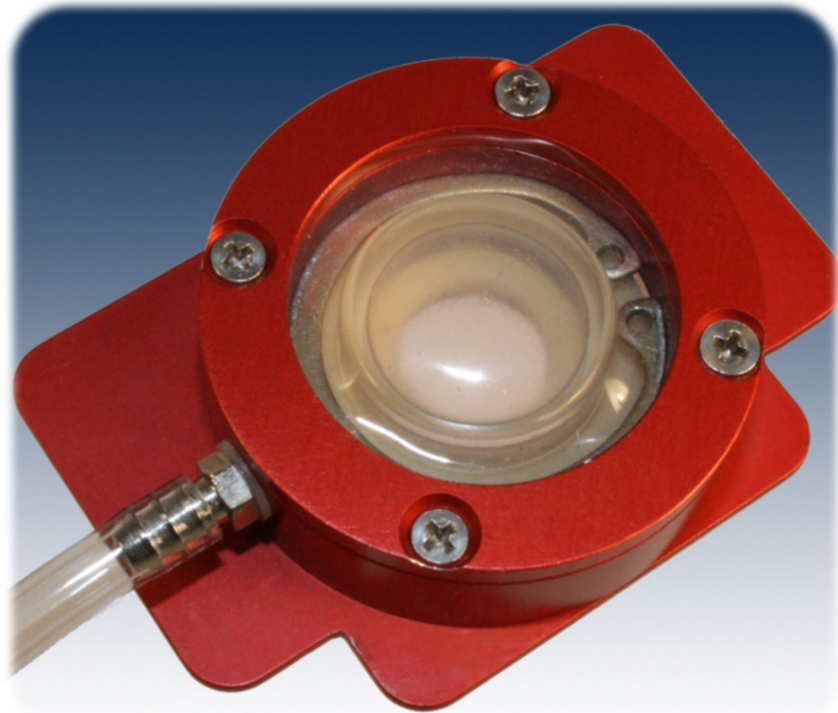




USER MANUAL

StageFlexer®



05-12-17
Rev 4.0

Culturing Cells in a Mechanically Active Environment™

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1. INTRODUCTION

The Flexcell® StageFlexer® (Fig. 1) in conjunction with the FX-5000™ or Flex Jr.™ Tension System allows users to observe cell stretching activity under a microscope. Cells are grown on a 42 mm diameter silicone membrane (StageFlexer® Membrane) which is clamped and sealed above a small cylindrical vacuum chamber. When a vacuum is applied, the membrane is pulled downward into this chamber applying strain to the cells growing on the membrane surface (Fig. 2). The membrane can be deformed freely in an open chamber or over a loading post to supply uniform biaxial strain to the cells (Fig. 2). The loading post comes in three different diameters (25 mm, 28 mm, and 31 mm) for varied growth surface area.

Cells in a StageFlexer® can be viewed on a standard microscope. The hole in the bottom of the StageFlexer® allows for illumination through the loading post.

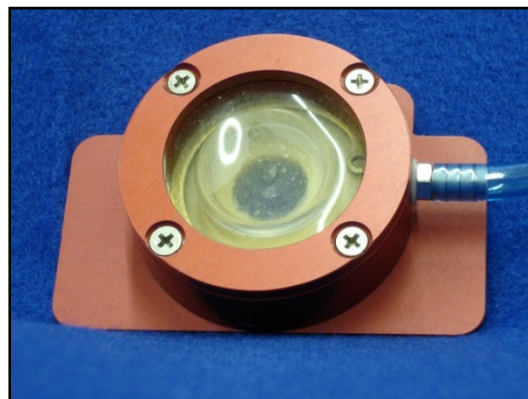


Figure 1. Flexcell® StageFlexer®

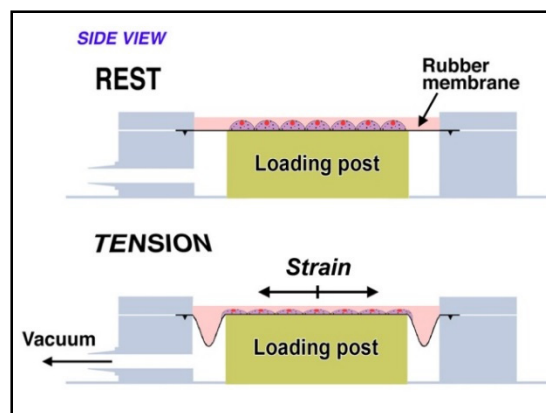


Figure 2. Schematic of strain application to cells in a StageFlexer®

2. STAGEFLEXER® ASSEMBLY

Assemble the eight main parts of the StageFlexer® in the following order (Fig. 3):

1. StageFlexer® body
2. Rubber gasket
3. Loading Station™
4. Retaining ring
5. O-ring
6. Silicone membrane
7. Top ring
8. Four top screws

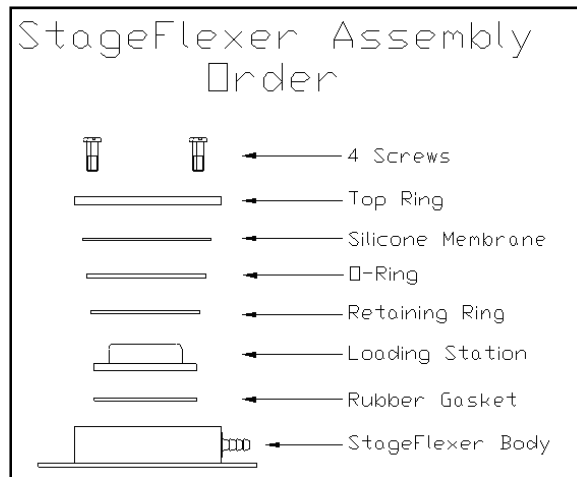


Figure 3. StageFlexer® assembly order



The Loading Station™ (i.e., loading post) is a small hollow acrylic piece that is inserted into the StageFlexer® vacuum chamber. It is used with a rubber gasket and a retaining ring that effectively seal the vacuum within the unit.

To insert the Loading Station™ into the StageFlexer®:

1. Coat the rubber gasket with a thin film of silicone grease on both sides and insert it into the bottom of the StageFlexer®.
2. When using the 25 mm Loading Station™, place it into the StageFlexer® first. Using retaining ring pliers to squeeze the retaining ring, close the ring enough to push it into the StageFlexer®. Push the ring down until it snaps into the milled ring hole at the bottom of the StageFlexer®. Getting the ring low enough may require additional vertical pressure from a regular screwdriver or similar tool.
3. When using the 28 mm or 31 mm Loading Stations™, use the retaining ring tool to squeeze the retaining ring around the bottom of the Loading Station™ into the milled ring area. Place the assembly into the StageFlexer® and push the ring down until it snaps into the milled ring hole at the bottom. As before, this may require the use of a regular screwdriver or similar tool.

To remove the 25 mm, 28 mm and 31 mm Loading Stations™, reverse the above process.

After assembly, use the Lubri-Sil™ lubricant supplied with the post to spread a generous, even layer of lubricant on top of the Loading Station™. The idea is to create a boundary

layer between the silicone membrane and Loading Station™ to reduce friction.

Next, place the O-ring over the ridge around the main body of the StageFlexer®. Then carefully place the membrane over top of the vacuum chamber and O-ring and center it using the screw holes around the edge of the StageFlexer®. Finally place the top ring over the membrane and O-ring, put the screws in place, and tighten the assembly to clamp the membrane.

Add medium to the cells as needed.

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.

Also included with the StageFlexer® is a larger aluminum plate which can be screwed onto the microscope stage. This plate replaces the small plate that is screwed to the bottom of the StageFlexer®. Simply remove the four screws on the bottom of the StageFlexer® and then the small plate. Screw the larger plate in its place such that the long end is facing away from the fitting on the front of the StageFlexer®. The larger base will have to be drilled at the back end to fit onto your particular microscope. The stage clips on your microscope should be held in place by two screws at the back of the stage. The holes to be drilled in the back of the larger base should be distanced apart exactly to fit the two screws that hold the stage clips in place. Once the holes are dimensioned and drilled in the larger StageFlexer® base, attach the base to the stage of the microscope with the screws. This will allow you to easily adjust the position of the StageFlexer® on your microscope and keep it in place over time.



3. PLATING AND VIEWING CELLS WHEN USING A LOADING STATION™

It should be noted that the only cells that are going to receive uniform strain are those attached to area of the membrane that is over the post when the membrane is in its fully stretched position. Therefore, it is best to attempt to plate cells only in the uniformly strained area or to view or test the cells that are in the uniformly strained area. To determine this area, the following equation can be used:

$$\text{Diameter} = (\text{Diameter of Loading Station}) / (1 + (\text{Max}\% \text{Elongation} / 100))$$

The "Max%Elongation" is the maximum % elongation that you plan to use in your regimen. The calculated diameter is the diameter of a circle at the center of the membrane. Any cells outside of this circle will not receive uniform strain.

4. USING THE STAGEFLEXER® WITH THE FX-5000™ OR FLEX JR.™ TENSION SYSTEM

Connect the front hose barb of the StageFlexer® to the FX-5000™ or Flex Jr.™ as shown in Figure 4. Connect the short blue tubing provided with the StageFlexer® to the front hose barb on the StageFlexer®. Then connect the quick disconnect at the end of the StageFlexer® to the quick disconnect at the end of the tubing shown below.

Also included with the system is a white PVC volume that should be used inline with the **FLEX IN** and **FLEX OUT** tubing. Because the StageFlexer® has such a low air volume, it is necessary that some extra volume be added to the system for stabilization. The PVC volume is simply added by cutting the larger clear tubing on the FX-5000™/ Flex Jr.™ tubing adapter at its midpoint, and connecting each cut end to the fitting on each end of the PVC volume.

MINIMUM LOADING STATION™ LIMITATION

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. For the 25mm Loading Station™, this level is -6.0 kPa or 1.6% elongation. For the 28mm Loading Station™, this level is -12.0 kPa or 1.9%. For the 31mm Loading Station™, this level is -16.0 kPa or 2.1%. Do not try stretching cells at a % elongation less than that listed for each Loading Station™ diameter.

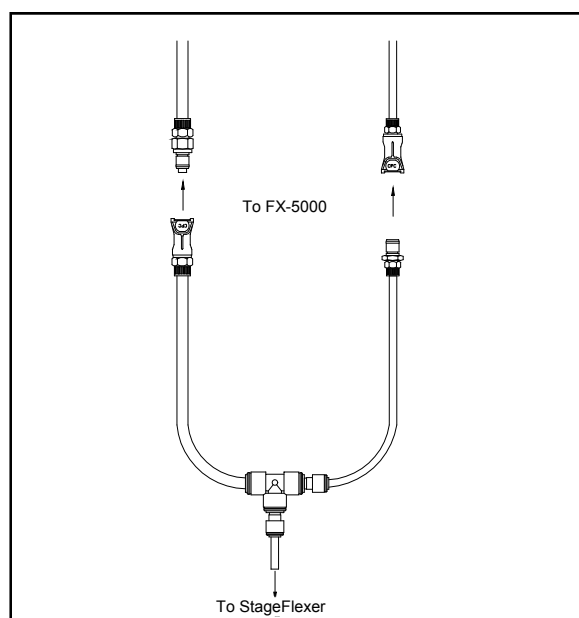


Figure 4. StageFlexer® to FX-5000™ Tension Unit Tubing Adapter



APPENDIX 1: STAGEFLEXER® 25 MM LOADING STATION™ CONVERSION CHART

<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>	<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>	<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>
0.00	0.0	0.000	21.35	5.1	0.051	54.32	10.2	0.102
0.28	0.1	0.001	21.90	5.2	0.052	55.05	10.3	0.103
0.57	0.2	0.002	22.45	5.3	0.053	55.79	10.4	0.104
0.86	0.3	0.003	23.01	5.4	0.054	56.53	10.5	0.105
1.16	0.4	0.004	23.57	5.5	0.055	57.28	10.6	0.106
1.46	0.5	0.005	24.13	5.6	0.056	58.02	10.7	0.107
1.77	0.6	0.006	24.70	5.7	0.057	58.77	10.8	0.108
2.09	0.7	0.007	25.28	5.8	0.058	59.52	10.9	0.109
2.41	0.8	0.008	25.86	5.9	0.059	60.28	11.0	0.110
2.74	0.9	0.009	26.44	6.0	0.060	61.03	11.1	0.111
3.08	1.0	0.010	27.03	6.1	0.061	61.79	11.2	0.112
3.42	1.1	0.011	27.62	6.2	0.062	62.56	11.3	0.113
3.76	1.2	0.012	28.21	6.3	0.063	63.32	11.4	0.114
4.11	1.3	0.013	28.81	6.4	0.064	64.09	11.5	0.115
4.47	1.4	0.014	29.41	6.5	0.065	64.86	11.6	0.116
4.83	1.5	0.015	30.02	6.6	0.066	65.63	11.7	0.117
5.20	1.6	0.016	30.63	6.7	0.067	66.41	11.8	0.118
5.57	1.7	0.017	31.25	6.8	0.068	67.18	11.9	0.119
5.95	1.8	0.018	31.87	6.9	0.069	67.96	12.0	0.120
6.33	1.9	0.019	32.49	7.0	0.070	68.74	12.1	0.121
6.72	2.0	0.020	33.12	7.1	0.071	69.53	12.2	0.122
7.12	2.1	0.021	33.75	7.2	0.072	70.31	12.3	0.123
7.52	2.2	0.022	34.38	7.3	0.073	71.10	12.4	0.124
7.93	2.3	0.023	35.02	7.4	0.074	71.89	12.5	0.125
8.34	2.4	0.024	35.67	7.5	0.075	72.69	12.6	0.126
8.75	2.5	0.025	36.31	7.6	0.076	73.48	12.7	0.127
9.18	2.6	0.026	36.96	7.7	0.077	74.28	12.8	0.128
9.60	2.7	0.027	37.62	7.8	0.078	75.08	12.9	0.129
10.04	2.8	0.028	38.27	7.9	0.079	75.88	13.0	0.130
10.47	2.9	0.029	38.93	8.0	0.080	76.68	13.1	0.131
10.92	3.0	0.030	39.60	8.1	0.081	77.49	13.2	0.132
11.36	3.1	0.031	40.27	8.2	0.082	78.30	13.3	0.133
11.82	3.2	0.032	40.94	8.3	0.083	79.11	13.4	0.134
12.27	3.3	0.033	41.61	8.4	0.084	79.92	13.5	0.135
12.74	3.4	0.034	42.29	8.5	0.085	80.73	13.6	0.136
13.21	3.5	0.035	42.97	8.6	0.086	81.55	13.7	0.137
13.68	3.6	0.036	43.66	8.7	0.087	82.36	13.8	0.138
14.16	3.7	0.037	44.35	8.8	0.088	83.18	13.9	0.139
14.64	3.8	0.038	45.04	8.9	0.089	84.00	14.0	0.140
15.13	3.9	0.039	45.73	9.0	0.090	84.82	14.1	0.141
15.62	4.0	0.040	46.43	9.1	0.091	85.65	14.2	0.142
16.12	4.1	0.041	47.13	9.2	0.092	86.47	14.3	0.143
16.62	4.2	0.042	47.84	9.3	0.093	87.30	14.4	0.144
17.13	4.3	0.043	48.54	9.4	0.094	88.13	14.5	0.145
17.64	4.4	0.044	49.26	9.5	0.095	88.96	14.6	0.146
18.15	4.5	0.045	49.97	9.6	0.096	89.79	14.7	0.147
18.67	4.6	0.046	50.69	9.7	0.097	90.62	14.8	0.148
19.20	4.7	0.047	51.41	9.8	0.098			
19.73	4.8	0.048	52.13	9.9	0.099			
20.26	4.9	0.049	52.86	10.0	0.100			
20.80	5.0	0.050	53.59	10.1	0.101			



APPENDIX 2: STAGEFLEXER® 28 MM LOADING STATION™ CONVERSION CHART

<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>	<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>	<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>
0.00	0.0	0.000	31.16	5.1	0.051	62.68	10.2	0.102
0.70	0.1	0.001	31.72	5.2	0.052	63.40	10.3	0.103
1.39	0.2	0.002	32.29	5.3	0.053	64.12	10.4	0.104
2.08	0.3	0.003	32.86	5.4	0.054	64.85	10.5	0.105
2.76	0.4	0.004	33.43	5.5	0.055	65.59	10.6	0.106
3.44	0.5	0.005	34.00	5.6	0.056	66.33	10.7	0.107
4.11	0.6	0.006	34.57	5.7	0.057	67.08	10.8	0.108
4.78	0.7	0.007	35.14	5.8	0.058	67.83	10.9	0.109
5.44	0.8	0.008	35.71	5.9	0.059	68.59	11.0	0.110
6.10	0.9	0.009	36.29	6.0	0.060	69.36	11.1	0.111
6.76	1.0	0.010	36.86	6.1	0.061	70.13	11.2	0.112
7.41	1.1	0.011	37.44	6.2	0.062	70.91	11.3	0.113
8.05	1.2	0.012	38.01	6.3	0.063	71.70	11.4	0.114
8.69	1.3	0.013	38.59	6.4	0.064	72.49	11.5	0.115
9.33	1.4	0.014	39.17	6.5	0.065	73.29	11.6	0.116
9.97	1.5	0.015	39.75	6.6	0.066	74.10	11.7	0.117
10.60	1.6	0.016	40.33	6.7	0.067	74.91	11.8	0.118
11.22	1.7	0.017	40.92	6.8	0.068	75.73	11.9	0.119
11.85	1.8	0.018	41.51	6.9	0.069	76.56	12.0	0.120
12.47	1.9	0.019	42.10	7.0	0.070	77.40	12.1	0.121
13.08	2.0	0.020	42.69	7.1	0.071	78.25	12.2	0.122
13.70	2.1	0.021	43.28	7.2	0.072	79.10	12.3	0.123
14.31	2.2	0.022	43.88	7.3	0.073	79.96	12.4	0.124
14.92	2.3	0.023	44.47	7.4	0.074	80.83	12.5	0.125
15.52	2.4	0.024	45.07	7.5	0.075	81.71	12.6	0.126
16.12	2.5	0.025	45.68	7.6	0.076	82.59	12.7	0.127
16.72	2.6	0.026	46.28	7.7	0.077	83.48	12.8	0.128
17.32	2.7	0.027	46.89	7.8	0.078	84.39	12.9	0.129
17.91	2.8	0.028	47.51	7.9	0.079	85.30	13.0	0.130
18.51	2.9	0.029	48.12	8.0	0.080	86.21	13.1	0.131
19.10	3.0	0.030	48.74	8.1	0.081	87.14	13.2	0.132
19.68	3.1	0.031	49.36	8.2	0.082	88.08	13.3	0.133
20.27	3.2	0.032	49.99	8.3	0.083	89.02	13.4	0.134
20.85	3.3	0.033	50.62	8.4	0.084	89.98	13.5	0.135
21.43	3.4	0.034	51.25	8.5	0.085	90.94	13.6	0.136
22.01	3.5	0.035	51.89	8.6	0.086			
22.59	3.6	0.036	52.53	8.7	0.087			
23.17	3.7	0.037	53.17	8.8	0.088			
23.75	3.8	0.038	53.82	8.9	0.089			
24.32	3.9	0.039	54.48	9.0	0.090			
24.89	4.0	0.040	55.13	9.1	0.091			
25.47	4.1	0.041	55.79	9.2	0.092			
26.04	4.2	0.042	56.46	9.3	0.093			
26.61	4.3	0.043	57.13	9.4	0.094			
27.18	4.4	0.044	57.81	9.5	0.095			
27.75	4.5	0.045	58.49	9.6	0.096			
28.32	4.6	0.046	59.17	9.7	0.097			
28.88	4.7	0.047	59.86	9.8	0.098			
29.45	4.8	0.048	60.56	9.9	0.099			
30.02	4.9	0.049	61.26	10.0	0.100			
30.59	5.0	0.050	61.97	10.1	0.101			

**APPENDIX 3: STAGEFLEXER® 31 MM LOADING STATION™
CONVERSION CHART**

<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>		<u>Press (-kPa)</u>	<u>% Elong</u>	<u>Strain</u>
0.00	0.0	0.000		54.51	5.0	0.050
0.37	0.1	0.001		55.87	5.1	0.051
0.79	0.2	0.002		57.22	5.2	0.052
1.25	0.3	0.003		58.56	5.3	0.053
1.76	0.4	0.004		59.89	5.4	0.054
2.31	0.5	0.005		61.21	5.5	0.055
2.91	0.6	0.006		62.51	5.6	0.056
3.54	0.7	0.007		63.80	5.7	0.057
4.22	0.8	0.008		65.07	5.8	0.058
4.94	0.9	0.009		66.33	5.9	0.059
5.70	1.0	0.010		67.56	6.0	0.060
6.49	1.1	0.011		68.78	6.1	0.061
7.32	1.2	0.012		69.98	6.2	0.062
8.18	1.3	0.013		71.15	6.3	0.063
9.08	1.4	0.014		72.31	6.4	0.064
10.01	1.5	0.015		73.44	6.5	0.065
10.98	1.6	0.016		74.54	6.6	0.066
11.97	1.7	0.017		75.62	6.7	0.067
13.00	1.8	0.018		76.67	6.8	0.068
14.05	1.9	0.019		77.69	6.9	0.069
15.13	2.0	0.020		78.69	7.0	0.070
16.23	2.1	0.021		79.65	7.1	0.071
17.36	2.2	0.022		80.58	7.2	0.072
18.51	2.3	0.023		81.48	7.3	0.073
19.69	2.4	0.024		82.35	7.4	0.074
20.89	2.5	0.025		83.18	7.5	0.075
22.11	2.6	0.026		83.97	7.6	0.076
23.34	2.7	0.027		84.72	7.7	0.077
24.60	2.8	0.028		85.44	7.8	0.078
25.87	2.9	0.029		86.12	7.9	0.079
27.16	3.0	0.030		86.76	8.0	0.080
28.46	3.1	0.031		87.35	8.1	0.081
29.78	3.2	0.032		87.90	8.2	0.082
31.11	3.3	0.033		88.41	8.3	0.083
32.45	3.4	0.034		88.87	8.4	0.084
33.80	3.5	0.035		89.29	8.5	0.085
35.16	3.6	0.036		89.66	8.6	0.086
36.53	3.7	0.037		89.98	8.7	0.087
37.90	3.8	0.038		90.25	8.8	0.088
39.28	3.9	0.039				
40.67	4.0	0.040				
42.05	4.1	0.041				
43.44	4.2	0.042				
44.84	4.3	0.043				
46.23	4.4	0.044				
47.62	4.5	0.045				
49.00	4.6	0.046				
50.39	4.7	0.047				
51.77	4.8	0.048				
53.14	4.9	0.049				