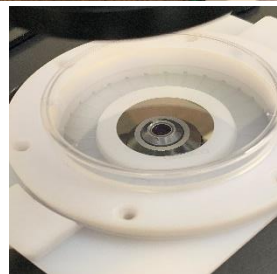
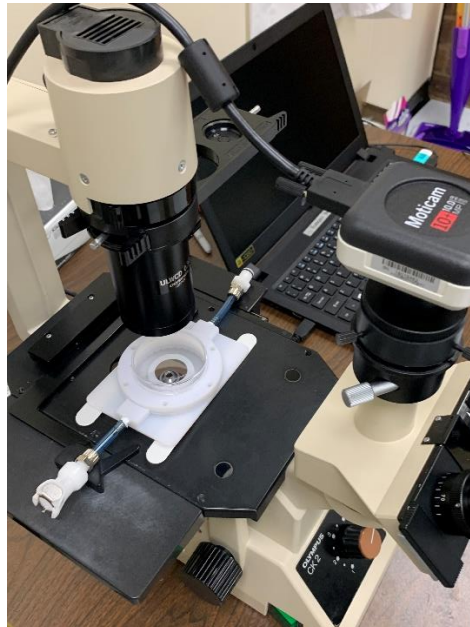




# USER MANUAL

## StageFlexer I®

For microscopes with inverted objectives



Rev 2.0

*Culturing Cells in a Mechanically Active Environment™*

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## 1. INTRODUCTION

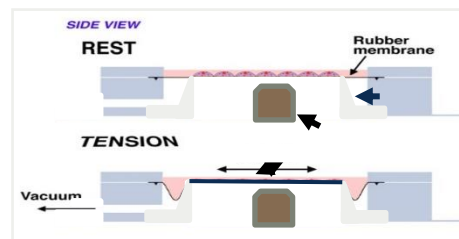
Flexcell®'s StageFlexer I® (Fig. 1) can be used in conjunction with the FX-6000™, FX-5000™, or Flex Jr.™ tension systems to observe cell stretching activity with an inverted objective microscope. Cells are grown on a 54 mm diameter silicone membrane (Stageflexer I® Membrane growth surface area = 22.9 cm<sup>2</sup>.) which is clamped and sealed to a cylindrical vacuum chamber. When vacuum is applied, the membrane translates across the chamber circumference, applying strain to the cells on the membrane surface (Fig. 2). The membrane deformation results in application of uniform, equibiaxial strain to the cells (Fig. 2).



**Figure 1.** Flexcell®'s StageFlexer I® for use with an inverted microscope objective.

Cells in a StageFlexer I® device can be viewed on a standard or inverted microscope. The opening at the bottom of the StageFlexer I® device allows for illumination of the membrane surface when using a standard microscope and provides clearance for most 10-40x objective lens, when using an inverted microscope.

**Figure 2.** Schematic of strain application to cells in a StageFlexer I®



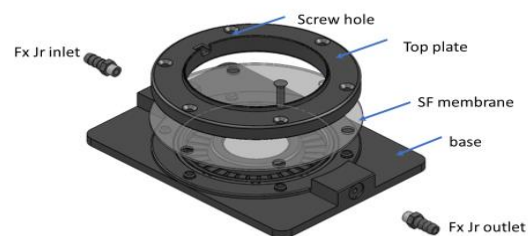
## 2. STAGEFLEXER I® ASSEMBLY

Figure 3 Stageflexer I component diagram.

Assembly of the StageFlexer I® will require a Phillips Head screwdriver. The provided components include (see Fig. 3):

1. StageFlexer I® Body
2. Sterile StageFlexer I® Membrane with protective Mylar
3. StageFlexer I® Top Ring
4. 60 mm culture plate lid
5. Top Screws (6x)

The StageFlexer I® device has the barbed connectors in place with tubing connected to each of the quick disconnect terminals.

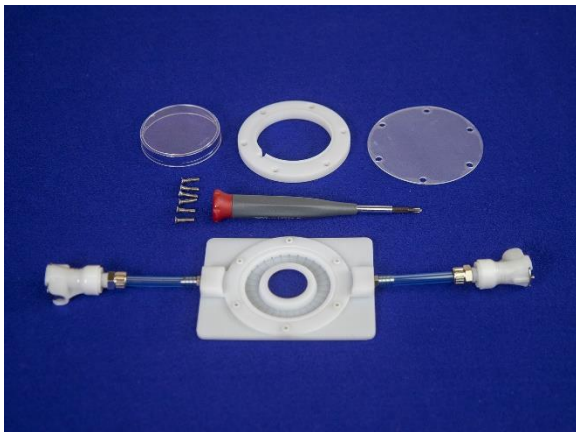


In a sterile environment, gather the StageFlexer I® components.

1. Coat the top surface of the central Loading Post Annulus with a thin film of silicone grease.



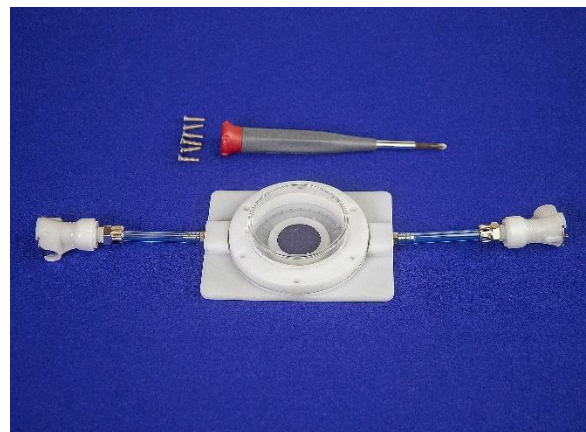
2. While holding the membrane with one set of forceps, carefully peel the Mylar protective layer off one side of the membrane with a second pair of forceps. Remove the other Mylar protective layer in the same manner.
3. Carefully align the holes in the membrane with the screw posts on the Inverted StageFlexer I® Body. Make sure the membrane lies flat across the Loading Post Annulus with slight tension. (See Fig. 4)
4. Align the StageFlexer I® Top Ring with the screw posts. Make sure the square notch in the Stageflexer I® Top Ring is facing upwards. (See Fig. 5)
5. Apply the 6 Top Screws to the Top Ring. Do not fully tighten the screws until they are all threaded into the Stageflexer I® Body. Tighten the screws in diagonal positions while evenly applying pressure around the perimeter of the Top Ring.
6. Place the 60 mm dish cover on the Stageflexer I® Top Ring to protect sterility.



**Figure 3.** StageFlexer I® components prepared for assembly.



**Figure 4.** StageFlexer I® device with SFI membrane atop the base.



**Figure 5.** StageFlexer I® with culture dish 60 mm top cover.



**Figure 6.** Fully assembled Stageflexer I®

Plate cells on the StageFlexer I® membrane in a 100 mm diameter dish according to your protocol. Transfer the membrane to the StageFlexer I® base as described above for



your stretch experiment. Add medium to the cells as needed.

The Stageflexer I® Body base should be situated directly over the objective lens on the

microscope base for proper viewing. Use the provided silicone elastomer sticky binders at each corner of the StageFlexer I® device to attach device to the microscope stage top.

### 3. PLATING AND VIEWING CELLS

Note that only the cells that are situated directly in the center of the membrane will receive uniform strain. Therefore, it is best to plate cells only in the uniformly strained area or to view or test the cells that are in the uniformly strained area.

Any cells outside of this circle will not receive uniform strain.

### 4. USING THE STAGEFLEXER® WITH A FLEXCELL® TENSION SYSTEM

To apply strain to the Stageflexer I®, connect the Flex In and Flex Out tubing segments from the Flexercell® Tension device to the corresponding fittings on the Stageflexer I®. Make sure the threads on the barbed fittings are securely fastened to the Stageflexer I® Body.

Connect the 1/4" blue tubing with BioFlex® adapter to the fitting on the front of the StageFlexer®. The base of the assembly should fit onto the microscope base for proper viewing.

It is important to note that there is a minimum vacuum level in which static friction is overcome on the Loading Stations™. The membrane does not begin to stretch until this point. This level is -6.0 kPa or 1.6% elongation. Do not try stretching cells at a % elongation less than that listed for each Loading Station™ diameter.

The vacuum seal is created by contact between the membrane and the Loading Post Annulus. Be sure the membrane is pulled taut across the annulus and is in contact with lubricant around the full annulus perimeter.

#### MINIMUM VACUUM LIMITATION

### APPENDIX 1: STAGEFLEXER I® CONVERSION CHART