A Linear Concentration Gradient Generator based on Multi-layered Centrifugal Microfluidics and Its Application in Antimicrobial Susceptibility Testing

Supplementary Information

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Table S1. Dimensions of metering chambers along the spiral channel in the first layer.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length (mm)	4	3.75	3.5	3.25	3	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1	1.25
Width (mm)	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	0.8
Height (mm)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Capacity (μl)	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Table S2. Dimensions of metering chambers along the spiral channel in the second layer.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length (mm)	1.25	1	1.5	2	2.5	3	3.5	4	2.25	2.5	2.75	3	3.25	3.5	3.75	4
Width (mm)	0.8	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4
Height (mm)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Capacity (μl)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Table S3. The measured dimensions of the metering chambers in the first layer PMMA mould.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length (mm)	4.02	3.78	3.48	3.25	3.01	5.48	4.98	4.42	4.06	3.56	2.99	2.49	1.98	1.52	0.99	1.28
Width (mm)	3.95	3.93	3.97	3.95	3.96	1.97	1.96	1.98	2.01	2.02	2.03	2.00	1.99	1.98	2.01	0.79
Height (mm)	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Capacity (μl)	15.56	14.56	13.54	12.58	11.68	10.58	9.57	8.58	8.00	7.05	5.95	4.88	3.86	2.95	1.95	1.03
Error	-3%	-3%	-3%	-3%	-3%	-4%	-4%	-5%	0	1%	-1%	-2%	-4%	-2%	-3%	3%

Table S4. The measured dimensions of the metering chambers in the second layer PMMA mould.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length (mm)	1.24	0.99	1.49	1.99	2.51	3.05	3.49	3.96	2.25	2.49	2.71	2.99	3.29	3.51	3.79	4.05
Width (mm)	0.78	2.01	1.98	1.95	1.99	2.00	2.01	2.03	3.98	3.99	3.95	3.99	3.96	4.00	4.01	3.93
Height (mm)	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03
Capacity (μl)	1.00	2.04	3.04	4.00	5.14	6.28	7.23	8.28	9.22	10.23	11.03	12.29	13.42	14.46	15.65	16.39
Error	0	2%	1%	0	3%	5%	3%	4%	2%	2%	0	2%	3%	3%	4%	2%

Table S5. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 0 hour. Each data point was obtained by taking the average of 5 measurements.

Number	1	2	3	4	5
1	2.345	2.388	2.365	2.362	2.381
2	2.315	2.318	2.334	2.339	2.304
3	2.333	2.333	2.331	2.3	2.326
4	2.348	2.355	2.361	2.345	2.357
5	2.387	2.41	2.351	2.352	2.358
6	2.375	2.429	2.375	2.413	2.389
7	2.378	2.398	2.395	2.365	2.375
8	2.365	2.378	2.391	2.398	2.407
9	2.42	2.41	2.389	2.421	2.395
10	2.365	2.359	2.373	2.4	2.354
11	2.348	2.346	2.357	2.387	2.379
12	2.365	2.35	2.375	2.339	2.387

13	2.396	2.397	2.39	2.364	2.399
14	2.4	2.388	2.37	2.406	2.399
15	2.342	2.333	2.349	2.362	2.348
16	2.388	2.358	2.405	2.413	2.341
17	2.344	2.357	2.386	2.373	2.401

Table S6. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 1 hour.

Number	1	2	3	4	5
1	2.356	2.32	2.325	2.414	2.335
2	2.368	2.367	2.349	2.302	2.328
3	2.31	2.308	2.326	2.313	2.318
4	2.372	2.403	2.372	2.378	2.348
5	2.367	2.4	2.415	2.403	2.387
6	2.335	2.373	2.392	2.358	2.402
7	2.365	2.3	2.356	2.379	2.395
8	2.354	2.388	2.349	2.409	2.387
9	2.401	2.428	2.389	2.398	2.38
10	2.368	2.377	2.375	2.396	2.384
11	2.4	2.402	2.383	2.387	2.4
12	2.393	2.398	2.375	2.388	2.398
13	2.372	2.412	2.386	2.401	2.395
14	2.4	2.409	2.398	2.403	2.388
15	2.383	2.418	2.386	2.395	2.429
16	2.418	2.372	2.38	2.373	2.384
17	2.356	2.348	2.371	2.368	2.357

Table S7. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 2 hour.

Number	1	2	3	4	5
1	2.273	2.289	2.206	2.238	2.217
2	2.215	2.264	2.27	2.282	2.3
3	2.277	2.248	2.338	2.257	2.268
4	2.308	2.271	2.315	2.34	2.276
5	2.285	2.311	2.301	2.304	2.335
6	2.311	2.325	2.321	2.341	2.337
7	2.327	2.336	2.298	2.301	2.312
8	2.338	2.342	2.345	2.32	2.311
9	2.355	2.395	2.365	2.397	2.374
10	2.32	2.297	2.351	2.361	2.358
11	2.328	2.339	2.345	2.357	2.36
12	2.35	2.367	2.358	2.361	2.378

13	2.298	2.313	2.353	2.361	2.368
14	2.335	2.301	2.368	2.398	2.371
15	2.357	2.349	2.37	2.353	2.359
16	2.358	2.378	2.364	2.367	2.361
17	2.358	2.371	2.367	2.318	2.355

Table S8. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 3 hour.

Number	**1**	**2**	**3**	**4**	**5**
1	2.14	2.17	2.102	2.161	2.081
2	2.155	2.157	2.158	2.171	2.146
3	2.203	2.218	2.207	2.187	2.167
4	2.21	2.228	2.275	2.209	2.251
5	2.204	2.252	2.253	2.19	2.208
6	2.307	2.241	2.242	2.304	2.314
7	2.278	2.251	2.333	2.301	2.257
8	2.271	2.315	2.342	2.351	2.329
9	2.329	2.334	2.308	2.325	2.361
10	2.341	2.357	2.331	2.342	2.337
11	2.318	2.339	2.357	2.348	2.351
12	2.328	2.357	2.361	2.351	2.317
13	2.347	2.398	2.378	2.367	2.351
14	2.289	2.312	2.371	2.351	2.35
15	2.351	2.346	2.357	2.371	2.365
16	2.352	2.401	2.371	2.361	2.358
17	2.356	2.361	2.381	2.379	2.39

Table S9. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 4 hour.

Number	**1**	**2**	**3**	**4**	**5**
1	1.994	2.088	1.958	2.036	2.096
2	2.082	2.091	2.134	2.078	2.127
3	2.178	2.168	2.171	2.098	2.078
4	2.18	2.179	2.209	2.218	2.212
5	2.213	2.197	2.17	2.155	2.231
6	2.222	2.211	2.275	2.31	2.299
7	2.291	2.251	2.3	2.305	2.264
8	2.317	2.349	2.351	2.338	2.35
9	2.366	2.32	2.405	2.367	2.343
10	2.395	2.411	2.388	2.357	2.375
11	2.329	2.35	2.379	2.387	2.399
12	2.338	2.321	2.385	2.384	2.371

13	2.383	2.35	2.313	2.389	2.354
14	2.304	2.347	2.321	2.354	2.337
15	2.32	2.367	2.314	2.338	2.351
16	2.357	2.368	2.329	2.409	2.366
17	2.351	2.371	2.384	2.356	2.373

Table S10. Measured transmitted optical intensity (mW) of 17 incubation chambers along the spiral channel at 5 hour.

Number	**1**	**2**	**3**	**4**	**5**
1	1.91	1.876	1.789	1.911	1.85
2	1.94	2.047	2.098	2.035	2.066
3	2.102	2.12	2.089	2.108	2.153
4	2.151	2.11	2.127	2.194	2.127
5	2.184	2.173	2.182	2.166	2.206
6	2.198	2.201	2.199	2.186	2.213
7	2.194	2.253	2.351	2.314	2.321
8	2.357	2.361	2.356	2.323	2.33
9	2.325	2.346	2.357	2.371	2.361
10	2.321	2.351	2.346	2.371	2.346
11	2.35	2.362	2.359	2.357	2.371
12	2.35	2.345	2.334	2.353	2.356
13	2.357	2.343	2.371	2.368	2.348
14	2.365	2.371	2.337	2.343	2.357
15	2.358	2.339	2.347	2.401	2.361
16	2.363	2.385	2.409	2.373	2.378
17	2.393	2.368	2.371	2.357	2.419

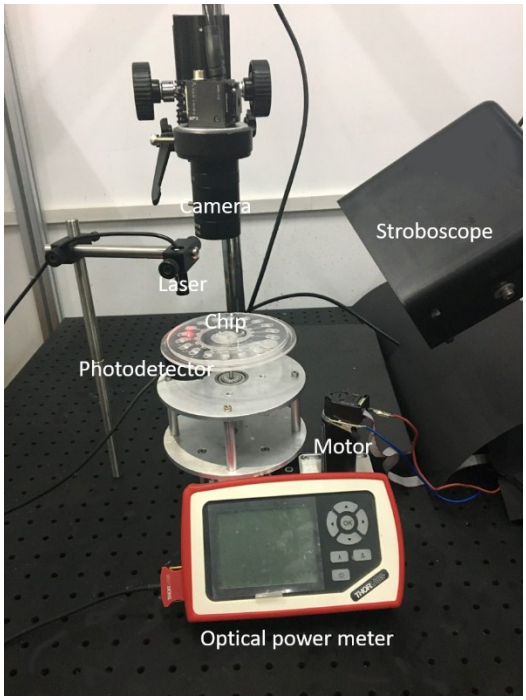

Figure S1. An image of our self-contained LOAD AST system comprising a laser, a photodetector, an optical power meter and the LOAD platform.

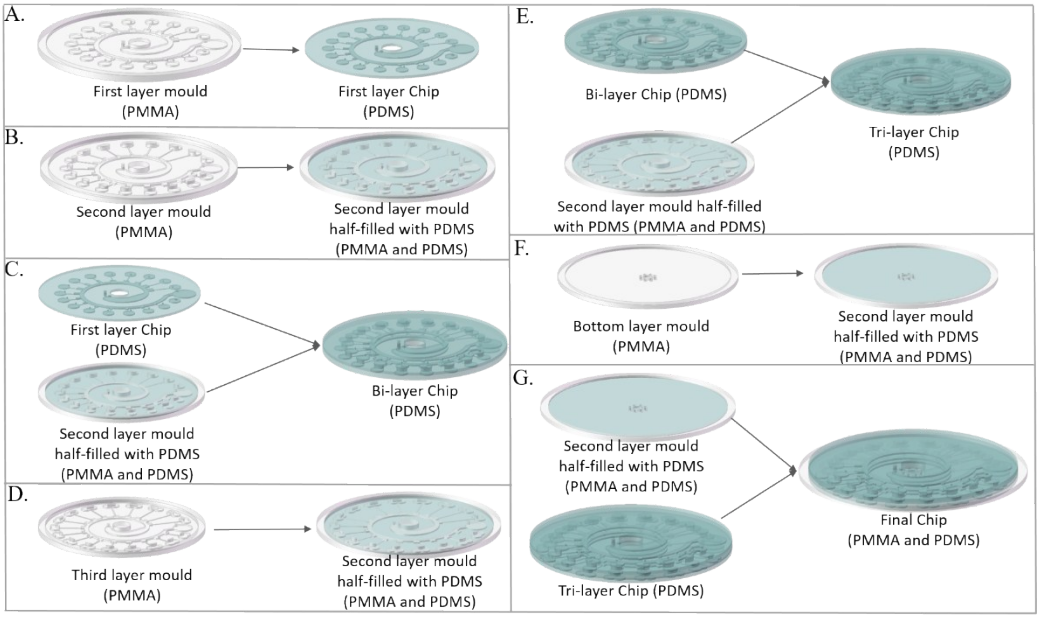

Figure S2. Fabrication procedures of our AST microfluidic chip: A. pour PDMS into the first layer mould and fill it fully; completely dry the PDMS and tear it out; B. pour PDMS into the second layer mould but do not fill it fully to ensure that the channel hole for cross-layer transfer is not blocked; wait until the PDMS in the mould is only partially cured; C. place the hardened PDMS layer over the

surface of the soft layer while it is still attached to the mould and add pressure to the hardened layer carefully from one end to the other to drive away any bubbles that might be trapped in the interface; wait until the bottom PDMS layer is cured completely and tear the bi-layer PDMS chip out of the mould; D. pour PDMS into the third layer mould but do not fill it fully to ensure that the channel hole for cross-layer transfer is not blocked; wait until the PDMS in the mould is only partially cured; E. place the hardened PDMS layer over the surface of the soft layer while it is still attached to the mould and add pressure to the hardened layer carefully from one end to the other to drive away any bubbles that might be trapped in the interface; wait until the bottom PDMS layer is cured completely and tear the tri-layer PDMS chip out of the mould; F. take a bottom layer mould and firstly the screw holes in the mould are sealed by adhesive tape; then, pour a small amount of PDMS into the mould until it is half full, wait until it is half cured; G. place the tri-layer chip on the surface of the bottom PDMS layer while it is still soft and apply pressure carefully from one end to the other again to drive away trapped air bubbles on the interface.

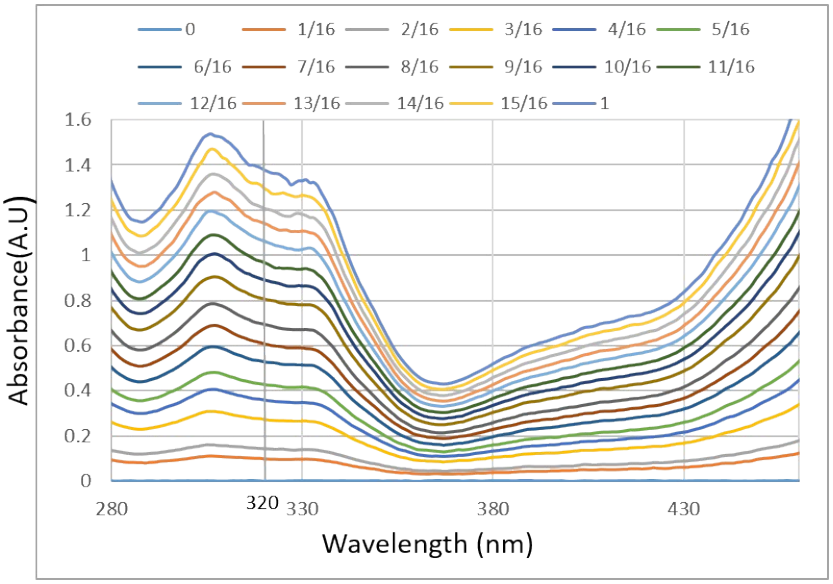

Figure S3. Experimental data shows that for this red dye solution (PILOT, diluted to 1/100 with DI water), the absorbance at 320nm is roughly proportional to its concentration. Here, 0, 1/16, 2/16…15/16, 1 represents that this red dye solution is diluted to 0, 1/16, 2/16…15/16, 1.

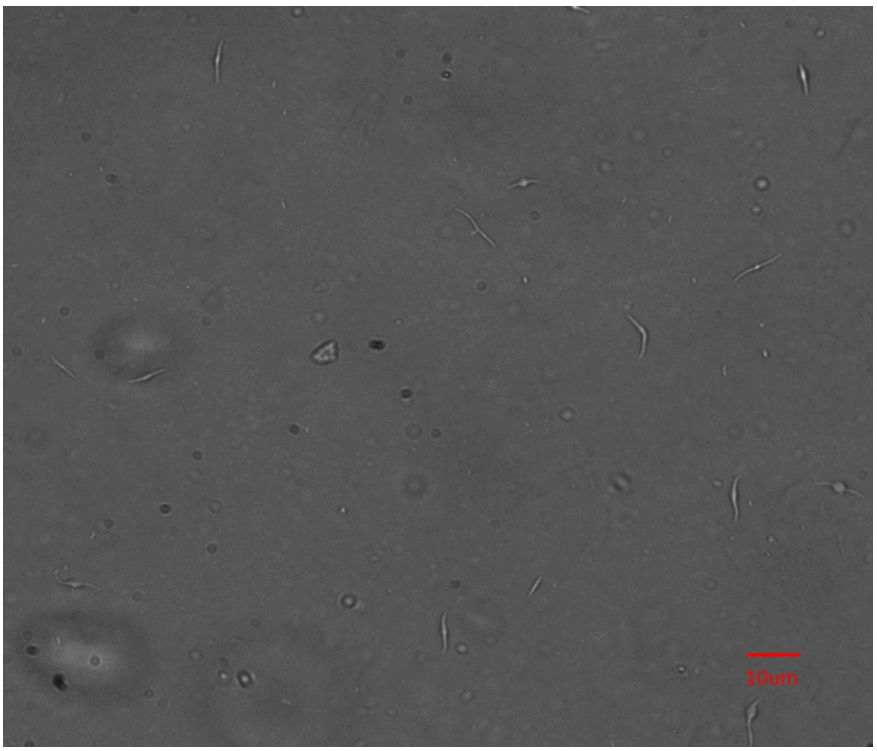

Figure S4. An image of *E.coli* suspension incubated in 3μg/ml ampicillin for 3 hours. Elongation and swelling of the cells indicate that they are no longer alive.