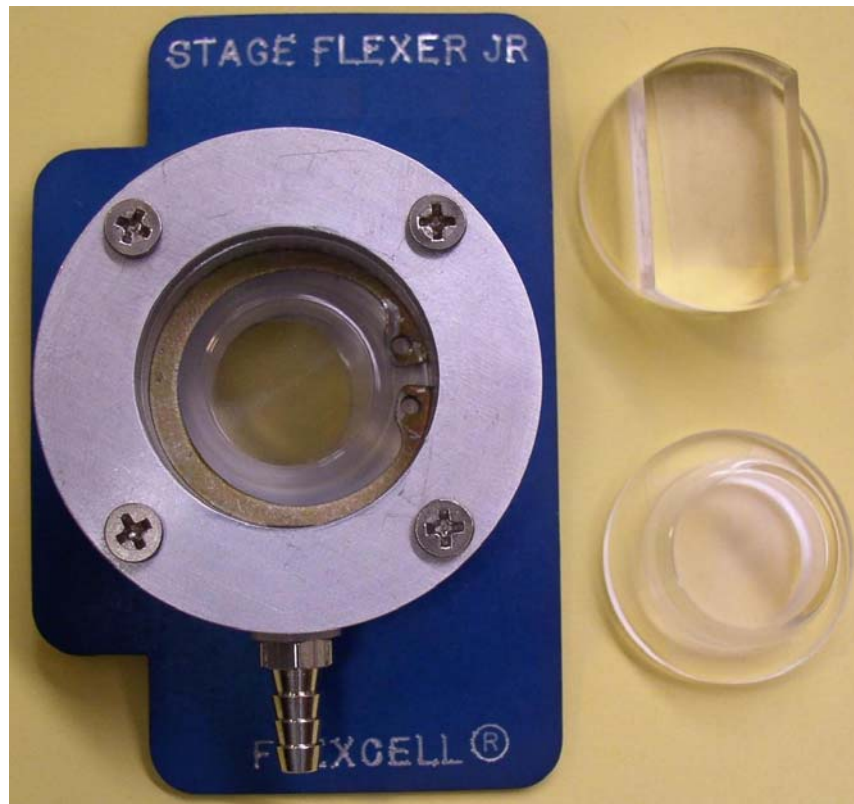




StageFlexer[®] Jr. User's Manual



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Rev 2.1

Culturing Cells in a Mechanically Active Environment[™]
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USING THE STAGEFLEXER® JR.

INTRODUCTION

The StageFlexer® Jr. (Fig. 1) is designed to allow users to remove a membrane from a BioFlex®, Tissue Train®, or UniFlex® culture plate well and continue observing cellular responses to strain under a microscope in real-time.



Figure 1. StageFlexer® Jr. with Arctriangle® and 18.5 mm cylindrical Loading Stations™.

The excised membrane is clamped and sealed above a small cylindrical vacuum chamber. When a vacuum is applied with the FX-5000™ or Flex Jr.™ Tension System, the membrane is pulled downward into this chamber applying strain to the cells growing on the membrane surface (BioFlex® or UniFlex® plate) or in 3-dimensional gels (Tissue Train® plate, Fig. 2). The membrane can be deformed freely in an open chamber, or over a Loading Station™ to supply uniform biaxial or uniaxial strain to the cells. The Loading Station™ comes in two different geometries (Fig. 1): cylindrical (18.5 mm diameter) for biaxial strain of a membrane from a BioFlex® plate and Arctriangle™ for uniaxial strain of a

membrane from a UniFlex® or Tissue Train® plate.

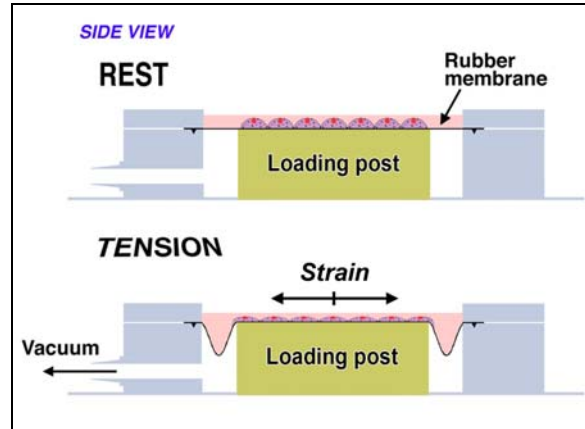


Figure 2. Schematic of how strain is applied to the cells cultured on the silicone membranes.

Cells in a StageFlexer® Jr. can be viewed on a standard microscope. The hole in the bottom of the StageFlexer® Jr. allows for illumination of the Loading Station™ from beneath.

STAGEFLEXER® JR. ASSEMBLY

Assemble the eight main parts of the StageFlexer® Jr. 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

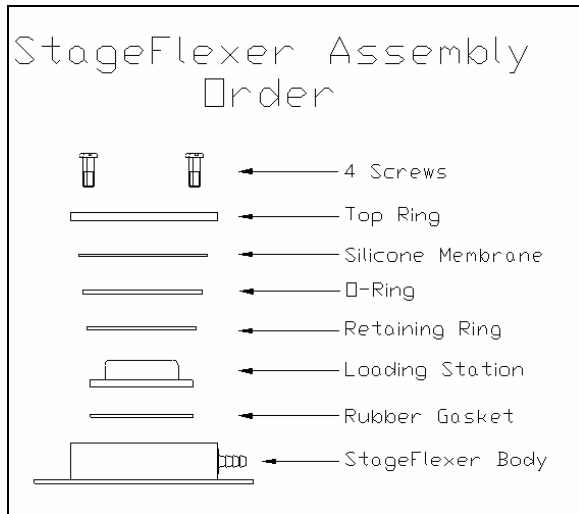


Figure 3. Assembly order for the StageFlexer® Jr.

The Loading Station™ (i.e., loading post) is a small hollow acrylic piece that is inserted into the StageFlexer® Jr. 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® Jr.:

1. Coat the rubber gasket with a thin film of silicone grease on both sides and insert it into the bottom of the StageFlexer® Jr.
2. Place the Loading Station™ into the StageFlexer® Jr. Using retaining ring pliers to squeeze the retaining ring, close the ring enough to push it into the StageFlexer® Jr. Push the ring down until it snaps into the milled ring hole at the bottom of the StageFlexer® Jr. Getting the ring low enough may require additional vertical pressure from a regular screwdriver or similar tool.

To remove the 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™. This creates 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® Jr. Then carefully place the membrane over top of the vacuum chamber and O-ring and center it using the screw holes on the edge of the StageFlexer® Jr. 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® Jr. The base of the assembly should fit onto the microscope base for proper viewing.

Also included with the StageFlexer® Jr. 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® Jr. Simply remove the four screws on the bottom of the StageFlexer® Jr. 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® Jr. 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® Jr. 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® Jr. 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 the 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 biaxial strain.

Please see *Tech Report 100: Tissue Train Culture System. A Method for Culture and Mechanical Loading of Cells in a Linear 3D Matrix* and *Tech Report 102: UniFlex Culture System. A Method to Apply Uniaxial Mechanical Loading to Monolayer Cell Cultures* for appropriate culture of cells in the Tissue Train® and UniFlex® plates, respectively.

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

Connect the front hose barb of the StageFlexer® Jr. to the FX-5000™ or Flex Jr.™ as shown in Figure 4. Connect the short blue tubing provided with the StageFlexer®

Jr. to the front hose barb on the StageFlexer® Jr. Then connect the open end to the tubing adapter. Next, connect the 3/8" (9.5 mm) and the 1/4" (6.4 mm) quick disconnected tubing, already attached to the adapter, to the respective **FLEX IN** and **FLEX OUT** tubing from the rear of the FX-5000™ or Flex Jr.™.

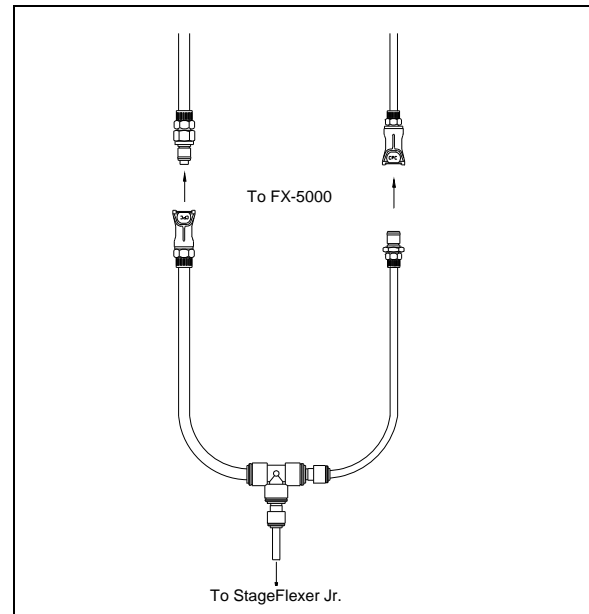


Figure 4. StageFlexer® Jr. to FX-5000™ Tension Unit tubing adapter

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® Jr. 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 18.5 mm Loading Station™, this level is -12.0 kPa or 1.8% elongation. For the Arcangle®

Loading Station™ with a Tissue Train® membrane, this level is -12.0 kPa or 2.5%. For the Arcangle® Loading Station™ with a UniFlex® membrane, this level is -16.0 kPa or 1.5%. Do not try stretching cells at a % elongation less than that listed for each Loading Station™ and corresponding membrane.



APPENDICES

Appendix 1: BioFlex® Membrane and 18.5 mm Loading Station™ Conversion Chart

Appendix 2: Tissue Train® Membrane with Arcangle™ Loading Station™ Conversion Chart

Appendix 3: UniFlex® Membrane with Arcangle™ Loading Station™ Conversion Chart



**APPENDIX 1: BIOFLEX® MEMBRANE AND 18.5 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	32.39	4.9	0.049	64.25	9.8	0.098
0.67	0.1	0.001	33.04	5.0	0.050	64.90	9.9	0.099
1.33	0.2	0.002	33.70	5.1	0.051	65.54	10.0	0.100
2.00	0.3	0.003	34.35	5.2	0.052	66.19	10.1	0.101
2.66	0.4	0.004	35.01	5.3	0.053	66.83	10.2	0.102
3.33	0.5	0.005	35.66	5.4	0.054	67.47	10.3	0.103
3.99	0.6	0.006	36.32	5.5	0.055	68.12	10.4	0.104
4.66	0.7	0.007	36.97	5.6	0.056	68.76	10.5	0.105
5.32	0.8	0.008	37.63	5.7	0.057	69.40	10.6	0.106
5.98	0.9	0.009	38.28	5.8	0.058	70.05	10.7	0.107
6.65	1.0	0.010	38.93	5.9	0.059	70.69	10.8	0.108
7.31	1.1	0.011	39.59	6.0	0.060	71.33	10.9	0.109
7.98	1.2	0.012	40.24	6.1	0.061	71.97	11.0	0.110
8.64	1.3	0.013	40.89	6.2	0.062	72.61	11.1	0.111
9.30	1.4	0.014	41.55	6.3	0.063	73.26	11.2	0.112
9.97	1.5	0.015	42.20	6.4	0.064	73.90	11.3	0.113
10.63	1.6	0.016	42.85	6.5	0.065	74.54	11.4	0.114
11.29	1.7	0.017	43.50	6.6	0.066	75.18	11.5	0.115
11.95	1.8	0.018	44.15	6.7	0.067	75.82	11.6	0.116
12.62	1.9	0.019	44.81	6.8	0.068	76.46	11.7	0.117
13.28	2.0	0.020	45.46	6.9	0.069	77.10	11.8	0.118
13.94	2.1	0.021	46.11	7.0	0.070	77.74	11.9	0.119
14.60	2.2	0.022	46.76	7.1	0.071	78.38	12.0	0.120
15.26	2.3	0.023	47.41	7.2	0.072	79.02	12.1	0.121
15.92	2.4	0.024	48.06	7.3	0.073	79.66	12.2	0.122
16.58	2.5	0.025	48.71	7.4	0.074	80.29	12.3	0.123
17.25	2.6	0.026	49.36	7.5	0.075	80.93	12.4	0.124
17.91	2.7	0.027	50.01	7.6	0.076	81.57	12.5	0.125
18.57	2.8	0.028	50.66	7.7	0.077	82.21	12.6	0.126
19.23	2.9	0.029	51.31	7.8	0.078	82.85	12.7	0.127
19.89	3.0	0.030	51.96	7.9	0.079	83.48	12.8	0.128
20.55	3.1	0.031	52.61	8.0	0.080	84.12	12.9	0.129
21.21	3.2	0.032	53.26	8.1	0.081	84.76	13.0	0.130
21.87	3.3	0.033	53.91	8.2	0.082	85.40	13.1	0.131
22.52	3.4	0.034	54.56	8.3	0.083	86.03	13.2	0.132
23.18	3.5	0.035	55.20	8.4	0.084	86.67	13.3	0.133
23.84	3.6	0.036	55.85	8.5	0.085	87.30	13.4	0.134
24.50	3.7	0.037	56.50	8.6	0.086	87.94	13.5	0.135
25.16	3.8	0.038	57.15	8.7	0.087	88.58	13.6	0.136
25.82	3.9	0.039	57.79	8.8	0.088	89.21	13.7	0.137
26.47	4.0	0.040	58.44	8.9	0.089	89.85	13.8	0.138
27.13	4.1	0.041	59.09	9.0	0.090	90.48	13.9	0.139
27.79	4.2	0.042	59.73	9.1	0.091			
28.45	4.3	0.043	60.38	9.2	0.092			
29.10	4.4	0.044	61.03	9.3	0.093			
29.76	4.5	0.045	61.67	9.4	0.094			
30.42	4.6	0.046	62.32	9.5	0.095			
31.07	4.7	0.047	62.96	9.6	0.096			
31.73	4.8	0.048	63.61	9.7	0.097			



APPENDIX 2: TISSUE TRAIN® MEMBRANE WITH ARCTANGLE™ 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	28.78	6.1	0.061	58.49	12.2	0.122
0.49	0.1	0.001	29.25	6.2	0.062	59.01	12.3	0.123
0.98	0.2	0.002	29.71	6.3	0.063	59.53	12.4	0.124
1.47	0.3	0.003	30.18	6.4	0.064	60.06	12.5	0.125
1.95	0.4	0.004	30.65	6.5	0.065	60.58	12.6	0.126
2.44	0.5	0.005	31.12	6.6	0.066	61.11	12.7	0.127
2.92	0.6	0.006	31.58	6.7	0.067	61.64	12.8	0.128
3.41	0.7	0.007	32.05	6.8	0.068	62.17	12.9	0.129
3.89	0.8	0.008	32.52	6.9	0.069	62.70	13.0	0.130
4.37	0.9	0.009	32.99	7.0	0.070	63.24	13.1	0.131
4.85	1.0	0.010	33.46	7.1	0.071	63.77	13.2	0.132
5.33	1.1	0.011	33.93	7.2	0.072	64.31	13.3	0.133
5.81	1.2	0.012	34.40	7.3	0.073	64.85	13.4	0.134
6.29	1.3	0.013	34.87	7.4	0.074	65.39	13.5	0.135
6.77	1.4	0.014	35.34	7.5	0.075	65.94	13.6	0.136
7.25	1.5	0.015	35.82	7.6	0.076	66.48	13.7	0.137
7.73	1.6	0.016	36.29	7.7	0.077	67.03	13.8	0.138
8.20	1.7	0.017	36.76	7.8	0.078	67.58	13.9	0.139
8.68	1.8	0.018	37.24	7.9	0.079	68.13	14.0	0.140
9.15	1.9	0.019	37.71	8.0	0.080	68.68	14.1	0.141
9.63	2.0	0.020	38.19	8.1	0.081	69.23	14.2	0.142
10.10	2.1	0.021	38.66	8.2	0.082	69.79	14.3	0.143
10.57	2.2	0.022	39.14	8.3	0.083	70.35	14.4	0.144
11.05	2.3	0.023	39.62	8.4	0.084	70.91	14.5	0.145
11.52	2.4	0.024	40.09	8.5	0.085	71.47	14.6	0.146
11.99	2.5	0.025	40.57	8.6	0.086	72.03	14.7	0.147
12.46	2.6	0.026	41.05	8.7	0.087	72.60	14.8	0.148
12.93	2.7	0.027	41.53	8.8	0.088	73.17	14.9	0.149
13.40	2.8	0.028	42.01	8.9	0.089	73.74	15.0	0.150
13.87	2.9	0.029	42.50	9.0	0.090	74.31	15.1	0.151
14.34	3.0	0.030	42.98	9.1	0.091	74.89	15.2	0.152
14.81	3.1	0.031	43.46	9.2	0.092	75.46	15.3	0.153
15.27	3.2	0.032	43.95	9.3	0.093	76.04	15.4	0.154
15.74	3.3	0.033	44.43	9.4	0.094	76.62	15.5	0.155
16.21	3.4	0.034	44.92	9.5	0.095	77.21	15.6	0.156
16.68	3.5	0.035	45.41	9.6	0.096	77.79	15.7	0.157
17.14	3.6	0.036	45.90	9.7	0.097	78.38	15.8	0.158
17.61	3.7	0.037	46.39	9.8	0.098	78.97	15.9	0.159
18.08	3.8	0.038	46.88	9.9	0.099	79.56	16.0	0.160
18.54	3.9	0.039	47.37	10.0	0.100	80.16	16.1	0.161
19.01	4.0	0.040	47.86	10.1	0.101	80.76	16.2	0.162
19.47	4.1	0.041	48.35	10.2	0.102	81.36	16.3	0.163
19.94	4.2	0.042	48.85	10.3	0.103	81.96	16.4	0.164
20.41	4.3	0.043	49.34	10.4	0.104	82.56	16.5	0.165
20.87	4.4	0.044	49.84	10.5	0.105	83.17	16.6	0.166
21.34	4.5	0.045	50.34	10.6	0.106	83.78	16.7	0.167
21.80	4.6	0.046	50.84	10.7	0.107	84.39	16.8	0.168
22.27	4.7	0.047	51.34	10.8	0.108	85.00	16.9	0.169
22.73	4.8	0.048	51.84	10.9	0.109	85.62	17.0	0.170
23.20	4.9	0.049	52.35	11.0	0.110	86.24	17.1	0.171
23.66	5.0	0.050	52.85	11.1	0.111	86.86	17.2	0.172
24.13	5.1	0.051	53.36	11.2	0.112	87.49	17.3	0.173
24.59	5.2	0.052	53.86	11.3	0.113	88.11	17.4	0.174
25.06	5.3	0.053	54.37	11.4	0.114	88.74	17.5	0.175
25.52	5.4	0.054	54.88	11.5	0.115	89.38	17.6	0.176
25.99	5.5	0.055	55.39	11.6	0.116	90.01	17.7	0.177
26.45	5.6	0.056	55.90	11.7	0.117			
26.92	5.7	0.057	56.42	11.8	0.118			
27.38	5.8	0.058	56.93	11.9	0.119			
27.85	5.9	0.059	57.45	12.0	0.120			
28.31	6.0	0.060	57.97	12.1	0.121			



**APPENDIX 3: UNIFLEX® MEMBRANE WITH ARCTANGLE™ 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		50.87	4.8	0.048
1.16	0.1	0.001		51.97	4.9	0.049
2.30	0.2	0.002		53.08	5.0	0.050
3.44	0.3	0.003		54.20	5.1	0.051
4.57	0.4	0.004		55.33	5.2	0.052
5.69	0.5	0.005		56.46	5.3	0.053
6.80	0.6	0.006		57.61	5.4	0.054
7.90	0.7	0.007		58.77	5.5	0.055
9.00	0.8	0.008		59.93	5.6	0.056
10.08	0.9	0.009		61.11	5.7	0.057
11.17	1.0	0.010		62.29	5.8	0.058
12.24	1.1	0.011		63.49	5.9	0.059
13.31	1.2	0.012		64.70	6.0	0.060
14.37	1.3	0.013		65.92	6.1	0.061
15.43	1.4	0.014		67.16	6.2	0.062
16.48	1.5	0.015		68.40	6.3	0.063
17.53	1.6	0.016		69.66	6.4	0.064
18.58	1.7	0.017		70.94	6.5	0.065
19.62	1.8	0.018		72.22	6.6	0.066
20.66	1.9	0.019		73.52	6.7	0.067
21.69	2.0	0.020		74.84	6.8	0.068
22.72	2.1	0.021		76.17	6.9	0.069
23.75	2.2	0.022		77.51	7.0	0.070
24.78	2.3	0.023		78.88	7.1	0.071
25.80	2.4	0.024		80.25	7.2	0.072
26.83	2.5	0.025		81.64	7.3	0.073
27.85	2.6	0.026		83.05	7.4	0.074
28.87	2.7	0.027		84.48	7.5	0.075
29.89	2.8	0.028		85.92	7.6	0.076
30.92	2.9	0.029		87.39	7.7	0.077
31.94	3.0	0.030		88.87	7.8	0.078
32.96	3.1	0.031		90.36	7.9	0.079
33.99	3.2	0.032				
35.02	3.3	0.033				
36.05	3.4	0.034				
37.08	3.5	0.035				
38.11	3.6	0.036				
39.15	3.7	0.037				
40.19	3.8	0.038				
41.23	3.9	0.039				
42.28	4.0	0.040				
43.33	4.1	0.041				
44.39	4.2	0.042				
45.46	4.3	0.043				
46.53	4.4	0.044				
47.60	4.5	0.045				
48.68	4.6	0.046				
49.77	4.7	0.047				