Malaria parasites: Blood, drugs and sex

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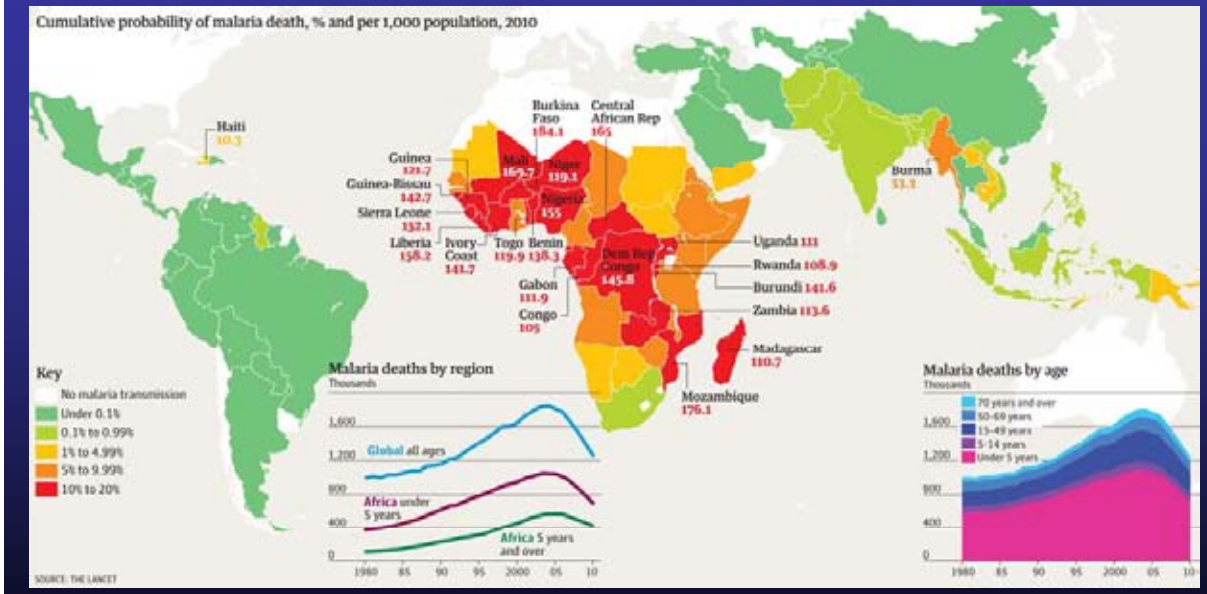
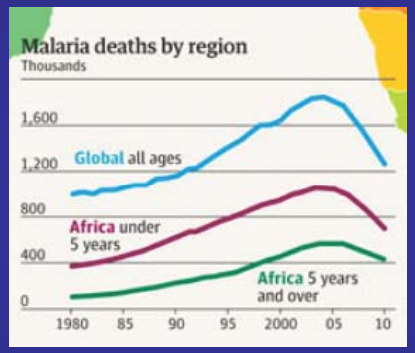
Malaria – a major human killer

- World-wide - 300 million cases/ year; ~ 0.7 – 1.2 million deaths/ year; (>1 death/ 1 minute) (mostly 0-5 yr olds)
- Asia Pacific region - 30 million cases/ year; 42,000 deaths / year

Institute for Health Metrics and Evaluation (IHME); The Lancet, 379, 413 - 31 (2012)



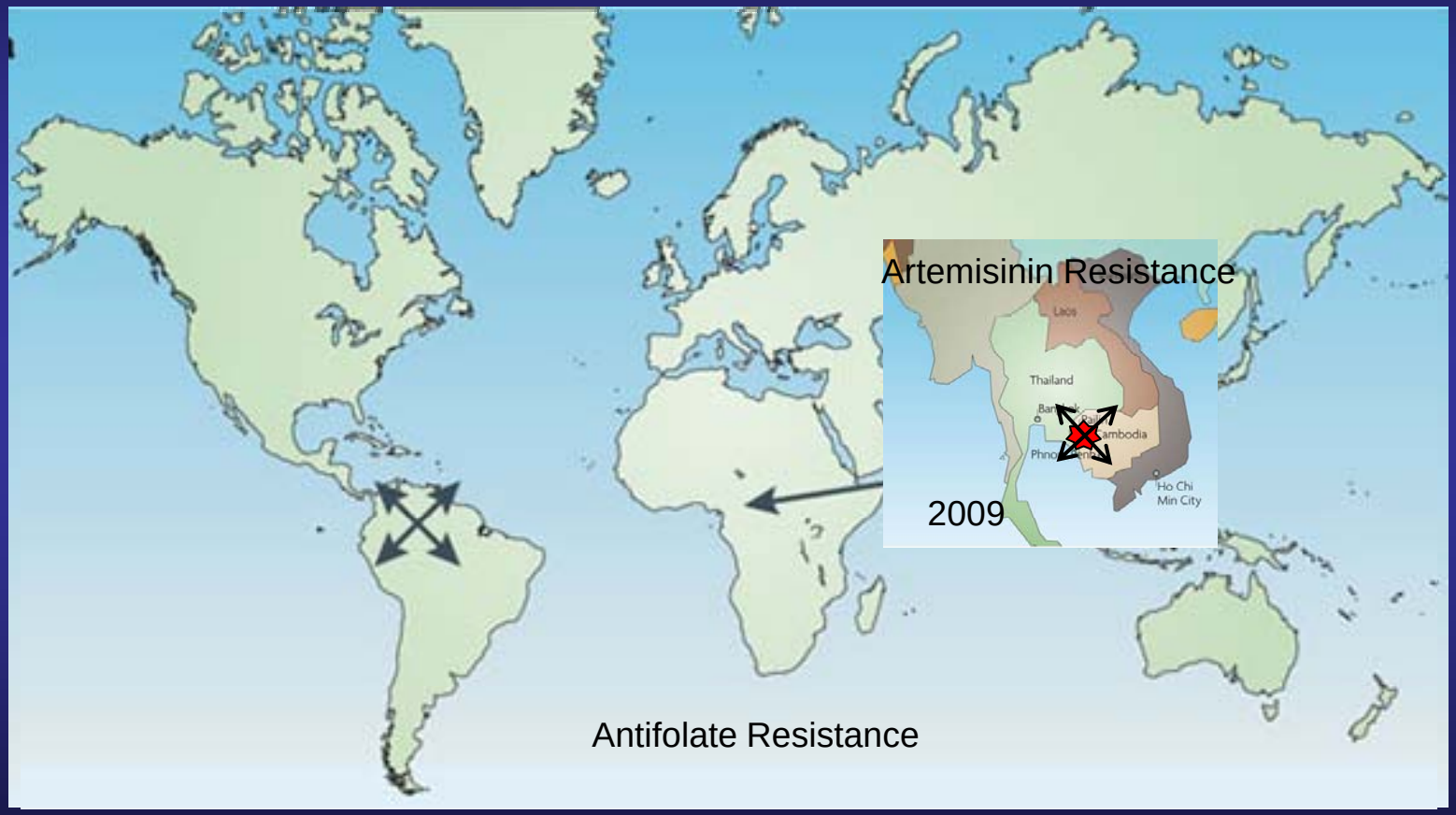


http://www.healthmetricsandevaluation.org/news-events/ihme-in-the-news/global-malaria-mortality-between-1980-and-2010-systematic-analysis

Malaria Treatments

- Vaccine trials have had limited success
- Drug treatment will be the major defence for the foreseeable future
- Drugs that have been used include quinolines, such as chloroquine, and antifolates, such as SP
- Resistance has developed to every drug so far employed
- Current treatment – artemisinin-based combination therapy

Spread of Antimalarial Drug Resistance



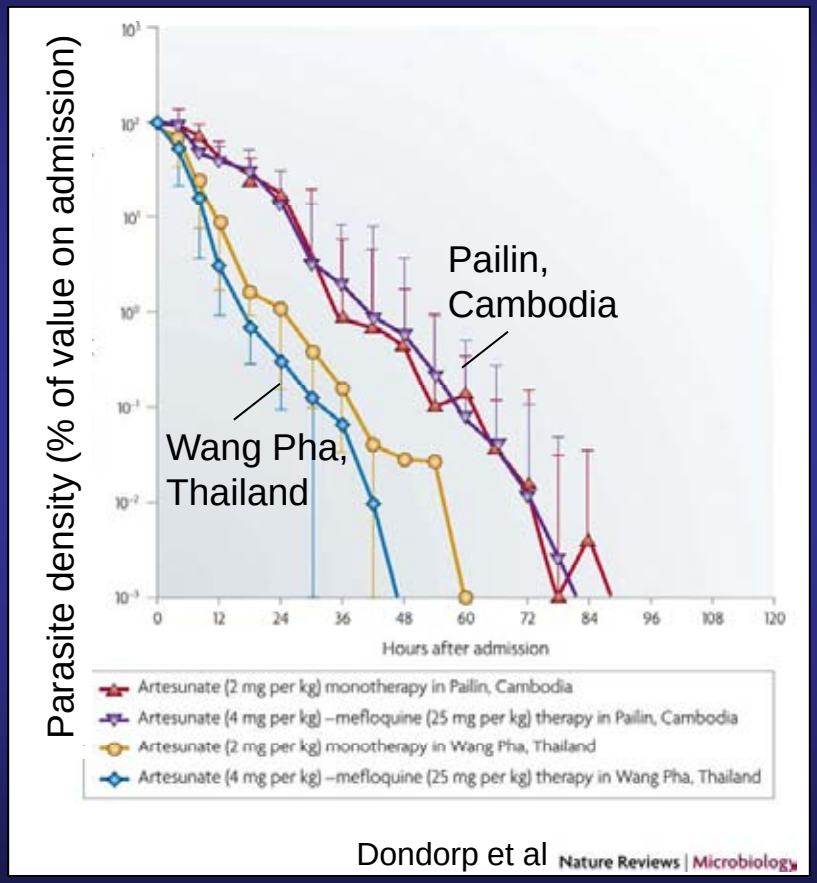
Dondorp et al. Nature Rev Micro (2010)

Artemisinin Resistance

- Current antimalarial control is highly dependent on Artemisinin-based Combination Therapy
- The WHO has launched a Global Plan for Artemisinin Resistance Containment (GPARC)

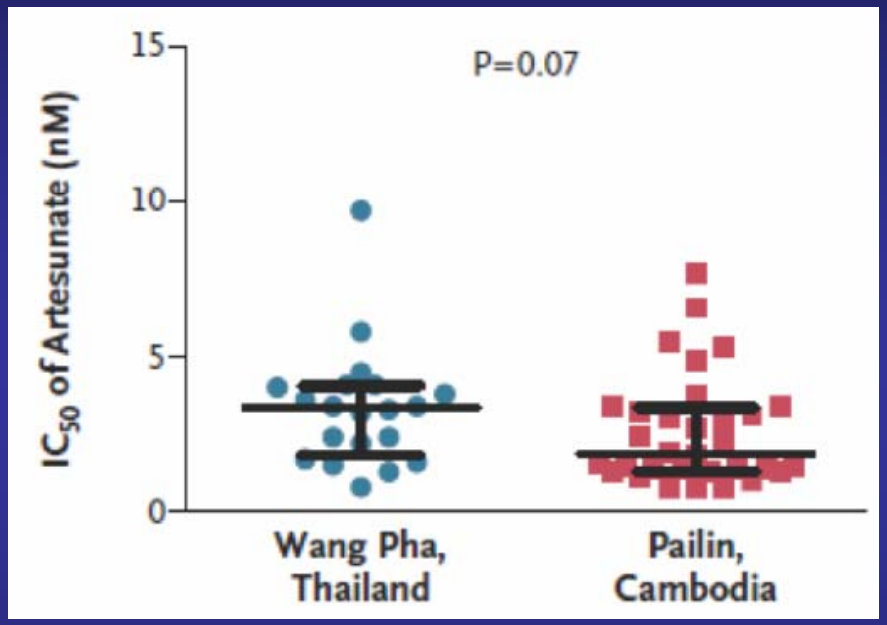
- "There is a finite window of opportunity to contain artemisinin resistance before it spreads. If the current foci of artemisinin-resistant parasites are not contained or eliminated, the costs, both human and financial, could be great".

Assaying Artemisinin Resistance in the Field



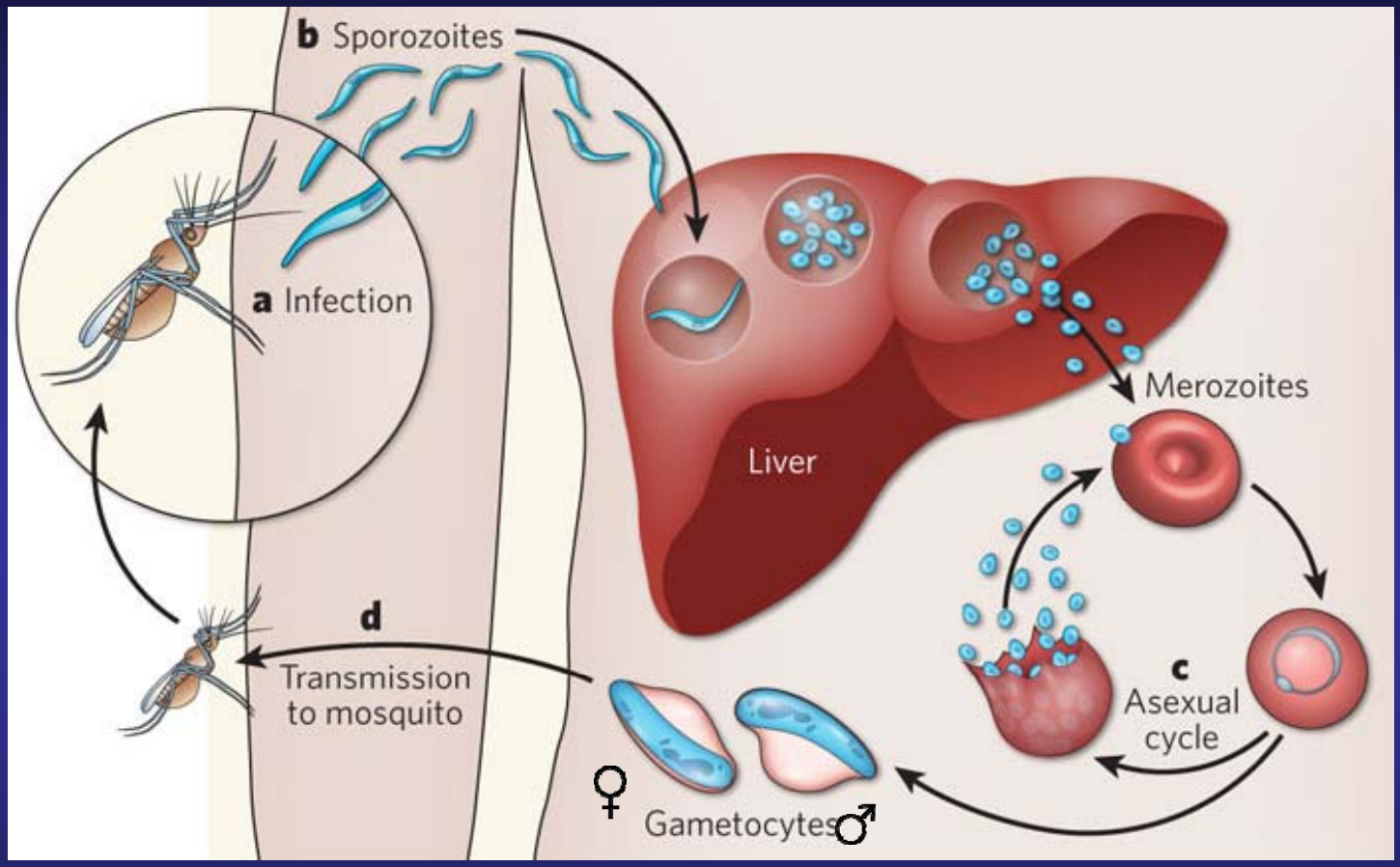
Dondorp et al Nature Reviews | Microbiology

Dondorp et al (2009) *N Engl J Med* **361:** 455-467



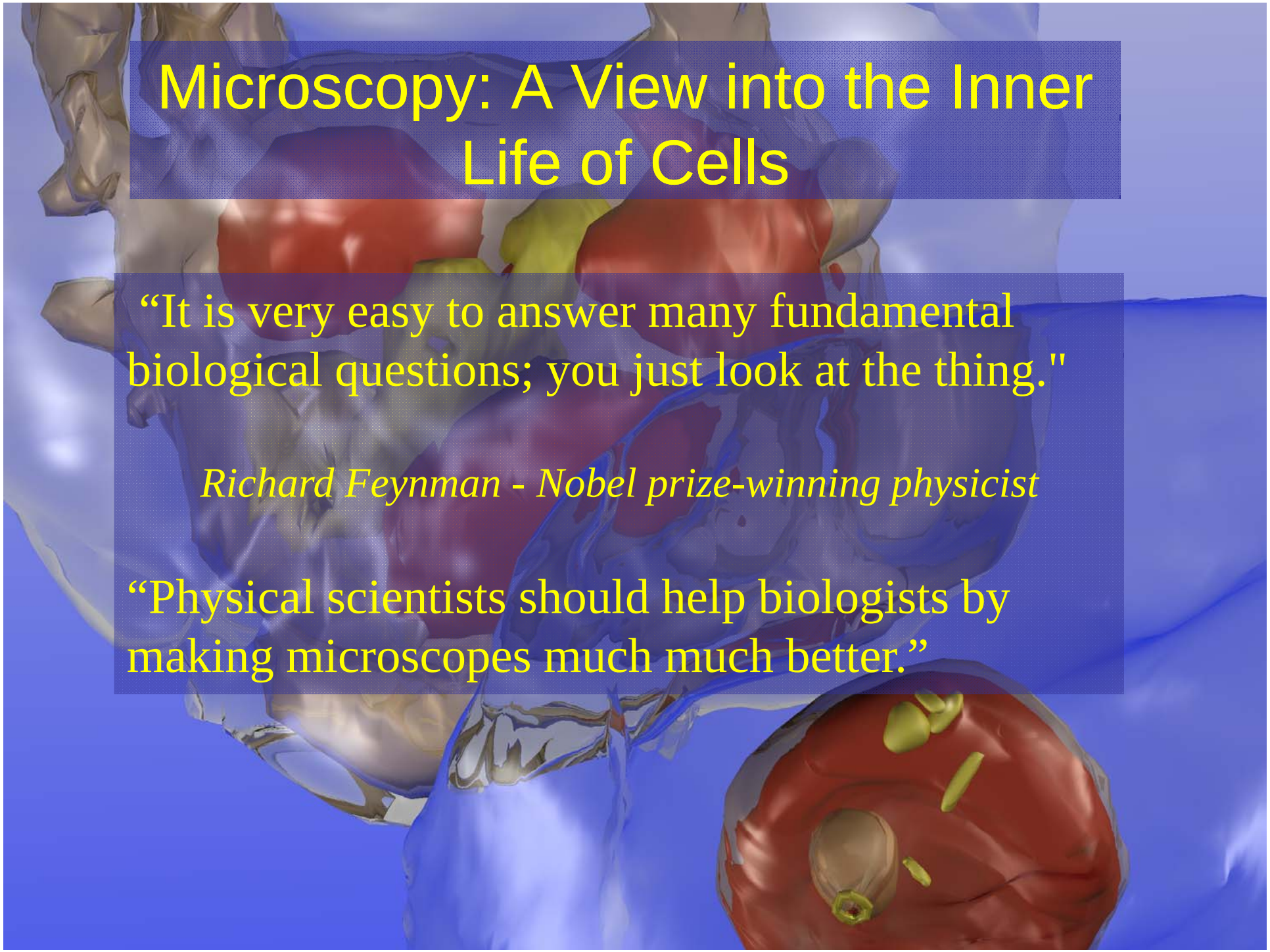
Inhibitory Concentrations for *in vitro* assay of killing over 48 h for strains showing normal and delayed clearance

Lifecycle of *P. falciparum*



Michalakis & Renaud (2009) *Nature*

Blood stages cause pathology and are most susceptible to attack by antimalarials



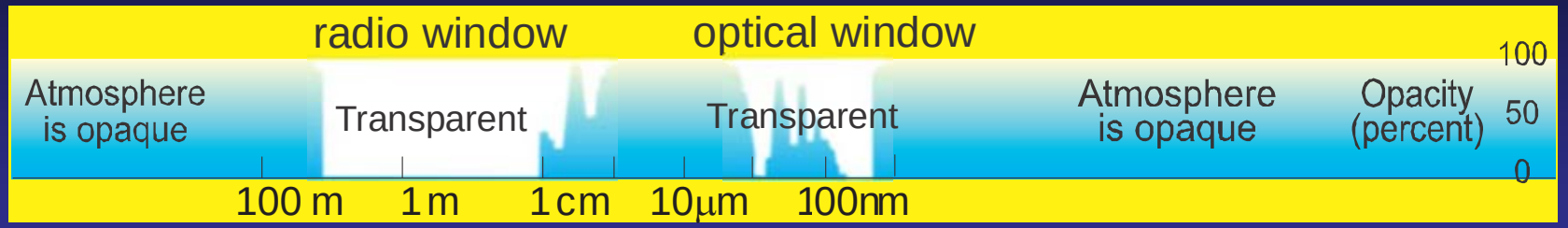
Microscopy: A View into the Inner Life of Cells

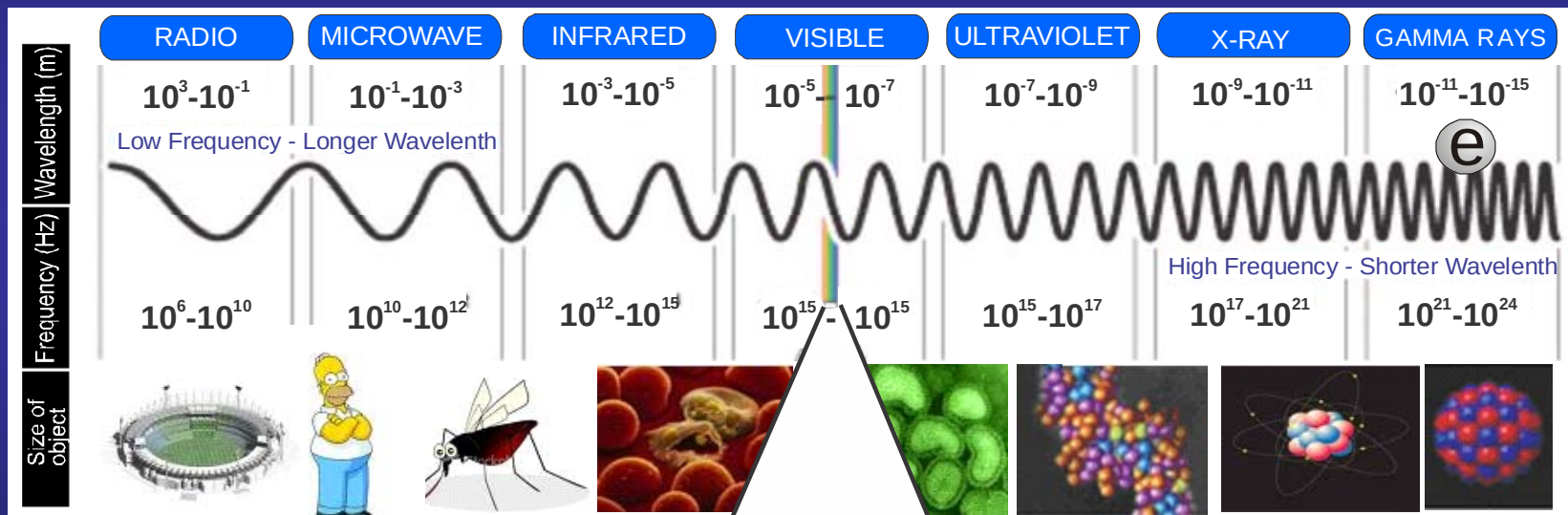
"It is very easy to answer many fundamental biological questions; you just look at the thing."

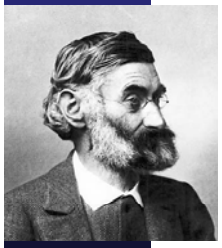
Richard Feynman - Nobel prize-winning physicist

"Physical scientists should help biologists by making microscopes much much better."

What Limits the Resolution of Microscopy?







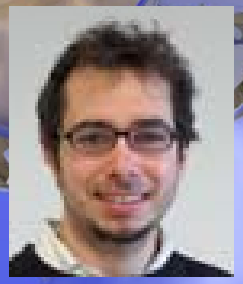
$$d = \frac{\lambda}{2A_N}$$

increasing radiation damage

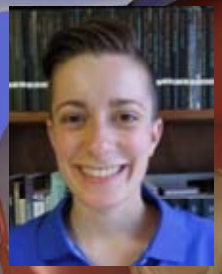
How does *P. falciparum* export and assemble the virulence proteins that modify the host RBC?


Matt Dixon


Coralie Millet


Mauro Maiorca


Steven Batinovic

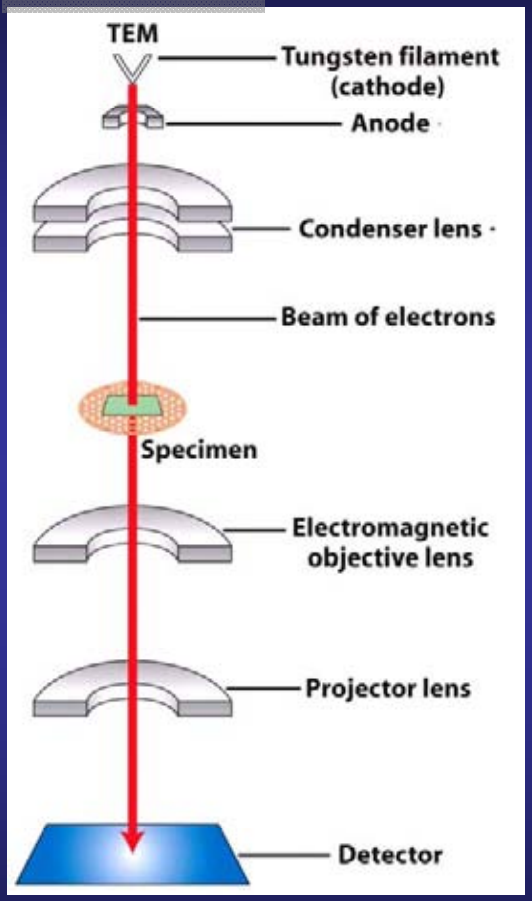

Emma McHugh


Eric Hanssen


Paul McMillan

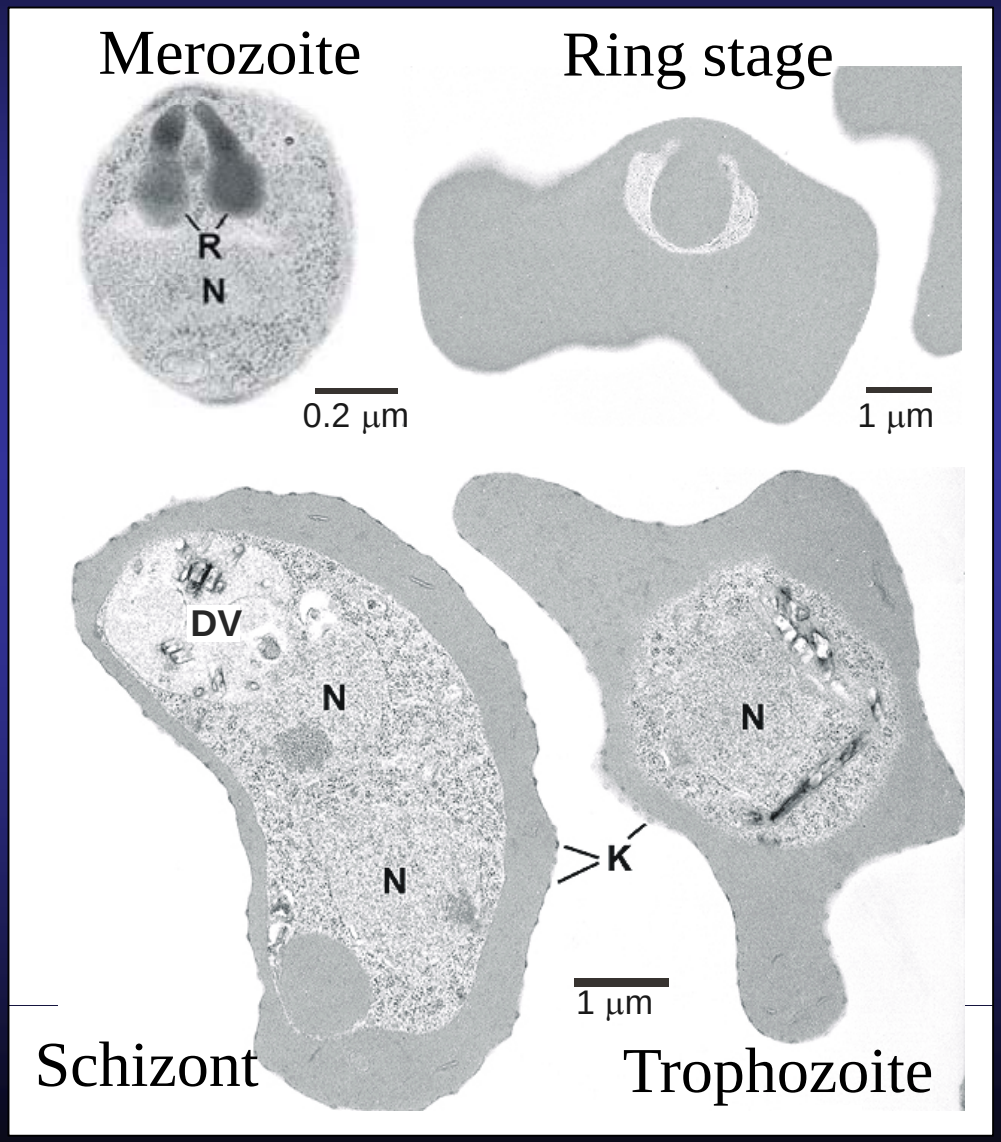
Electron Microscopy

Molecular Cell Biology, Freeman & Co..

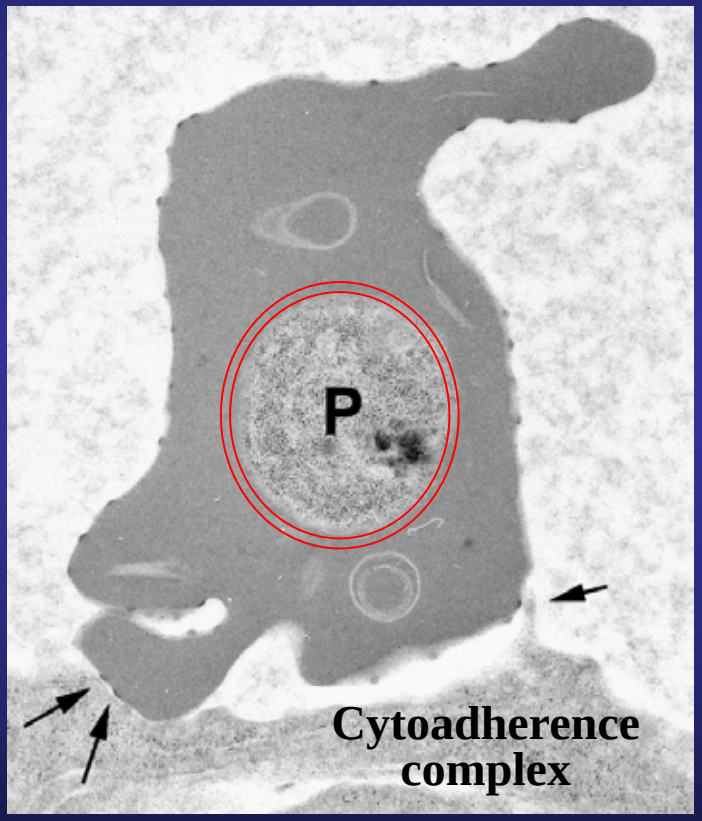


FEI Tecnai G2 F30 (up to ~300 keV)
Advanced Microscopy Facility, Bio21 Institute, Melbourne.

Electron Micrographs showing Parasite Development



(70 nm slices)



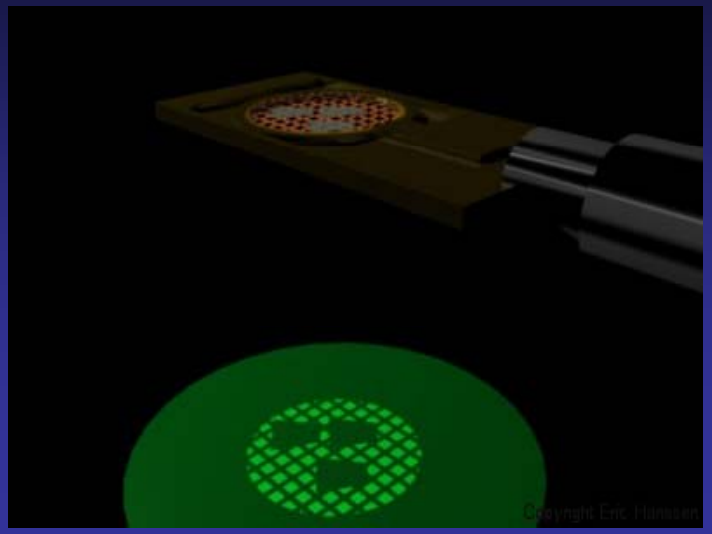
(David Ferguson, Oxford)

Electron Tomography

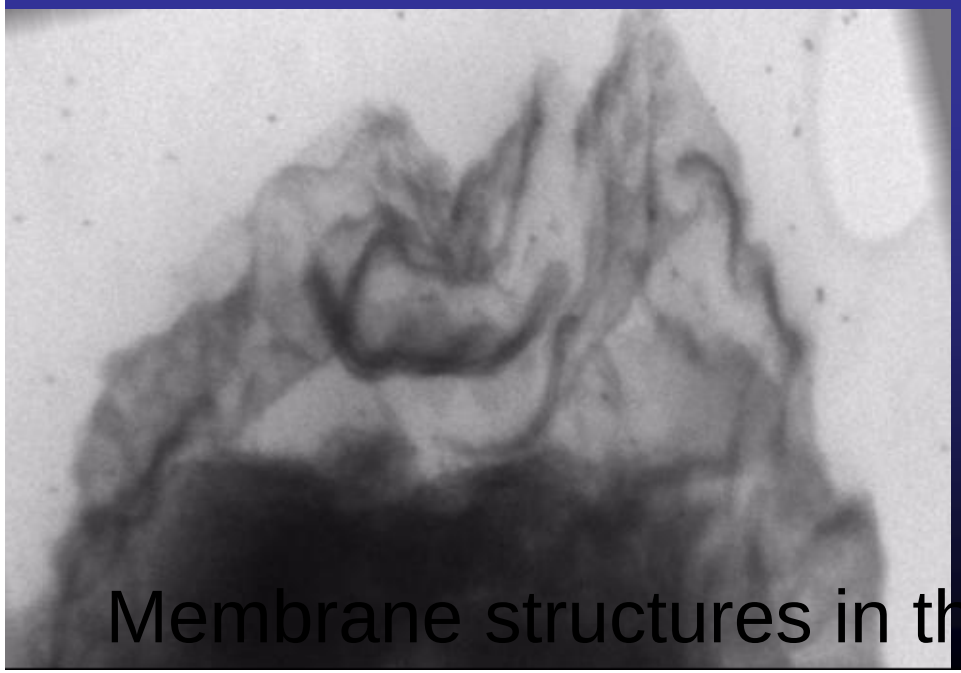
Can thicker samples be imaged using electrons?

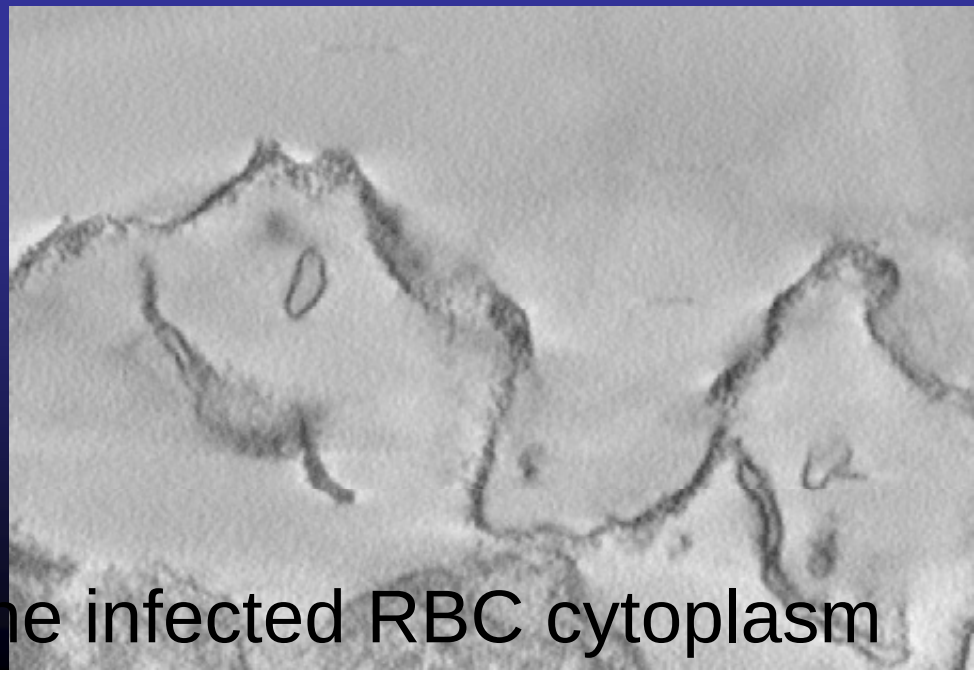
FEI Tecnai G2 F30 (up to ~300 keV; tiltable cryo stage, liquid N_2)
Advanced Microscopy Facility, Bio21 Institute, Melbourne

Electron Tomography

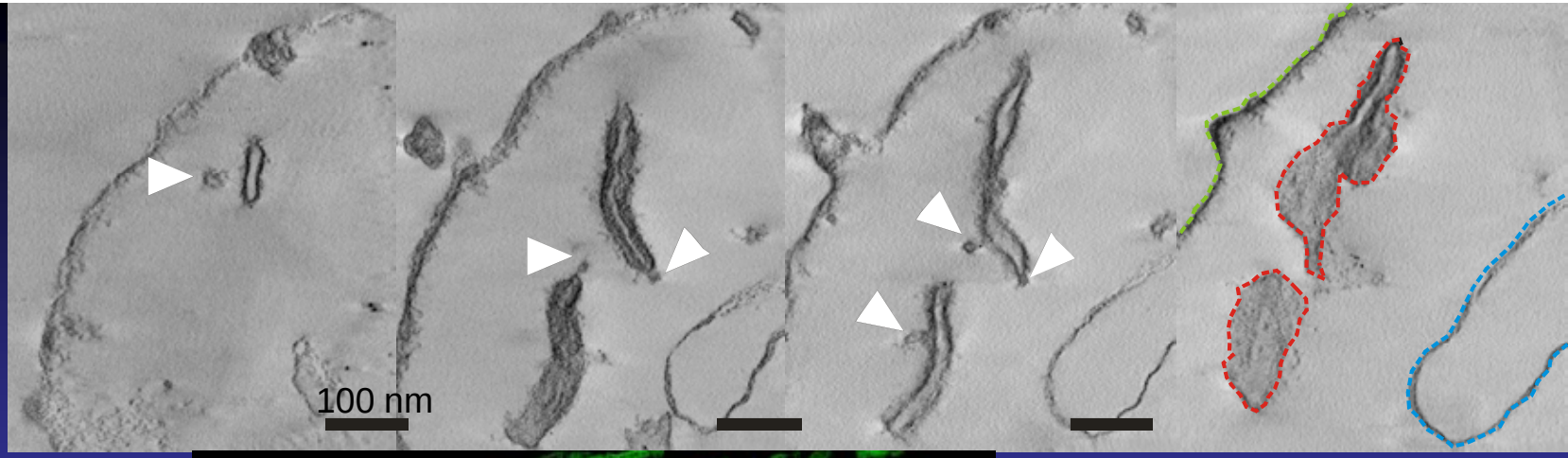


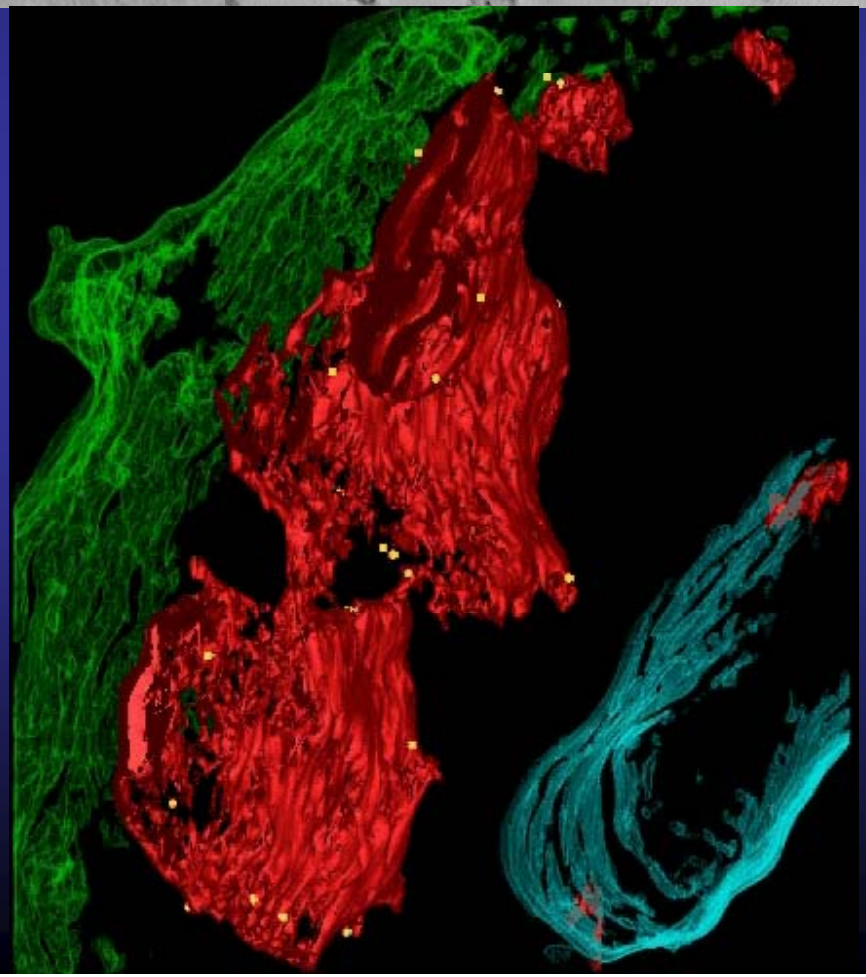
Eric Hanssen,
Bio21 Institute





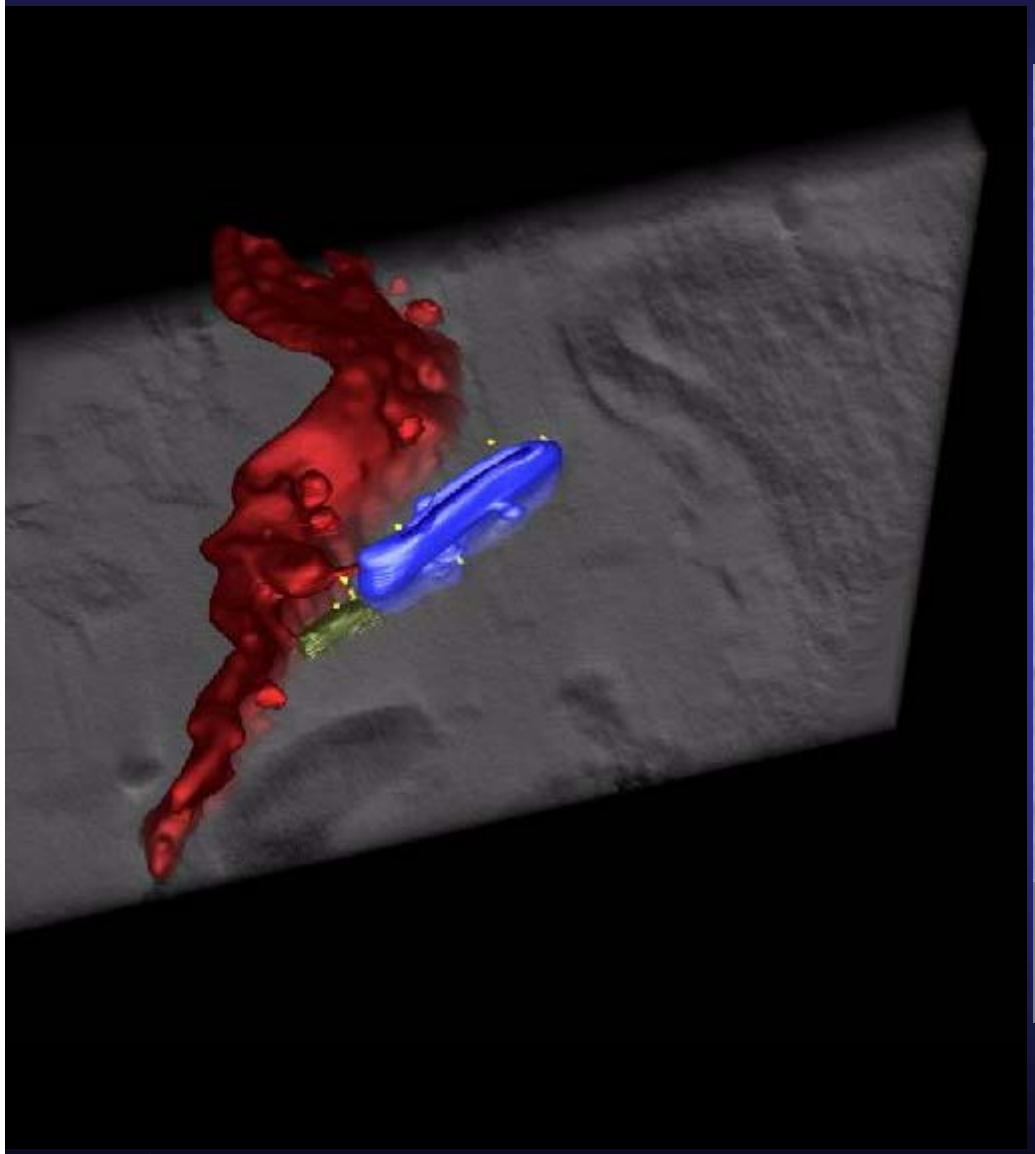
Membrane structures in the infected RBC cytoplasm



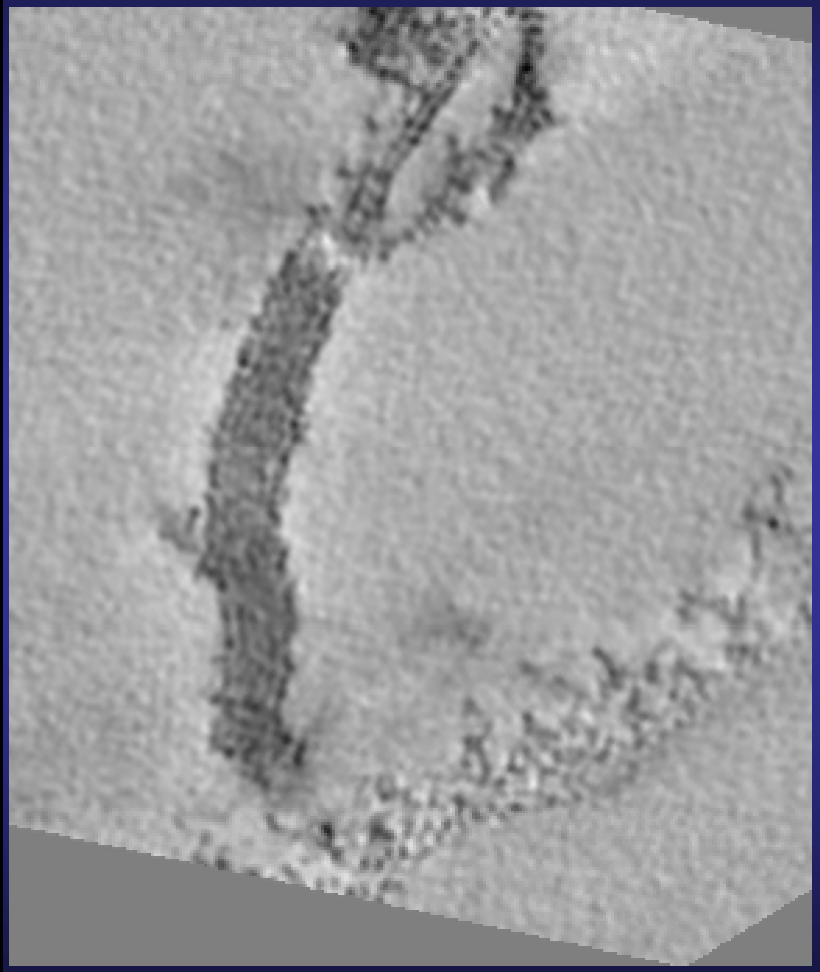


Rendered tomograms of membrane structures in the infected RBC cytoplasm - with immunogold labelling

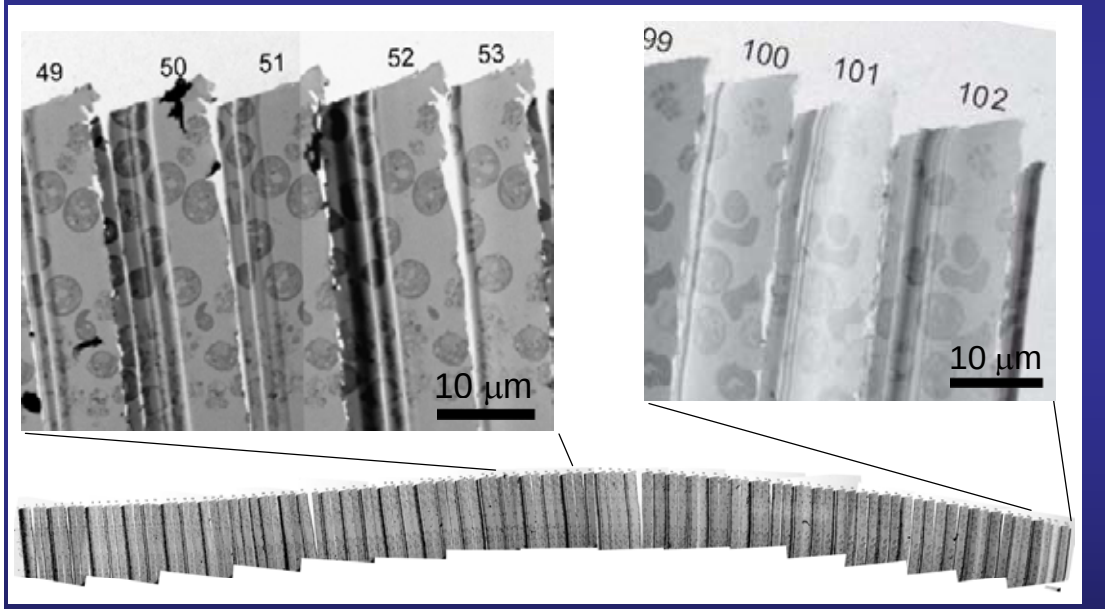
RBC connections

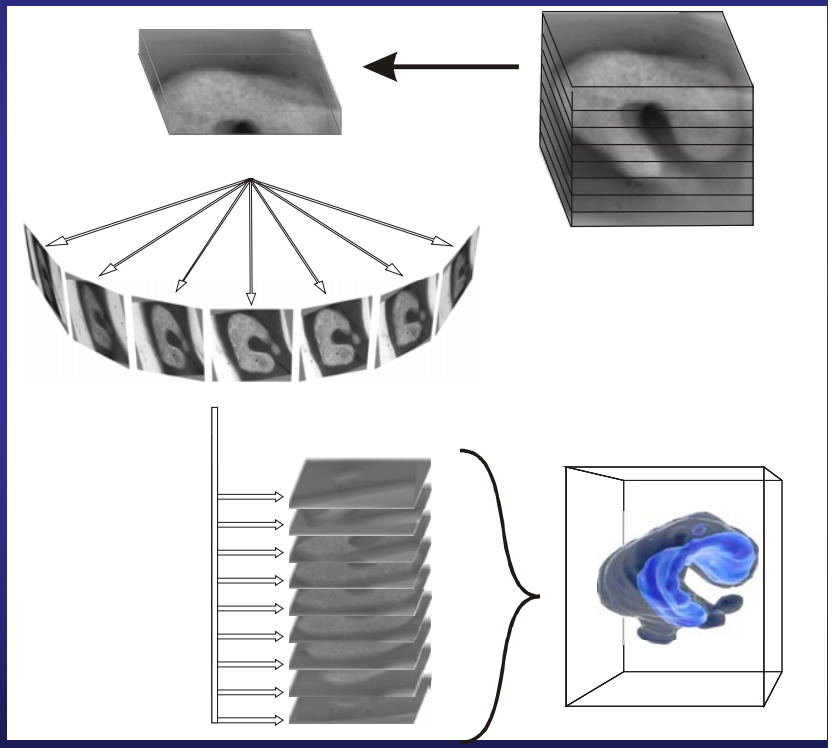


Anti-PfEMP1B (adhesion protein)



Serial Sectioning Electron Tomography

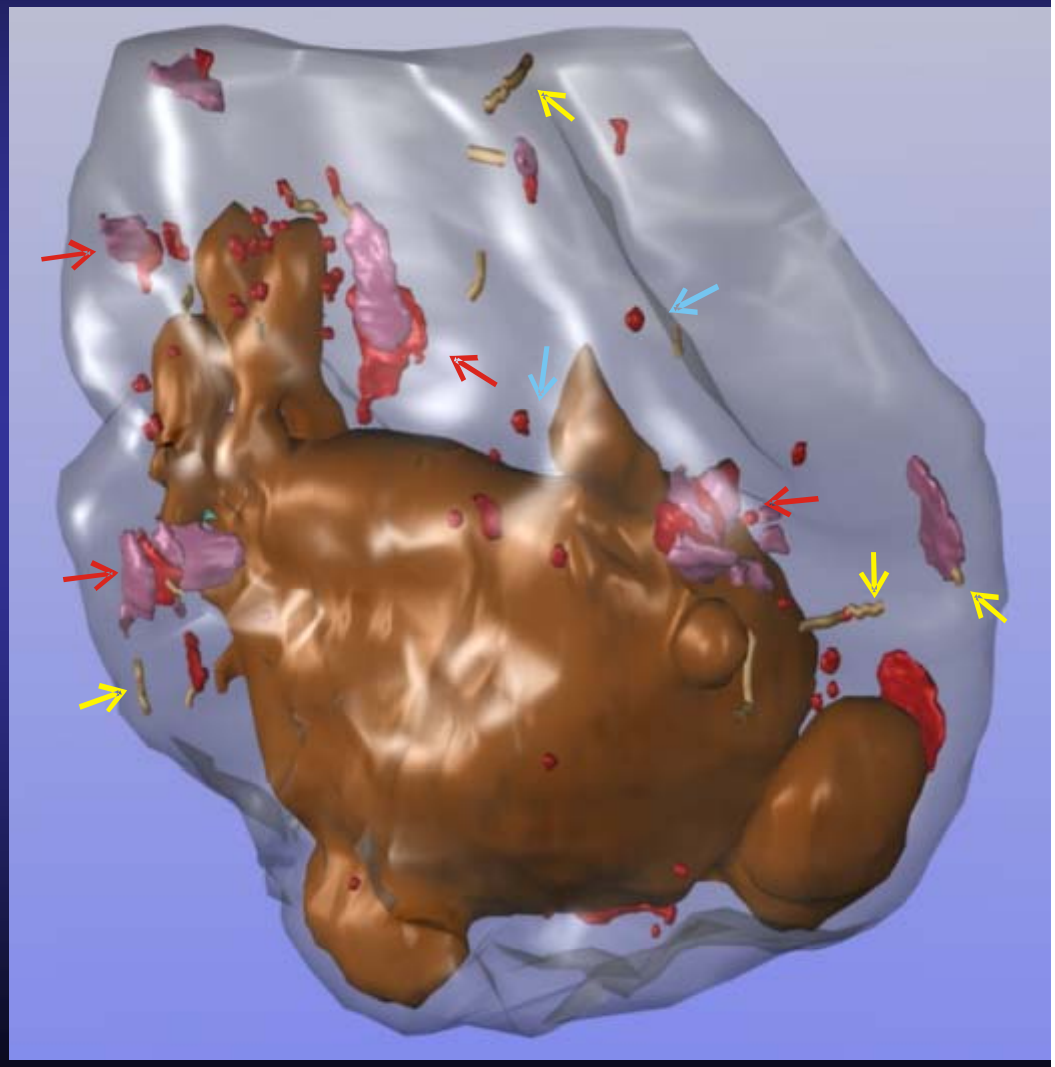




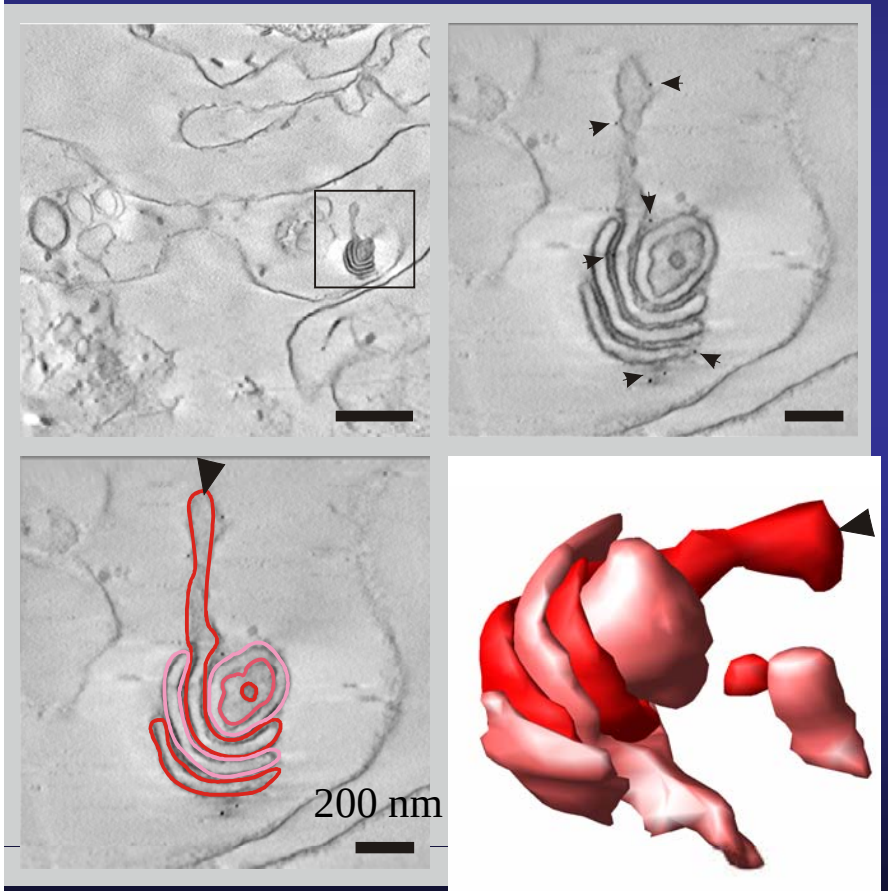
(300 nm slices)

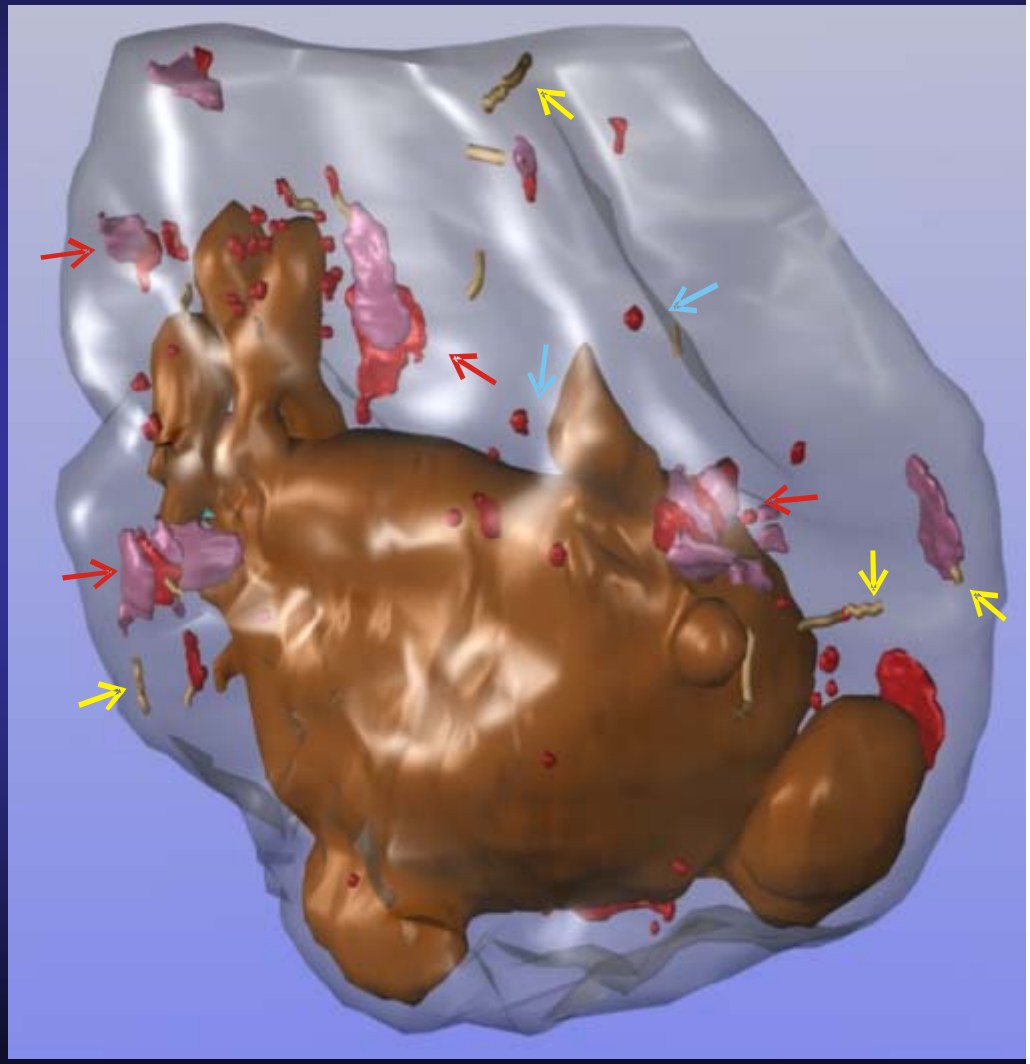
Eric Hanssen, Megan Dearnley, Coralie Millet, Mauro Maiorca

Serial Section Electron Tomography of an Entire Infected RBC (25 sections of 300 nm, 7.5 μm)

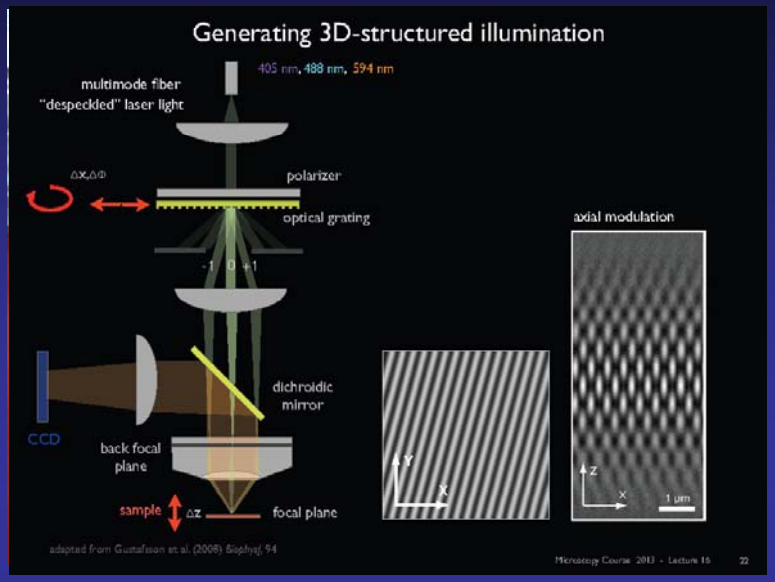


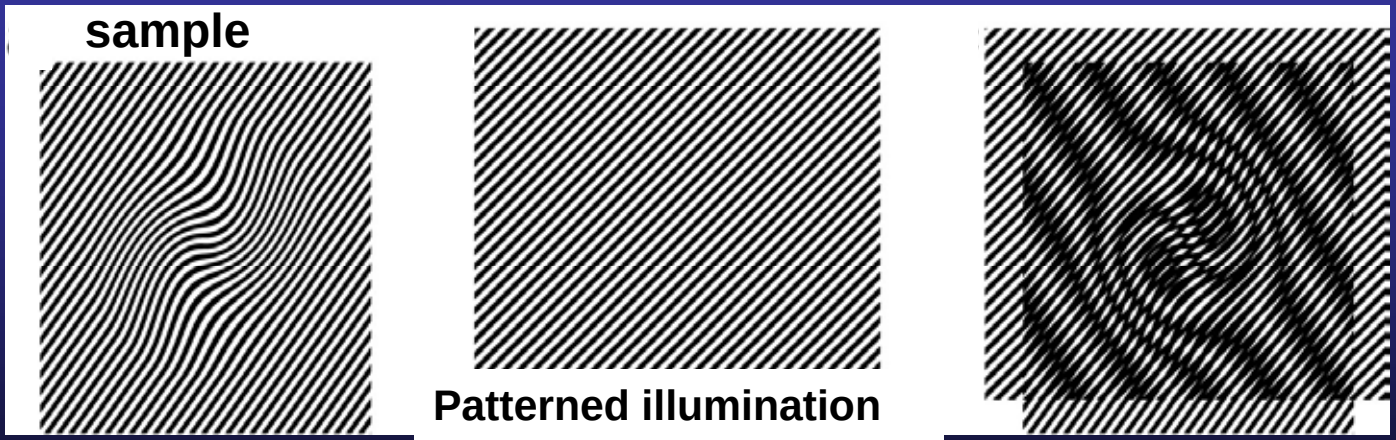
Imaging Structures in Whole Infected RBCs





Can the Resolution of Light Microscopy be Improved?





Moiré fringe patterns

Super-Resolution Optical Microscopy

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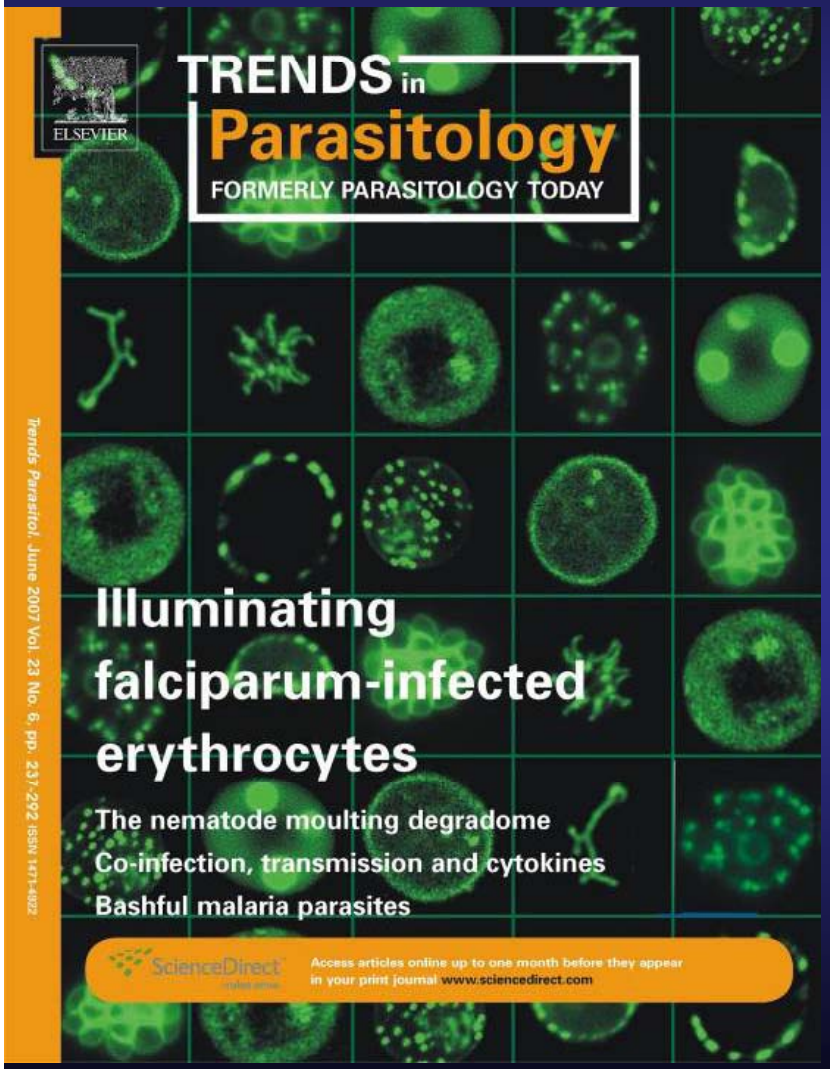
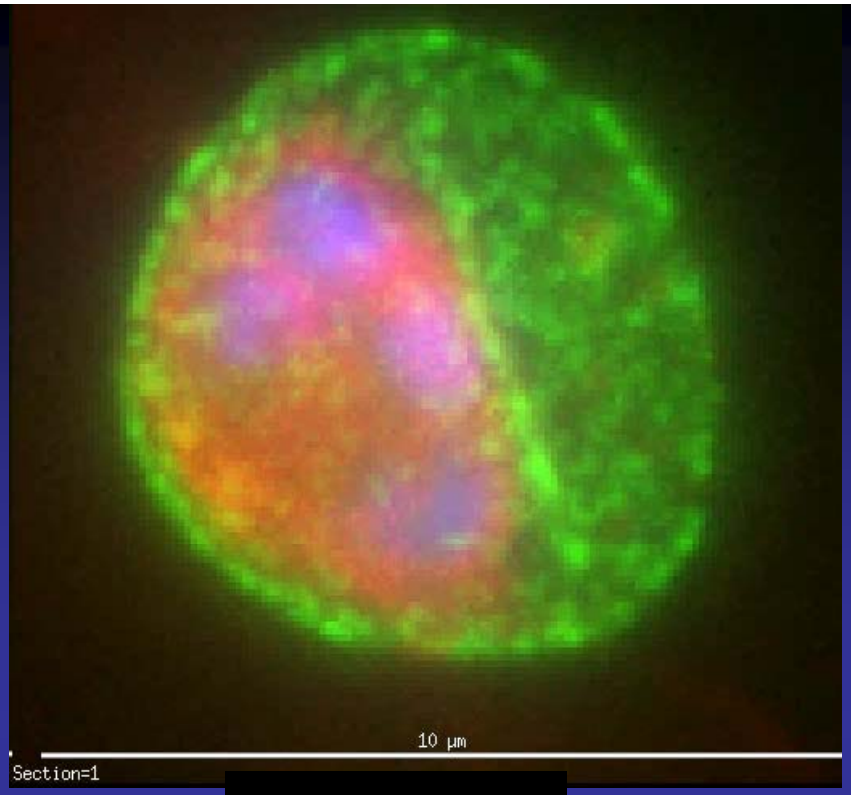
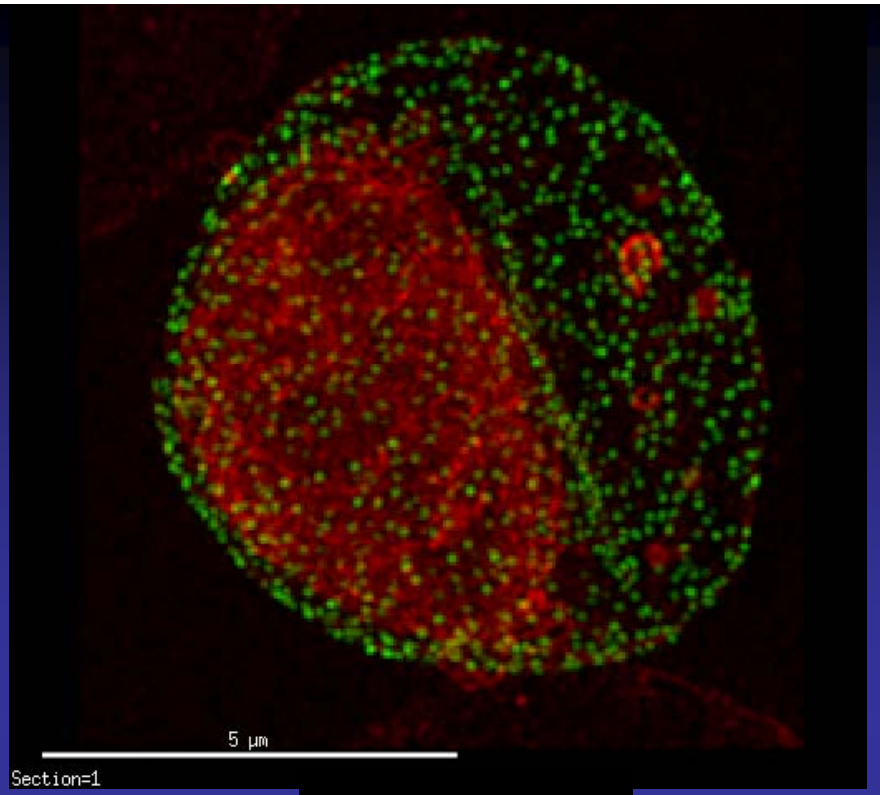
Green Fluorescent Protein is used to Visualise Proteins in Cells



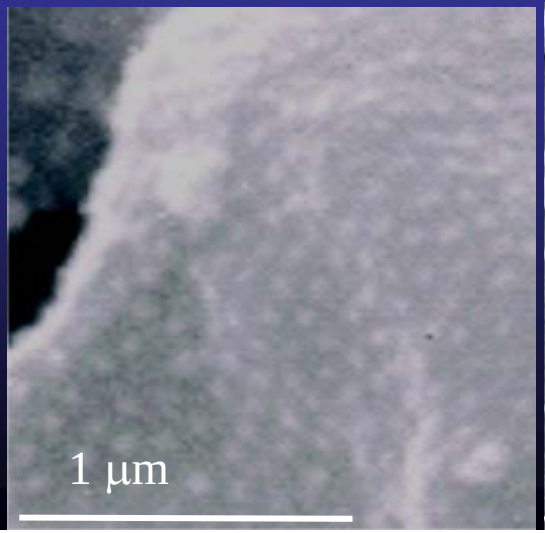


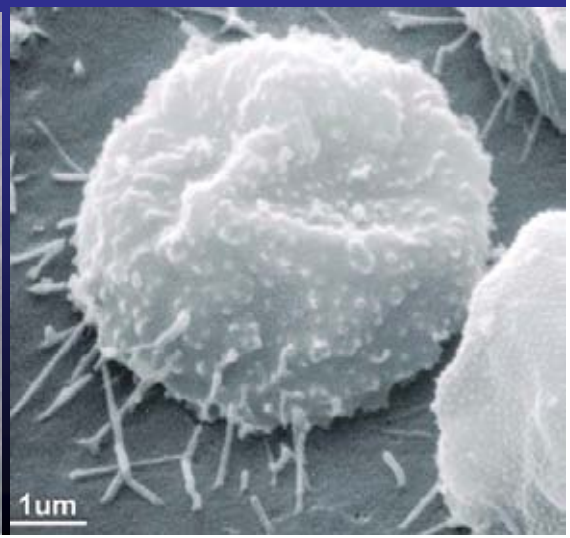
Widefield



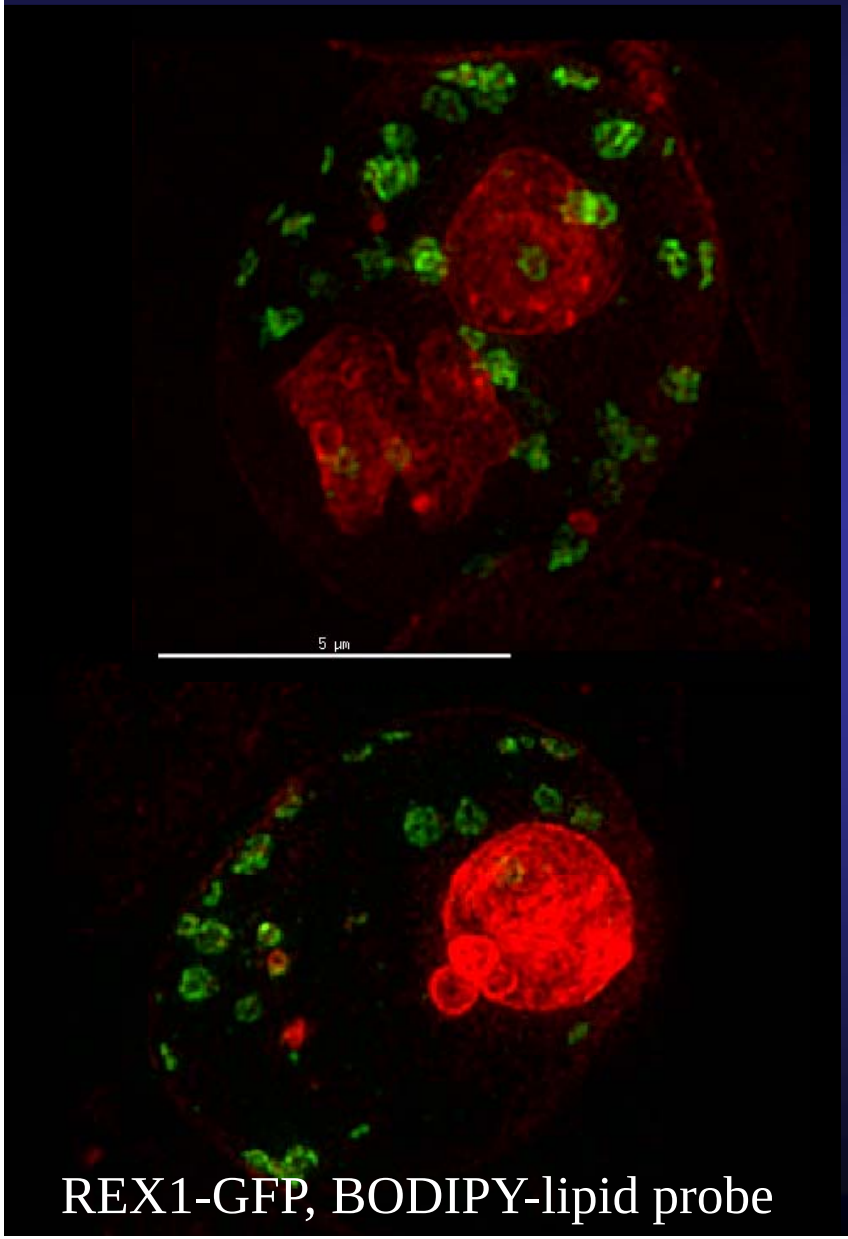
3D-SIM

Super-Resolution Imaging of a GFP-labelled knob protein. Knobs: 90 nm diameter

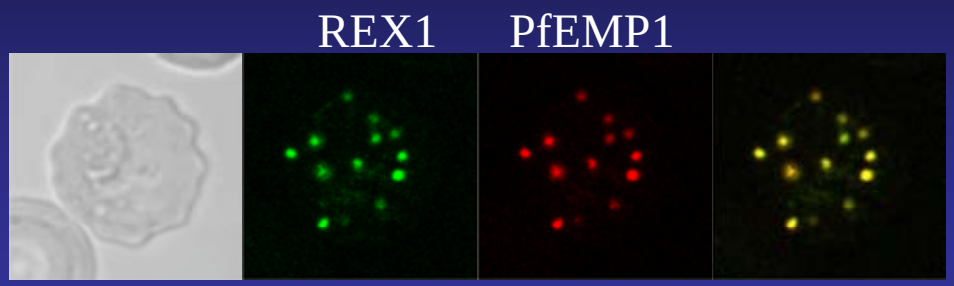


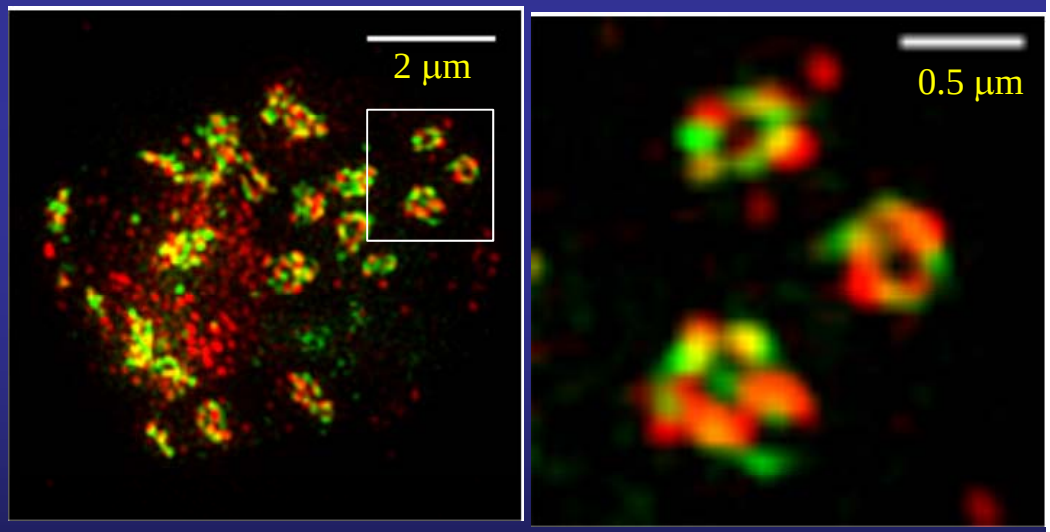


How is the virulence adhesion protein trafficked to the RBC surface?



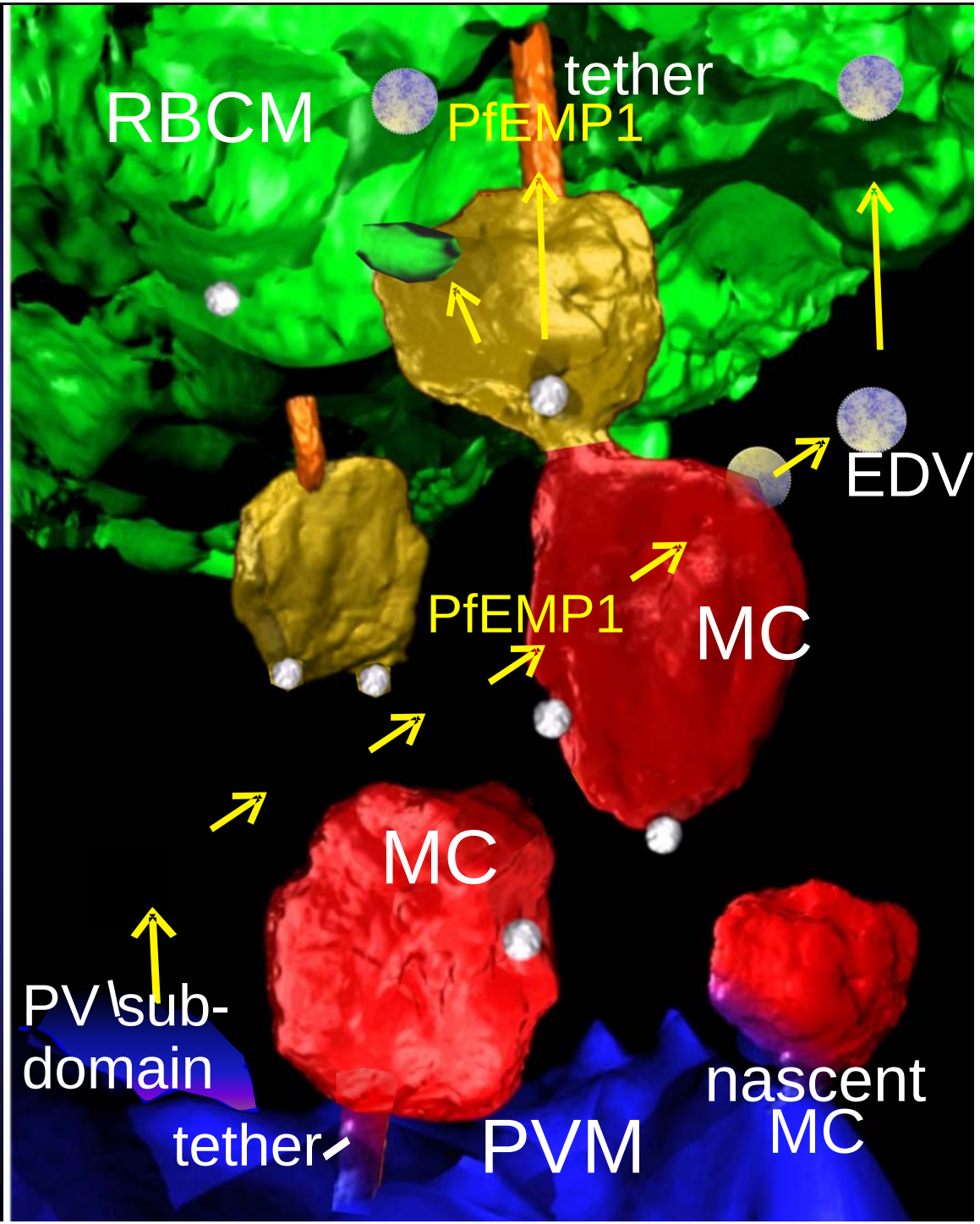
REX1-GFP, BODIPY-lipid probe





PfEMP1(red), REX1 (green)

An exomembrane system mediates the transfer of the adhesion protein, PfEMP1, from the parasite to the RBC membrane.



Images on Front Covers of Journals

Can High Resolution Microscopy Help Design Better Antimalarials?


Nick Klonis

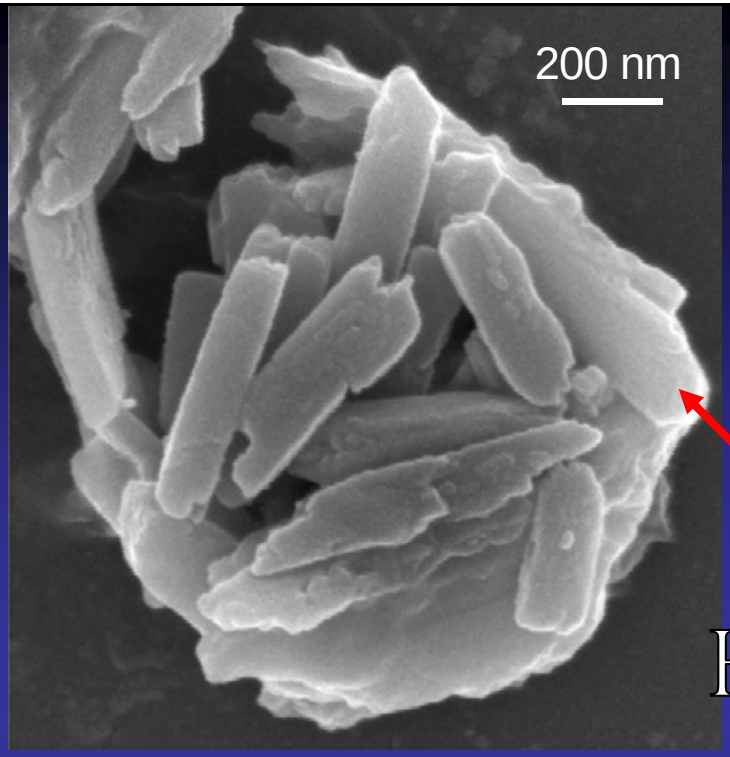

Stanley Xie

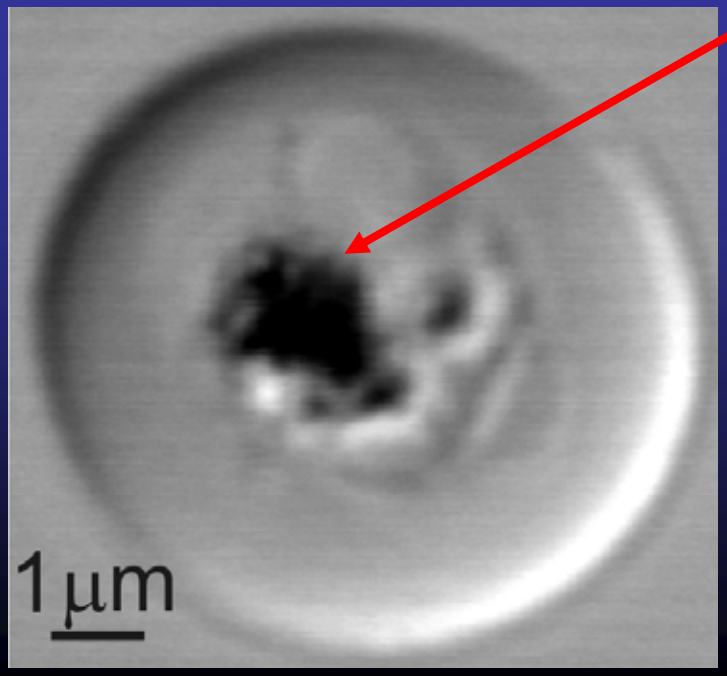

Con Dogovski


Silvia Teguh

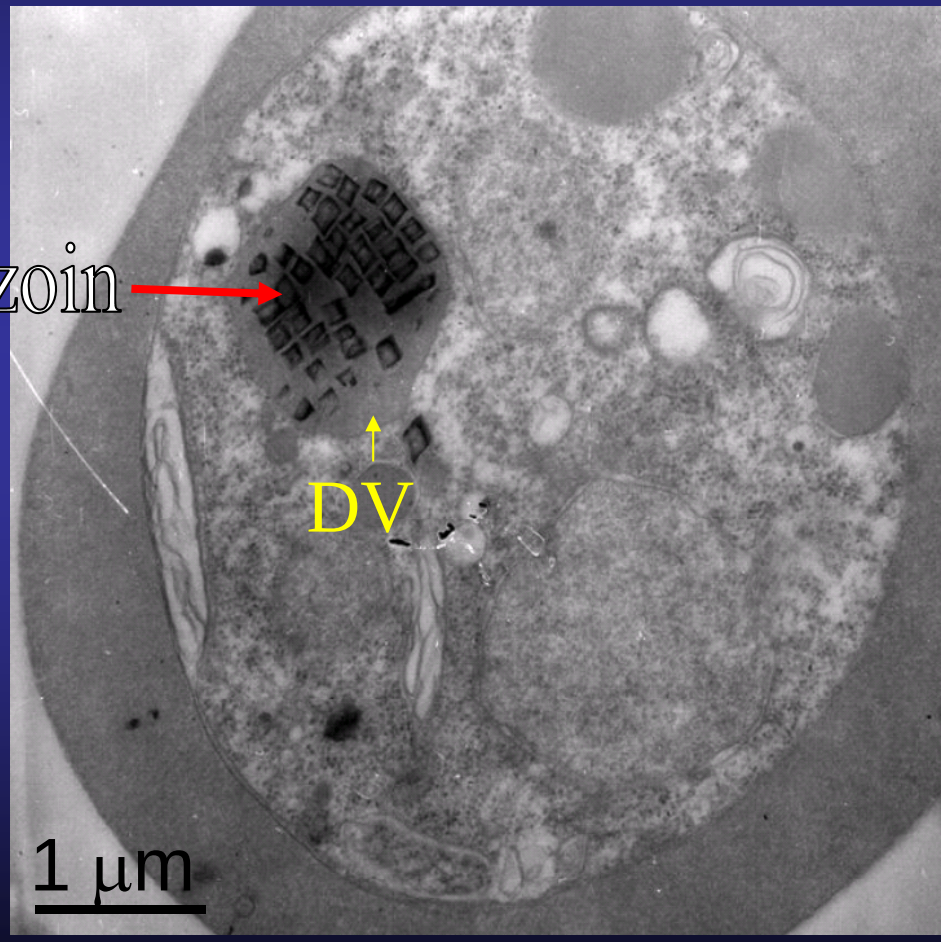

Eric Hanssen

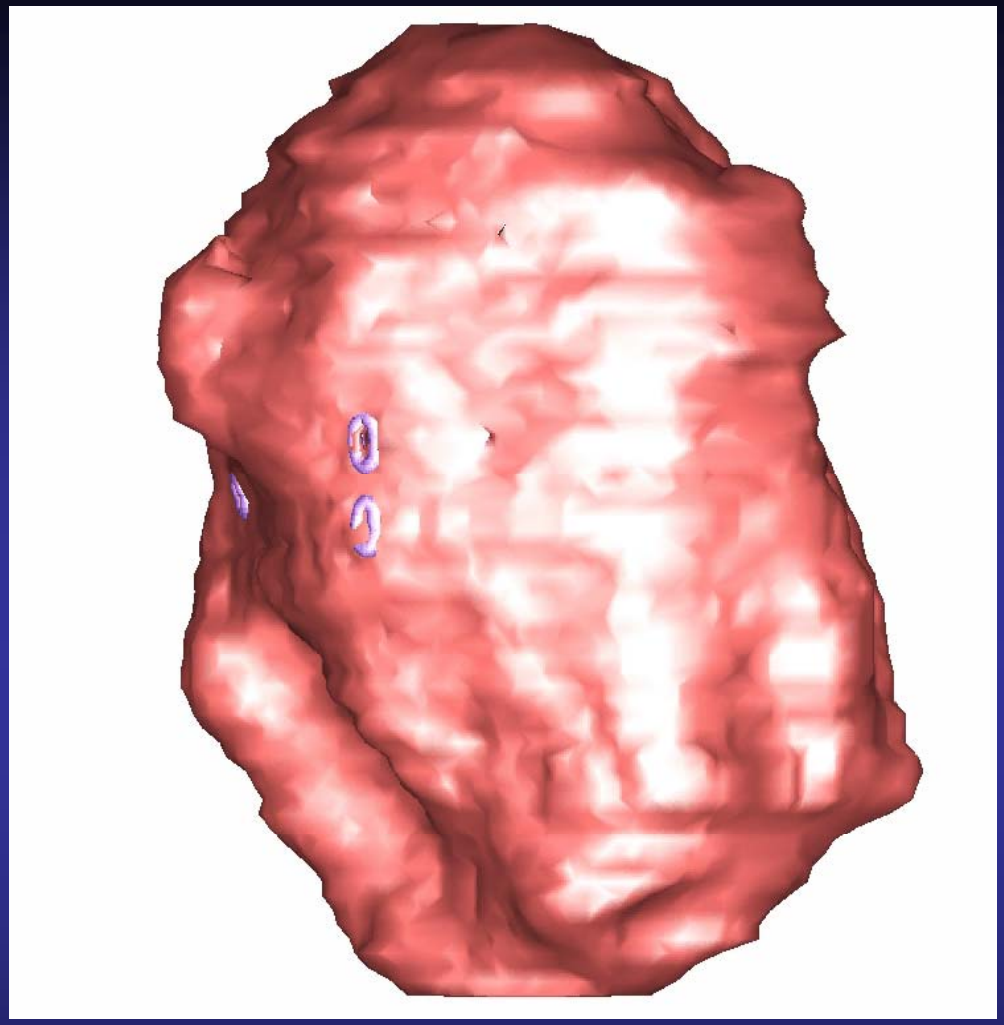
Haemoglobin Degradation: An Achilles' heel?

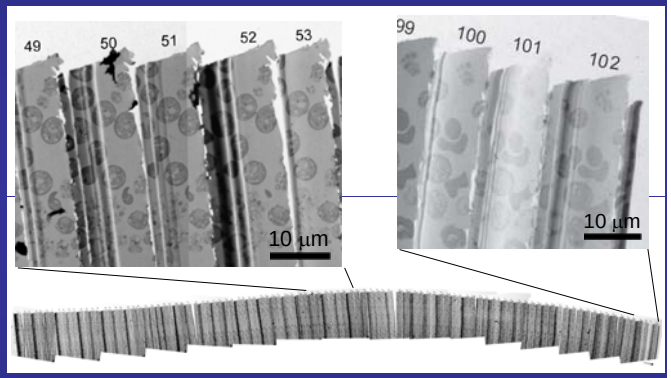




Haemozoin



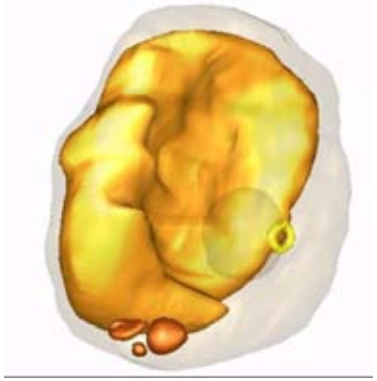


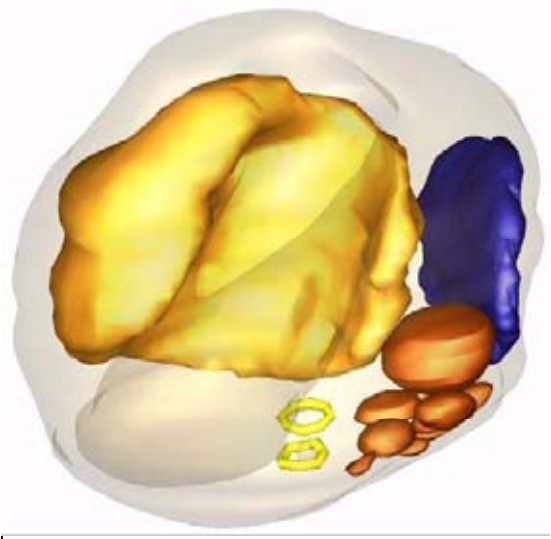


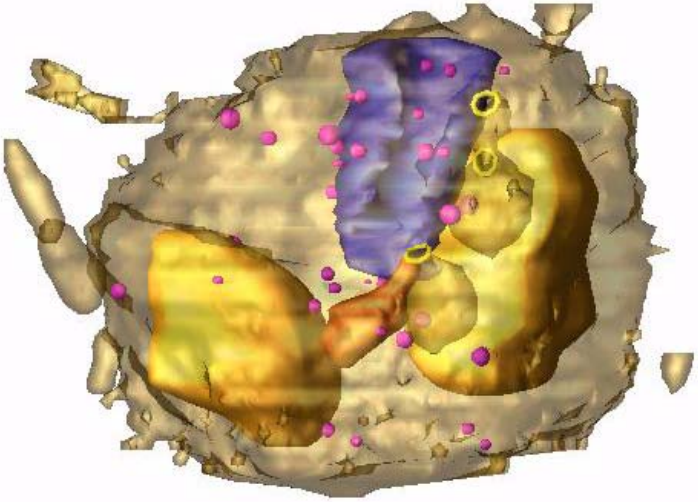
Multi-Section Electron Tomography of Intact Infected RBC

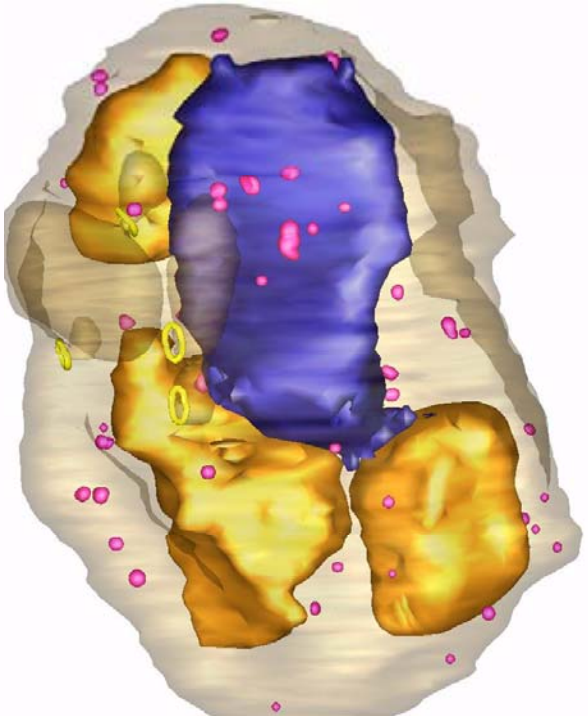
Klonis, Hanssen

Electron tomography of different stages of infected RBCs

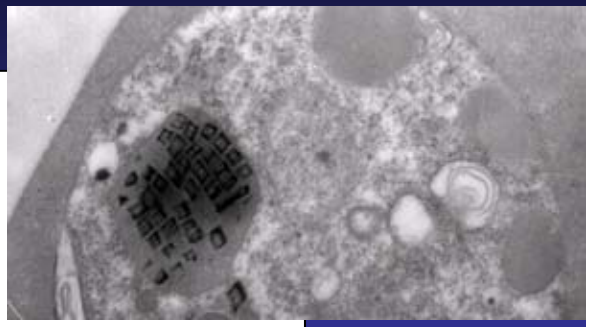








Mechanism of Action of Artemisinin

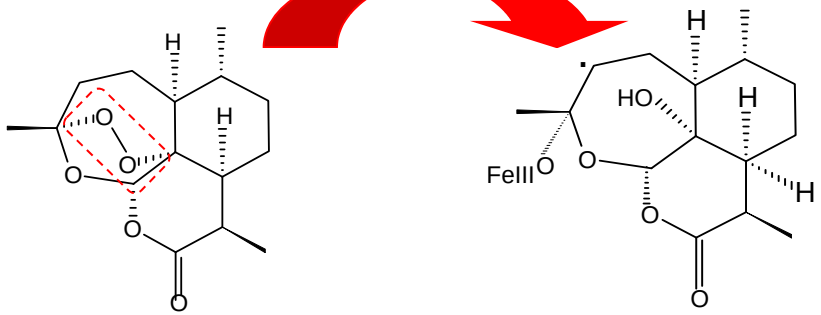


Parasite
Digestive Vacuole

Proteases

Hemoglobin → **Amino acids**

Free heme/ Fe(II)

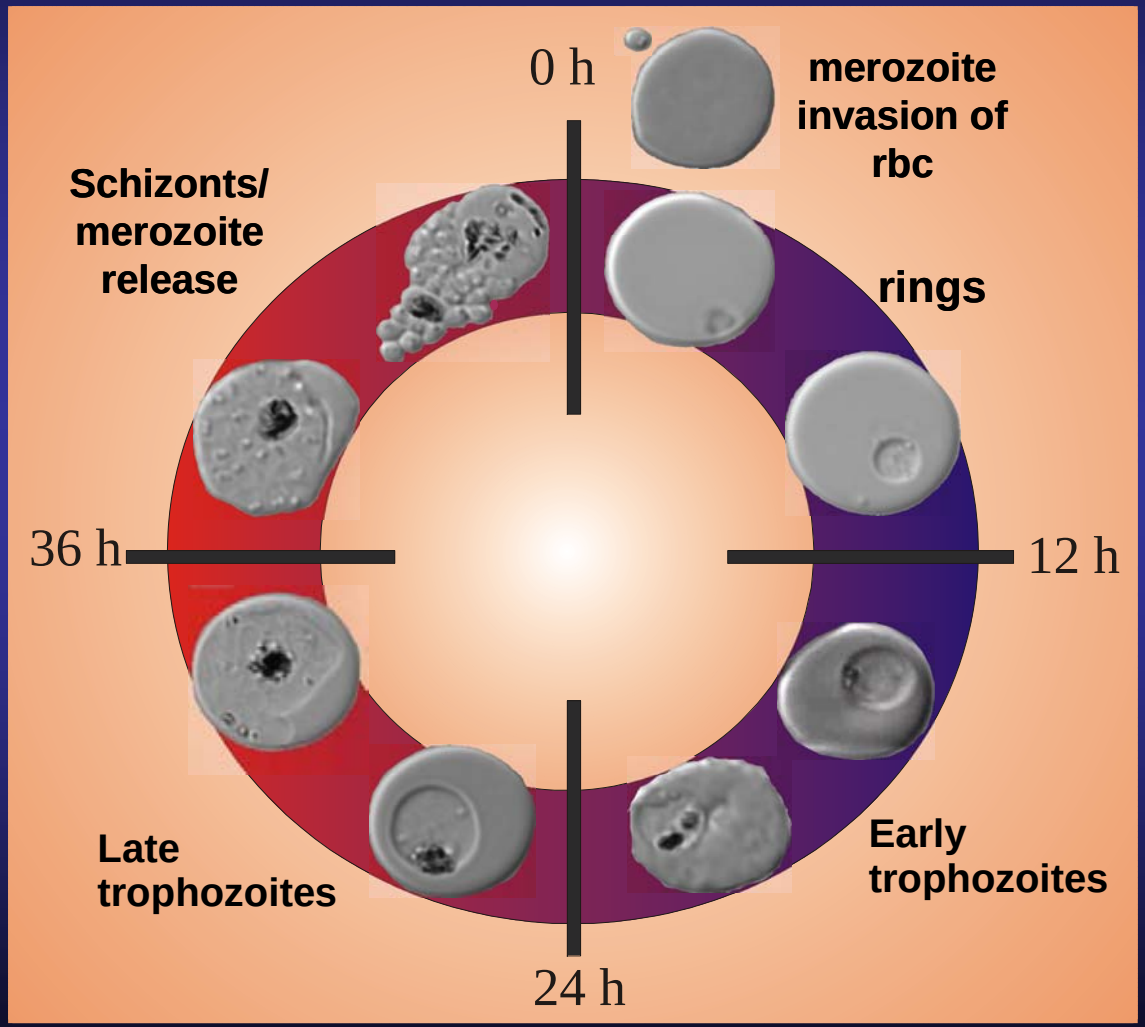


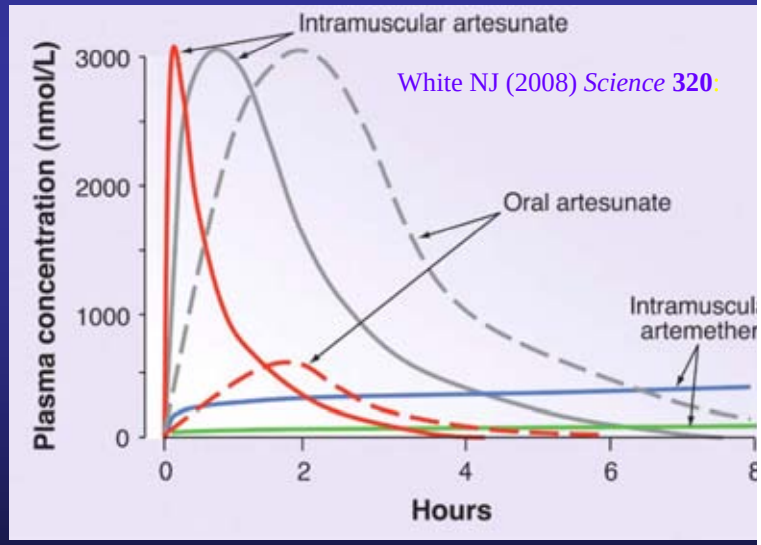
Activated Artemisinin

→ Damage to parasite proteins

Haemoglobin digestion => heme => activates ART

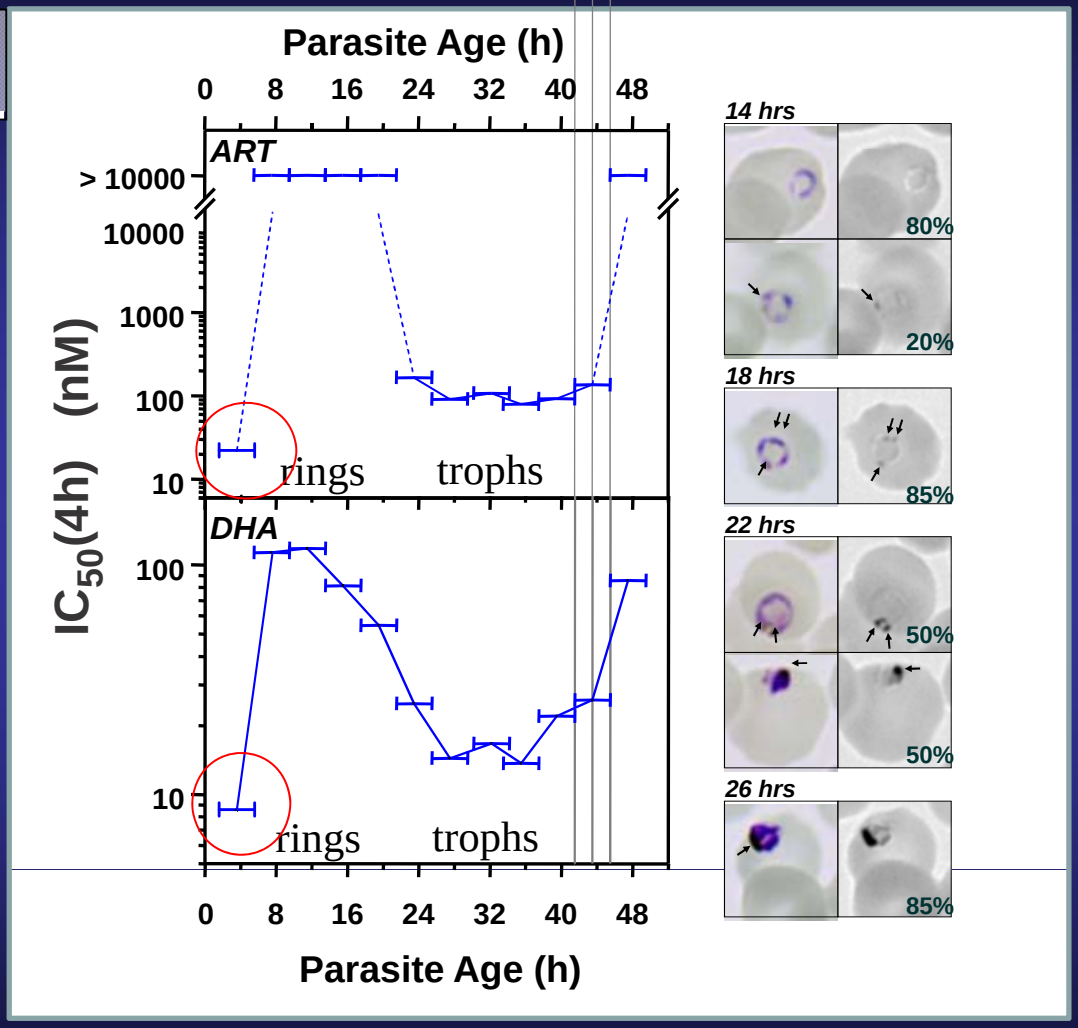
Immature Parasites should be less Susceptible to Artemisinin





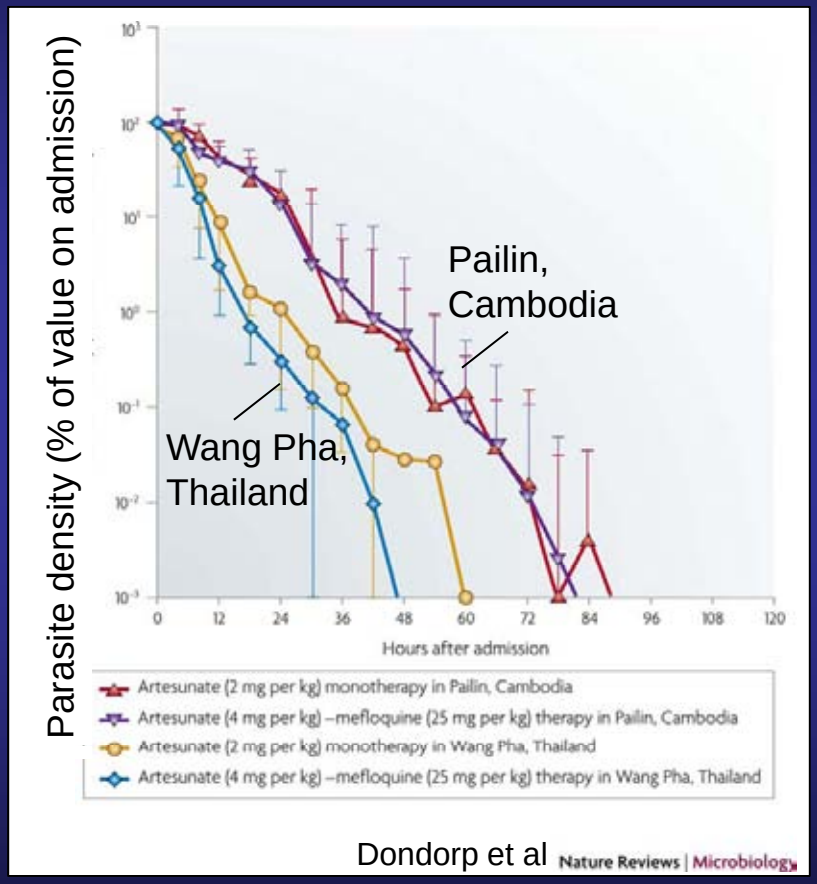
Stage Dependence of Artemisinin Response

1 h window
4 h drug pulse, 3D7

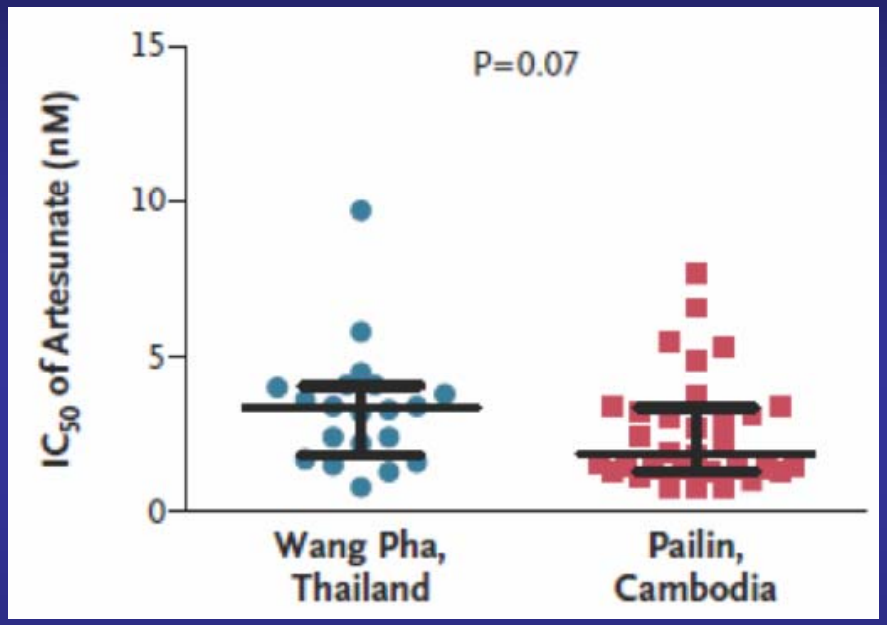


Very early ring stage parasites shows hyper-sensitivity to short pulses of ART

Assaying Artemisinin Resistance in the Field

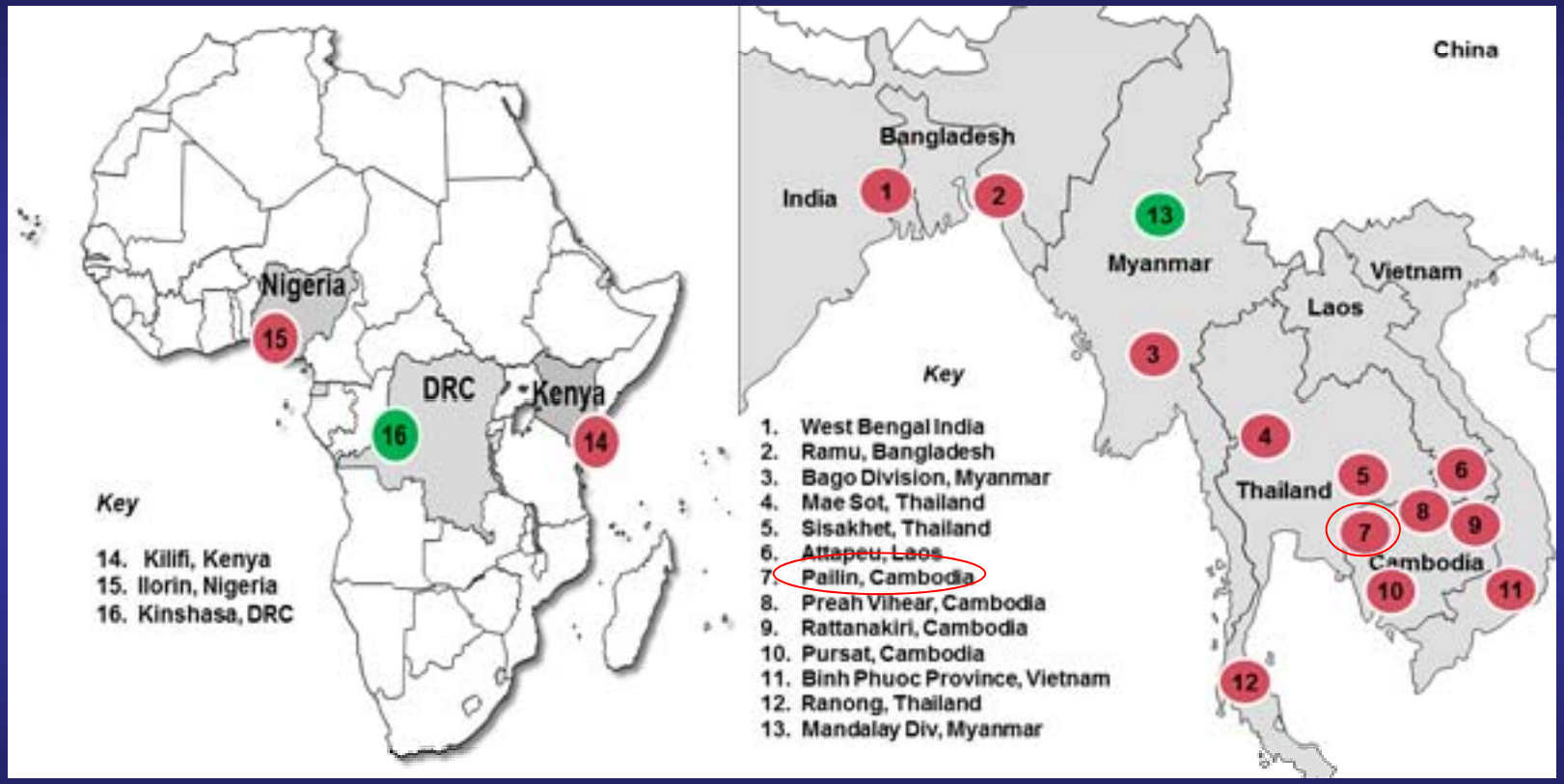


Dondorp et al Nature Reviews | Microbiology



Inhibitory Concentrations for *in vitro* assay of killing over 48 h for strains showing normal and delayed clearance

Tracking Artemisinin Resistance



UK Government Department for International Development (DFID). Partners include: Mahidol Oxford Tropical Medicine Research Programme (MORU); London School of Hygiene and Tropical Medicine (LSHTM); Liverpool School of Tropical Medicine (LSTM); WHO Global Malaria Programme; and the WorldWide Antimalarial Resistance Network (WWARN).

Malaria Control in the Field

Convergence Science

- Physical and biological sciences working together
⇒ **Advanced microscopy & quantitative biology**

- Interactions with clinical and field scientists
⇒**Translation of the research findings**

- Hypothesis-driven basic research to underpin and drive discovery science

Why we Need more Women making Careers in Research Science

- Discovery science needs diverse inputs from people with different ideas/ different perspectives => collective intelligence

- Convergence and translation science relies on co-operation and communication

- To lose trained scientist to the leaky pipeline is to lose important experience

- Passionate women in science are needed to help drive collaborative efforts

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Helen Sexton, Lilian Alexander, Elizabeth O'Hara,
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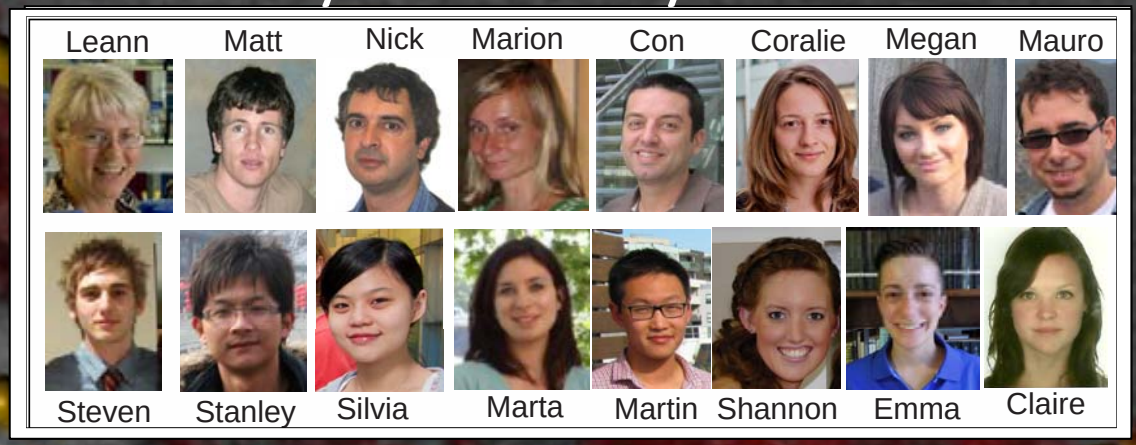
Jane Gunn, Judith Whitworth, Ruth Bishop AO

The Tilley Laboratory



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MEGA, UoM: Julie Simpson, James McCaw
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