Supplementary Information

Tables:

Figures:

Supplementary Table 1: Bills Summary

Component	Unit	Unit Cost (USD)	Subtotal	Source of Material	Model type/Material
Arduino Mega 2560 R3	1	$48.40	$48.40	https://www.sparkfun.com/products/11061	DEV-11061 RoHS
CD-40CO2/Temperature /Humidity Sensor	1	$44.95	$44.95	https://www.adafruit.com/product/5187	STEMMA QT / Qwiic
FeatherS2 - ESP32	1	$24.95	$24.95	https://www.adafruit.com/product/4769	S2
Cavro Centris Pump	1	$5,000	$5,000	https://partnering.tecan.com/cavro-centrispump-for-oem-liquid-handling	Cavro
PTC Heating Element AC DC 12V Aluminum Shell	1	$7.51	$7.51	https://www.amazon.com/Aluminum-Temperature-Thermostatic-Applicable-Miniature/dp/B07GBNGB4L/	Aluminum
Uxcell 10K NTC Thermistor Probe	1	$8.49	$8.49	https://www.amazon.com/uxcell-Thermistor-Sensitive-Temperature-Conditioner/dp/B07LGLCYFP?th=1	Epoxy
W1209 12V DC Digital Temperature Controller	1	$8.49	$8.49	https://www.amazon.com/HiLetgo-Temperature-Controller-Thermostat-One-Channel/dp/B07CH7W3XP?th=1	B07CH7W3XP
Dino-Lite Edge	1	$899.00	$899.00	https://www.auroraprosci.com/Digital-Microscope-AM4115T-GRFBY	AM4115TGRFBY
ALITOVE 12V 5A 60W Power Supply Adapter	1	$12.99	$12.99	https://www.amazon.com/dp/B078RY6YY3	AL12V5AT
100ml Thick Glass Media Storage	1	$6.99	$6.99	https://www.amazon.com/Storage-Bottles-Graduations-Scientific-Containers/dp/B07Y65GN1S/	CQ15707
TAILONZ PNEUMATIC 1/4 Inch NPT 12V/24V/110V/220V	1	$17.99	$17.99	https://www.amazon.com/Tailonz-Pneumatic-Electric-Solenoid-2W025-08/dp/B07X287BSX/?th=1	2W025-08
Raspberry Pi 4 Model B	1	$77.99	$77.99	https://www.amazon.com/Raspberry-Model-2019-Quad-Bluetooth/dp/B07TC2BK1X/	RAS-4-4G
ELECROW 5 Inch Raspberry Pi Screen Touchscreen	1	$43.99	$43.99	https://www.amazon.com/Elecrow-800x480-Interface-Supports-Raspberry/dp/B013JECYF2/	ILI9486L
ph Kit Atlas Scientific	1	$149.00	$149.00	https://atlas-scientific.com/kits/ph-kit/	#KIT-101P
Mini Lab Grade pH Probe	1	$60.99	$60.99	https://atlas-scientific.com/probes/miniphprobe/	#ENV-20-pH
SYLGARD™ 184 Silicone Elastomer Kit	1	$154	$154	https://krayden.com/productdetail/dc4019862/dc-184-sylgard-05kg-11lb-kit	Silicone
Plus Microscope Slides	1	$211.50	$211.50	https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-microscope-slides-2/1255015	Glass
Model Resin Formlabs	1	$149	$149	https://dental.formlabs.com/store/materials/model-resin/	Resin
CO2 Stainless Steel Diffuser	1	$15.99	$15.99	https://www.amazon.com/YOU-Stainless-Diffuser-Regulator-Freshwater/	Stainless Steel
NeoPixel Ring	1	$16.95	$16.95	https://www.adafruit.com/product/1586/	5050 RGB LED
Adjustable Precision Mount	1	$199.00	$199.00	https://www.dinolite.us/products/accessories	Stainless Steel
Polyvinylidene Fluoride (PVDF) Filter	1	$2.69	$2.69	https://www.perkinelmer.com/product/filterpvdf-syringe-25mm-22um-pk-100-02542918	Polyvinylidene fluoride

Supplementary Table 2: Components of the platform

Number	Descriptor	Repository
1	Arduino_mega_pH_Solenoid.ino	braingeneersdifussionproject/Arduino/
2	ImageProcessing.m	braingeneersdifussionproject/MATLAB/
3	ObtainVideoFrames.m	braingeneersdifussionproject/MATLAB/
4	ObtainVideoFrames_V2.m	braingeneersdifussionproject/MATLAB/
5	ReduceVideoSize.m	braingeneersdifussionproject/MATLAB/
6	CO2TempHumidityRecording.py	braingeneersdifussionproject/Python/Enviroment_Sensor/
7	MainPumpProgramExe.py	braingeneersdifussionproject/Python/Servo_Pump/Apps/
8	PDMS_Mold_V1.stl	braingeneersdifussionproject/Print_Files/
9	Hole_Base.stl	braingeneersdifussionproject/Print_Files/
10	BioChamber_Base.stl	braingeneersdifussionproject/Print_Files/
11	Cap_Biochamber.stl	braingeneersdifussionproject/Print_Files/
12	EnviromentalSensor_BaseCase.stl	braingeneersdifussionproject/Print_Files/
13	EnviromentalSensor_TopCase.stl	braingeneersdifussionproject/Print_Files/
14	GFP_Chamber.stl	braingeneersdifussionproject/Print_Files/
15	GFP_Chamber_Cap.stl	braingeneersdifussionproject/Print_Files/
16	Heater_Holder.stl	braingeneersdifussionproject/Print_Files/
17	Heater_Holder_Biochamber.stl	braingeneersdifussionproject/Print_Files/
18	Holder_Biochamber_Left.stl	braingeneersdifussionproject/Print_Files/
19	Holder_Biochamber_Rigth.stl	braingeneersdifussionproject/Print_Files/
20	Microscope_Holder.stl	braingeneersdifussionproject/Print_Files/
21	Pump_Holder_.stl	braingeneersdifussionproject/Print_Files/
22	RaspberryPi_Holder.stl	braingeneersdifussionproject/Print_Files/
23	ReservoirCap_Left.stl	braingeneersdifussionproject/Print_Files/
24	ReservoirCap_Rigth.stl	braingeneersdifussionproject/Print_Files/
25	SourcePower_Holder.stl	braingeneersdifussionproject/Print_Files/
26	pHController_Botom.stl	braingeneersdifussionproject/Print_Files/
27	pHController_Top.stl	braingeneersdifussionproject/Print_Files/
28	CO2_Holder.stl	braingeneersdifussionproject/Print_Files/
29	RawData	braingeneersdifussionproject/RawData/

Supplementary Table 3: Comprehensive metabolic panel

Test	**Unit**	**Fresh Media**	**Platform D3**					**Incubator D6**					**Platform D6**				
			B1	B2	B3	AVG	S.DEV	B1	B2	B3	AVG	S.DEV	B1	B2	B3	AVG	S.DEV
pH	-	7.82	7.88	7.85	7.44	7.72	0.25	7.83	7.82	7.81	7.82	0.01	7.59	7.69	7.21	7.50	0.25
cGlu	g/L	2.92	2.93	3.44	2.96	3.11	0.00	2.63	3.20	2.78	2.87	0.28	2.77	3.36	3.10	3.08	0.01
cK	mM	3.87	3.90	6.40	6.00	5.43	1.34	4.10	5.60	4.40	4.70	0.79	4.90	5.80	6.00	5.57	0.59
cNa^+	mM	146	146	174	157	159	14.11	152	218	185	185	33	138	169	182	163	22.61
cCa^{2+}	mM	0.80	0.85	0.87	0.78	0.83	0.05	0.86	1.08	0.91	0.95	0.12	0.79	0.89	0.84	0.84	0.05
cCl^-	mM	123	125	149	129	134.33	12.86	130	184	141	151.67	28.54	119	144	135	132.67	12.66

Supplementary Table 4. Statistical comparison of metabolite differences between our life support platform and standard tissue culture conditions

Metabolite	Chi-Square	df	p-Value
pH	4.79	3	0.1874
cGlu	2.93	3	0.4012
cK	7.32	3	0.0623
cNa+	3.55	3	0.2073
cCa2+	5.12	3	0.1630
cCl-	4.19	3	0.1781
***The margin of error presented here corresponds to a 95% confidence interval**			

Supplementary Table 5: Organoid cross-sectional measures batch 1 in mm^2.

Days	PG0	PG1	PG2	PG3	Average PG	Standard Deviation
15	0.35	0.34	0.37	0.35	0.35	0.012
16	0.39	0.34	0.39	0.38	0.38	0.02
17	0.42	0.40	0.43	0.42	0.42	0.01
18	0.43	0.40	0.43	0.43	0.42	0.01
19	0.44	0.42	0.44	0.43	0.43	0.01
20	0.45	0.42	0.49	0.46	0.45	0.03

Supplementary Table 6: Organoid cross-sectional measures batch 2 in mm^2.

Days	PG0	PG1	Average PG	Standard Deviation
15	2.02	2.03	0.006	2.02
16	2.26	2.06	0.146	2.16
17	2.35	2.11	0.175	2.23
18	2.48	2.11	0.264	2.29
19	2.51	2.14	0.261	2.32
20	2.71	2.28	0.304	2.50

Supplementary Table 7: Organoid cross-sectional measures batch 3 in mm^2.

Days	PG0	PG1	PG2	Average PG	Standard Deviation
15	1.16	1.27	1.47	0.162	1.30
16	1.36	1.49	1.61	0.122	1.49
17	1.43	1.57	1.63	0.099	1.54
18	1.50	1.68	1.73	0.121	1.63
19	1.50	1.68	1.75	0.129	1.64
20	1.52	1.69	1.78	0.135	1.66

Supplementary Table 8: Organoid cross-sectional measures batch 4 in mm^2.

Days	PG0	PG1	PG2	PG3	PG4	Average PG	Standard Deviation
15	0.38	0.31	0.18	0.39	0.48	0.39	0.047
16	0.44	0.35	0.22	0.54	0.39	0.39	0.066
17	0.45	0.37	0.24	0.60	0.35	0.41	0.057
18	0.47	0.34	0.25	0.61	0.37	0.40	0.085
19	0.45	0.35	0.26	0.63	0.37	0.40	0.070
20	0.47	0.35	0.22	0.68	0.38	0.41	0.083

Supplementary Table 9: Organoid cross-sectional measures multiwell v1 batch in mm^2.

Days	PG0	PG1	Average PG	Standard Deviation
15	0.53	0.54	0.53	0.001
16	0.49	0.59	0.54	0.069
17	0.54	0.61	0.58	0.047
18	0.55	0.71	0.63	0.108
19	0.62	0.73	0.68	0.077
20	0.67	0.77	0.72	0.067

Supplementary Table 10: Organoid cross-sectional measures multiwell v2 batch in mm^2.

Days	PG0	PG1	PG2	PG3	PG4	Average PG	Standard Deviation
15	0.33	0.43	0.33	0.36	0.30	0.38	0.067
16	0.35	0.43	0.34	0.37	0.31	0.39	0.059
17	0.37	0.44	0.36	0.39	0.33	0.40	0.046
18	0.38	0.44	0.36	0.40	0.33	0.41	0.048
19	0.38	0.46	0.38	0.42	0.34	0.42	0.060
20	0.39	0.46	0.37	0.43	0.34	0.43	0.05

Supplementary Table 11: Normalized measures.

Day	Z1	Z1	Z1	Z1	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	-1.57	-3.00	4.99	-0.43	-0.22	0.22	-11.11	-2.33	13.44	-10.36	-48.21	12.98
2	10.70	-2.14	12.41	8.70	11.78	1.56	4.90	14.83	23.76	-1.25	-36.82	52.53
3	18.97	12.70	22.11	20.97	16.28	4.08	10.36	20.45	25.37	4.44	-31.13	71.31
4	23.82	14.98	23.54	21.83	22.55	4.12	15.29	28.99	32.99	-1.82	-27.43	73.02
5	25.25	18.97	25.53	23.54	23.88	5.66	15.44	29.45	34.76	0.74	-25.44	78.43
6	28.96	18.69	39.80	30.96	33.86	12.62	16.75	29.99	37.22	0.46	-35.97	92.94

Day	ZMW1	ZMW1	ZMW2	ZMW2	ZMW2	ZMW2	ZMW2
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	-0.19	0.19	-4.95	22.08	-6.66	2.45	-12.92
2	-7.99	10.22	-1.54	22.37	-2.39	6.72	-10.64
3	1.86	14.31	6.15	24.64	1.31	12.69	-6.09
4	3.90	32.34	7.00	26.35	2.73	14.68	-6.09
5	15.61	35.87	7.57	31.76	7.00	18.95	-3.53
6	25.09	42.75	11.27	32.04	6.72	21.80	-2.68

Day	AVG1	AVG2	AVG3	AVG4	AVGZMW1	AVGZMW2
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00	0.00	0.00
2	7.42	6.67	14.49	10.36	1.12	2.90
3	18.69	10.18	18.73	14.34	8.09	7.74
4	21.04	13.34	25.76	16.62	18.12	8.94
5	23.32	14.77	26.55	17.59	25.74	12.35
6	29.60	23.24	27.99	20.15	33.92	13.83

Supplementary Table 12: Kruskal-wallis analysis for cross-sectional area measurements per day (by batch).

Day	H	DF	SSB	p-Value	Residuals(Fist three)
15	15.976215	6	933.6500	0.013882	[-11.5, 2.0, 2.0]
16	14.668664	6	856.6500	0.022996	[-7.6, 2.30, 6.8].
17	14.846049	6	867.9000	0.021489	[-8.5, 4.0, 5.5]
18	14.926395	6	872.0000	0.020837	[-5.0, 0.0, 5.0]
19	15.600624	6	912.0125	0.016066	[-3.4, 3.1, 4.1]
20	14.275821	6	834.8500	0.026702	[-3.2, 9.8, -8.7]

Supplementary Table 13: Calibration curve at 0.52 W/mm^2 power intensity.

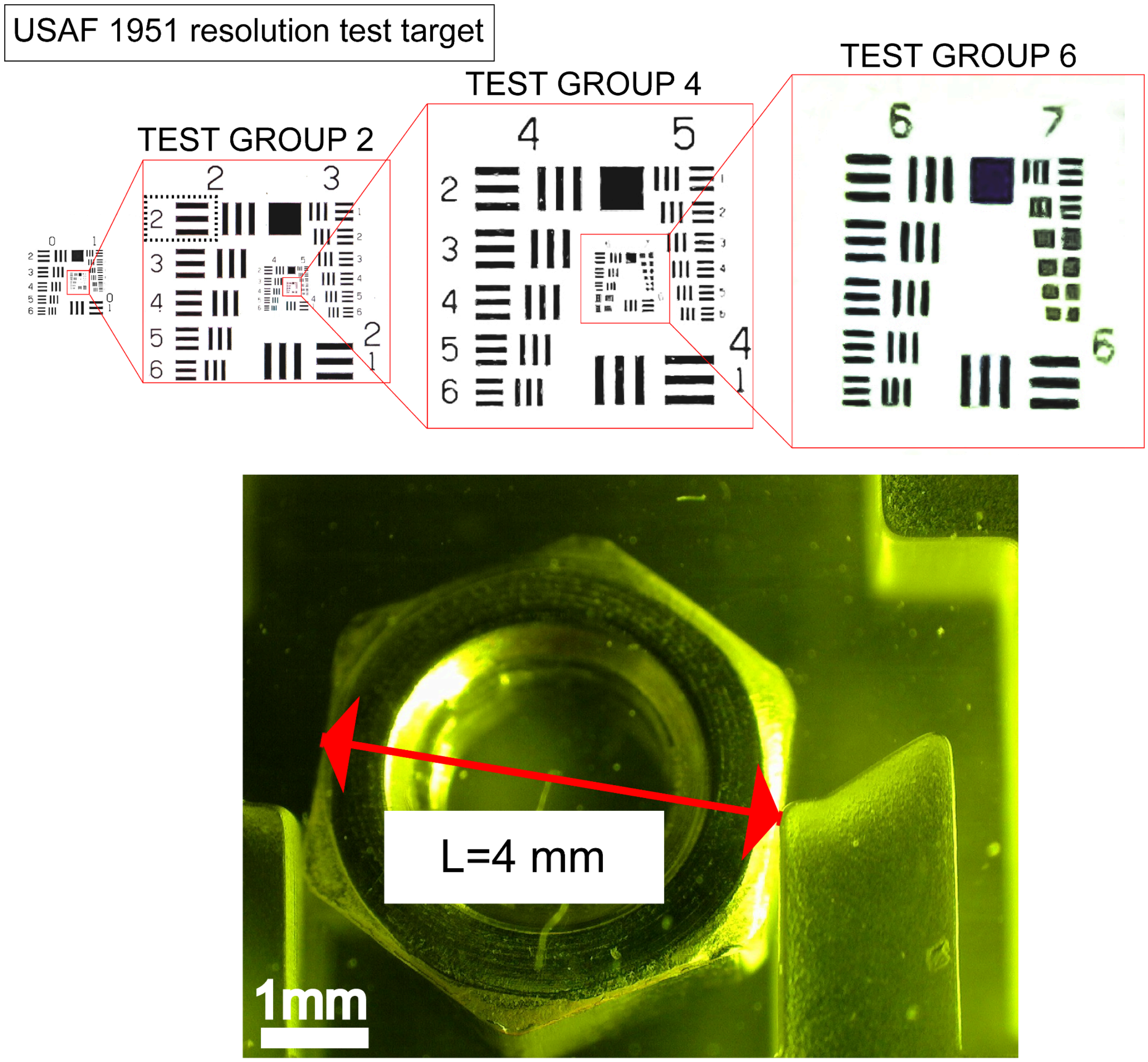
Con. (mM)	**M1**		**M2**		**M3**		**Standard Deviation**	**Power Intensity Average**
	Pixel Intensity	**Power Intensity**	**Pixel Intensity**	**Power Intensity**	**Pixel Intensity**	**Power Intensity**		
0	6.460	0.146	4.474	0.101	4.526	0.102	0.026	0.117
1	8.110	0.183	4.544	0.103	4.504	0.102	0.047	0.129
2	9.770	0.221	12.822	0.290	12.786	0.289	0.040	0.267
3	11.420	0.258	7.666	0.173	7.620	0.172	0.049	0.201
4	13.070	0.296	11.063	0.250	10.993	0.249	0.027	0.265
5	14.730	0.333	12.799	0.289	12.716	0.288	0.026	0.303
6	16.380	0.370	18.004	0.407	18.012	0.407	0.021	0.395
7	18.030	0.408	20.081	0.454	20.170	0.456	0.027	0.439
8	19.680	0.445	20.062	0.454	20.031	0.453	0.005	0.451
9	21.340	0.483	20.621	0.466	20.561	0.465	0.010	0.471
10	22.990	0.520	21.460	0.485	21.388	0.484	0.020	0.496

Supplementary Table 14: Calibration curve at 0.79 W/mm^2 power intensity.

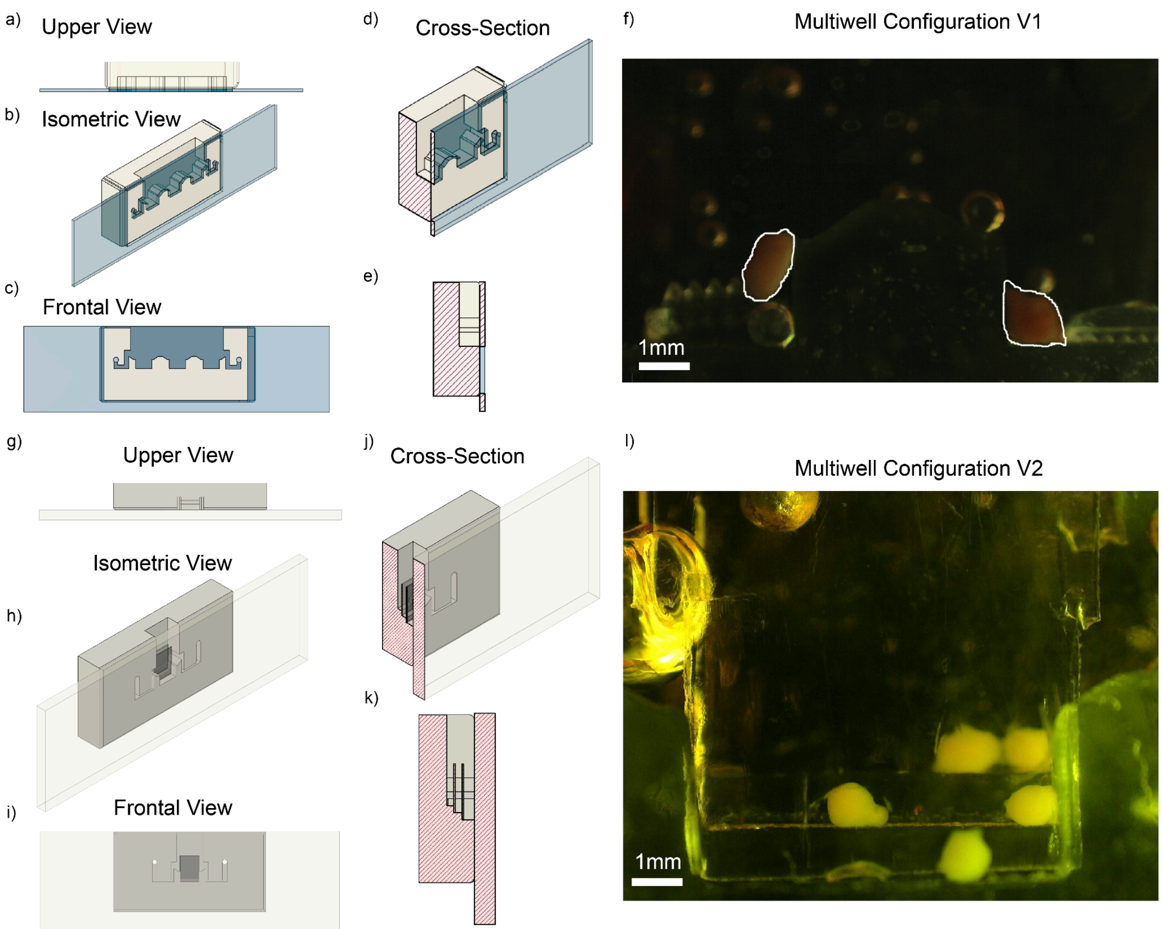
Con. (mM)	M1		M2		M3		Standard Deviation	Power Intensity Average
	Pixel Intensity	Power Intensity	Pixel Intensity	Power Intensity	Pixel Intensity	Power Intensity		
0	8.913	0.147	8.389	0.139	8.861	0.147	0.005	0.144
1	12.798	0.212	10.178	0.168	9.413	0.156	0.029	0.179
2	16.683	0.276	13.115	0.217	14.011	0.232	0.031	0.242
3	20.568	0.340	20.651	0.342	20.510	0.339	0.001	0.340
4	24.453	0.404	24.227	0.401	23.251	0.385	0.011	0.397
5	28.337	0.469	30.524	0.505	30.891	0.511	0.023	0.495
6	32.222	0.533	36.171	0.598	36.663	0.606	0.040	0.579
7	36.107	0.597	37.482	0.620	37.538	0.621	0.013	0.613
8	39.992	0.661	42.667	0.706	43.418	0.718	0.030	0.695
9	43.877	0.726	40.388	0.668	40.023	0.662	0.035	0.685
10	47.762	0.790	47.361	0.783	47.865	0.792	0.004	0.788

Supplementary Table 15. CFD Initial Conditions.

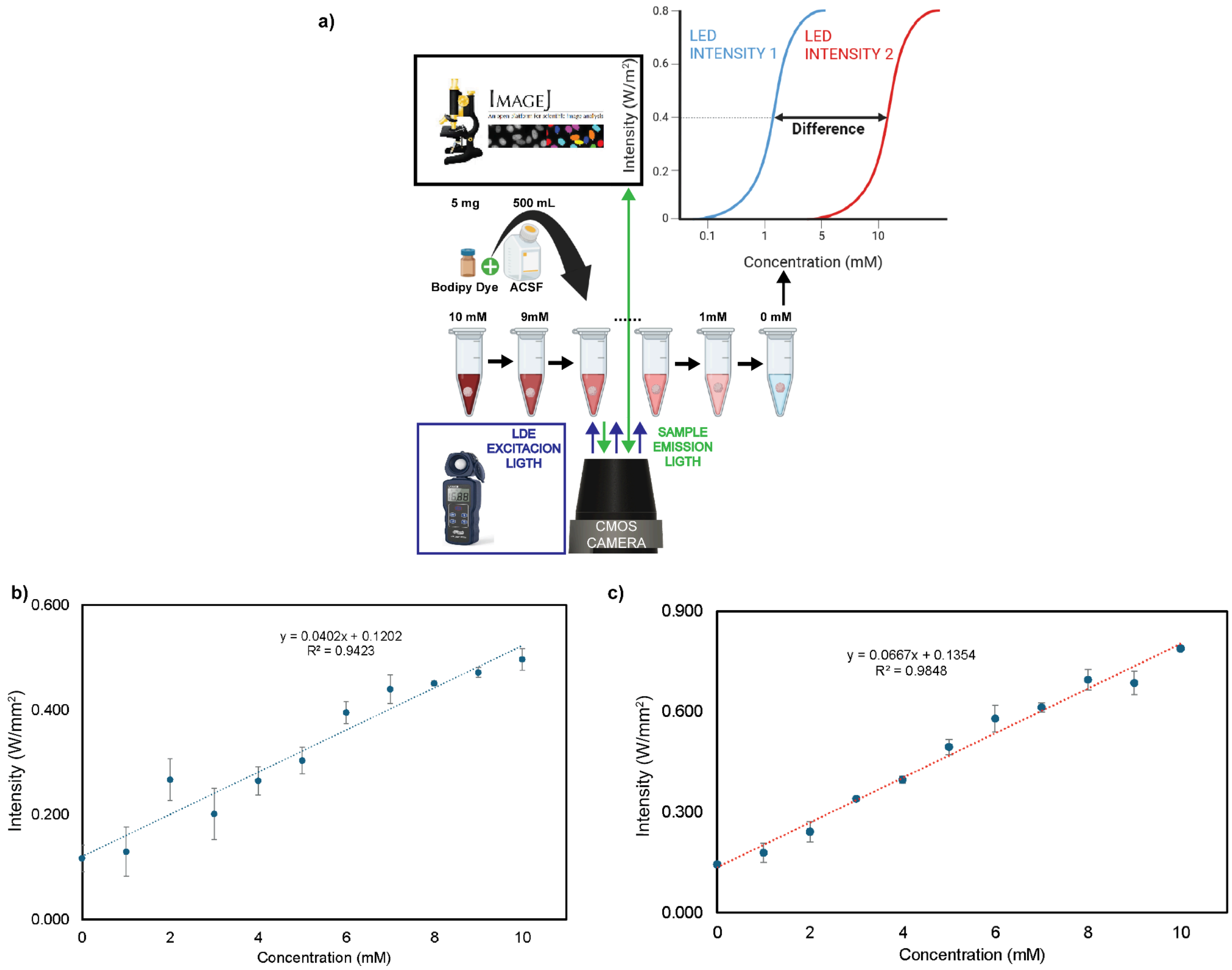
Variable	Value	Units
Inlet-Outlet Initial Velocities	0.02	m/s
Diffusion Coefficient[11]	1.00×10^{-10}	m^2/s
Initial Concentration	10	mol/m^3
Density	1000	kg/m^3
Dynamic Viscosity	0.002-0.003	Pa.s
Porosity	0.15	a.u.
Number of elements	519.830	Elements



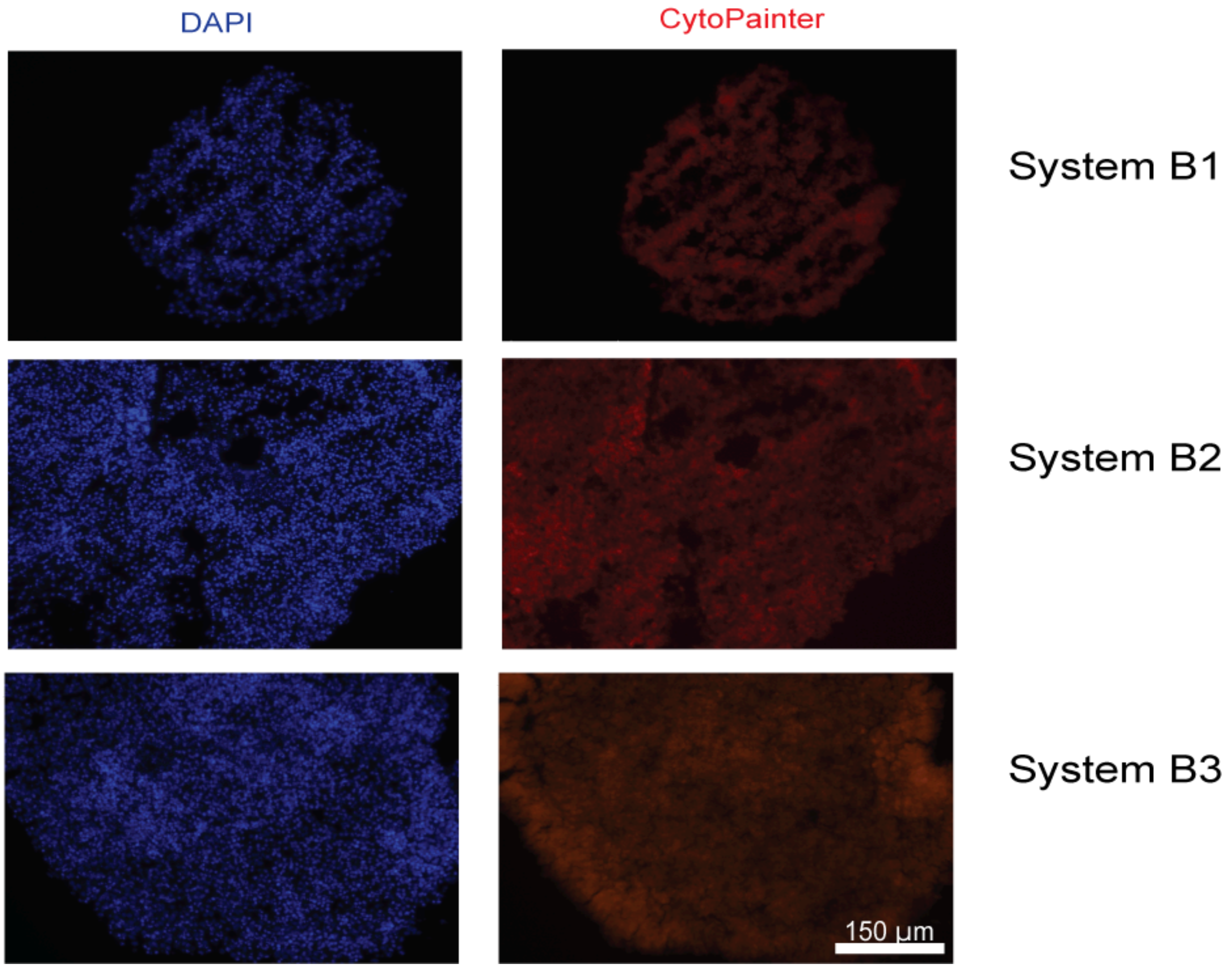
Supplementary Figure 1. Resolution and Field of View (FOV) Assessment. To assess the imaging resolution, a USAF 1951 resolution test target was used. Based on the captured images, the system resolved features up to Group 2, Element 2 (black dotted square), corresponding to 4.00 line pairs per millimeter (lp/mm), or approximately 4 mm resolution. The image below illustrates the total field of view (FOV), with the central region highlighted—this area was used for resolution analysis.



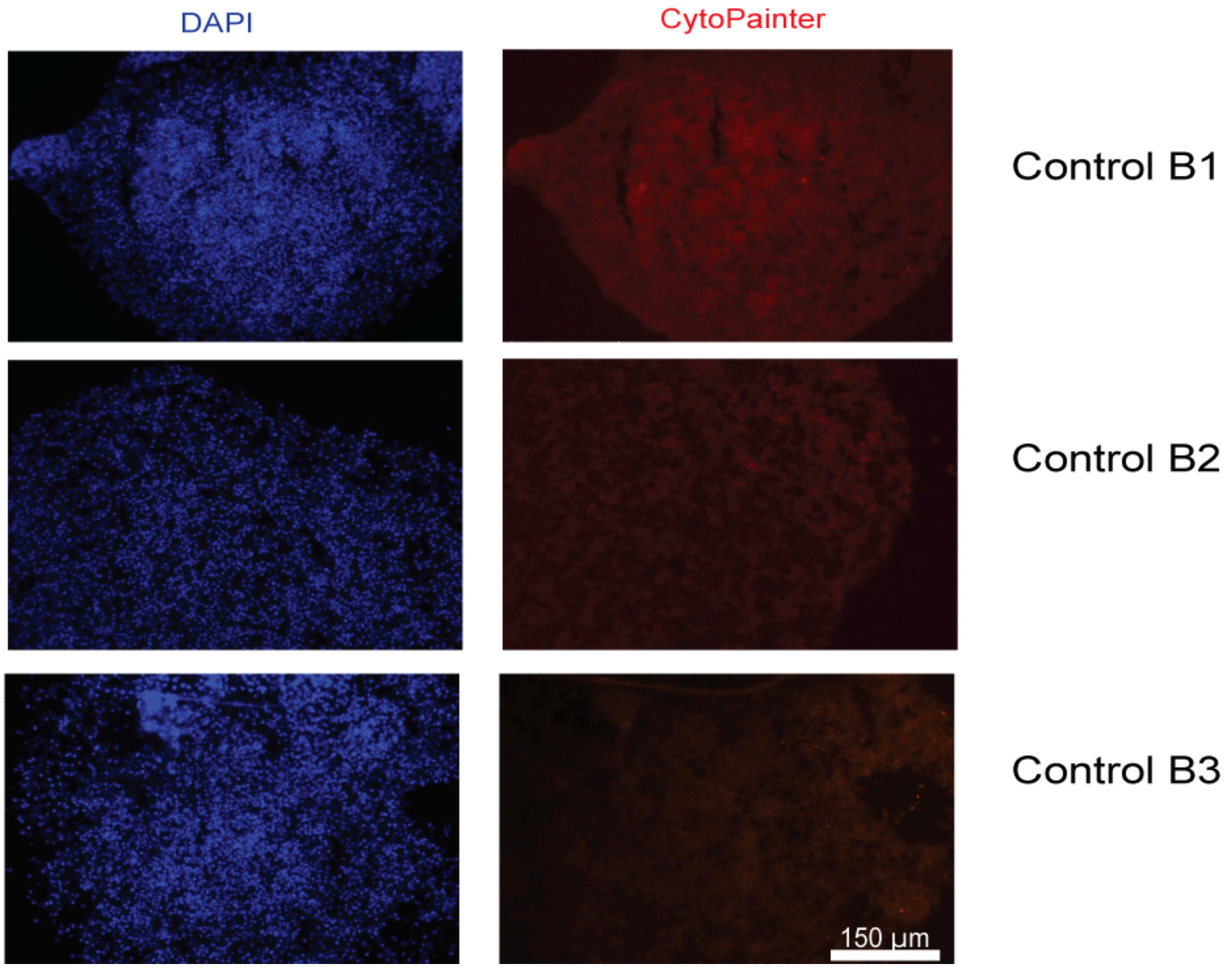
Supplementary Figure 2. Multiwell PDMS/Glass chip design and experimental validation for 3D organoid culture, for Multiwell v1 (Serial configuration) and Multiwell v2 (Parallel configuration). a) Side view showing chamber depth and channel arrangement. b) Transparent 3D overview highlighting the central chamber and side channels. c) Sectional 3D cutaway revealing vertical geometry and inlet-outlet positioning for media circulation. d) Detailed vertical cross-section through the chamber, indicating the stair-stepped architecture for organoid trapping and media perfusion. e) Top-down view of the whole device layout, displaying inlet ports and chamber configuration. f) Bright field image of the fabricated microfluidic chip seeded with cerebral organoids. The chamber contains multiple trapped organoids arranged along the stepped geometry. g) Side view showing chamber depth and channel arrangement. h) Transparent 3D overview highlighting the central chamber and side channels. i) Sectional 3D cutaway revealing vertical geometry and inlet-outlet positioning for media circulation. j) Detailed vertical cross-section through the chamber, indicating the stair-stepped architecture for organoid trapping and media perfusion. k) Top-down view of the whole device layout, displaying inlet ports and chamber configuration. l) Bright field image of the fabricated microfluidic chip seeded with cerebral organoids. The chamber contains multiple trapped organoids arranged along the stepped geometry. The 1 mm scale bar confirms chamber compatibility with tissue sizes in the range of ~500-800 µm, enabling confined long-term culture under continuous flow conditions.



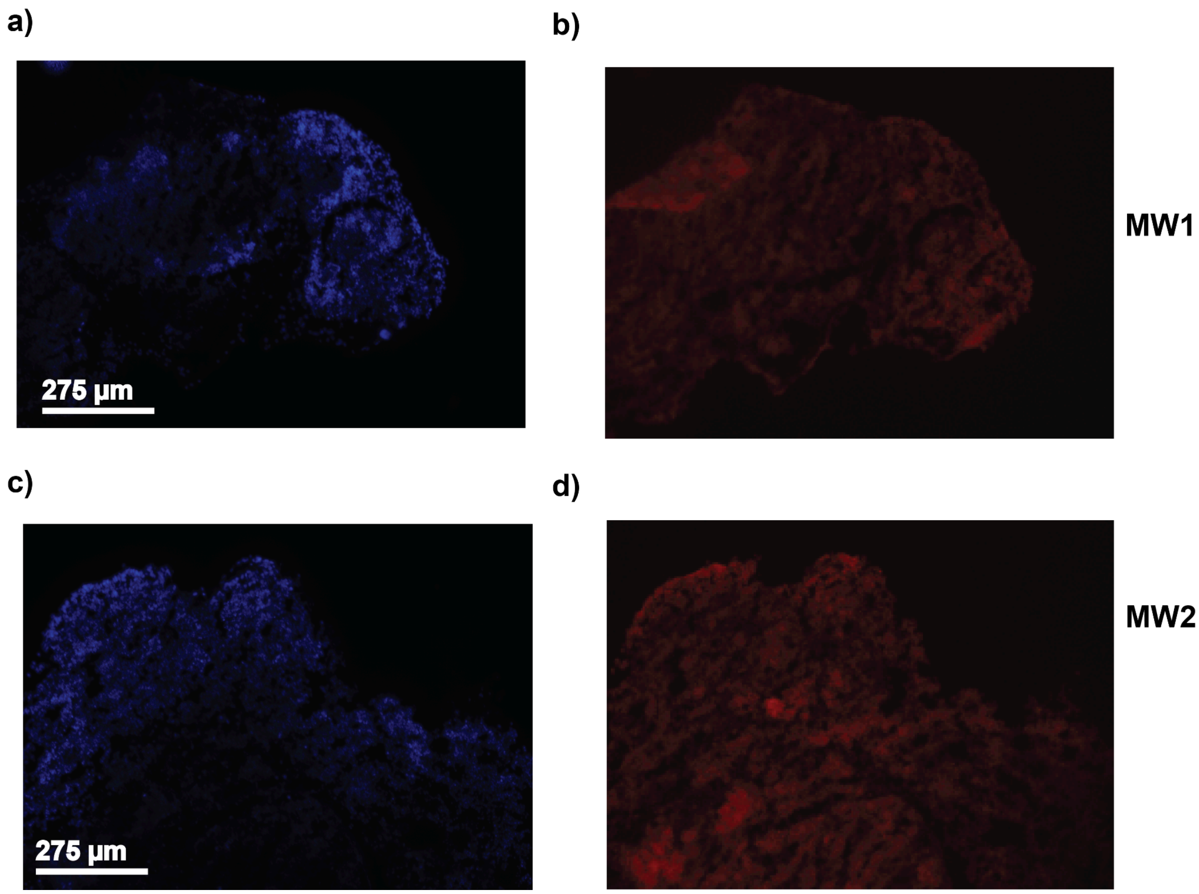
Supplementary Figure 3. illustrates the calibration and recording procedure for absorbing the green Bodipy dye. Panel a) The green BODIPY dye was diluted in ACSF medium to achieve concentrations ranging from 0 to 10 mM. The dye was then imaged using the AM4115T-GRFBY Dinolite, which provides camera capture and light source generation capabilities. The light intensity was measured in lux (LX) using a LX1332B light meter corresponding to the blue square. The microscope could switch between six intensity levels, with the two highest levels selected for constructing the calibration curves. ImageJ measured the intensity levels from the microscope camera, corresponding to the black square. LX was converted to lumens and power intensity (W/mm²). The calibration curve was generated using ten concentration points and replicated thrice. Panel b) shows the calibration curve for the 0.52 W/mm² value, corresponding to the second-highest intensity. Panel c) shows the highest intensity of 0.79 W/mm². Other intensity points were not considered due to their lack of emission. The process of concentration intensity calibration for the highest intensities is shown in supplementary tables ~~8~~13 and ~~9~~14, respectively. Figure 3a was produced in Biorender, Image J, photograph taken by the authors, and edited in Adobe Illustrator.



Supplementary Figure 4. Viability of cortical organoids grown in our life support platform. Representative images of D20 cortical organoids stained with CytoPainter after 120 hours are on our life support platform. A representative image from the three batches (b) analyzed is shown.

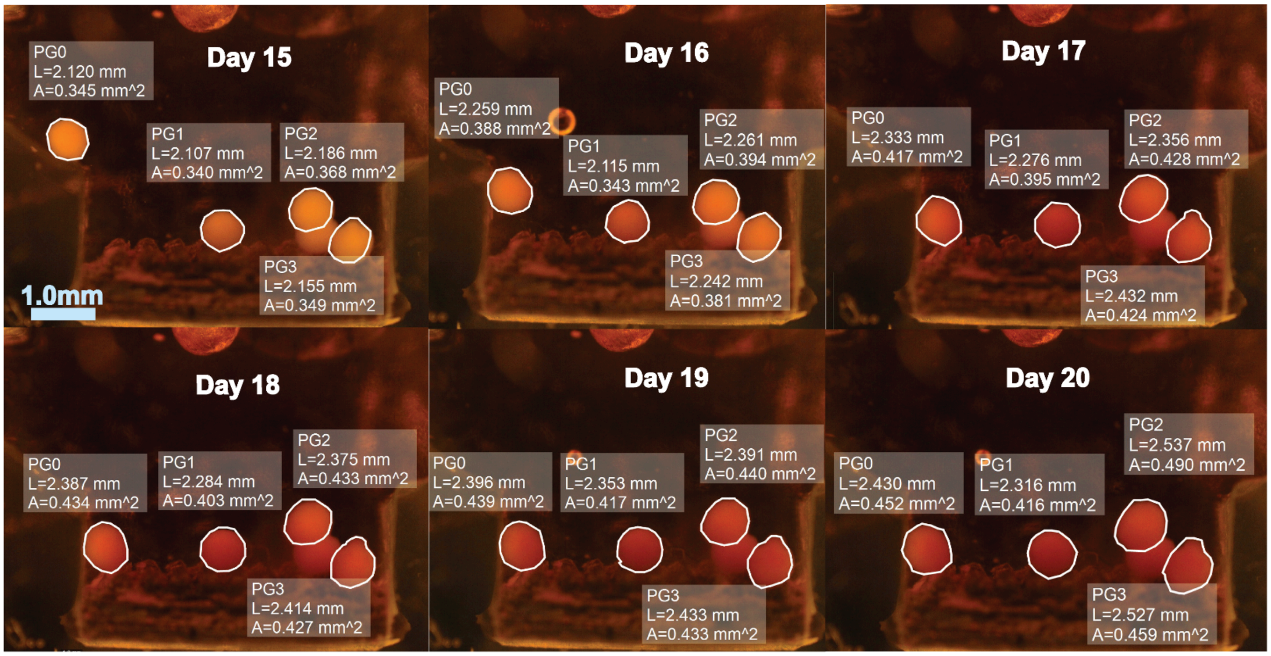


Supplementary Figure 5. Viability of cortical organoids grown in a standard tissue culture incubator. Representative images of D20 cortical organoids stained with CytoPainter. Organoids were grown in standard tissue culture conditions. A representative image from the three batches (b) analyzed is shown.

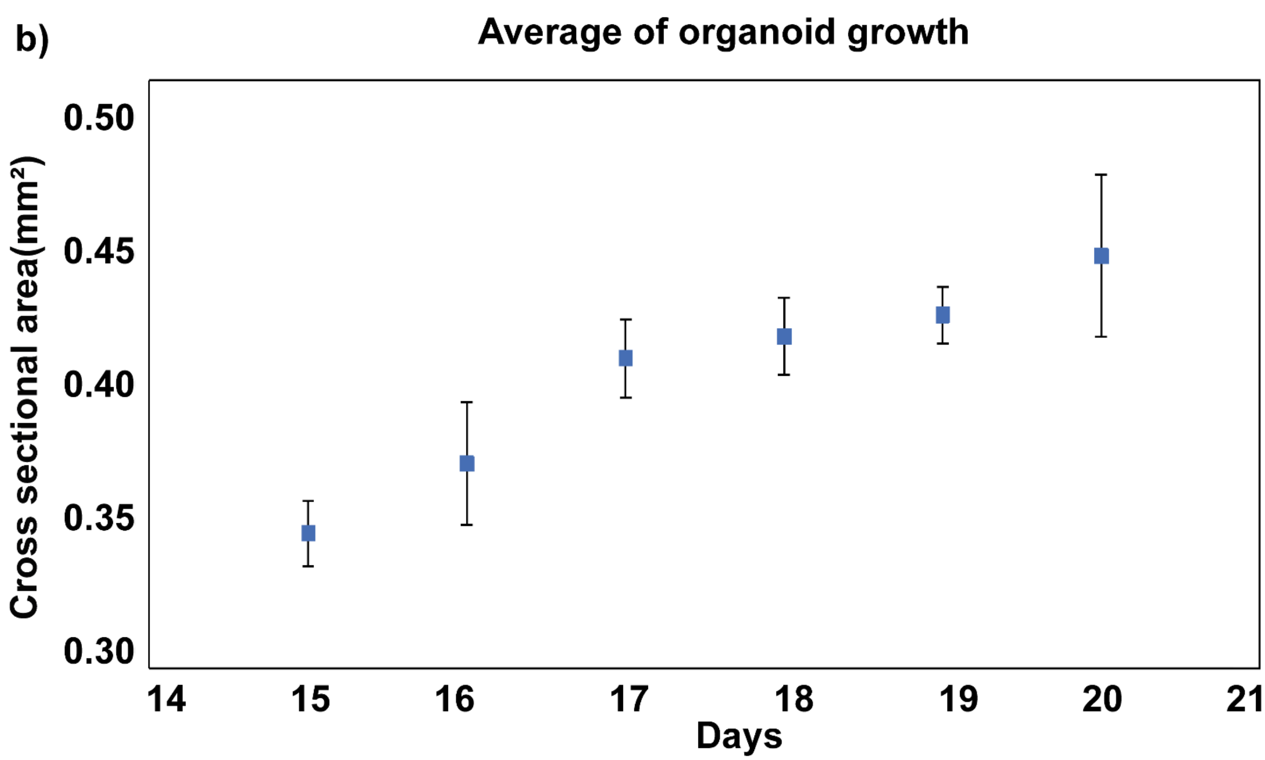


Supplementary Figure 6. Viability of cortical organoids grown in a standard tissue culture incubator. Representative images of D20 cortical organoids stained with CytoPainter. Organoids were grown in standard tissue culture conditions. A representative image from well 3 in the multiwell v1 chip approach, (a) staining in DAPI, and (b) stained with CytoPainter, and v2 (c) staining in DAPI, and (d) stained with CytoPainter

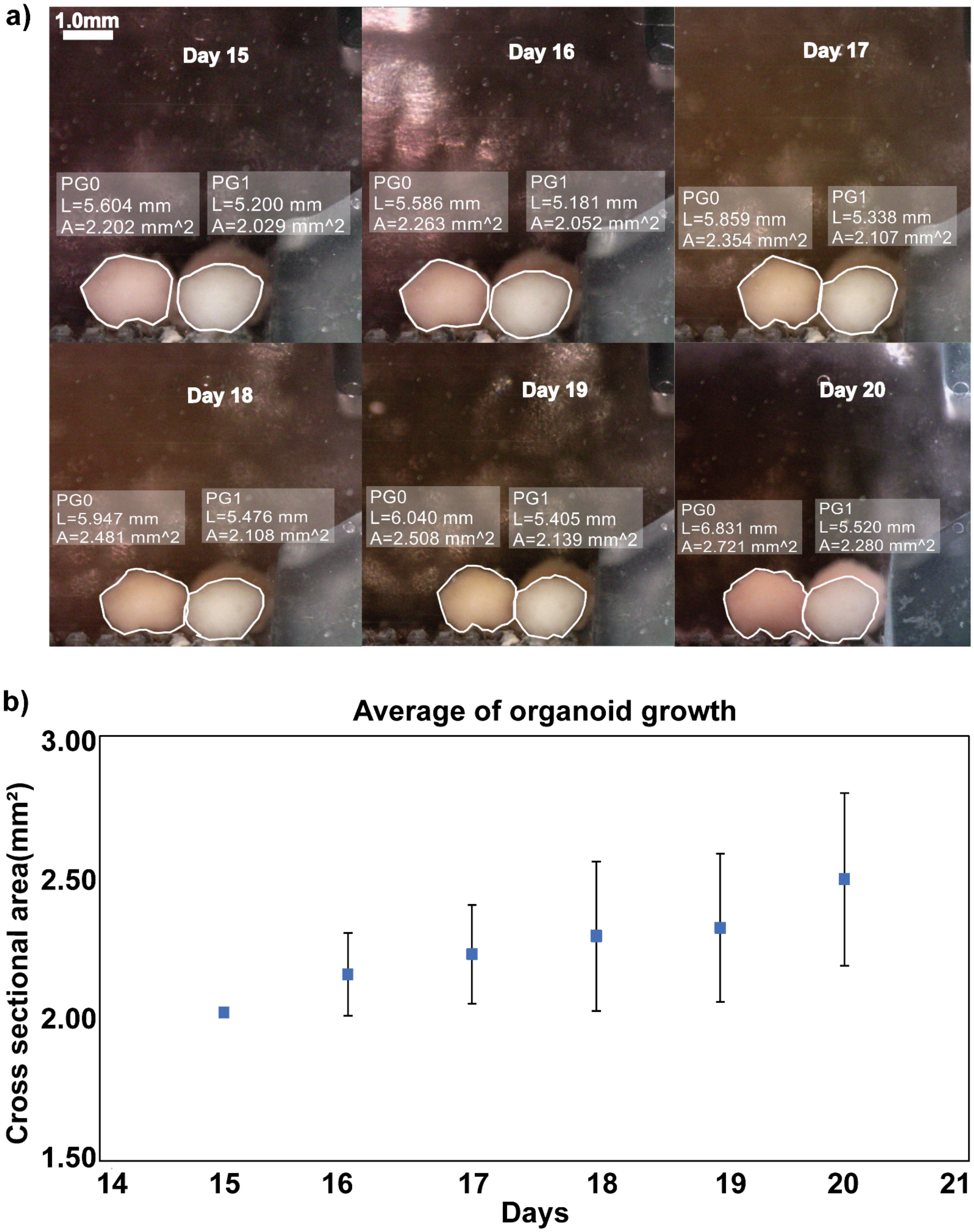
a)



b)

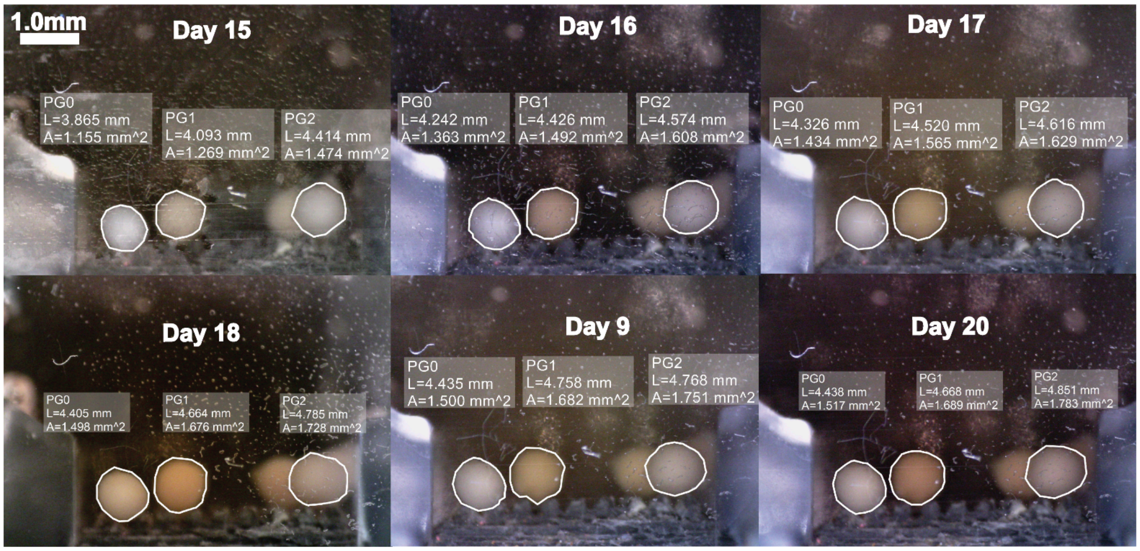


Supplementary Figure 7. Longitudinal growth of cortical organoid in batch 1. a) Estimated area. b) adjusted growth curve over 6 days.

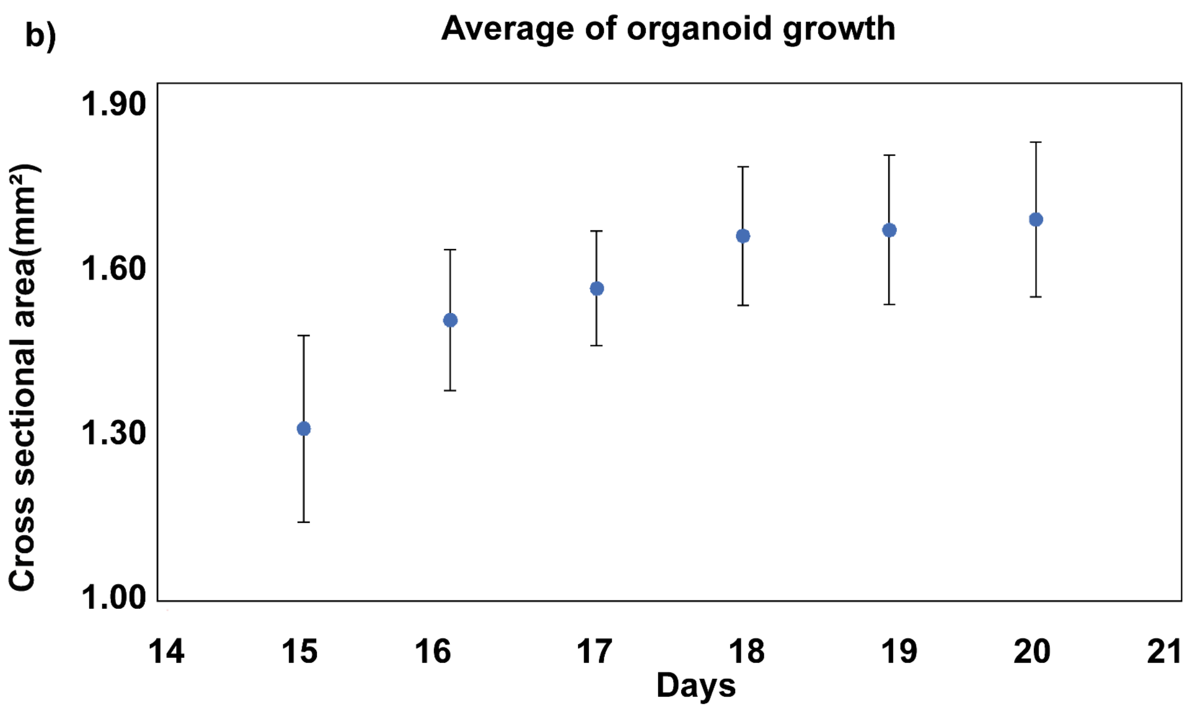


Supplementary Figure 8. Longitudinal growth of cortical organoid in batch 2. a) Estimated area. b) adjusted growth curve over 6 days.

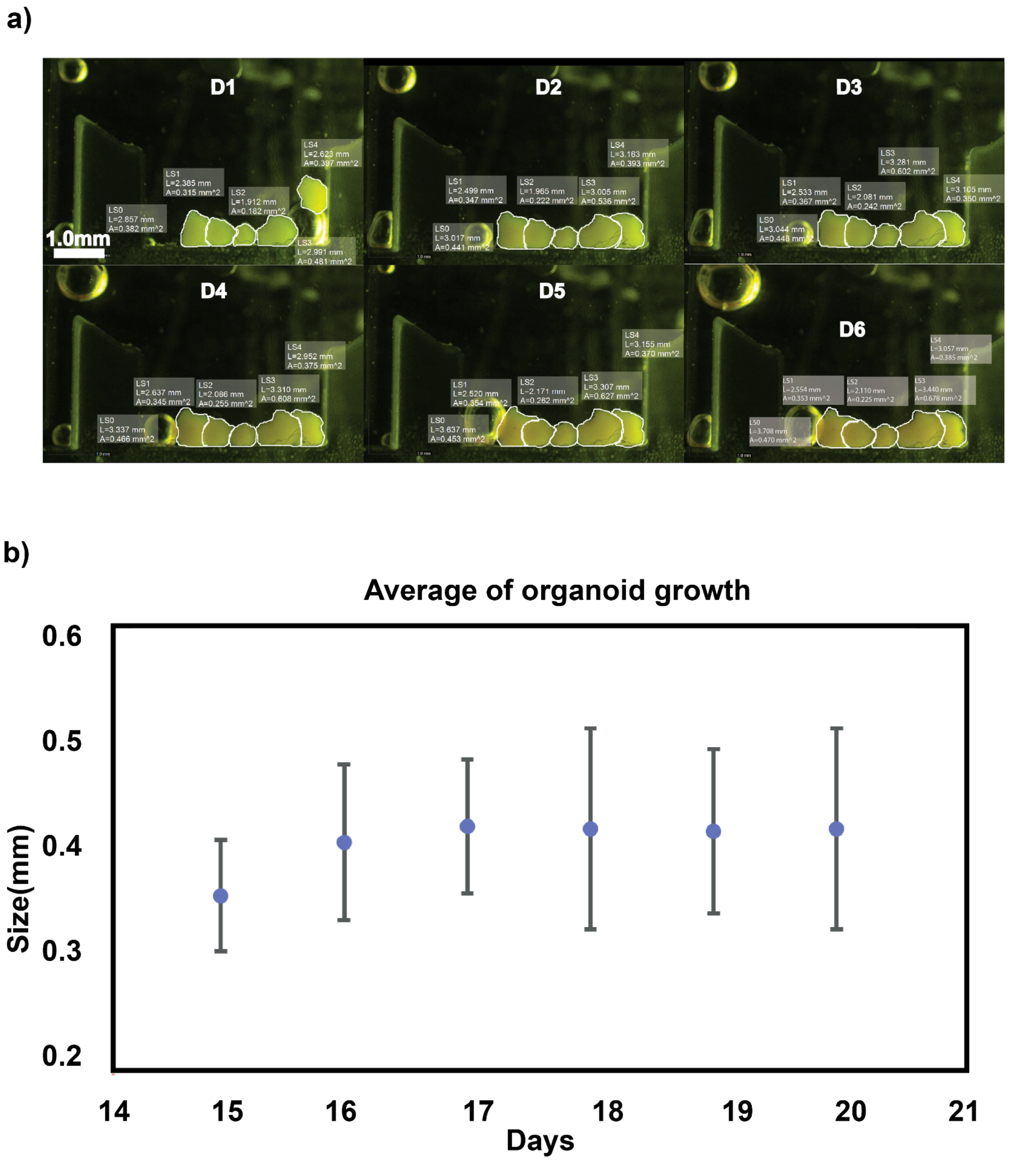
a)



b)

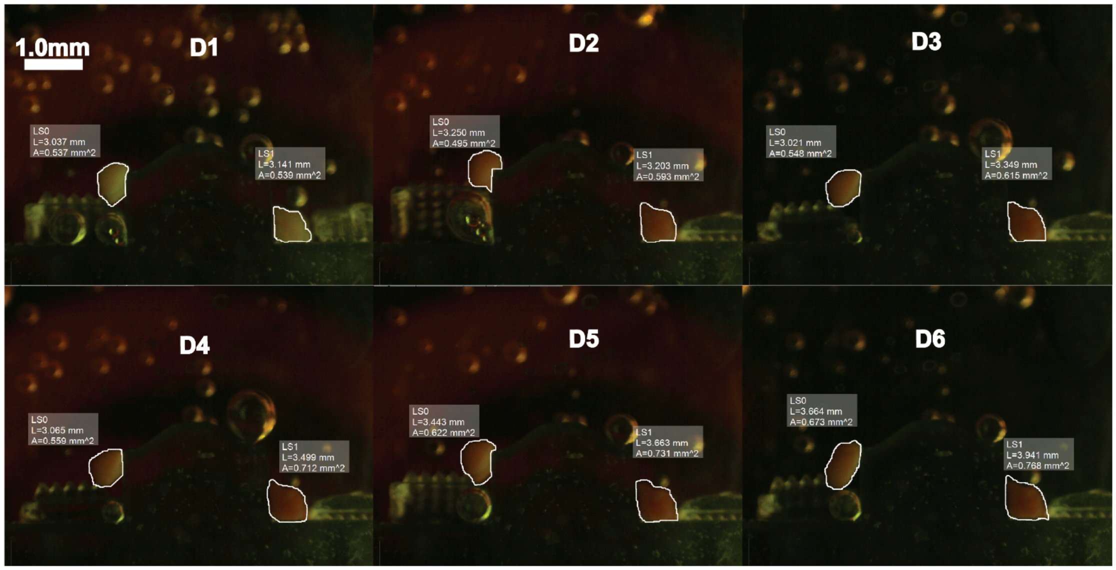


Supplementary Figure 9. Longitudinal growth of cortical organoid in batch 3. a) Estimated area. b) adjusted growth curve over 6 days.

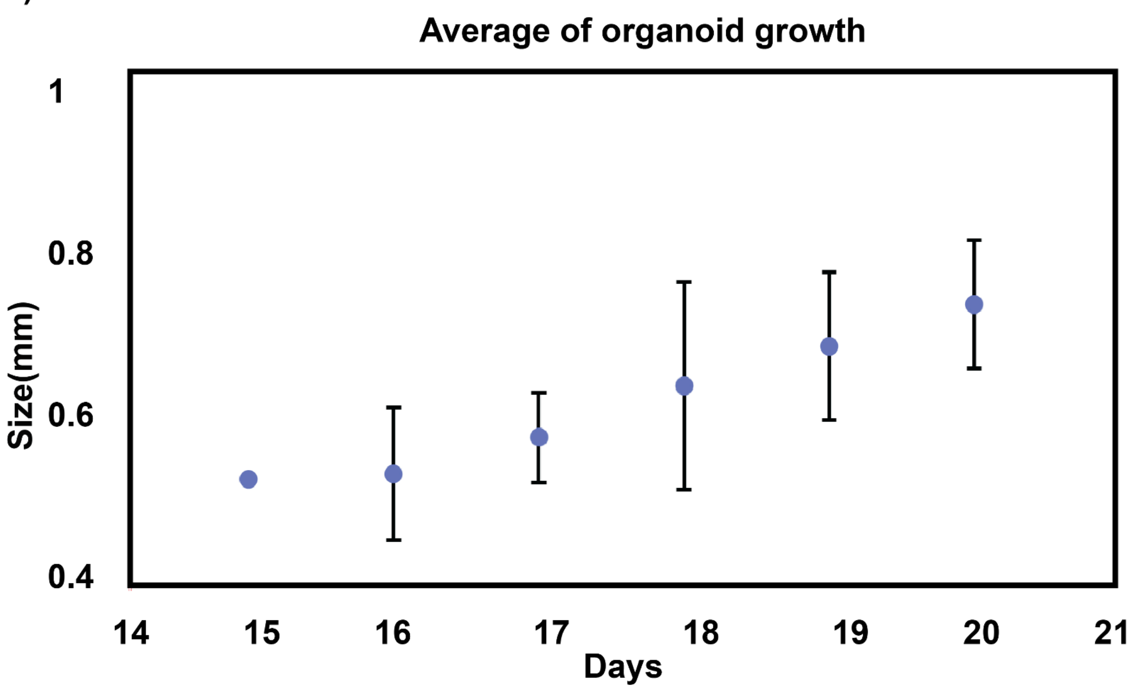


Supplementary Figure 10. Longitudinal growth of cortical organoid in batch 4. a) Estimated area. b) adjusted growth curve over 6 days.

a)

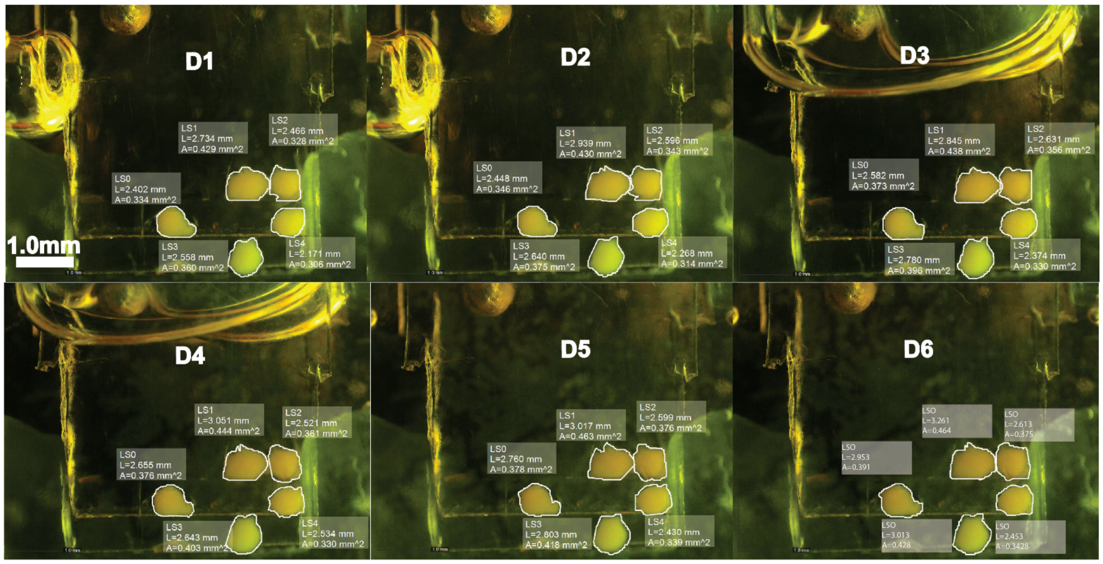


b)

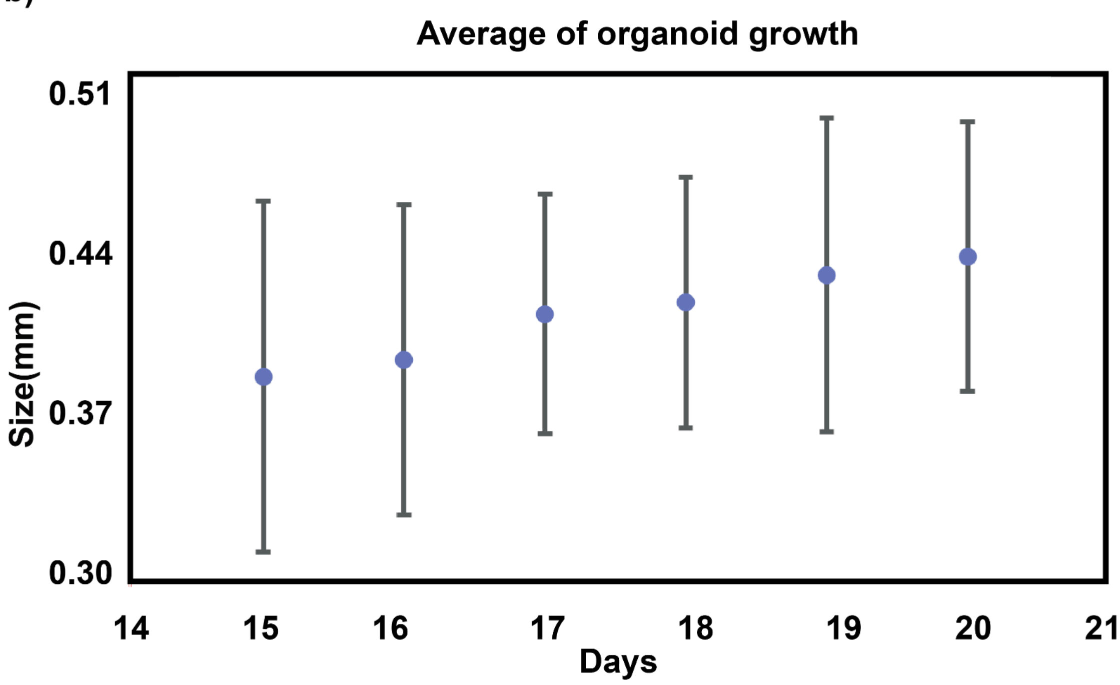


Supplementary Figure 11. Longitudinal growth of cortical organoid in batch multiwell v1. a) Estimated area. b) adjusted growth curve over 6 days.

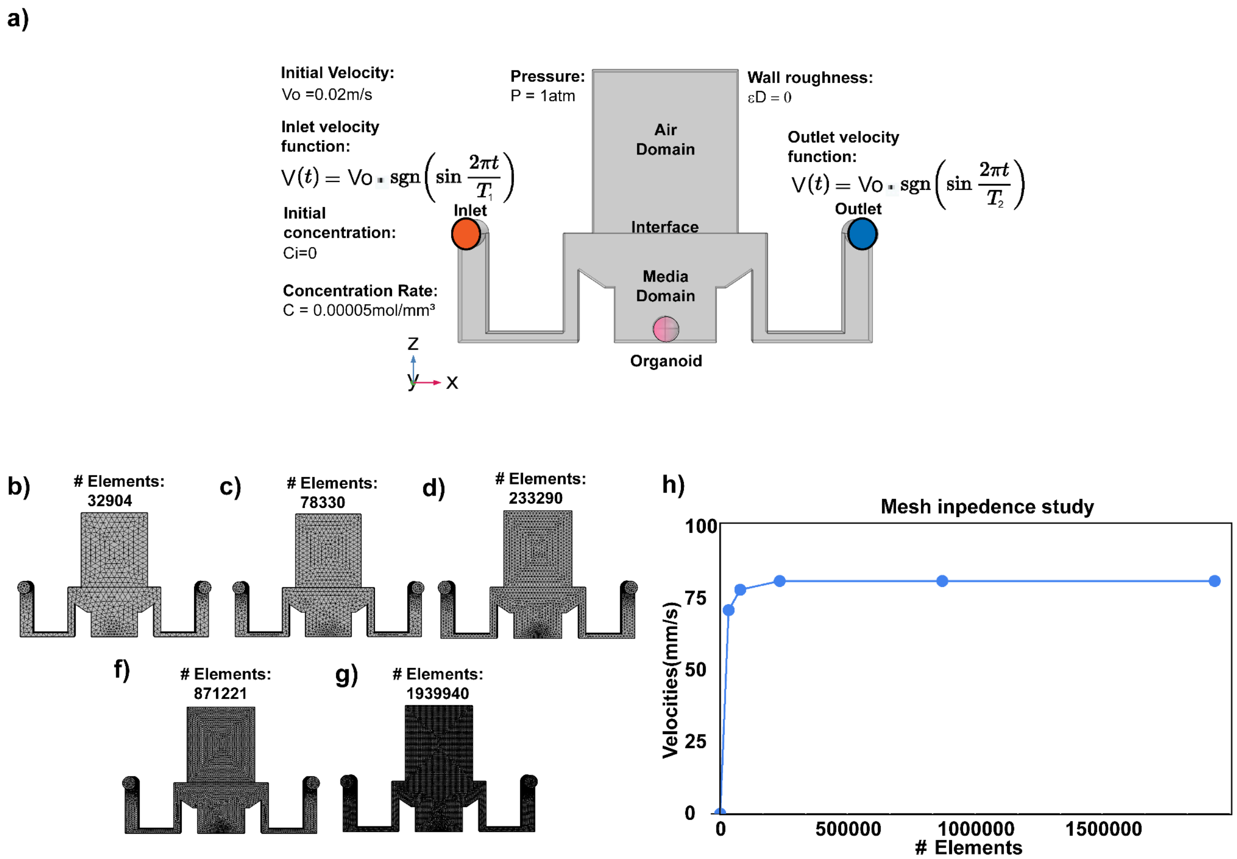
a)



b)



Supplementary Figure 12. Longitudinal growth of cortical organoid in batch multiwell v2. a) Estimated area. b) adjusted growth curve over 6 days.



Supplementary Figure 13**.** Computational domain definition, governing equations, boundary conditions, and mesh independence validation for the simulation model. **(a)** Schematic of the computational domain, comprising two phases: the air domain (top) and the media domain (bottom), separated by an interface. Solute transport occurs within the media domain. Volume of Fluid (VOF)-based Navier-Stokes and interface-tracking equations governing the two-phase flow. The left side depicts the incompressible momentum conservation and continuity equations, while the right side presents the phase field equation for the evolution of the interface. The boundary conditions applied to the inlets, described by time-dependent sign functions, are used to induce anti-phase pulsatile flow on the left and right sides with periods T_1 and T_2, respectively. **(b-g)** Meshes used for the grid sensitivity analysis, ranging from coarse to fine, capture geometric features of both the media and air domains. Each mesh is tailored to resolve the interface, inlet curvature, and chamber core. **(h)** Mesh convergence study. The graph shows that the simulation result stabilizes beyond ~400,000 elements, confirming mesh independence of the numerical solution. These results validate the suitability of the mesh resolution used in the main simulations.