Cell biology revealed with OMX super resolution imaging

Dr Lynne Turnbull
Cellular Imaging and Analysis
GE Healthcare

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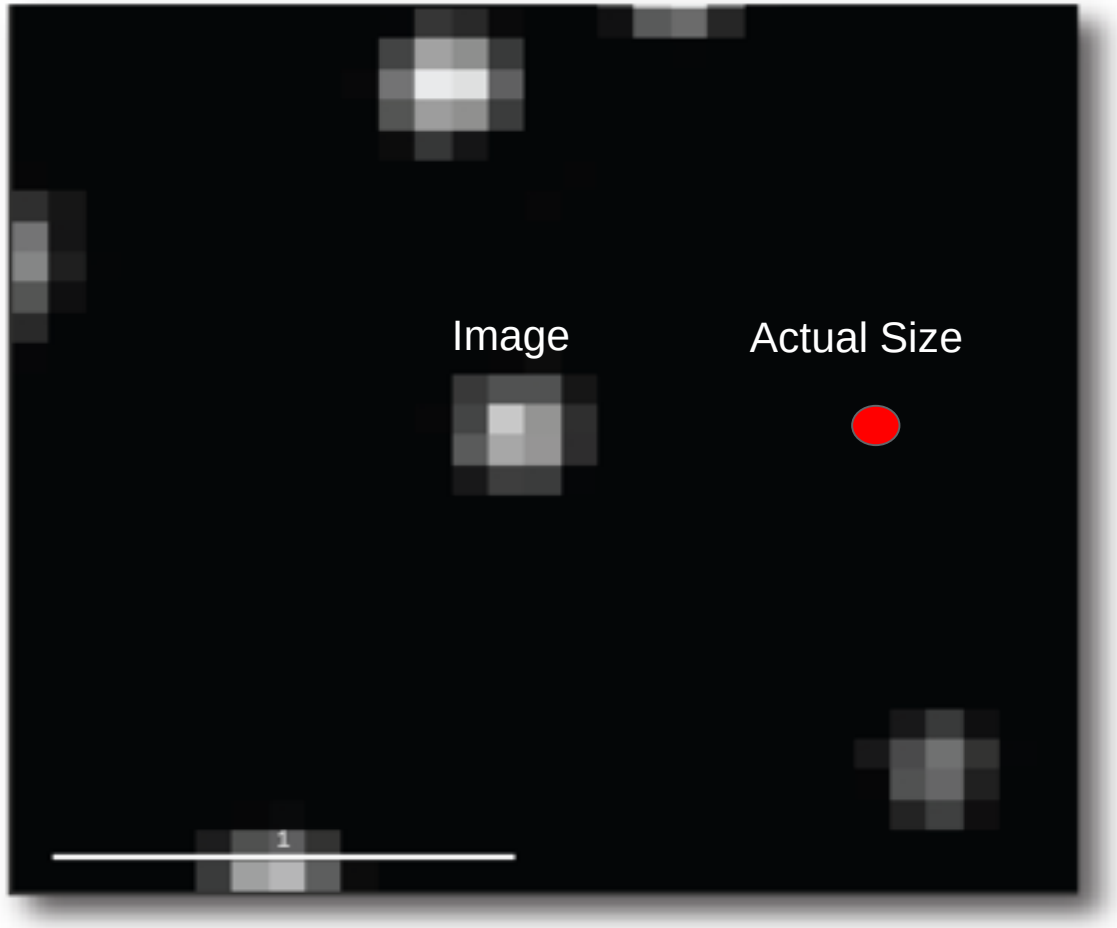
OMX SR Workshop

Beijing China

Types of imaging for biological specimens

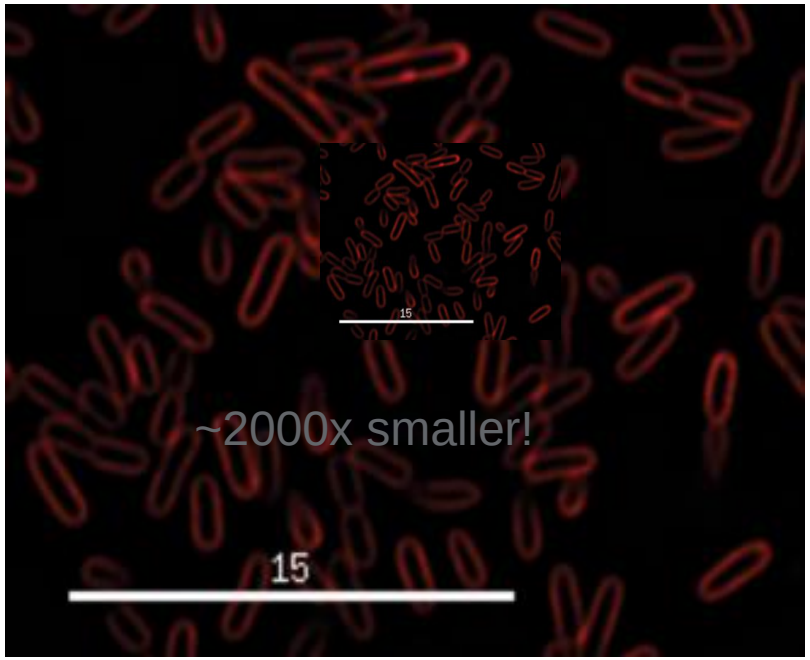
- Electron Microscopy
  - Transmission, Scanning
  - Specimen must be dead and cannot move
  - Lots of preparation needed before you can image
  - Great resolution!
  - Great for structures but harder to specifically identify features
- Light (Optical) Microscopy
  - Transmitted - Brightfield and related techniques
  - Fluorescence
  - Less resolution than EM but can use live and fully hydrated samples
  - Can stain and label specific structures, even multiple

Resolution



Size is important!

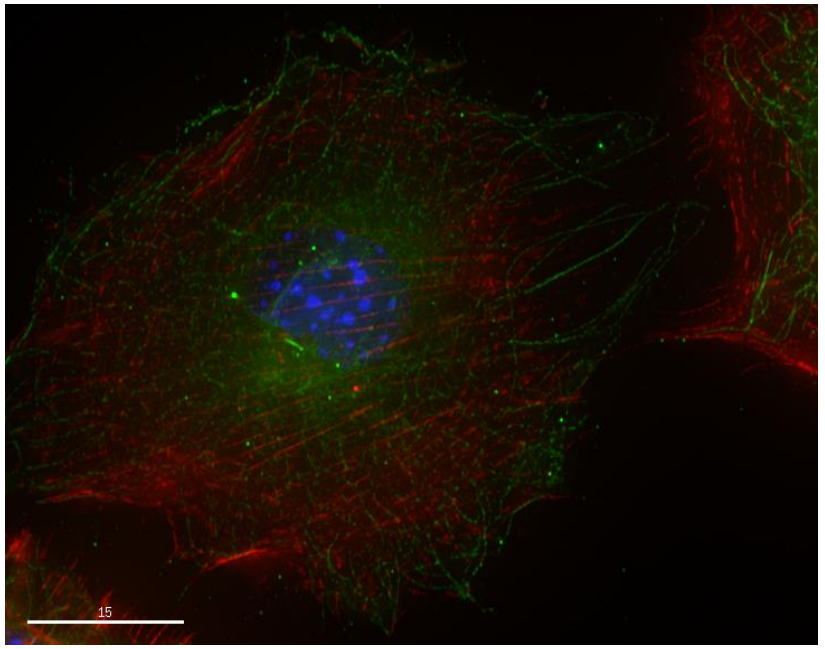
•Historically bacterial cell biology has been limited by small size of bacterial cells and resolution of conventional microscopy



Pseudomonas fluorescens cells

FM464 lipid stain

(3 μm long x 1μm diameter)

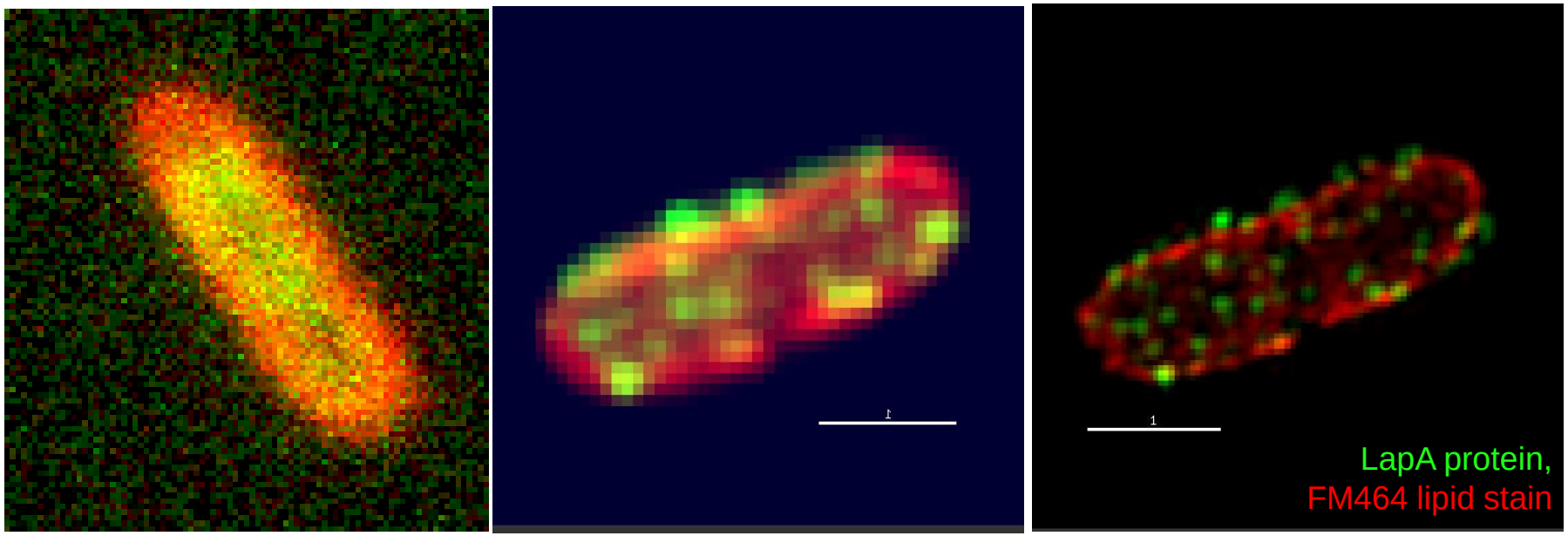


3T3 bone cells

Actin, tubulin,
DAPI

(40 μm long x 30 μm wide x 15 μm high)

Conventional vs OMX 3D-SIM

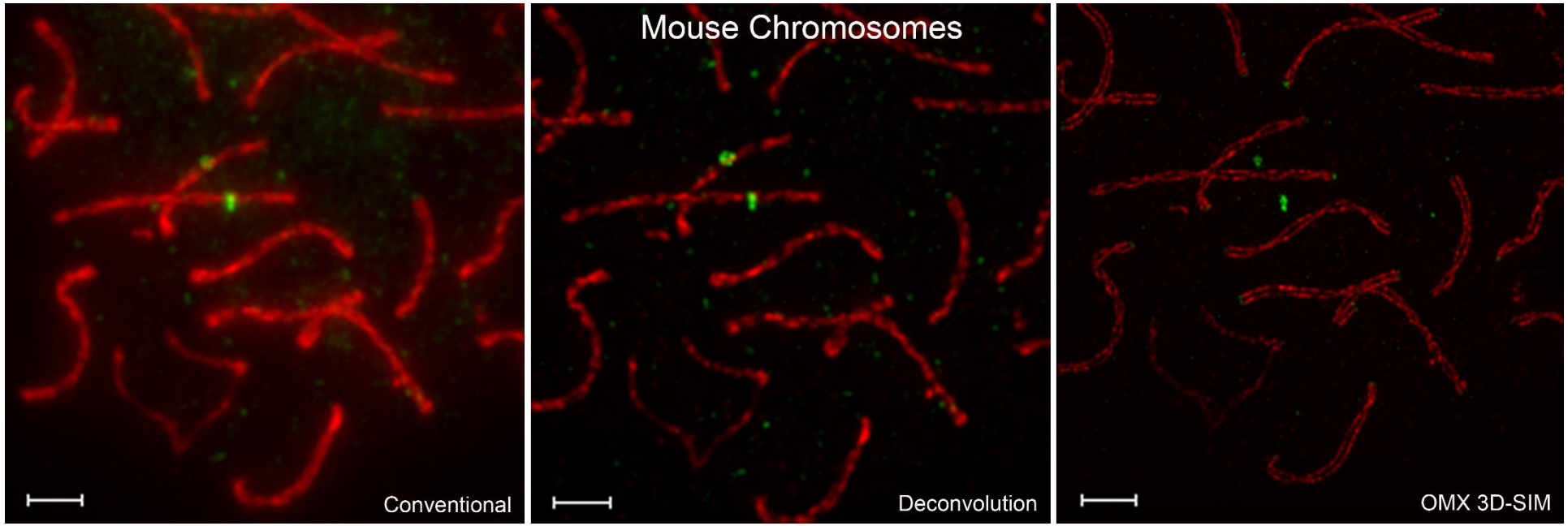


Confocal | Wide-field deconvolved | OMX 3D-SIM

- Subcellular localisation of protein secretion in *P. fluorescens*
- Regularly spaced along cell, not at poles

G. O'Toole, Dartmouth; Whitchurch, Turnbull, UTS

Conventional vs OMX 3D-SIM

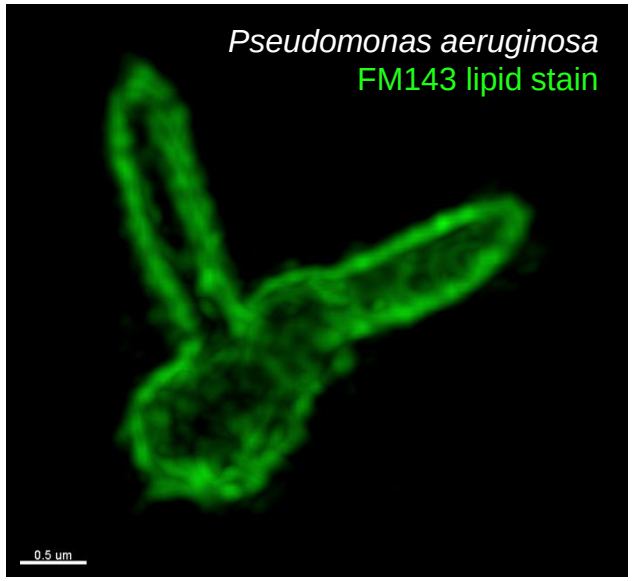


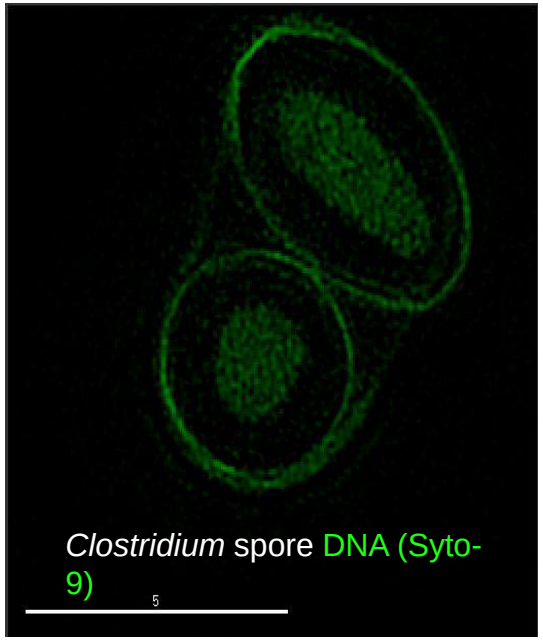
Mouse testes spreads showing synaptonemal complex protein 3 (SCP3) and KASH5

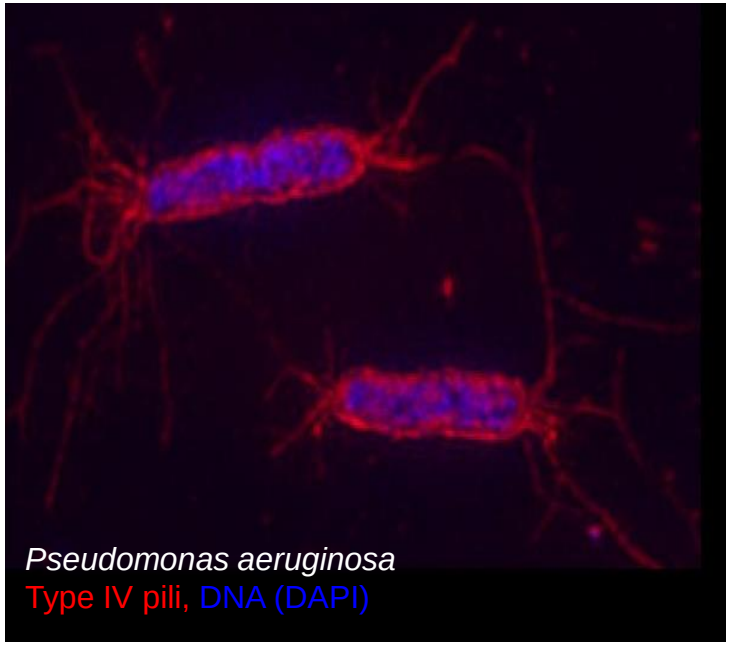
Horn, Wright, Burke, Turnbull, IMB Singapore, U

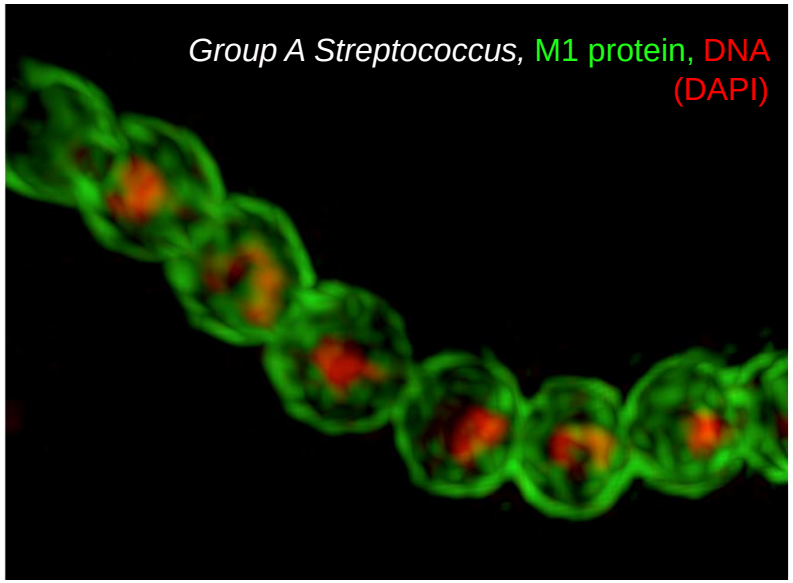
Biology using fixed samples

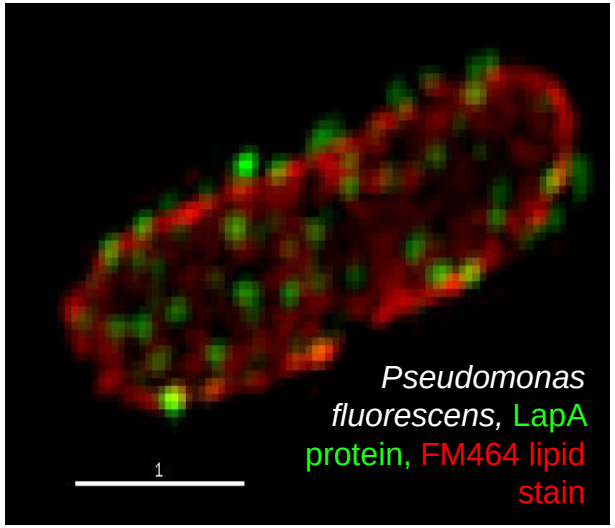
Bacterial cell biology



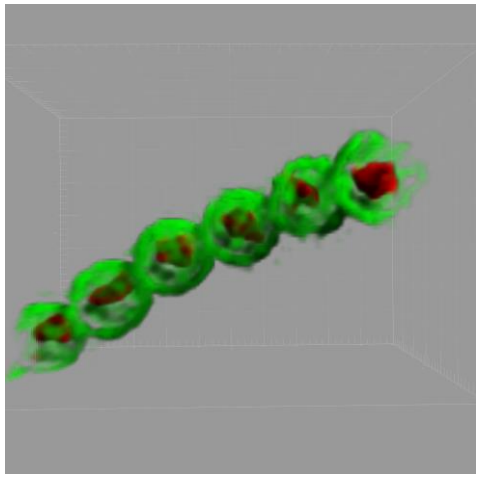




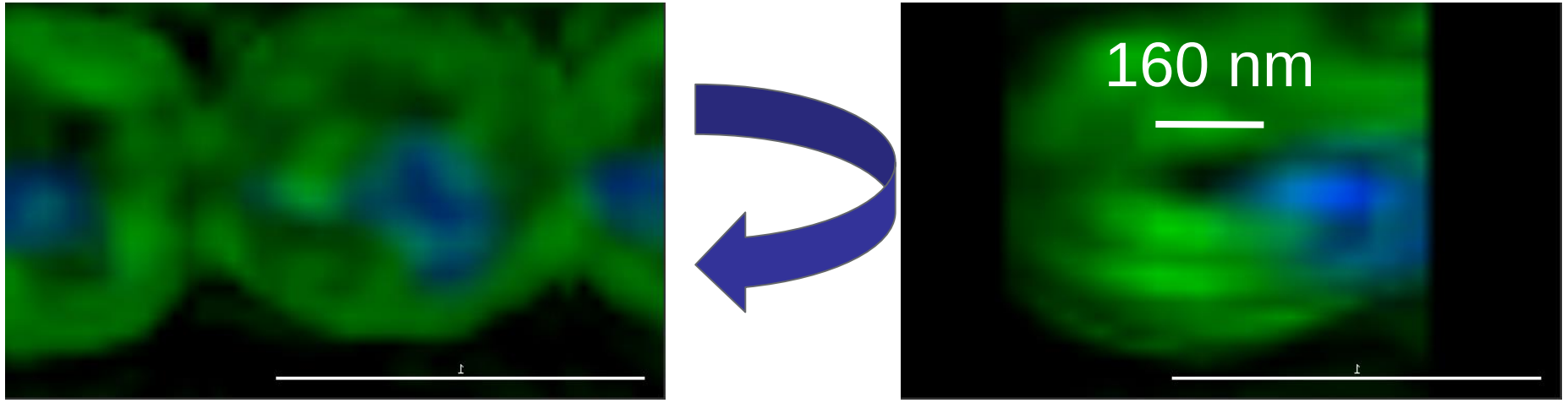




Novel features in bacteria



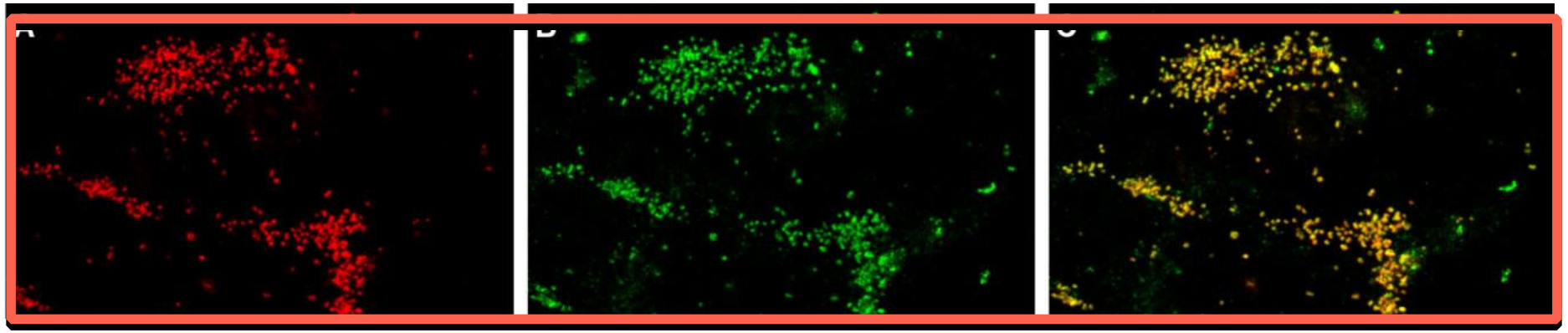
Cell wall proteins in Streptococci



M1 cell wall protein, DAPI

M. Walker UoW (UQ); C. Whitchurch, L. Turnbull, UTS

Surface protein interaction of pathogen and host



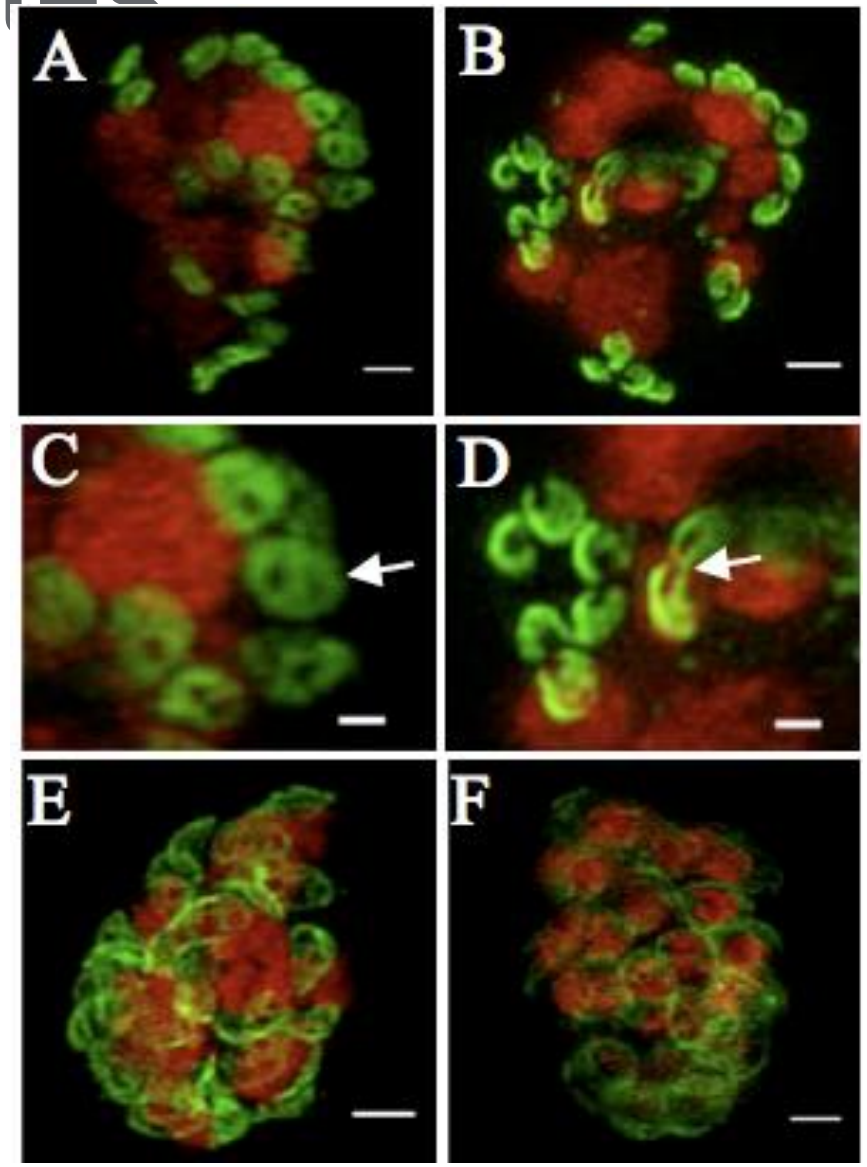
Mycoplasma hyopneumoniae

Fibronectin, P97 surface protein, DNA (DAPI)

Raymond, *et al.,* (2014) Proteolytic processing of the cilium adhesin MHJ_0194 (P123J) in *Mycoplasma hyopneumoniae* generates a functionally diverse array of cleavage fragments that bind multiple host molecules, *Cellular Microbiology*

Plasmodium falciparum merozoites

- Malaria GAP50-GFP-labelled protein in developing schizonts (asexual stage inside red blood cell, co-labelled for DNA)
- The inner membrane complex forms around an ellipsoid pore (~110 x 140 nm) in developing schizont (A, C)
- During division, these separate into claw-like structures with a 130 x 230 nm cavity (B, D)
- In mature schizont, GAP50 is distributed around periphery (E, F)

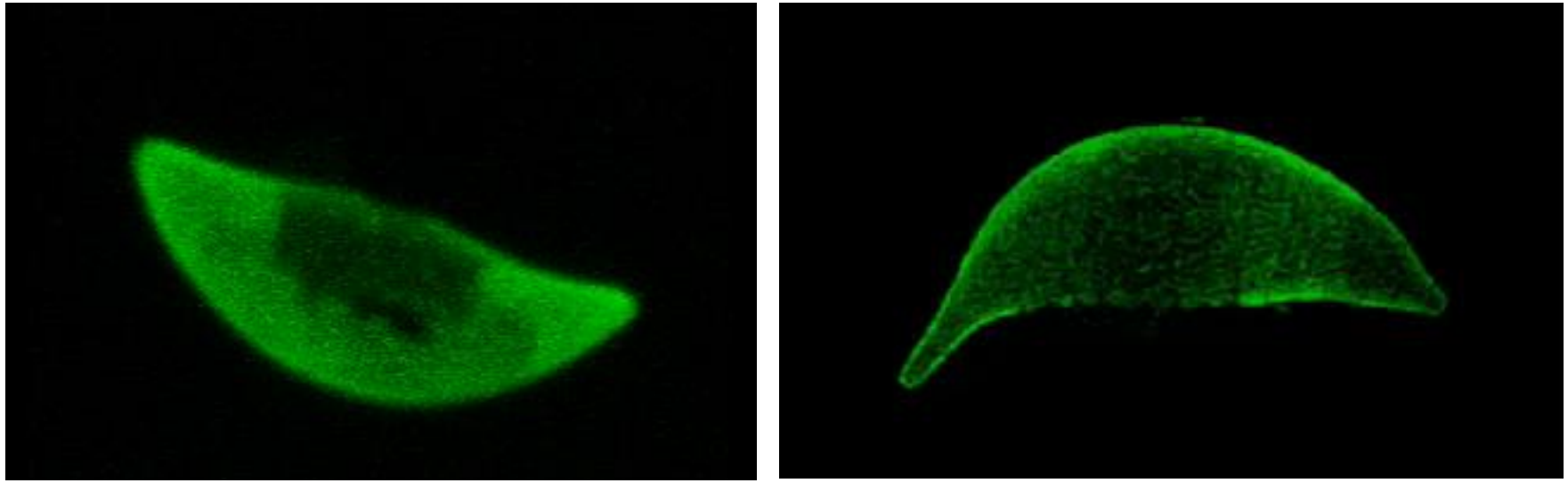


Yeoman *et al.*, (2011) Tracking glideosome-associated protein-50 reveals the development and organization of the inner membrane complex of *P. falciparum, Eukaryotic Cell* **10 (4):** 556-64 .

Plasmodium falciparum gametocytes

- OMX 3D-SIM has revealed novel features of malaria parasites

Confocal v 3D-SIM



Malaria Glidosome Associated Protein-50 (GAP50)-GFP-labelled Inner Membrane Complex - has ~100 nm striations in late stage gametocytes (sexual stage)

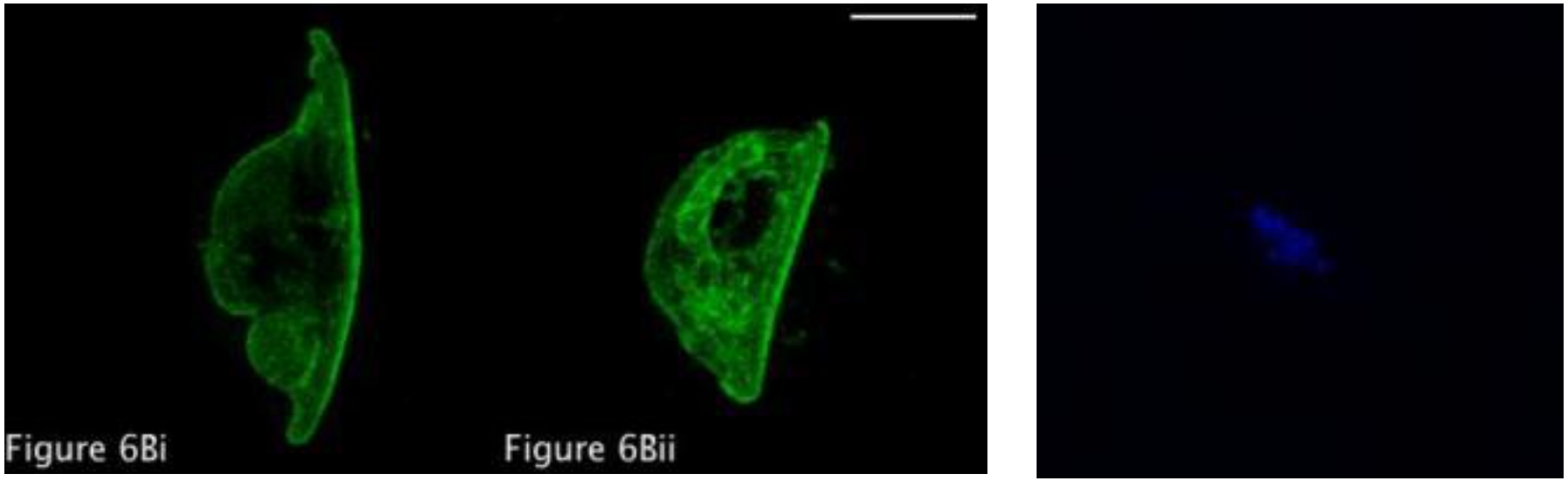
Dearnley *et al.,* (2012) Origin, composition, organization and function of the inner membrane complex of *Plasmodium falciparum* gametocytes, *J. Cell Science*, 125 2053-63.

Plasmodium falciparum gametocytes

- GAP50 redistribution in developing gametocyte linked to microtubule formation

STAGE III

STAGE IV

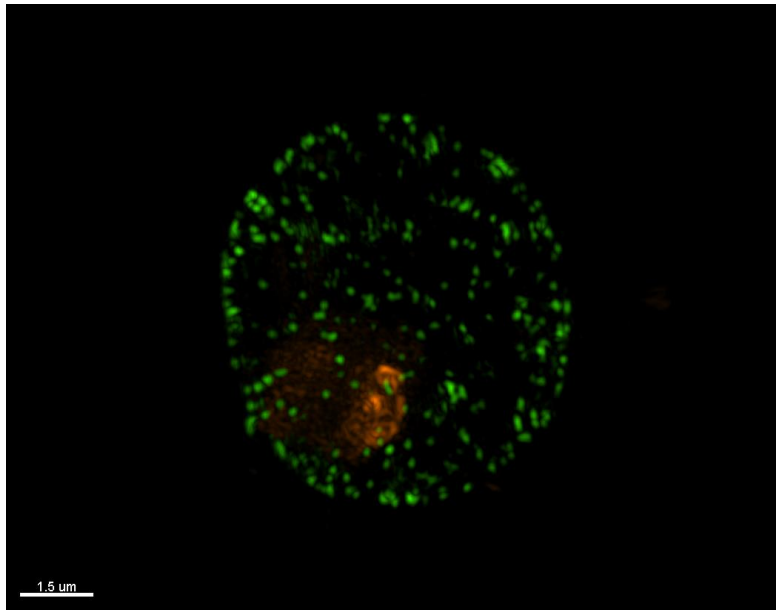


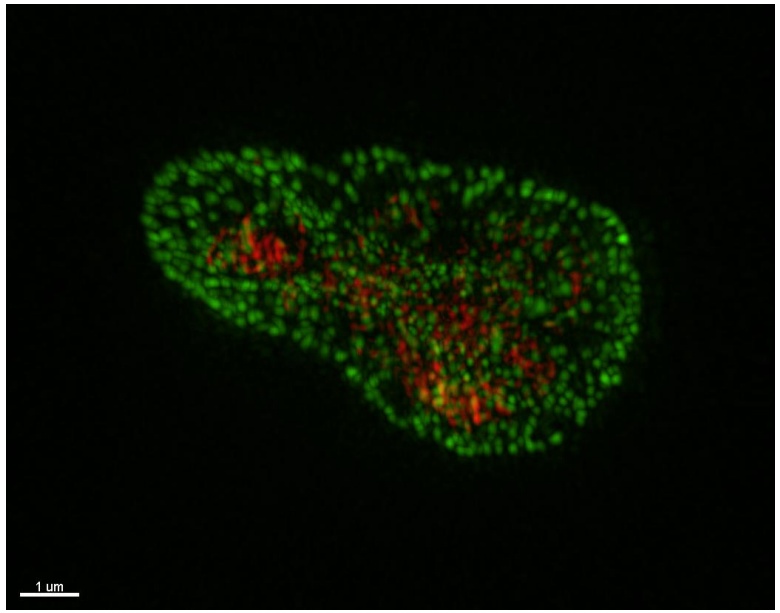
GAP50, tubulin, DNA (DAPI)

Dearnley *et al* (2012) Origin, composition, organization and function of the inner membrane complex of *Plasmodium falciparum* gametocytes, *J. Cell Science*, 125: 2053-63.

Malaria inside red blood cells

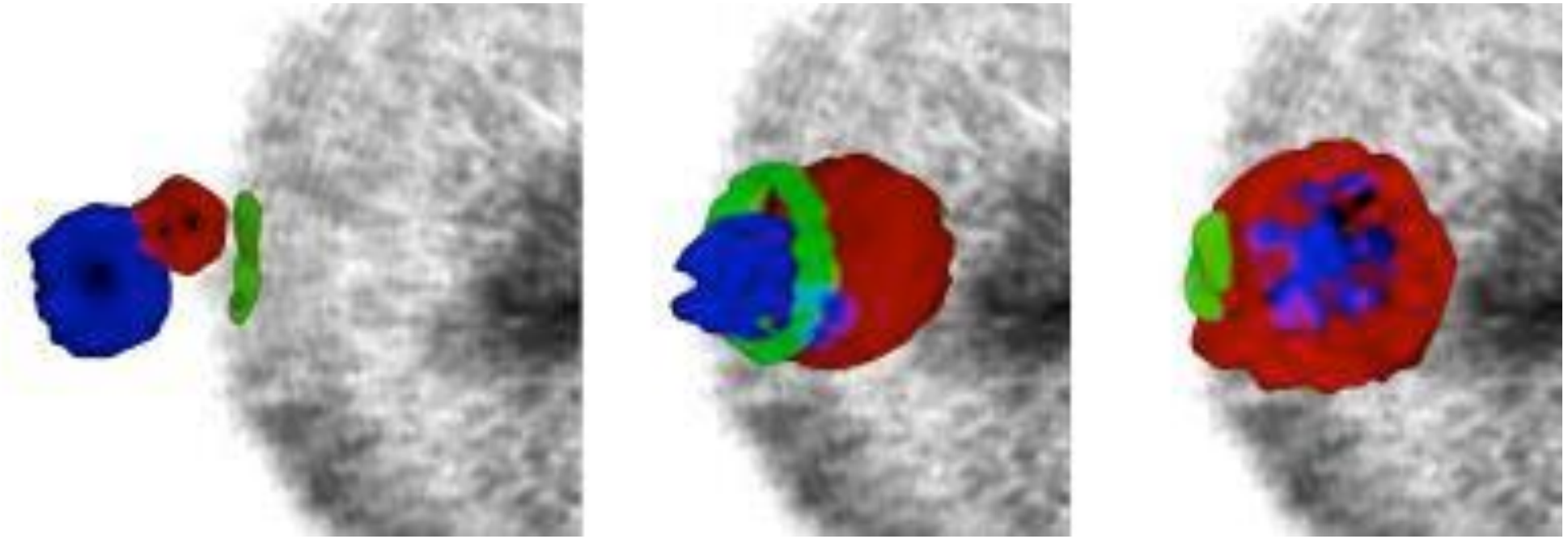
- Malaria KHARP protein trafficked to periphery of red blood cell
- Component of “knob” complex
- Causes deformation of red blood cell and “stickiness”
- OMX 3D-SIM has enabled observation of KHARP distribution in 3D
- Density increases during infection
- DNA labelled with DAPI (red)





Malaria invasion of red blood cell

- Invasion process revealed by OMX-3D SIM



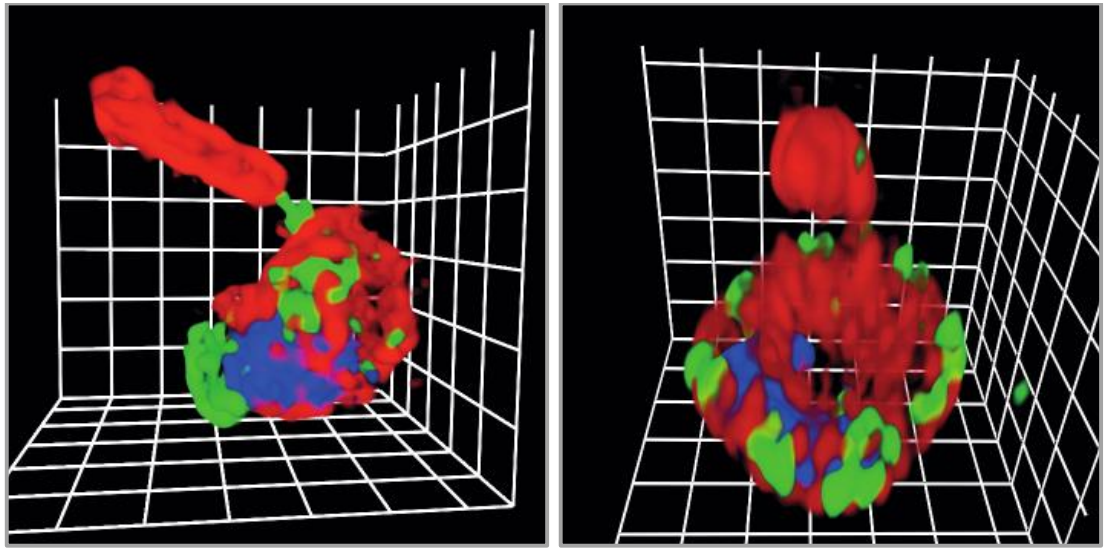
Parasite DNA (DAPI), rhoptry protein that becomes vacuole, tight junction between parasite & red blood cell

Riglar *et al.* (2011) Super resolution dissection of coordinated events behind malaria parasite invasion of the human erythrocyte, *Cell Host Microbe* **9(1):** 9-20

Spatial localisation of proteins in *P. falciparum*

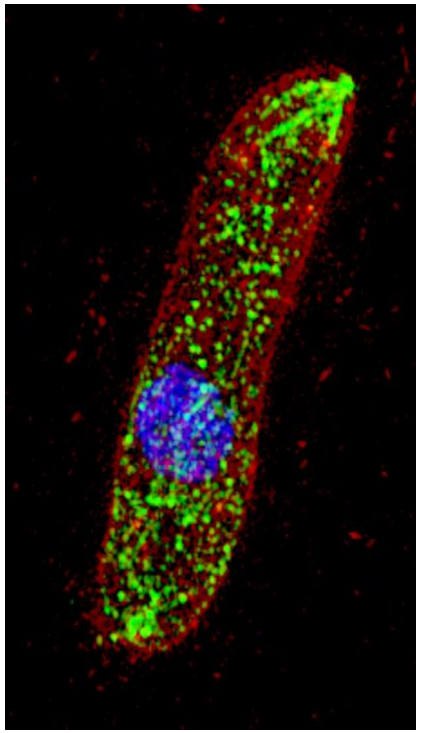
<12 min

60-90 min



Putative translocon (EXP2)
Vacuole (RAP1) Nucleus (DAPI)

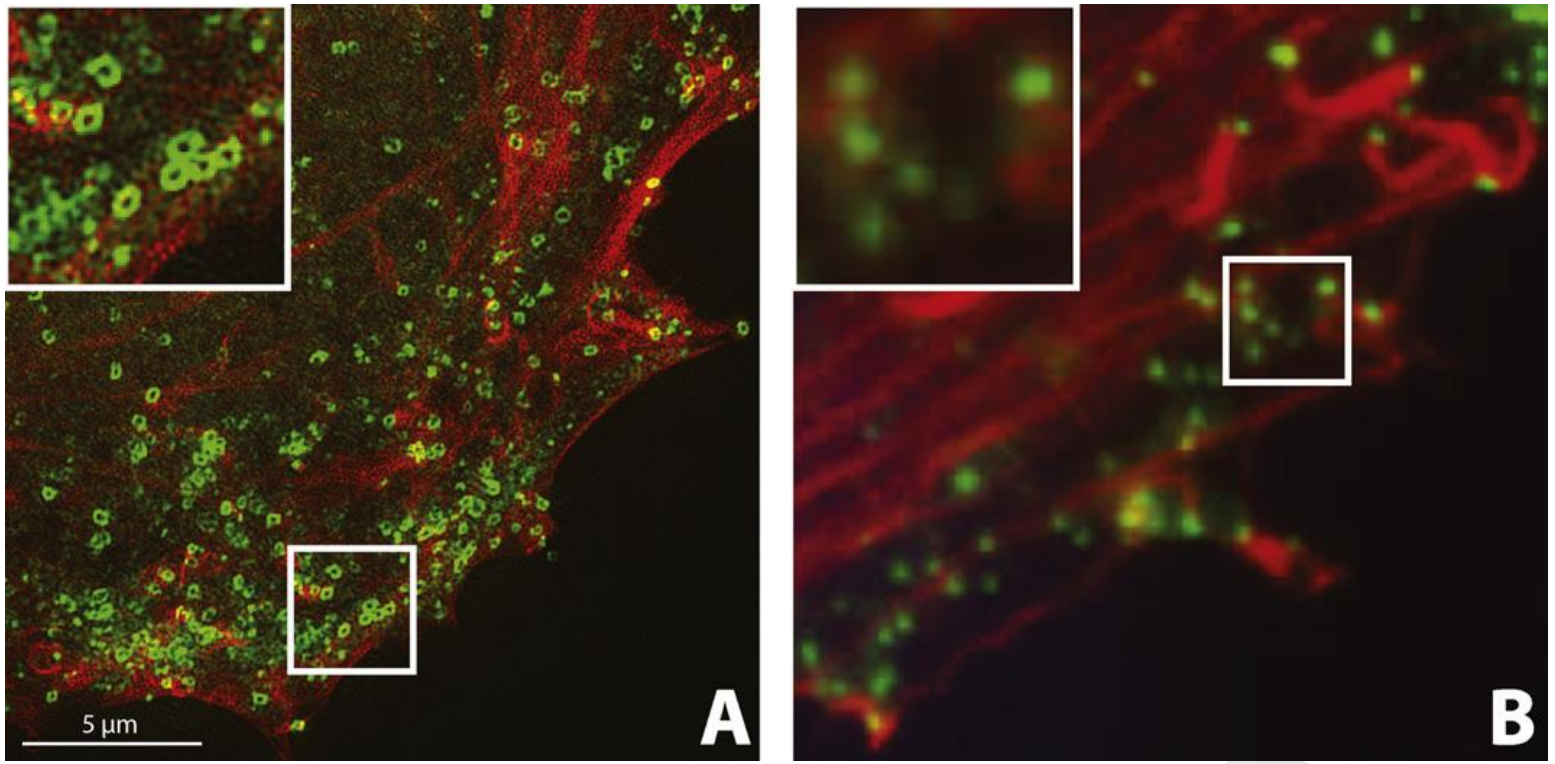
Riglar, *et al.* (2013) Spatial association with PTEX complexes defines regions for effector export into *Plasmodium falciparum*-infected erythrocytes, *Nature Communications*, **4:** 1415



Parasite actin, MPB28 (surface marker), Nucleus (DAPI)

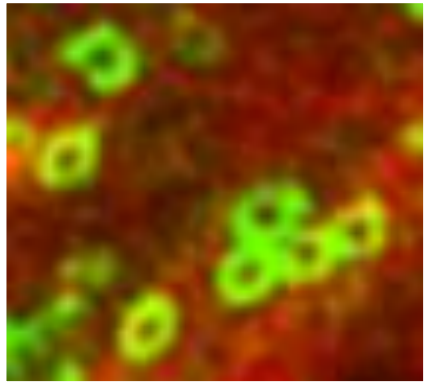
Angrisano, *et al.* (2012) Spatial localisation of actin filaments across developmental stages of the malaria parasite, *PLoS One*: **7(2):** e32188

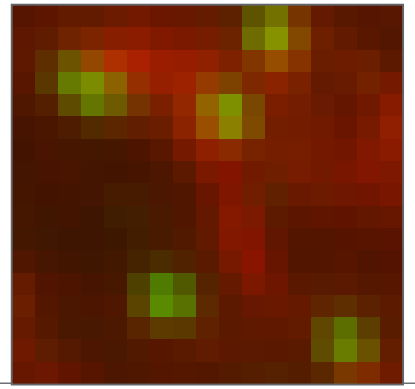
Vaccinia virus egress from a cell



B5

Actin

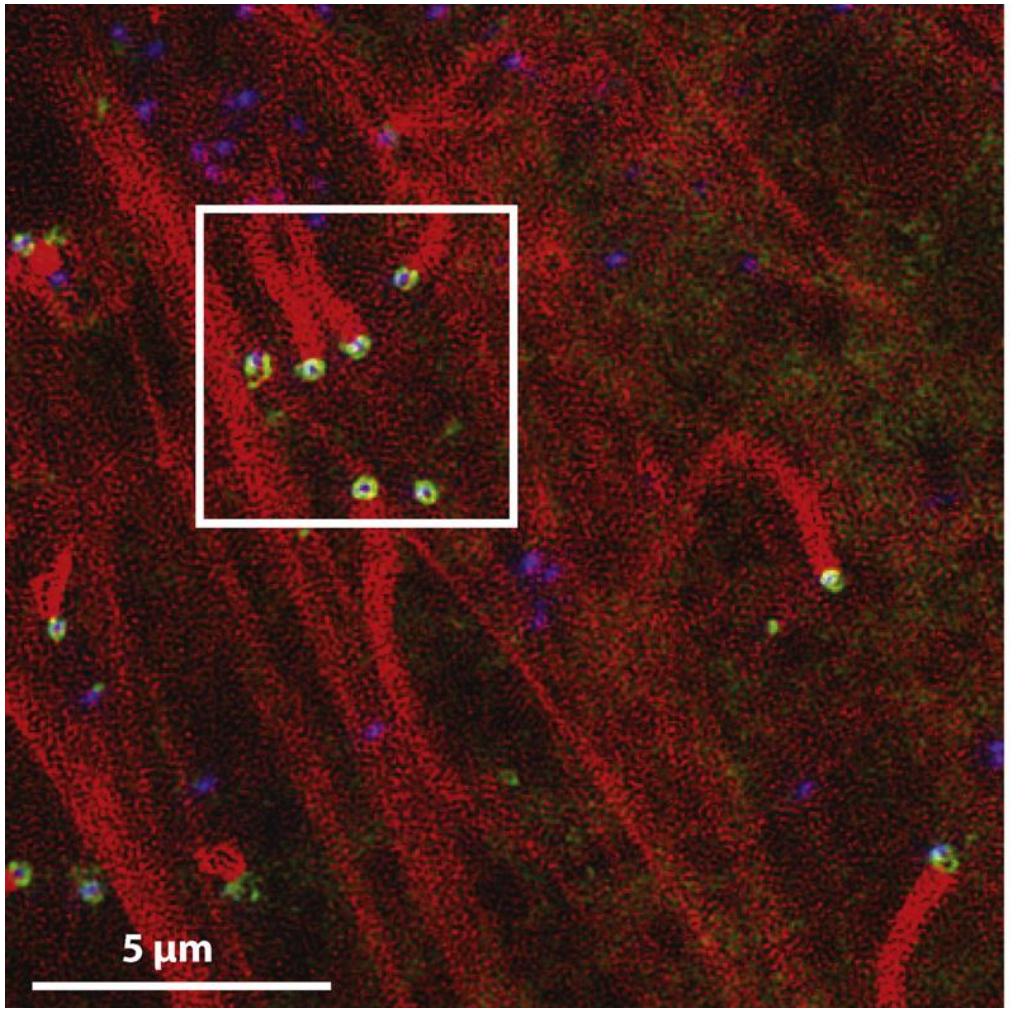


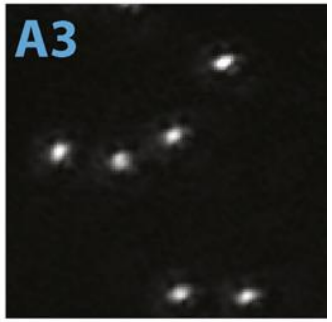


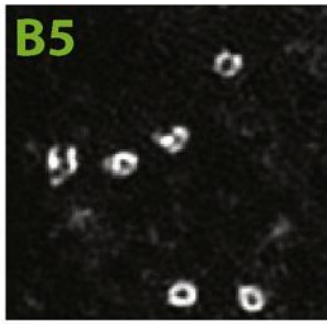
Horsington, *et al.,* (2012) Sub-viral imaging of vaccinia virus using super-resolution microscopy, *J Virological Methods*, 186(1-2): 132-136.

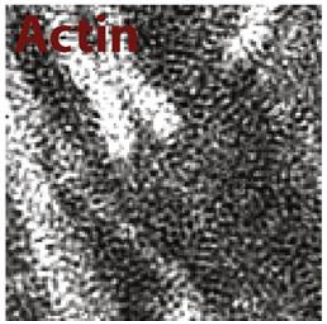
Vaccinia locomotion

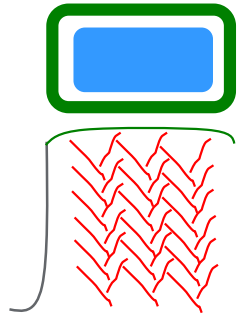
Actin (Lifeact) **B5** **A3**





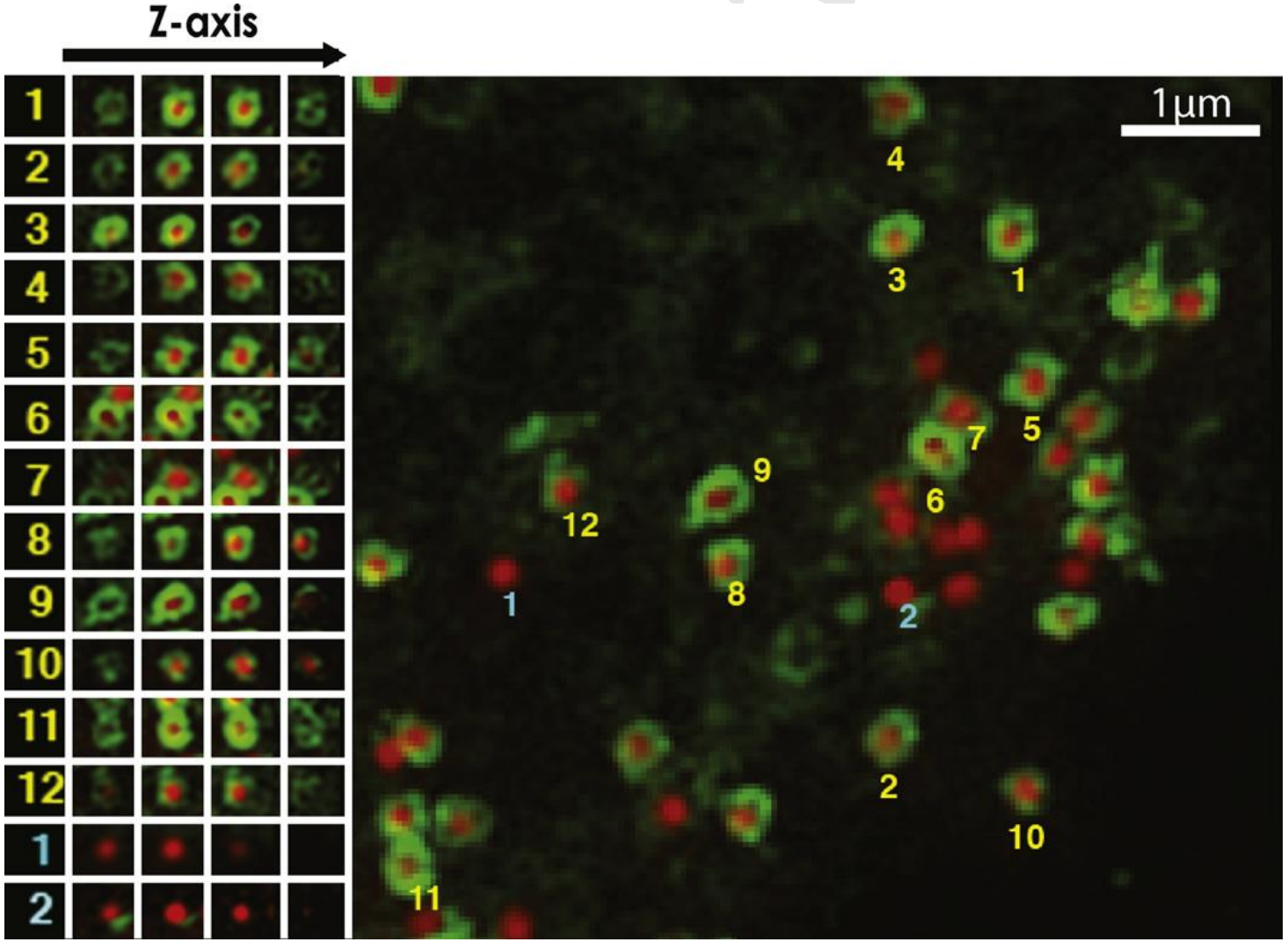






Horsington, *et al.,* (2012) Sub-viral imaging of vaccinia virus using super-resolution microscopy, *J Virological Methods*, 186(1-2): 132-136.

Virus maturation

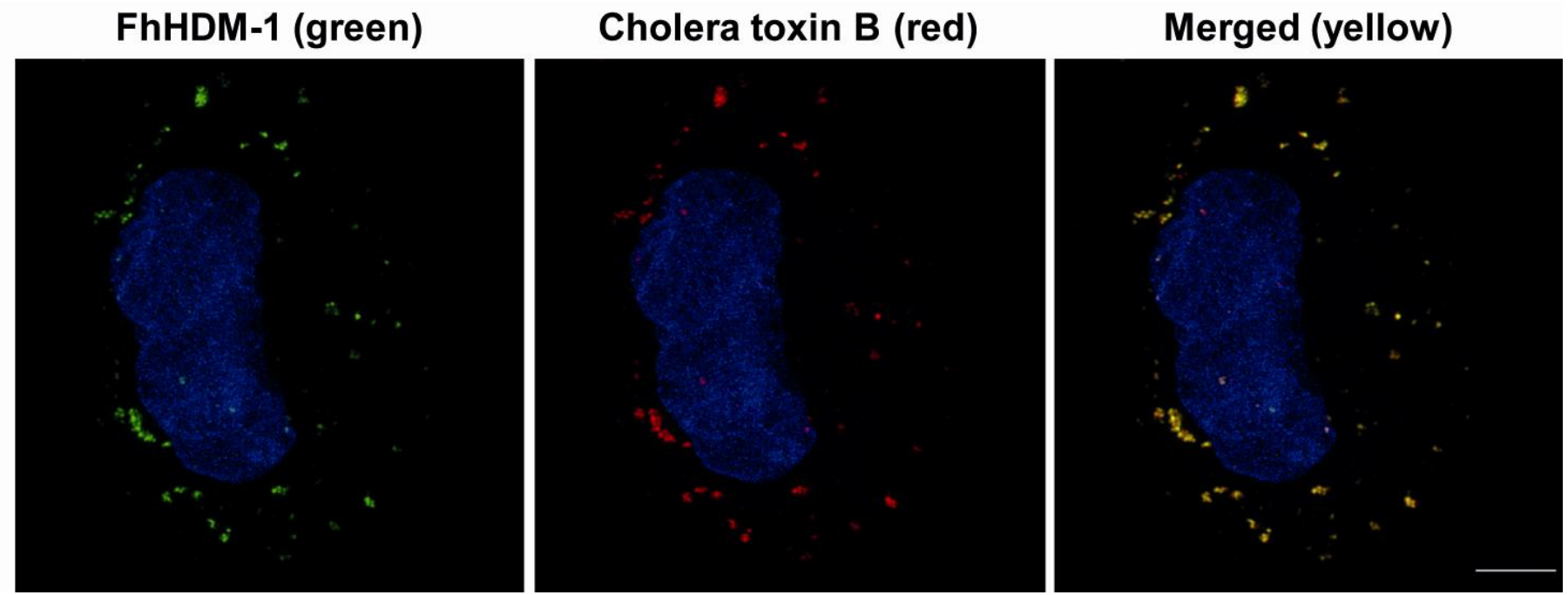


MV A3

WV B5

Horsington, J. *et al.*, (2013) A36-dependent actin filament nucleation promotes release of vaccinia virus, *PLoS Pathogens*, 9 (3): e1003239

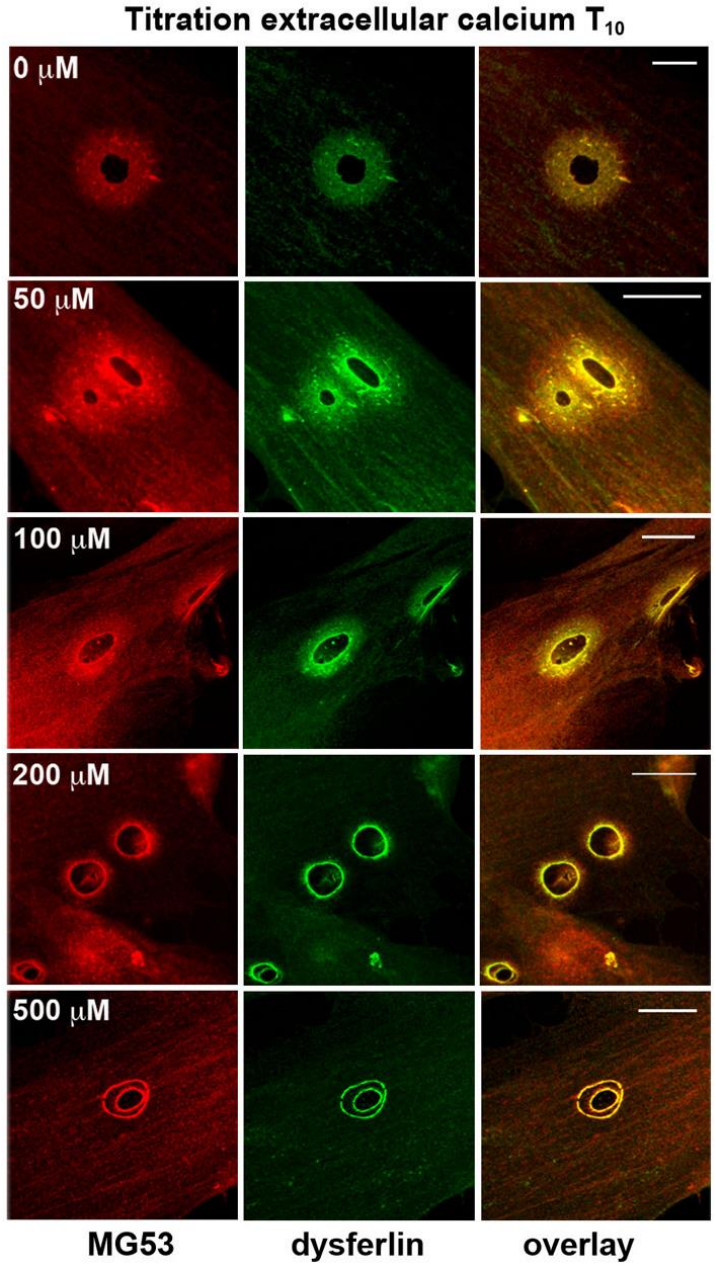
Helminth proteins interact with lipid rafts



Fasciola hepatica helminth defence molecule 1 (FhHDM-1)-treated macrophages were immunostained for the presence of FhHDM-1 (green) and stained with Alexa Fluor 594-CT-B conjugate (lipid raft marker; red) and with DAPI (blue) to detect nuclei. Scale bar = 3 μm.

Robinson *et al* (2012) A helminth Cathelicidin-like peptide prevents antigen processing and presentation in macrophages via inhibition of lysosomal vATPase, *FASEB J*, 26 (11): 4614-27.

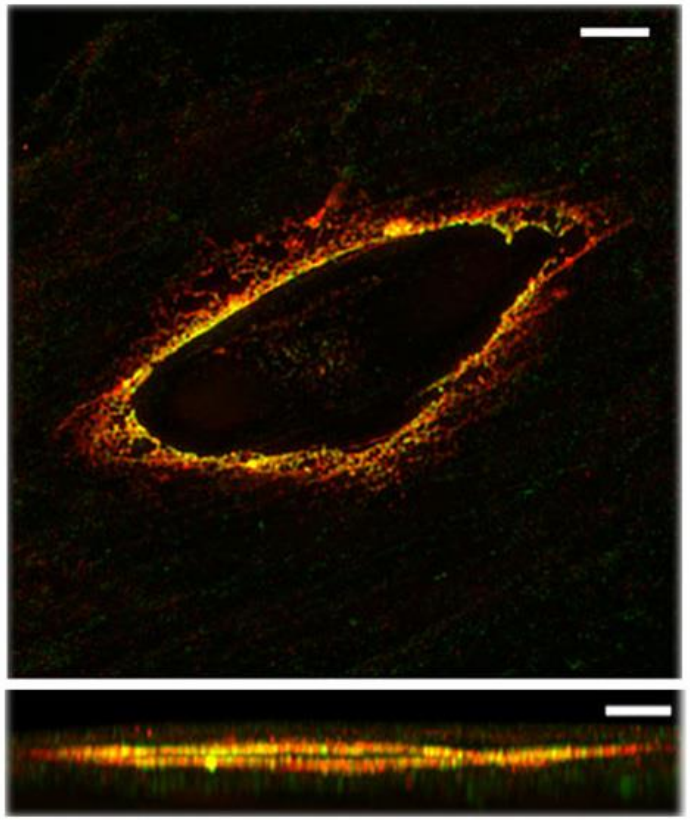
Muscular Dystrophy



- Dysferlin was discovered as a recessive cause of Limb-girdle Muscular Dystrophy (LGMD) in 1999
- Patients are typically symptom free until their late teens, after which they show rapid deterioration. Following the onset of deterioration, patients are often wheelchair bound within 10 years
- Dysferlin is a vesicle fusion protein with a role in membrane repair. Dysferlinopathy is due to an inability of muscle cells to effectively repair small sites of damage accrued during muscle contraction
- **Dysferlin and MG53 transition from a 'diffuse halo' to concentrated rings labelling the circumference of injury sites with ≥ 200 mM extracellular calcium**

Lek, *et al.* (2013) Calpains, cleaved mini-dysferlin$_{C72}$ and L-type channels underpin calcium-dependent muscle membrane repair, *Journal of Neuroscience*, **33 (12):** 5085-94

Calcium regulates wound closure by dysferlin and MG53



MG53 Dysferlin

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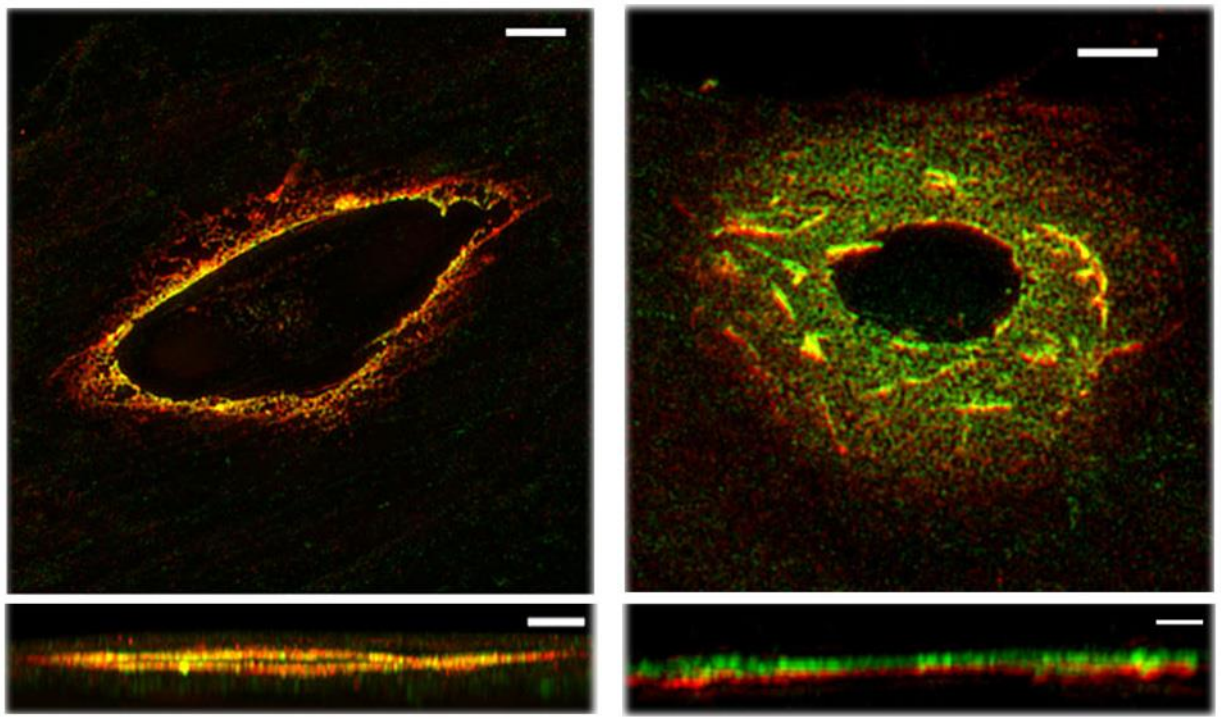
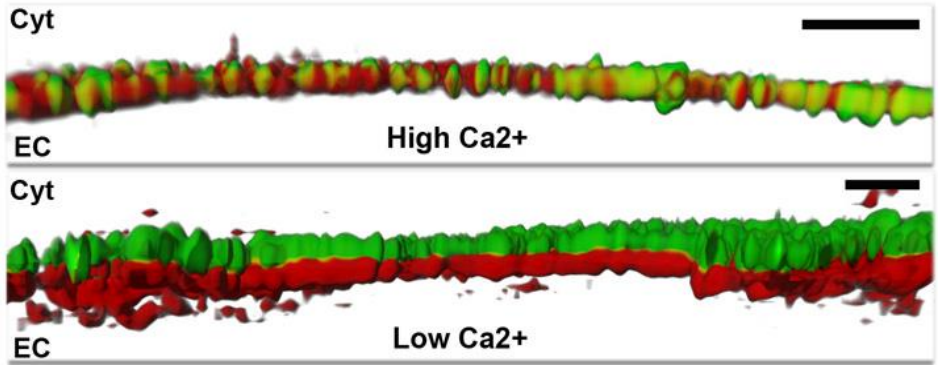


MG53 Dysferlin



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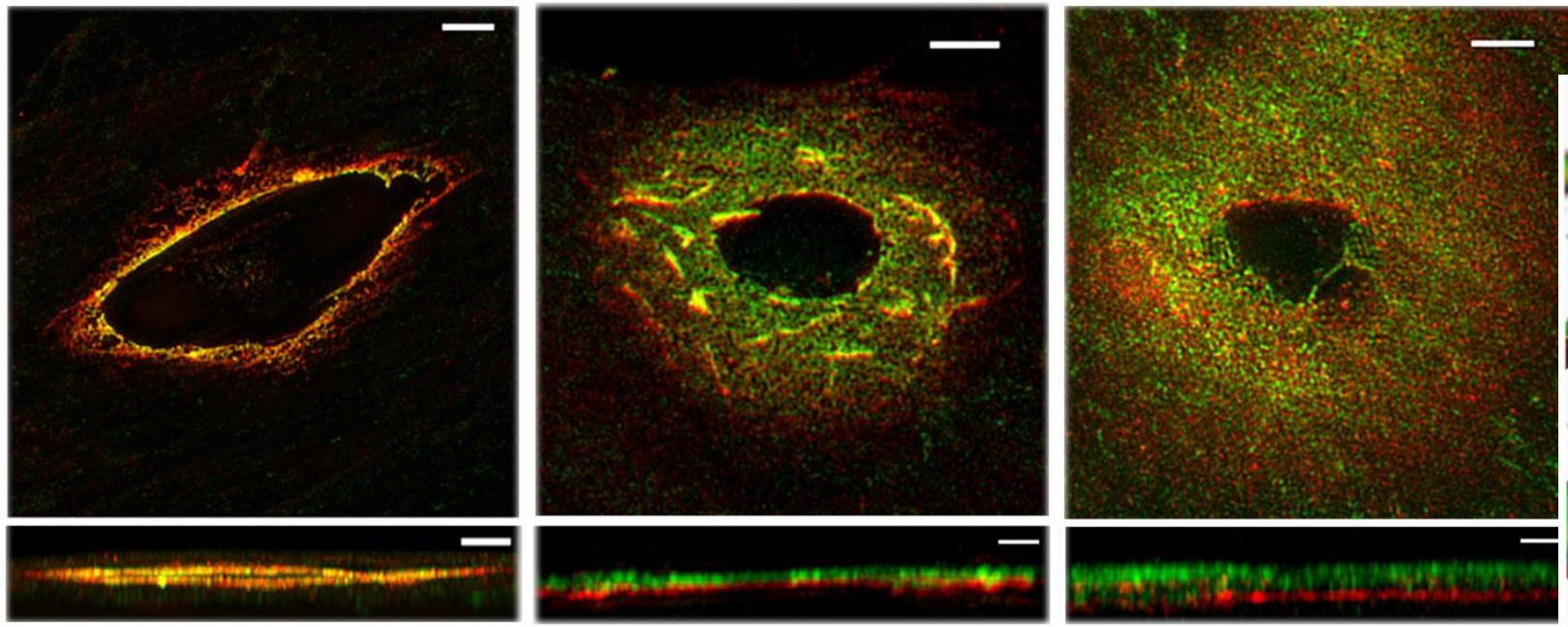
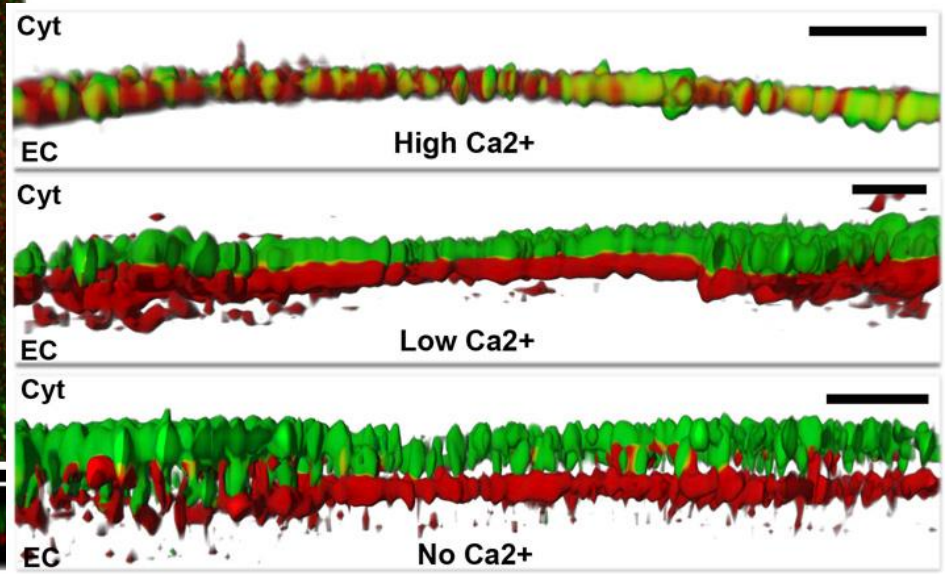


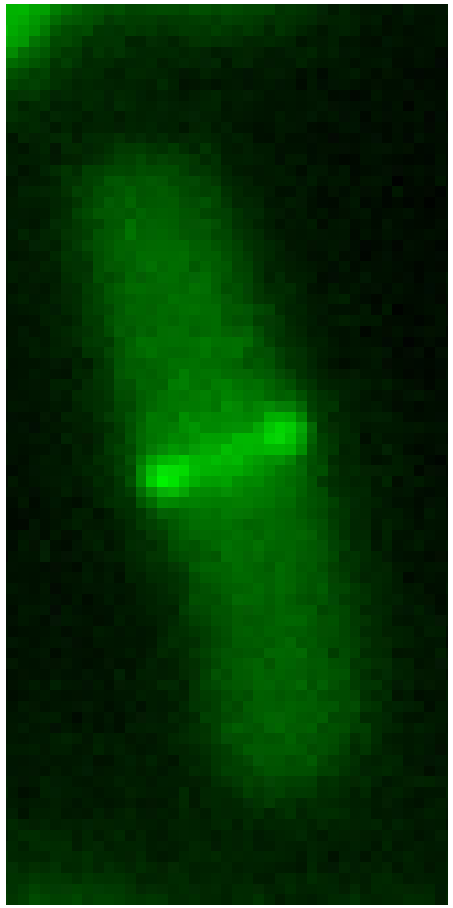


MG53 **Dysferlin**

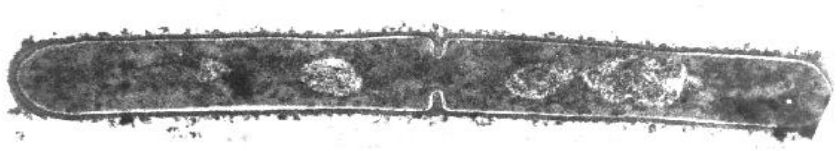
Lek, *et al.* (2013) Calpains, cleaved mini-dysferlin$_{C72}$ and L-type channels underpin calcium-dependent muscle membrane repair, *Journal of Neuroscience*, **33 (12):** 5085-94

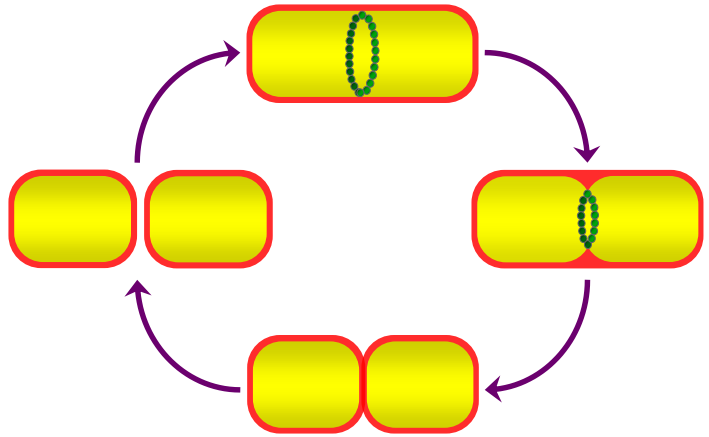
Biology using live samples

Bacterial cell division – the Z-ring



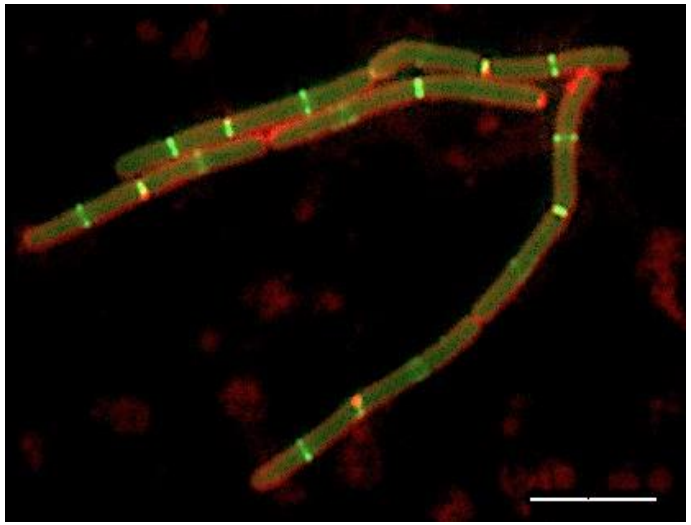
FtsZ-GFP in *Bacillus subtilis*

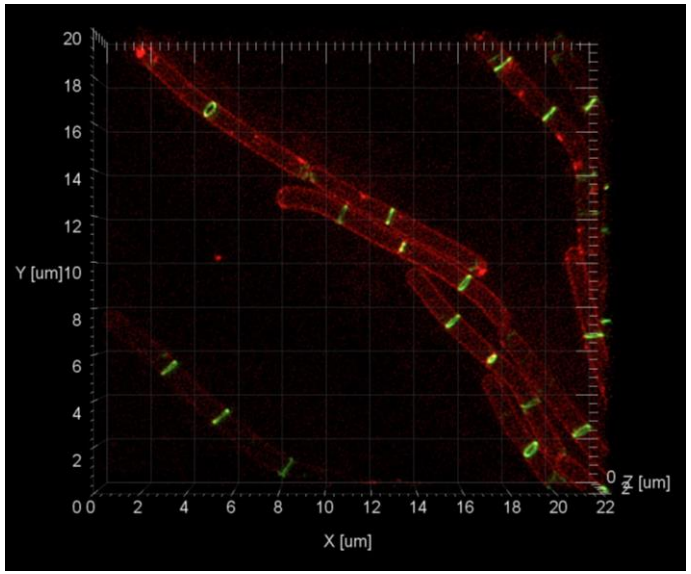


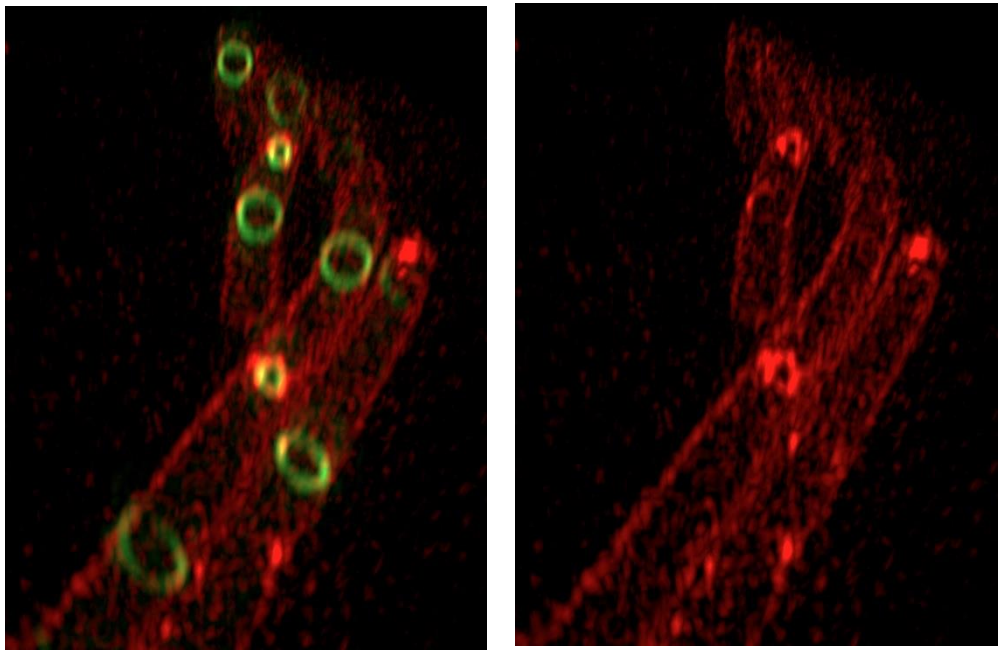


L. Harry, M. Strauss, C. Whitchurch, L. Turnbull, UTS

The Z ring - FtsZ-GFP





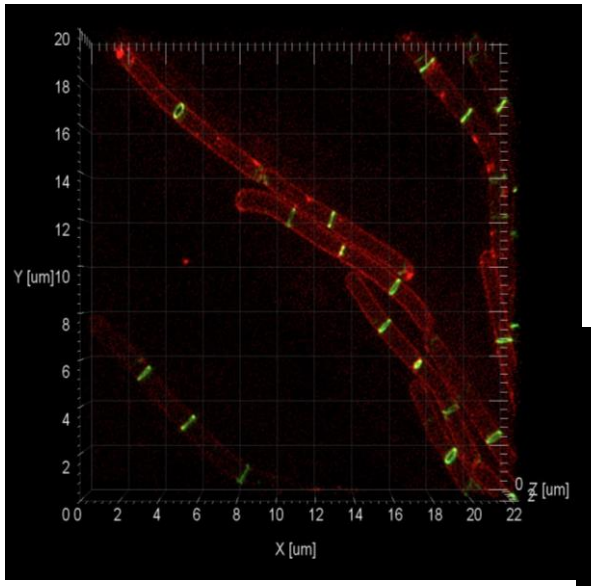


Bacillus subtilis, FtsZ-GFP, FM143 lipid stain

Strauss, *et al.* (2012) 3D-SIM super resolution microscopy reveals a bead-like arrangement for FtsZ and the division machinery: implications for triggering cytokinesis *PLoS Biology* **10(9):** e1001389

Bacterial cell division – the Z-ring

- Heterogeneous distribution of FtsZ-GFP (live and fixed)
- Gaps in the Z-ring → discontinuous structure



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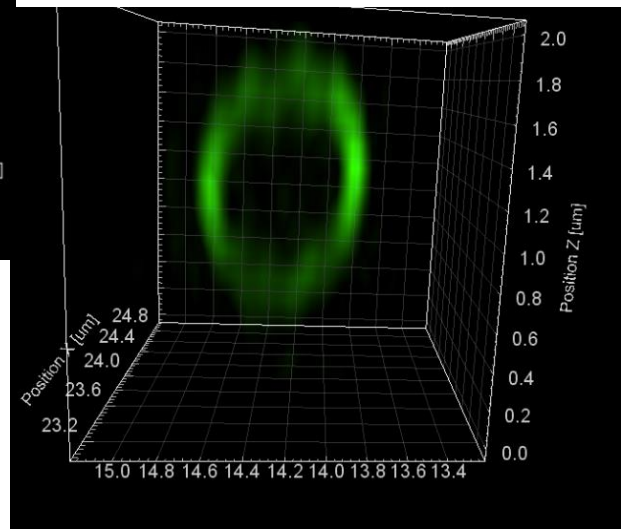
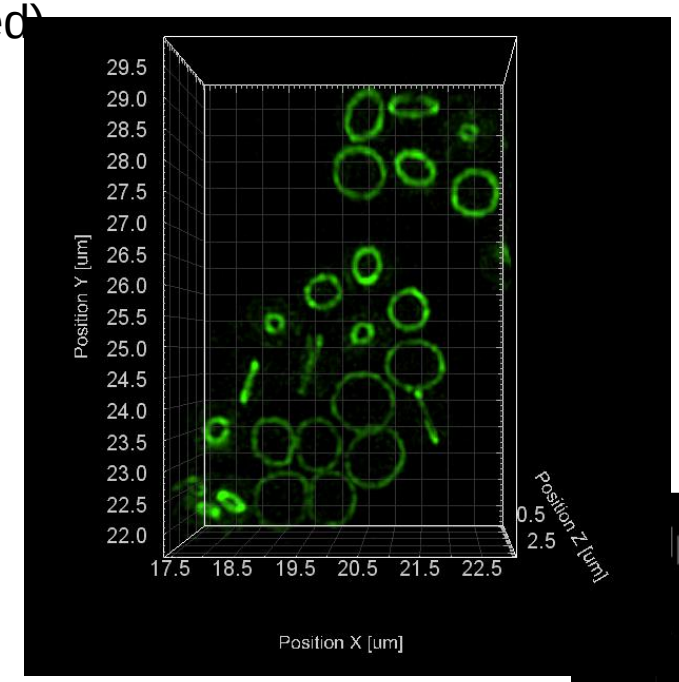
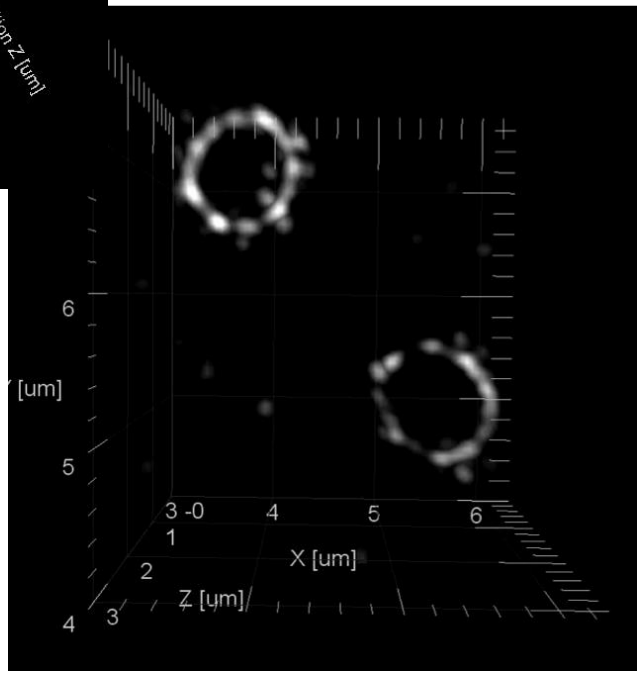




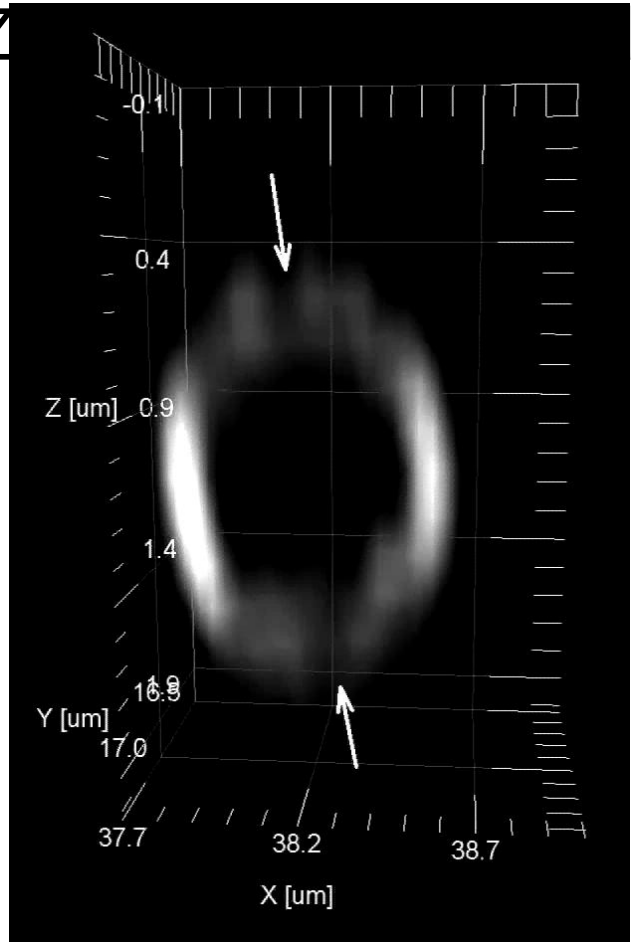
Staphylococcus aureus, FtsZ-GFP,

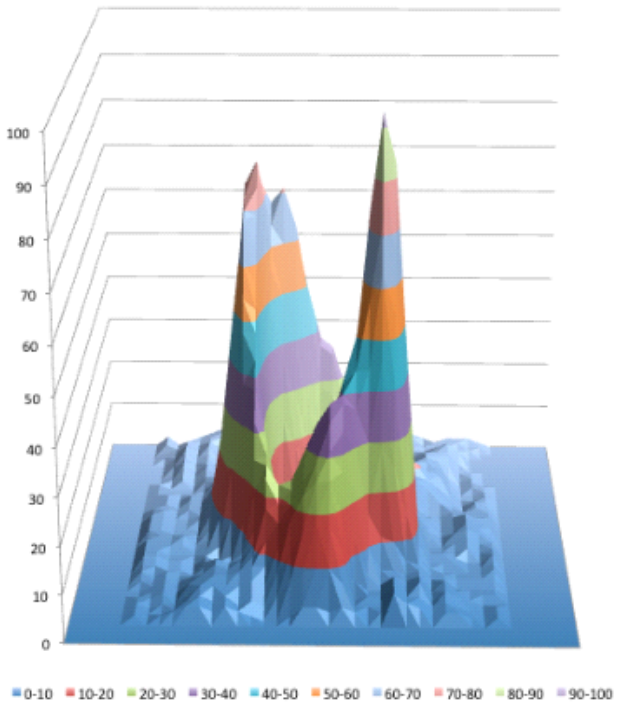


Strauss, *et al.* (2012) 3D-SIM super resolution microscopy reveals a bead-like arrangement for FtsZ and the division machinery: implications for triggering cytokinesis *PLoS Biology* **10(9):** e1001389

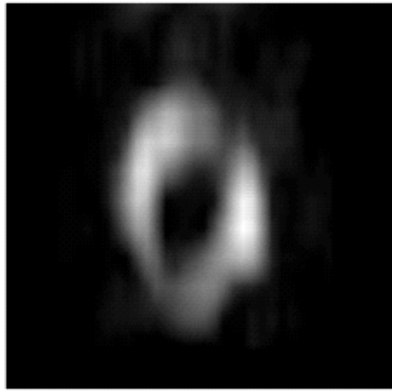
Bacterial cell division – the Z-ring

- Fast acquisition (3D-SIM image capture every 5 – 10 sec).
- FtsZ the Z rin





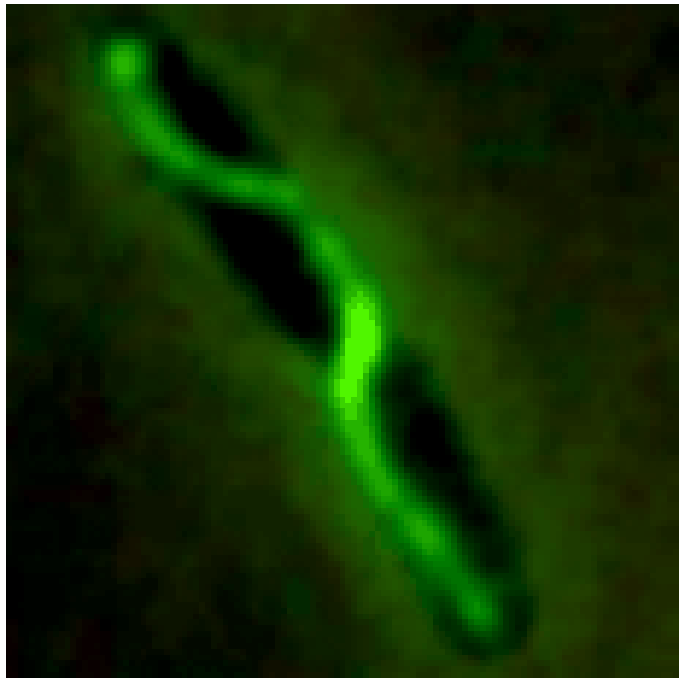
Time = 0 sec



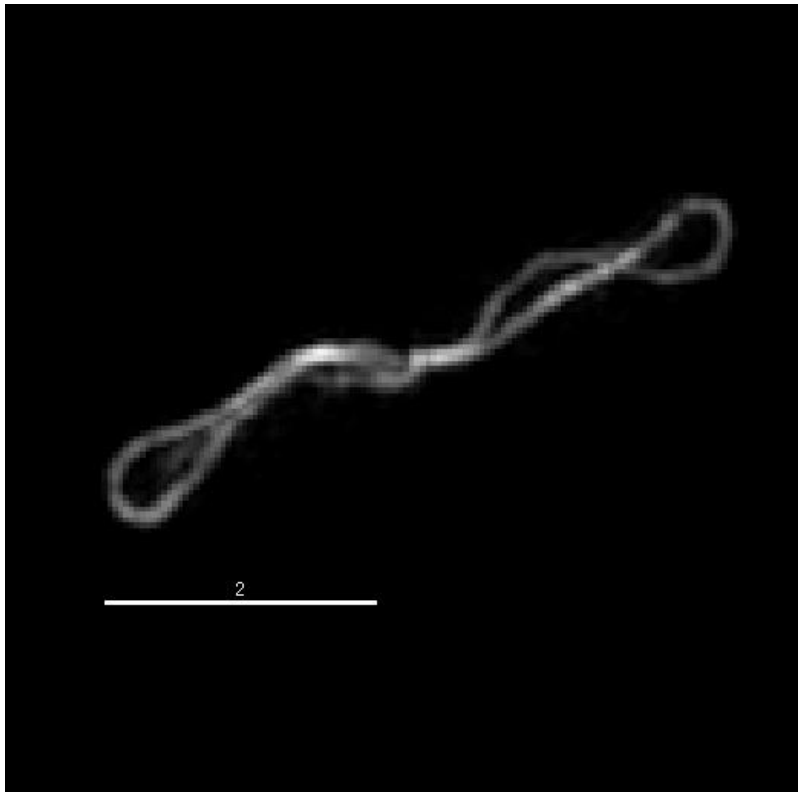
Strauss, *et al.* (2012) 3D-SIM super resolution microscopy reveals a bead-like arrangement for FtsZ and the division machinery: implications for triggering cytokinesis *PLoS Biology* **10(9):** e1001389

Live imaging of bacterial cells

Conventional



OMX 3D-SIM

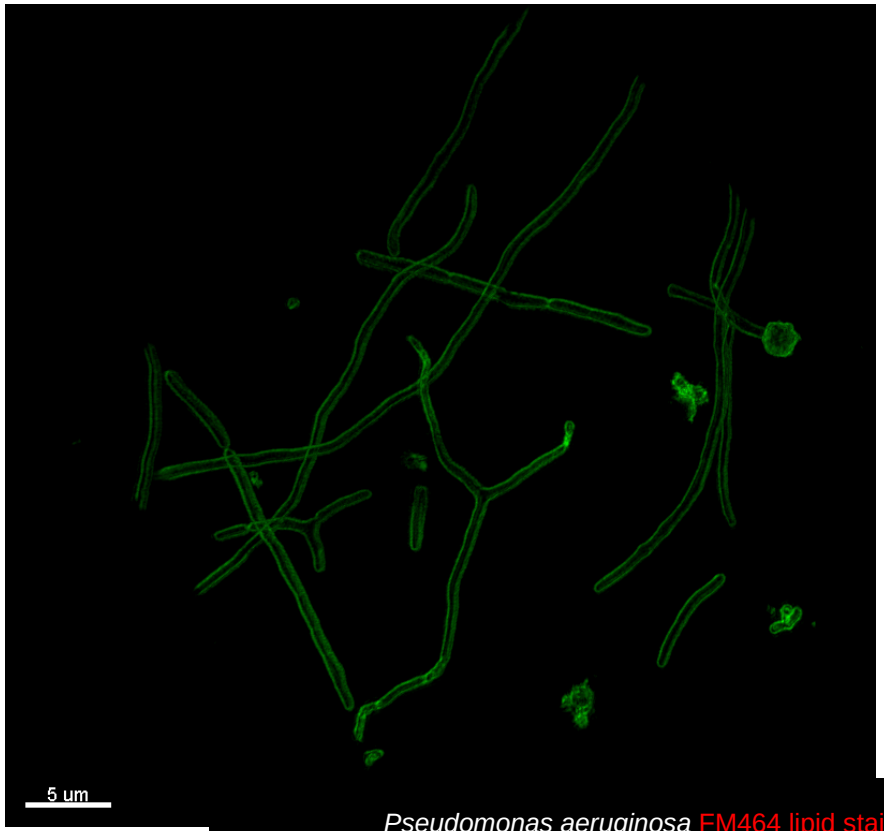


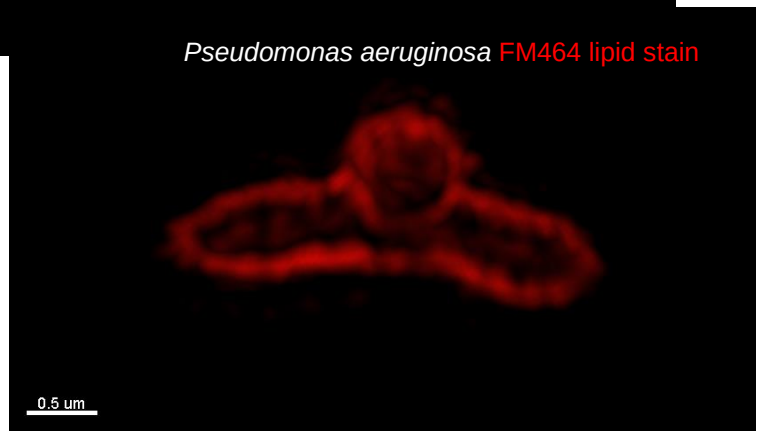
ParM is a twisted loop

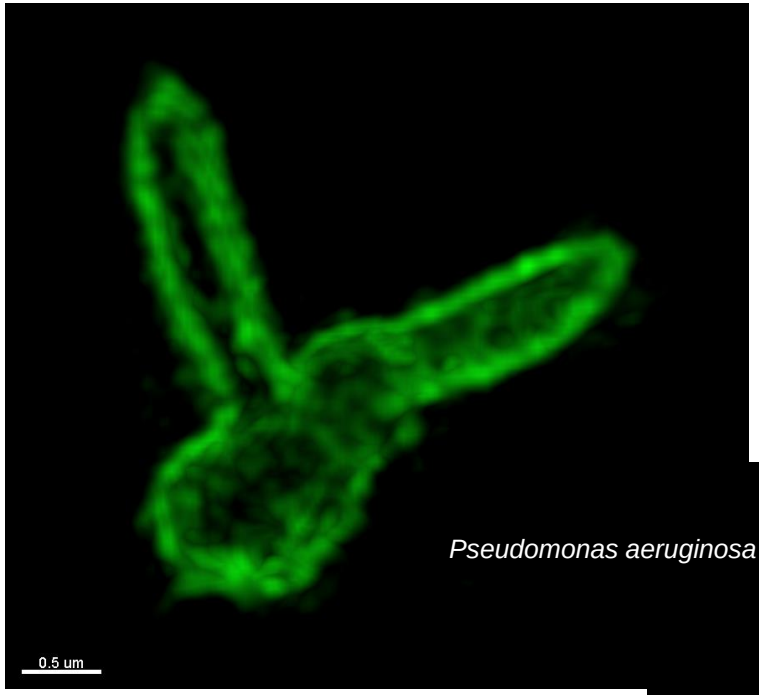
Par M (plasmid partitioning protein) in *E. coli*

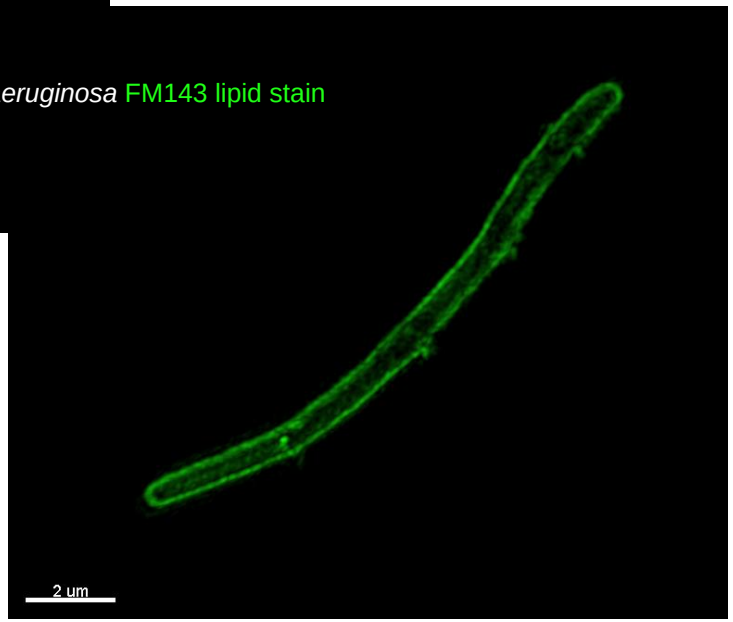
N. Firth, S. Jensen, U Syd; C. Whitchurch, L. Turnbull, UT

Unusual bacterial cell morphology





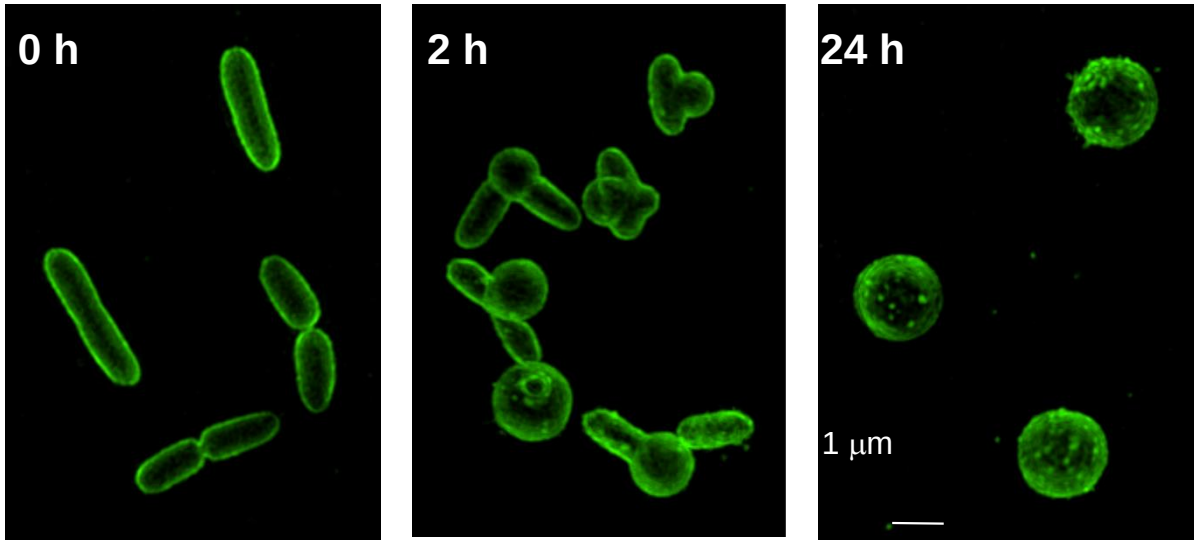




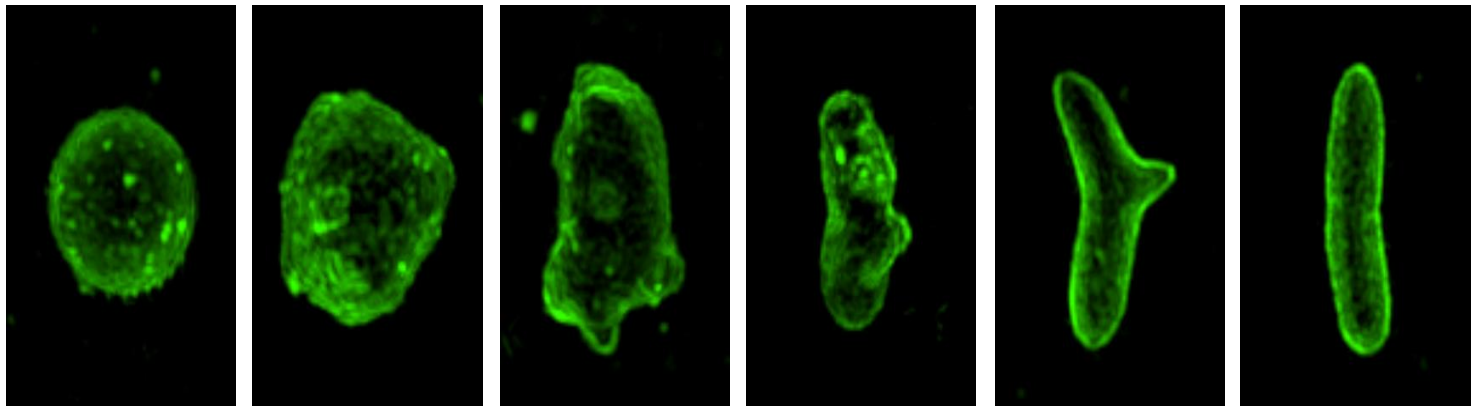
Whitchurch, Turnbull, Monahan, Osvath, UTS

Live interactions of cells with β-lactam antibiotics

Pseudomonas aeruginosa treatment with meropenam FM143 lipid stain



Pseudomonas aeruginosa after removal of meropenam FM143 lipid stain



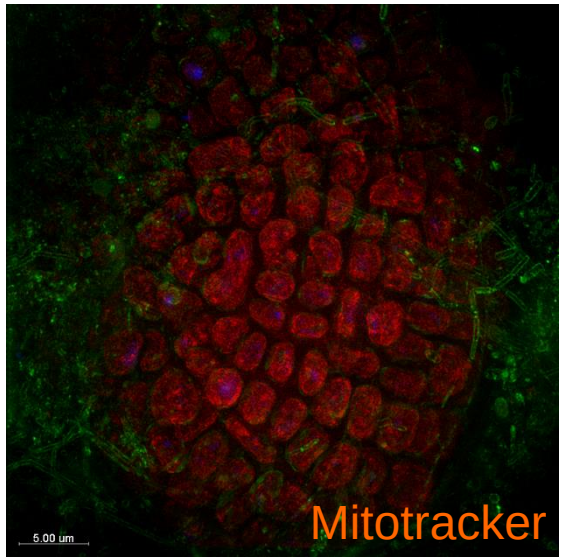
Monahan *et al.*,(2014) Rapid conversion of *Pseudomonas aeruginosa* to a spherical cell morphotype facilitates tolerance to carbapenems and penicillins but increases susceptibility to antimicrobial peptides, *Antimicrobial Agents Chemo*, 58(4): 1956-62

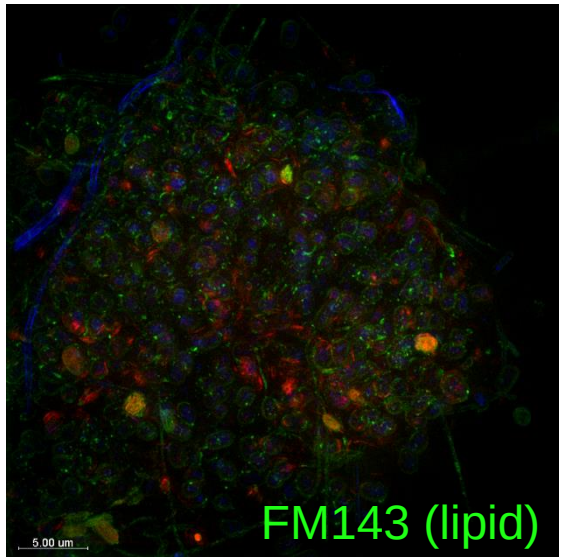
Stromatolite Communities

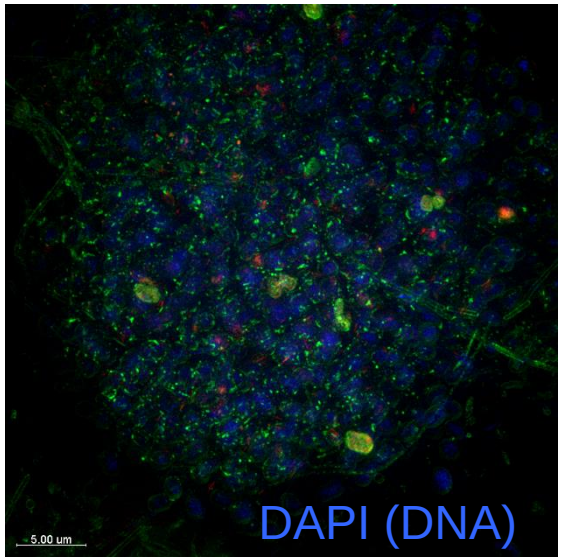
Living fossils
Shark Bay, WA

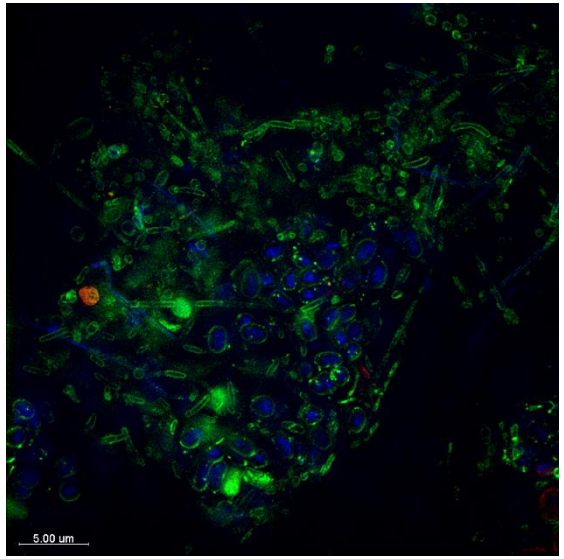
Cyanobacteria

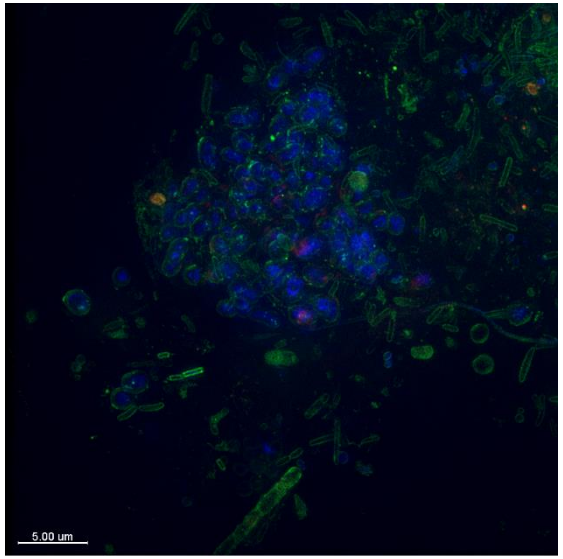
Stromatolite Communities

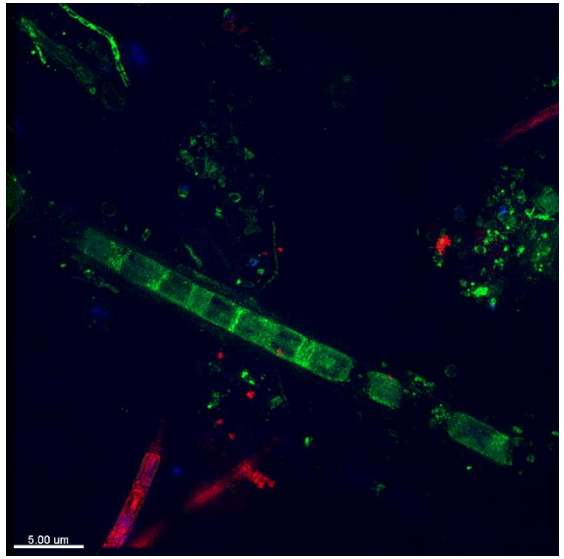












Schliep, Larkum, Ralph, Whitchurch, Turnbull, UTS

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