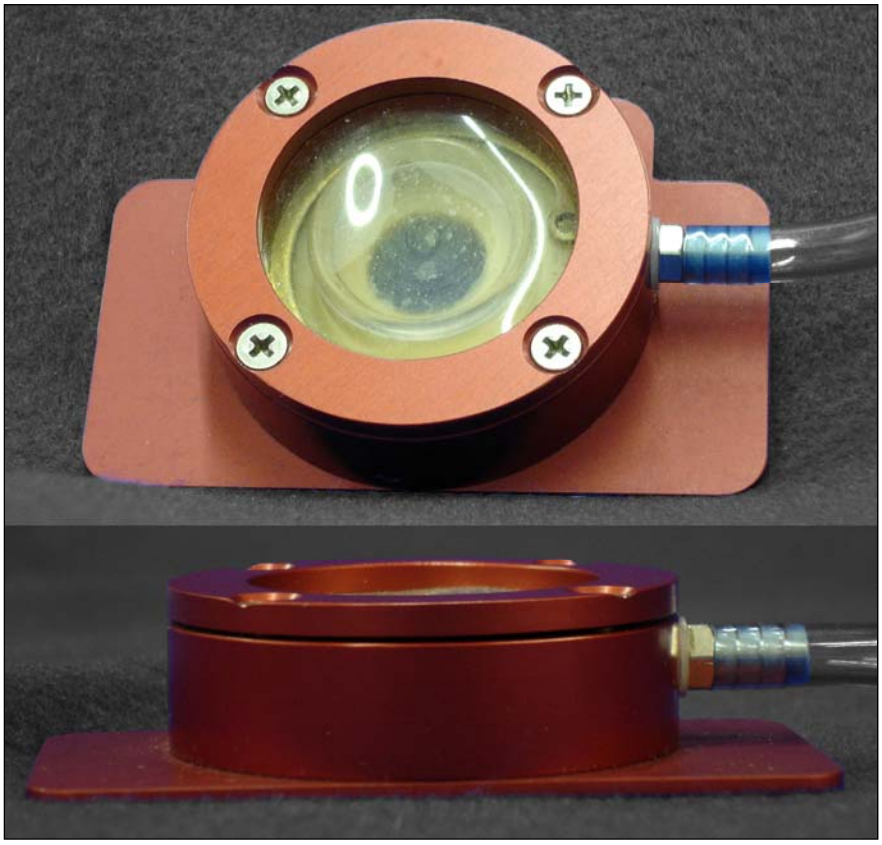
StageFlexer®
User's Manual



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Culturing Cells in a Mechanically Active Environment™

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TABLE OF CONTENTS

USING THE STAGEFLEXER®

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.

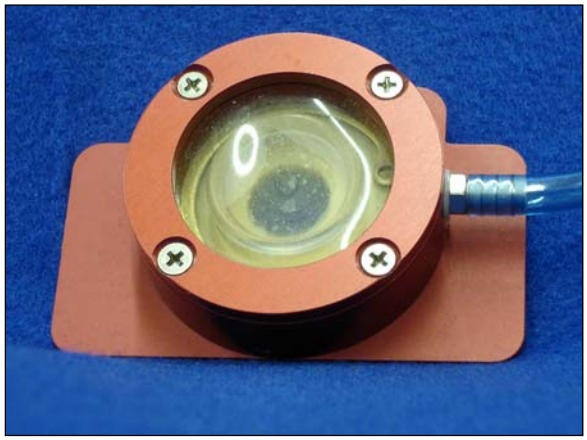

Figure 1. Flexcell® StageFlexer®

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.

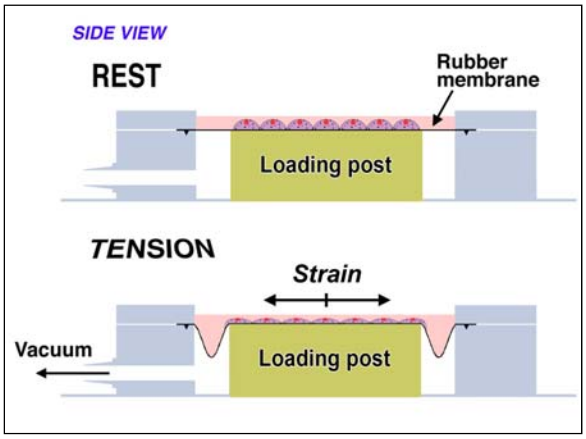

Figure 2. Schematic of strain application to cells in a StageFlexer®

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

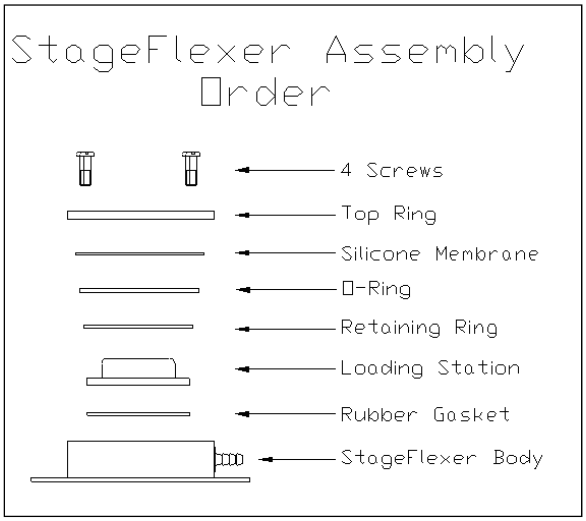

Figure 3. StageFlexer® assembly order

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.

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:

Diameter = (Diameter of Loading Station)/(1 + (Max%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.

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.

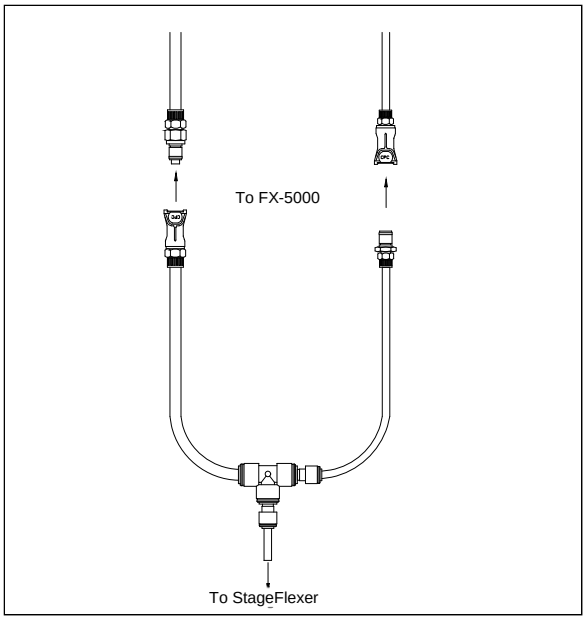

Figure 4. StageFlexer® to FX-5000™ Tension Unit Tubing Adapter

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.

APPENDICES

Appendix 1: StageFlexer® 25 mm Loading Station™ Conversion Chart

Appendix 2: StageFlexer® 28 mm Loading Station™ Conversion Chart

Appendix 3: StageFlexer® 31 mm Loading Station™ Conversion Chart

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

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

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

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

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

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