controller board
  - Microprocessor sampling rate for display data transducer: 200 Hz
  - Microprocessor sampling rate for feedback/error (valve adjustment) transducer: 1 KHz
- 2 proportional-solenoid valves
- Ethernet port (RJ45)

**FX5K™ TENSION FLEXLINK® CALIBRATION SPECIFICATIONS**

**Unconstrained Static Performance (low side transducers):**

Maximum allowable waveform variance from static line:  $\pm 0.25\%$  elongation  
Maximum % elongation error (actual vs. programmed):  $\pm 0.6\%$  elongation  
Maximum allowable transducer inaccuracy:  $\pm 1.0$  kPa

**Unconstrained Dynamic Performance (low side transducers):**

Maximum % elongation error (actual vs. programmed):  $\pm 0.5\%$  elongation, at the following frequencies:  
<5% elongation, 0.5 and 1.0 Hz; 10 % elongation, 0.5 and 0.75Hz; >10% elongation, 0.5 Hz  
Maximum allowable transducer inaccuracy:  $\pm 1.0$  kPa

**BioFlex 25mm Loading Station Static Performance (high side transducers):**

Maximum allowable waveform variance from static line:  $\pm 0.25\%$  elongation  
Maximum % elongation error (actual vs. programmed):  $\pm 0.5\%$  elongation  
Maximum allowable transducer inaccuracy:  $\pm 2.0$  kPa

**BioFlex 25mm Loading Station Dynamic Performance (high side transducers):**

Maximum % elongation error (actual vs. programmed):  $\pm 0.75\%$  elongation, at the following frequencies:  $\leq 15\%$  elongation, 0.5 and 1.0 Hz;  $> 17.5\%$  elongation, 0.5Hz  
Maximum allowable transducer inaccuracy:  $\pm 2.0$  kPa

**LOADING STATIONS™ SPECIFICATIONS**

**25mm Diameter Loading Stations™ (equibiaxial strain):**

Minimum achievable % elongation with the FX-5000™: 0.8%  
Maximum achievable % elongation with the FX-5000™: 21.8%

**28mm Diameter Loading Stations™ (equibiaxial strain):**

Minimum achievable % elongation with the FX-5000™: 1.0%  
Maximum achievable % elongation with the FX-5000™: 15.9%

**31mm Diameter Loading Stations™ (equibiaxial strain):**

Minimum achievable % elongation with the FX-5000™: 0.8%  
Maximum achievable % elongation with the FX-5000™: 6.0%

**Tissue Train® with Arctangle® Loading Stations™ (uniaxial strain):**

Minimum achievable % elongation with the FX-5000™: 1.6%  
Maximum achievable % elongation with the FX-5000™: 20.8%

**UniFlex® with Arctangle® Loading Stations™ (uniaxial strain):**

Minimum achievable % elongation with the FX-5000™: 1.1%

Maximum achievable % elongation with the FX-5000™: 12.2%

**HT 24 Well Plate with Cylindrical Loading Stations™ (equibiaxial strain):**

Minimum achievable % elongation with the FX-5000™: 1.2%

Maximum achievable % elongation with the FX-5000™: 15.0%

**FX-5000™ TENSION PHYSICAL SPECIFICATIONS**

|                                    |                     |                                                |
|------------------------------------|---------------------|------------------------------------------------|
| <b>FX-5000™ Computer:</b>          | Size, W x H x D:    | 3.65" x 12.45" x 13.40" (9.3 x 31.6 x 34.0 cm) |
|                                    | Power Requirements: | 115/230V – 8.8/4.3A or 4.3/2.2A – 60/50 Hz     |
|                                    | Weight (unit only): | 15.0 lbs (6.8 kg)                              |
| <b>FX5K™ Tension FlexLink®:</b>    | Size, W x H x D:    | 10.5" x 7.0" x 18.5" (26.7 x 17.8 x 46.9 cm)   |
|                                    | Power Requirements: | 115/230V – 6/3A – 60/50 Hz                     |
|                                    | Weight (unit only): | 22.15 lbs (10.06 kg)                           |
| <b>Monitor 17" LCD Flat Panel:</b> | Size, W x H x D:    | 14.6" x 15.7" x 7.1" (37.1 x 39.7 x 18.0 cm)   |
|                                    | Power Requirements: | 115V - 60 Hz Auto switch to 230 V              |
|                                    | Weight (unit only): | 11.22 lbs (5.1kg)                              |

**FX-5000™ TENSION VACUUM REQUIREMENTS****To achieve the maximum system capability, the minimum vacuum source requirements are:**

- Maximum Vacuum: : -100 kPa
- Free Airflow Rate: 5.7 cfm (161 L/min)



## Appendix A

# FX-5000™ TENSION SYSTEM DUAL BASEPLATE OPERATION



When using the FX-5000™ Tension System in dual baseplate operation, the user may notice a drop in the overall programmed % elongation, depending upon the desired % elongation and frequency of the regimen. This drop is due to the vacuum source being shared between both baseplates.

It is, therefore, recommended that users reduce the frequency of such a regimen to 0.5 Hz or lower. A lower frequency will allow the FX-5000™ Tension System the correct amount of time to intake and exhaust the vacuum, which, in turn, gives the system enough time to reach the desired % elongation.

For example, if an FX-5000™ Tension System is connected in the dual baseplate configuration using the BioFlex 25 mm Loading Stations and a regimen is programmed with the following variables:

Waveform = Sine  
% Elongation = 20%  
Frequency = 1 Hz

The maximum % elongation achieved might only be 10%. Reducing the frequency of the regimen to 0.5 Hz will produce an achievable % elongation of 18%.

The maximum allowable **% elongation** for dual baseplate operation with the FX-5000™ Tension System is as follows:

| Platform                | Frequency     |               |
|-------------------------|---------------|---------------|
|                         | <i>1.0 Hz</i> | <i>0.5 Hz</i> |
| BioFlex: 25 mm          | 10.0 %        | 18.0 %        |
| BioFlex: 28 mm          | 6.5 %         | 11.0 %        |
| BioFlex: 31 mm          | 2.0 %         | 3.5 %         |
| Tissue Train: Arctangle | 10.0 %        | 16.0 %        |
| UniFlex: Arctangle      | 5.0 %         | 8.5 %         |
| HT BioFlex              | 7.0 %         | 11.5 %        |



### **NOTICE**

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