



Movie S1. Trimming of an ROI frustum from a block



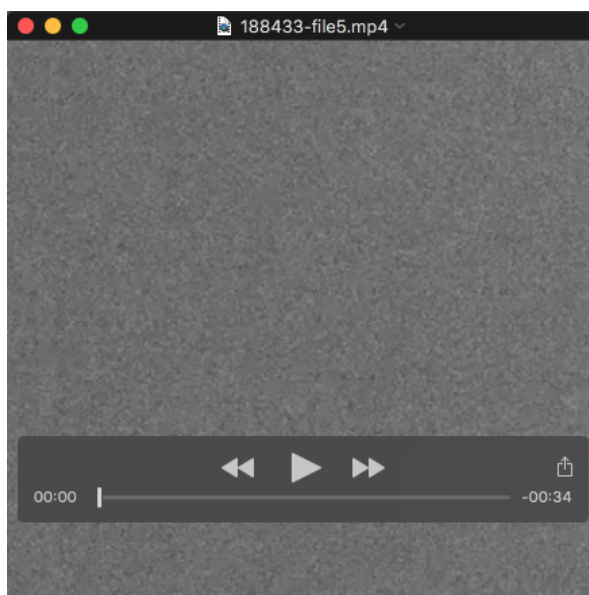
Movie S2. Mounting of a frustum on to a pin.

A small blob of conductive epoxy glue is first placed on the pin. After gently dislodging the frustum from the Parafilm™ (with the back of the frustum pointed upwards), a toothpick is used to lift it out of the Parafilm™ by placing a tip coated with conductive glue onto the back (i.e. opposite from the cell containing face). The frustum is then inverted so the face points upward, and the back placed onto the blob of conductive glue on the pin. A small amount of the glue was then brought up around the block sides (not shown).



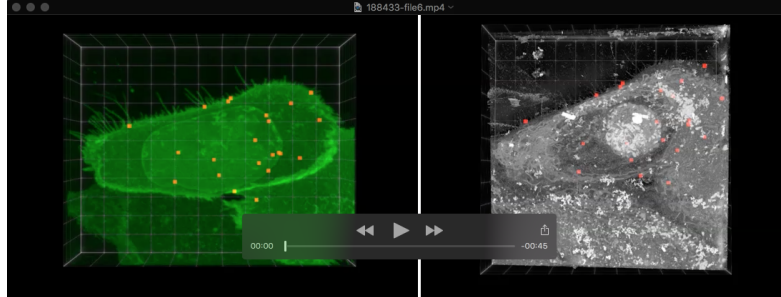
Movie S3. SBF SEM of Vero E6 cells with isotropic 10 nm voxels.

See <https://figshare.com/s/33a422c43fde70ac8580> for larger file.



Movie S4. SBF SEM of a cell-in-a-cell structure.

See <https://figshare.com/s/33a422c43fde70ac8580> for larger file.



Movie S5. 3D reconstructions of a cell-in-a-cell structure. The raw live FM (GFP-PLCd-PH; top left) and SBF SEM data (top right) show the landmarks used to align the FM to the SBF SEM data in BigWarp. H264 compression. 5 frames per second.

Table S1. Fluorescence light microscopy imaging parameters

	Fig 4-5, Mtb (Lerner et al., 2016)	Fig 6, Mtb	Fig 7, Entosis	Fig 8, HIV (Nkwe et al., 2016)	Recommended
Live-/Fixed-cell imaging	Live and fixed confocal	Fixed confocal	Live confocal	Fixed widefield	Live and fixed confocal
Objective lens	63x	63x	40x	20x	40x or 63x
NA	1.4	1.4	1.4	0.3	1.4
Pinhole	1 AU, 95.4 μm	1 AU, 95.4 μm	1 AU, 32 μm	n/a	1 AU
Pixel dimensions	1024 x 1024	1024 x 1024	512 x 512	1392 x 1040	1024 x 1024
Zoom	2	1	2.2	n/a	1 or 2
Line averaging	2	4	1	n/a	4
Pixel dwell time	2.1 μs (400 Hz)	2.1 μs (400 Hz)	2.55 μs	n/a	-
Pixel size (μm)	0.12	0.24	0.189	0.46	Nyquist sampling
Optical section thickness (μm)	0.771	0.771	1.7	n/a	set by pinhole ($\approx 0.7 \mu\text{m}$ for 1 AU)
Number of slices	5	1	50	n/a	for whole cell volume
Step size (μm)	1.19	n/a	0.373	n/a	(1/2 of optical section thickness)
HFW (xy μm)	123.02 x 123.02	246.03 x 246.03	96.92 x 96.92	640.32 x 478.4	-
Volume (xyz μm)	123.02 x 123.02 x 5.95	n/a	96.92 x 96.92 x 18.63	n/a	-

NA – numerical aperture of the objective lens

AU – Airy unit

HFW – horizontal field width

n/a – not applicable

Table S2. SBF SEM imaging parameters

	Fig 3A, MD4 Cell 15 nm cuts	Fig 3B-C, Vero Cell 10 nm voxel	Fig 4-5, Mtb (Lerner et al., 2016)	Fig 6, Mtb	Fig 7, Entosis	Fig 8, HIV (Nkwe, et al., 2016)
Vacuum (Pa)	High Vacuum	5	10	10	6	5
Voltage (kV)	2	2	1.8	1.8	2	2
Aperture (µm)	30	30	20	20	20	20
High current mode	No	No	Yes	Yes	Yes	Yes
Pixel dwell time (µs)	2	3	2	2	2	2
Pixel dimensions	2048 x 2048	8192 x 8192	8192 x 8192	8192 x 8192	8192 x 8192	8192 x 8192
Lateral pixel size (nm)	15	10	3	3.1	7	4
Slice thickness (nm)	15	10	50	50	50	50
Slices	1000	200	68	37	341	300
Volume (xyz µm)	30.7 x 30.7 x 15	81.9 x 81.9 x 2	24.3 x 24.3 x 3.4	25.3 x 25.3 x 1.85	57.1 x 57.1 x 17.05	32.8 x 32.8 x 15

Slice thickness – SBF SEM microtome thickness setting
Slices – number of slices in the dataset

Table S3. Mtb 3D reconstruction model quantitation

Mtb	Mtb Volume (μm^3)	Closest distance to surrounding membrane (nm)
1	0.497	271*
2	0.440	271*
3	0.454	345
4	0.283	866
5	0.549	271*
6	0.346	271*
7	0.396	271*
8	0.371	271*
9	0.703	271*
10	0.606	271*
11	0.430	271*
12	0.421	857
13	0.528	271*
14	0.299	347
15	0.386	271*
16	0.470	271*
17	0.318	271*
18	0.394	610
19	0.297	271*
20	0.469	271*
21	0.404	277
22	0.515	271*
23	0.824	445
24	0.361	655
25	0.346	271*
26	0.370	271*
27	0.385	539
28	0.354	271*
29	0.315	852
30	0.332	271*
31	0.432	574
32	1.018	452
33	0.442	271*
34	0.436	639
35	0.364	728
36	0.298	495
37	0.412	271*
38	0.386	271*
39	0.521	271*

Average volume 0.440

*The real proximity of bacteria shown as 271 nm is equal to or less than this value (since the surrounding membrane was originally segmented as a 270 nm thick object, with the outer face following the electron density of the surrounding membrane, in order to prevent artefactual holes appearing in the surface, see Lerner et al., 2016).

Table S4. IPMC 3D reconstruction model quantitation

	IPMC Membrane area		Virus buds		Virus buds/ μm^2 of membrane
	Area μm^2	%	number	% of total	
Whole IPMC	1,444	-	15,624	-	10.8
Compact domain (Fig.8I,K; arrow)	223	15.5	5,674	36.3	25.4
Convolutd domain (Fig.8I,K; arrowhead)	1,220	84.5	9,950	63.7	8.2

Table S5. Mtb BigWarp landmarks

Landmark	Landmark positions (μm)					
	Moving (LM)			Target (SBF SEM)		
	x	y	z	x	y	z
Pt-0	72.265	63.175	3.821	13.848	6.176	0.988
Pt-1	70.266	65.604	3.821	15.707	10.222	0.792
Pt-2	69.530	62.601	2.617	17.287	5.407	1.312
Pt-3	72.738	69.392	2.500	10.887	14.861	2.051
Pt-4	69.749	67.094	1.704	15.442	12.102	1.981
Pt-5	70.355	63.577	1.640	15.686	6.388	2.000
Pt-6	75.106	70.211	2.748	7.079	15.329	2.108

Table S6. Entosis BigWarp landmarks

Landmark†	Landmark positions (µm)					
	Moving (LM)			Target (SBF SEM)		
	x	y	z	x	y	z
Pt-0	45.571	38.328	2.551	26.316	15.651	5.370
Pt-1	52.014	36.194	2.309	32.520	13.018	4.223
Pt-2	71.733	34.783	1.921	51.385	9.352	1.157
Pt-3	27.016	42.888	2.648	8.992	21.646	8.339
Pt-4	52.990	59.062	2.890	36.781	34.734	6.125
Pt-5	51.402	36.719	5.309	32.061	13.587	5.839
Pt-6	38.215	56.713	3.852	21.936	33.877	8.569
Pt-9	58.704	61.538	7.133	43.687	36.518	8.361
Pt-10	38.360	49.570	13.668	22.756	26.681	15.878
Pt-11	63.257	50.245	11.002	48.633	22.205	9.360
Pt-12	64.647	49.555	10.498	48.925	21.330	8.776
Pt-13	74.746	41.195	7.467	56.124	16.466	4.787
Pt-14	61.223	40.107	6.502	41.726	14.423	5.857
Pt-15	67.434	37.173	6.208	46.493	12.282	4.690
Pt-16	69.843	50.849	7.879	52.136	25.513	6.441
Pt-17	49.153	55.442	10.986	34.916	30.961	13.056
Pt-18	47.958	51.469	14.437	33.846	26.778	13.835
Pt-19	64.692	49.753	6.390	48.341	22.692	5.663
Pt-21	58.479	46.449	8.516	40.833	21.811	7.961
Pt-22	59.399	52.308	8.053	42.403	26.206	7.988
Pt-23	61.707	54.182	6.749	45.715	28.706	6.835
Pt-24	62.077	41.364	9.538	44.828	15.580	7.828

†Landmarks 7, 8 and 20 were removed during refinement of the alignment