SIR WILLIAM DUNN
SCHOOL OF PATHOLOGY

Advanced EM techniques: 3D and Correlative light and electron microscopy

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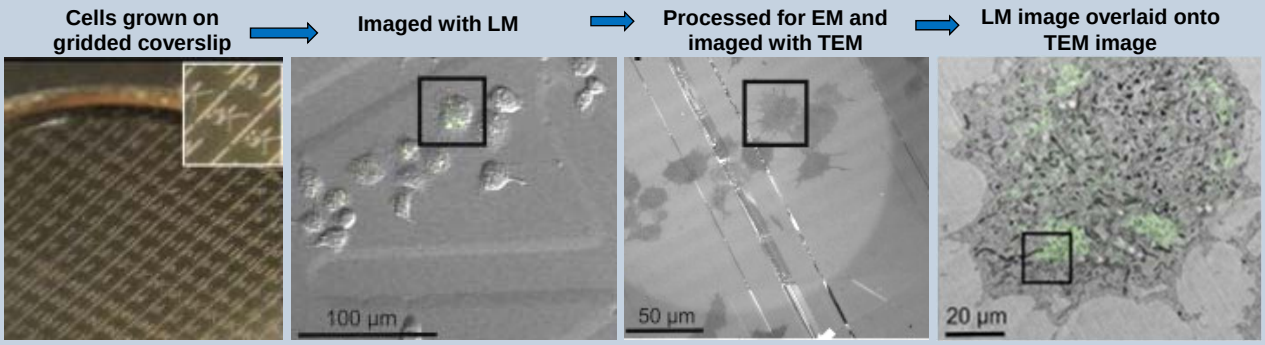
Correlative microscopy

Correlative Light & Electron Microscopy (CLEM)
Overview

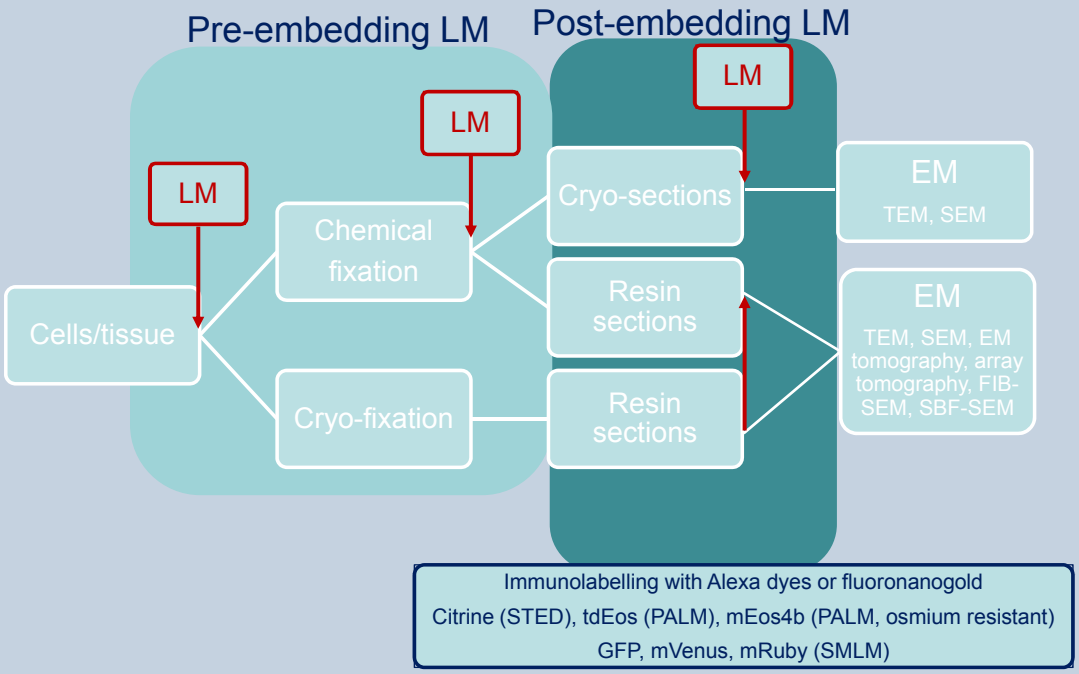
- Using Correlative Light and Electron Microscopy (CLEM) techniques, the same cell/tissue area is imaged with Light Microscopy (LM) *and* Electron Microscopy (EM). In this way, proteins, organelles or cells of interest can be localised within an ultrastructural context.



CLEM analysis of Simian Foamy Virus infection in C6/36 mosquito culture cells expressing ZsGreen-nsP3 fusion
From: Hellström et al (2015) Methods, in press

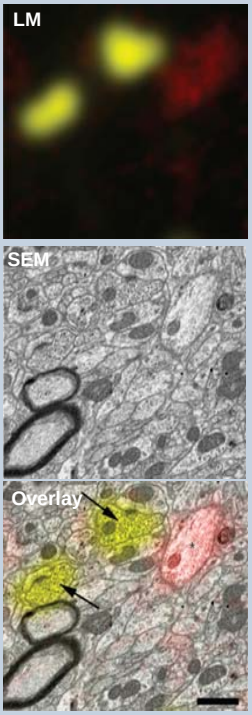
Correlative Light & Electron Microscopy (CLEM)

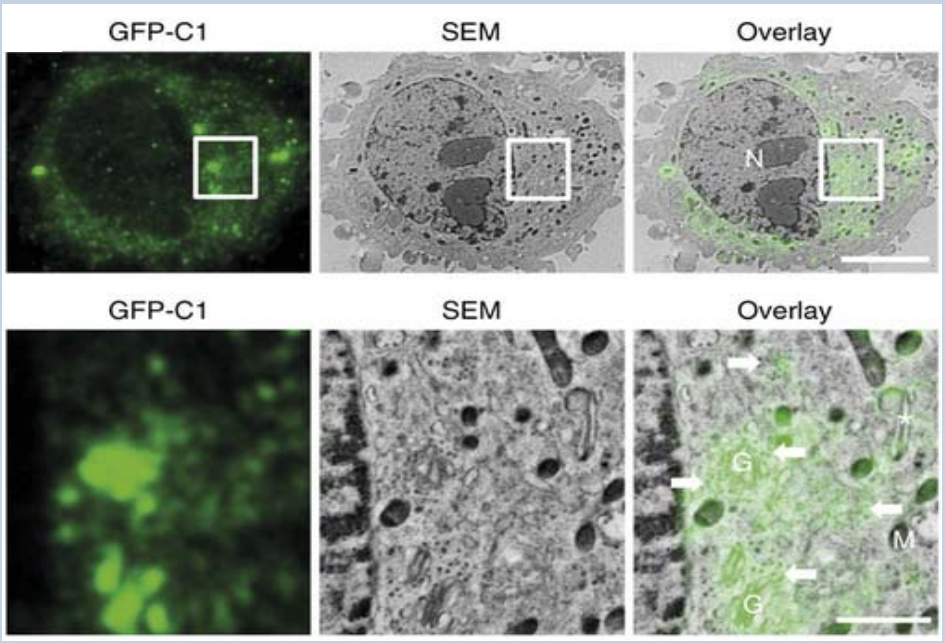
Overview



CLEM applications

Pinpointing specific cells and localising proteins

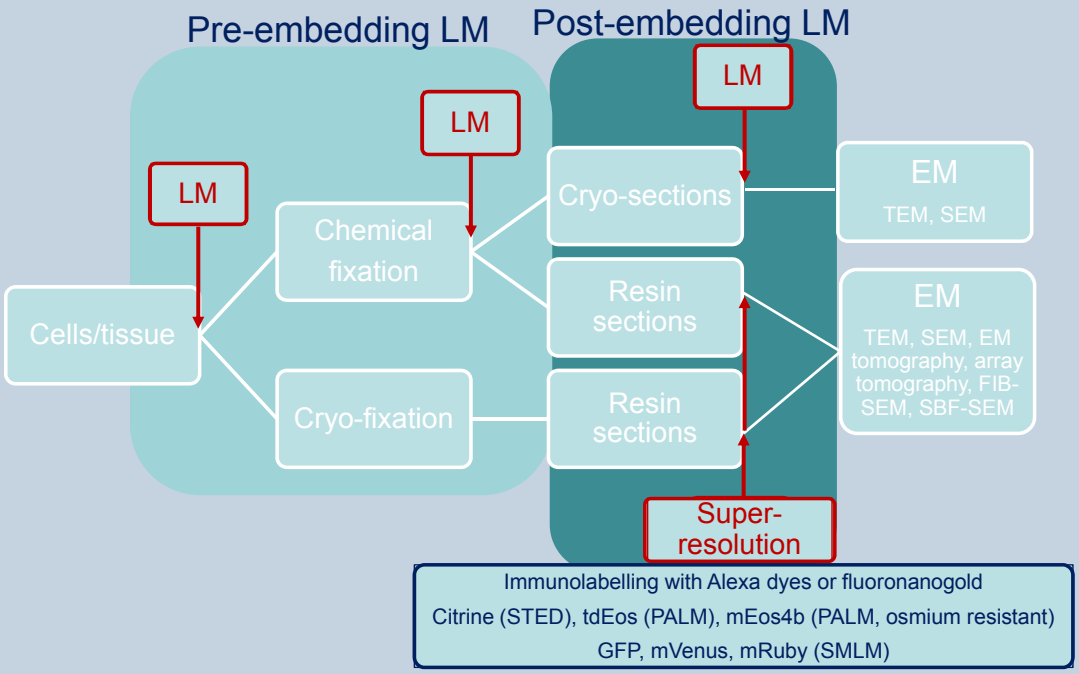




Left: CLEM of two different projection neuron populations in Zebra Finch brain tissue (Oberti et al. 2011, Front Neurosci)
Right: CLEM of GFP-C1 in resin sections of HeLa cells (C Peddie & L Collinson, Francis Crick Institute)

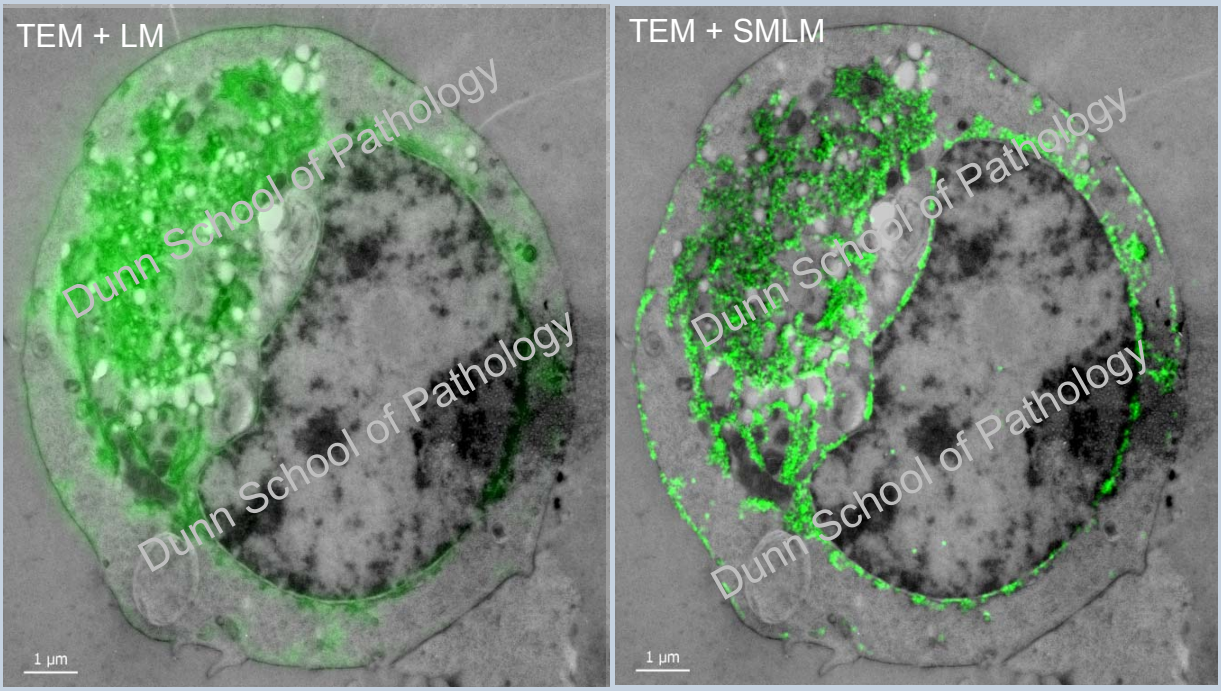
Correlative Light & Electron Microscopy (CLEM)

Overview



CLEM applications

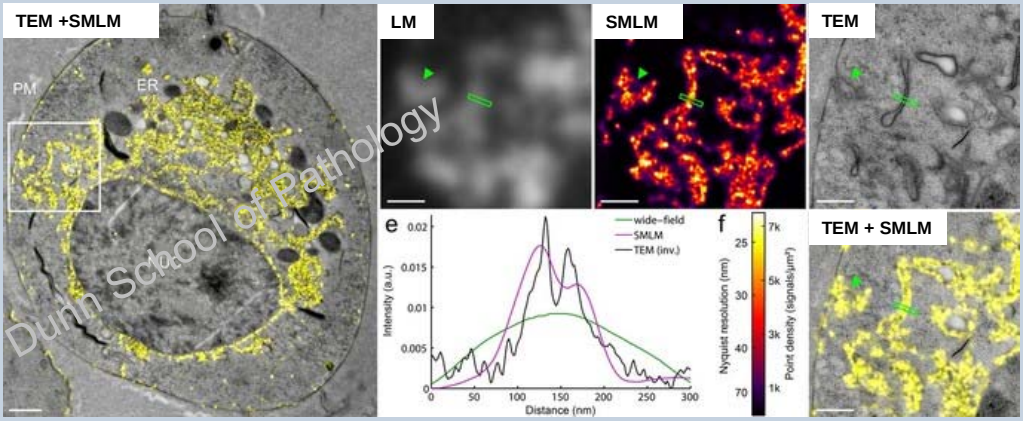
Protein localisation with Super-resolution microscopy



CLEM of HEK cells expressing EphA2-mVenus (E Johnson & R Kaufmann)

CLEM with super-resolution microscopy

SMLM with standard fluorescent proteins

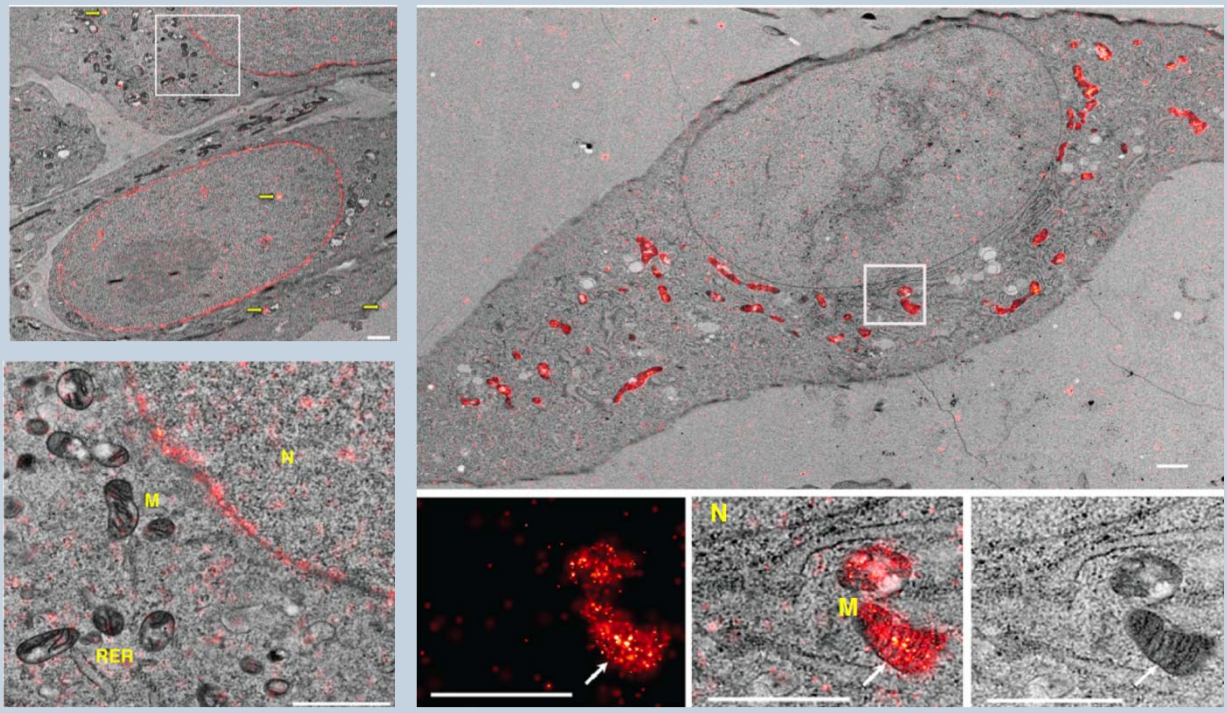


- Blinking/switching capability of standard FPs (GFP, YFP, mRuby) can be preserved using an optimised EM sample preparation protocol
  - Excellent structural resolution (40-50 nm) at fluorescence level with SMLM
  - Very good ultrastructural preservation and good contrast
  - Allows highly precise correlation between LM and TEM images

From: Johnson et al (2015) Scientific Reports, 5

CLEM with super-resolution microscopy

PALM with osmium resistant fluorescent proteins



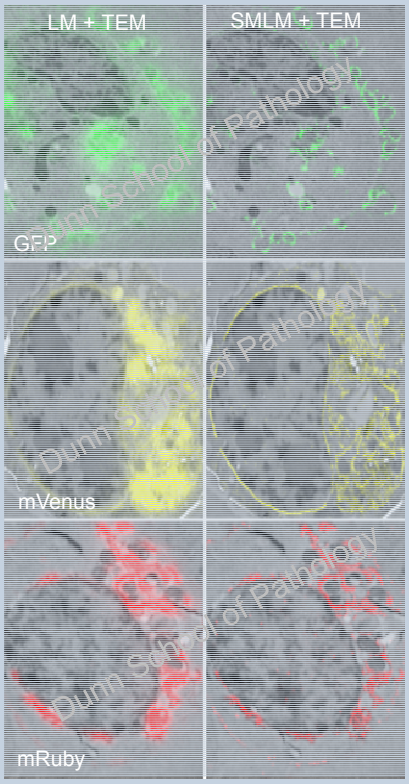
Correlative PALM and TEM imaging of mEos4b-lamin A (left) and mitochondrial mEos4a (right)in resin sections of 3T3 cells treated with 0.5% OsO_4

From: Paez-Segala et al (2015) Nature Methods

CLEM with super-resolution microscopy

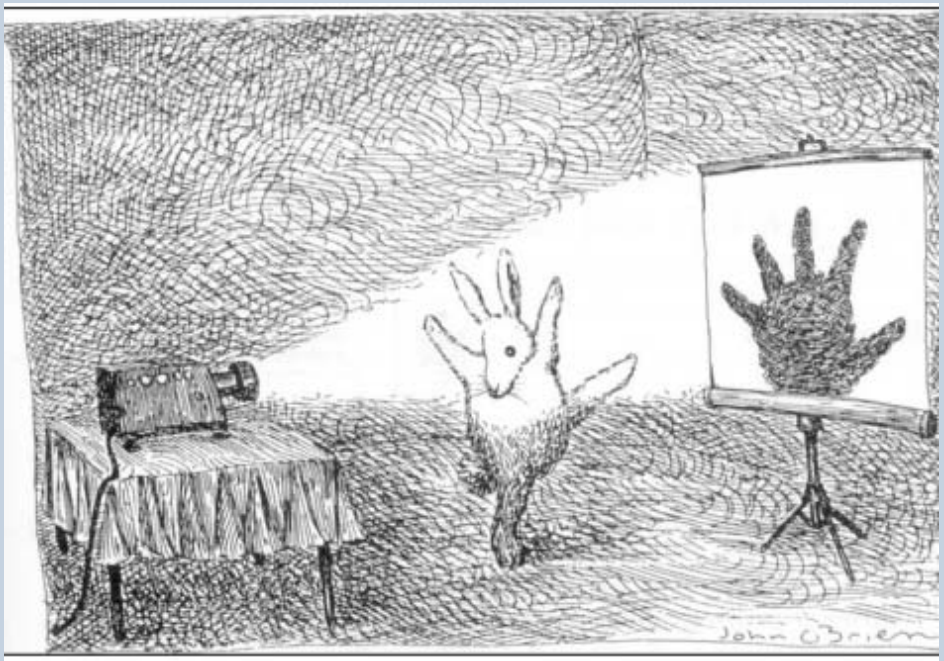
Summary

- CLEM puts fluorescently labelled organelles/proteins within their ultrastructural context
- Combining super-resolution microscopy and TEM is a highly precise method for correlating fluorescence and ultrastructure
- There are many different ways to perform CLEM, most are available here at Oxford



Johnson & Kaufmann, unpublished

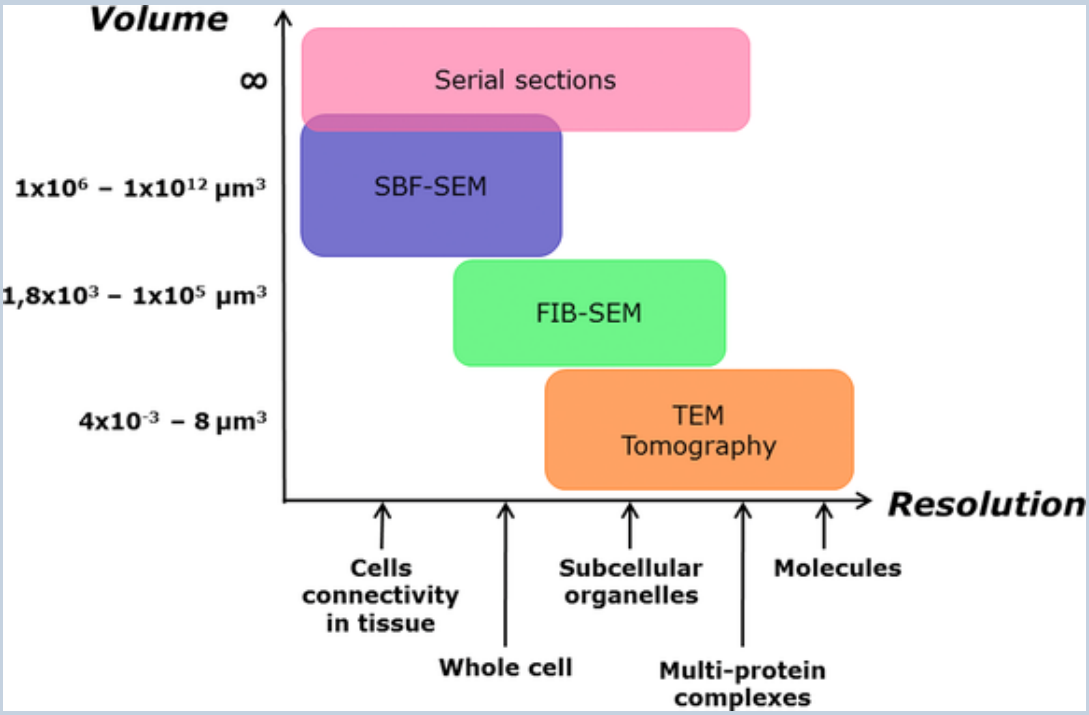
3D EM Techniques



Drawing by John O'Brien, The New Yorker Magazine (1991)

3D EM techniques

Overview

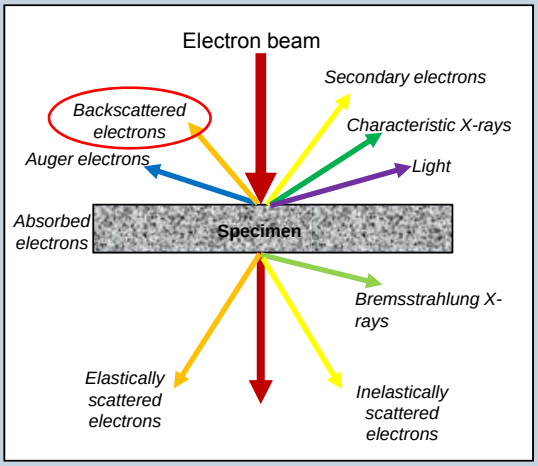


From: Kizilyaprak et al (2014) J Microscopy, 254(3).

3D EM Techniques

SEM - Serial Block Face Sectioning with Gatan 3View

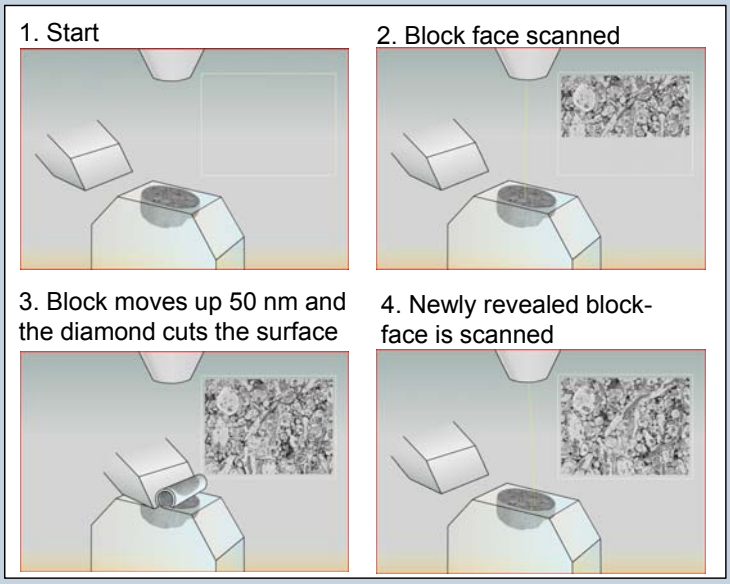
SEM can be used to generate 'TEM' images by detecting backscattered electrons, beam electrons that have been elastically scattered/deflected by high atomic number elements (heavy metals) in the sample



3D EM Techniques

SEM - Serial Block Face Sectioning with Gatan 3View

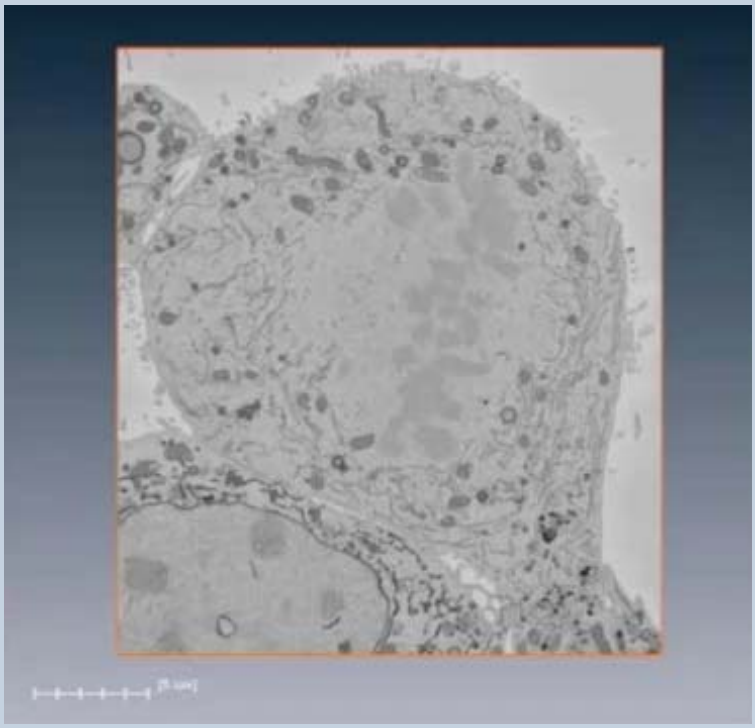
One method for generating a 3D high resolution image stack is to use serial block face sectioning with the Gatan 3View system



Courtesy Gatan UK

3D EM Techniques

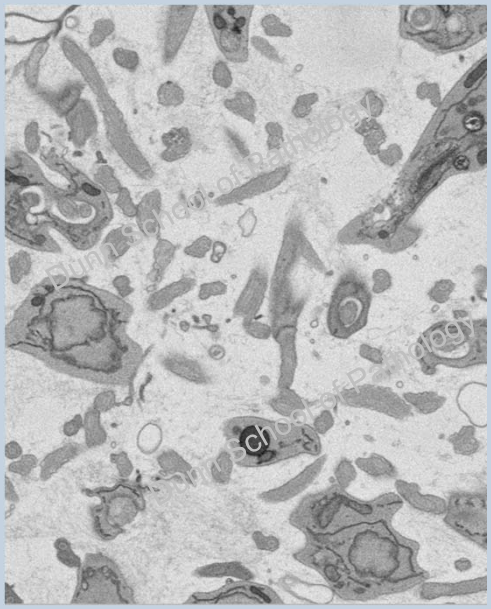
SEM - Serial Block Face Sectioning with Gatan 3View

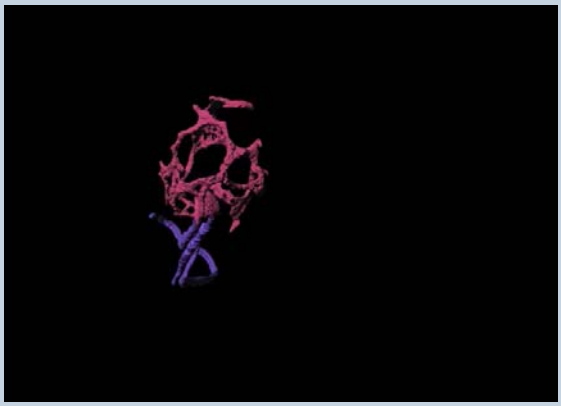


Huh-7 cell in early-metaphse with chromosomes and ER segmented and modelled
Puhka et al (2012) Mol Biol Cell 23(13)

3D EM Techniques

SEM - Serial Block Face Sectioning with Gatan 3View

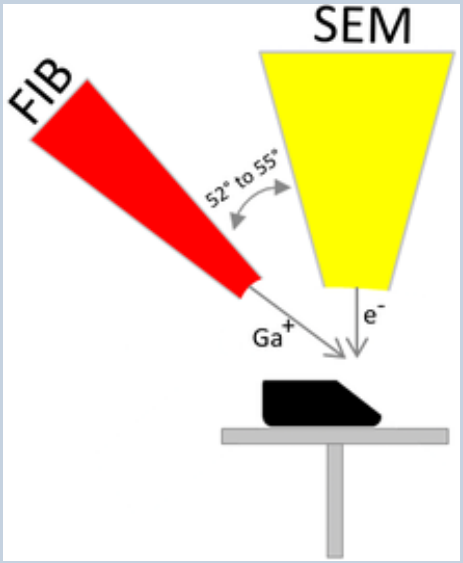




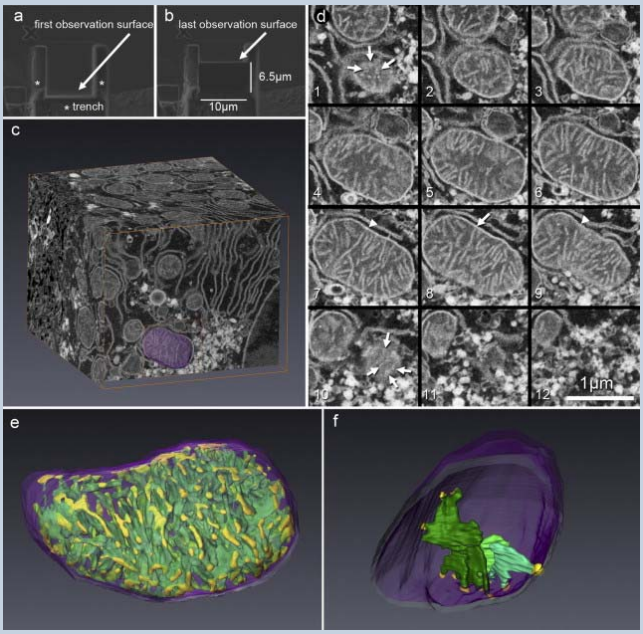
Leishmania Mexicana, promastigote form
Pixel size: 4.6 nm, Slice thickness: 50 nm, Volume: 9.8 µm x 12.2 µm x 16.1 µm, aligned, J Valli & E Johnson

3D EM techniques

SEM - Serial Block Face Sectioning with FIB



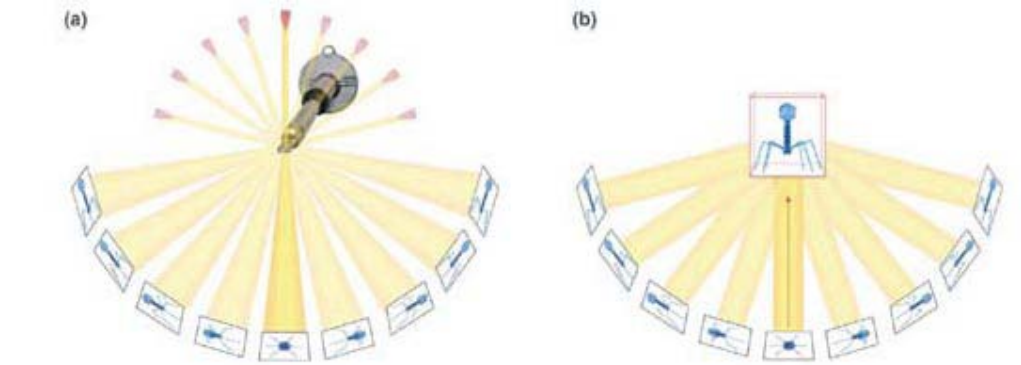
Schematic of a dual beam microscope with Focussed Ion Beam (FIB) and SEM columns (Kizilyaprak et al (2014) J Microscopy, 254(3).



FIB serial-sectioning of resin-embedded hepatocyte (Ohta et al (2012) Micron, 43(5): 612-620)

3D EM Techniques
TEM - Electron tomography

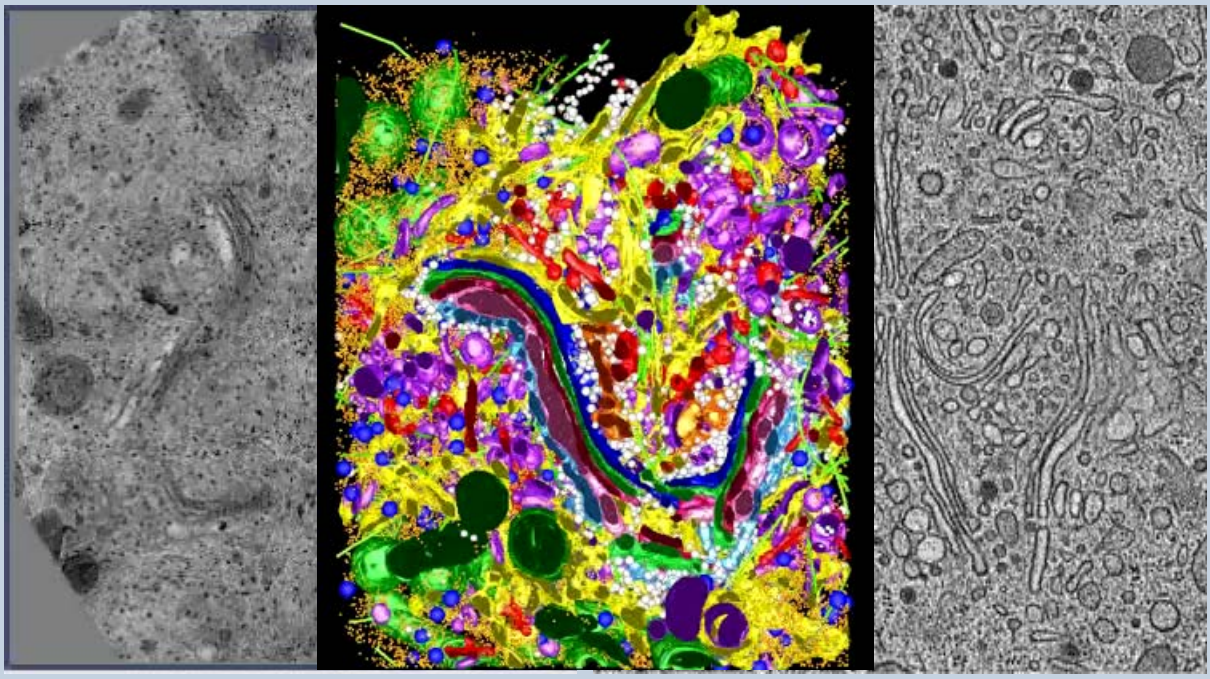
- Thicker sections (150-300 nm) on filmed slot grids with gold fiducial markers
- Use specialised tomography holder for dual axis tilting of the specimen
- Reconstruct using modelling software



Principles of Electron Tomography. (a) A biological specimen, in this case a bacteriophage contained in an EM sample holder, can be imaged from several orientations by tilting the holder in the electron microscope. (b) Process of computed backprojection, in which each tilted view is used to reconstruct to three-dimensional information of the original structure. [McIntosh, et al. (2005) Trends Cell Biol. 15:43-51].

3D EM Techniques

TEM - Electron tomography

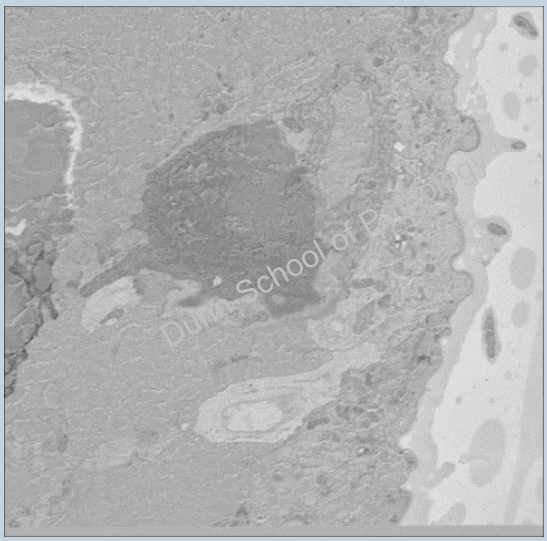


3D ultrastructure in the Golgi region of a pancreatic beta cell line.
Volume: ~3.1 × 3.2 × 1.2 µm, *Marsh et al (2001) PNAS, 98.*

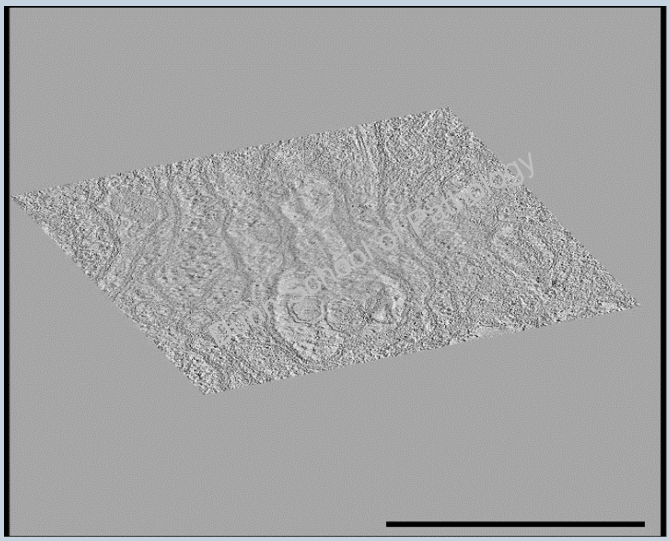
3D EM techniques

Comparing 3View and TEM tomography

3View



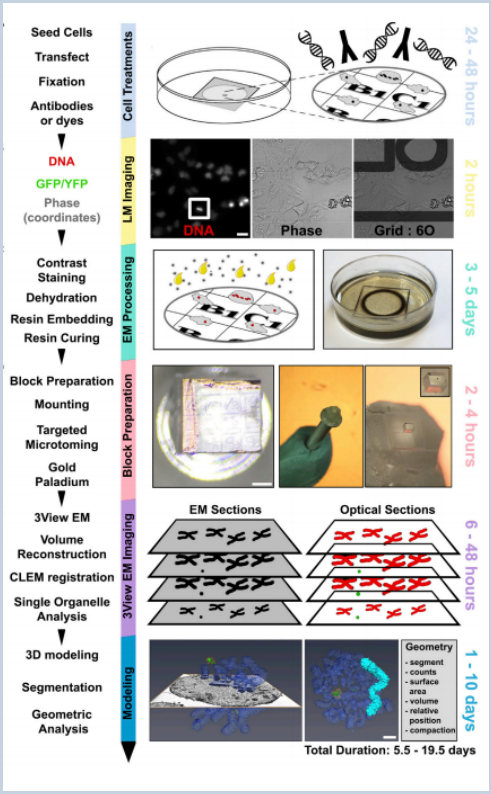
TEM tomography



Sensory cilia in Drosophila notum tissue imaged on the Zeiss Merlin Compact 3View SEM (left) and with serial section EM tomography on the Tecnai12 TEM (H Roque/E Johnson)

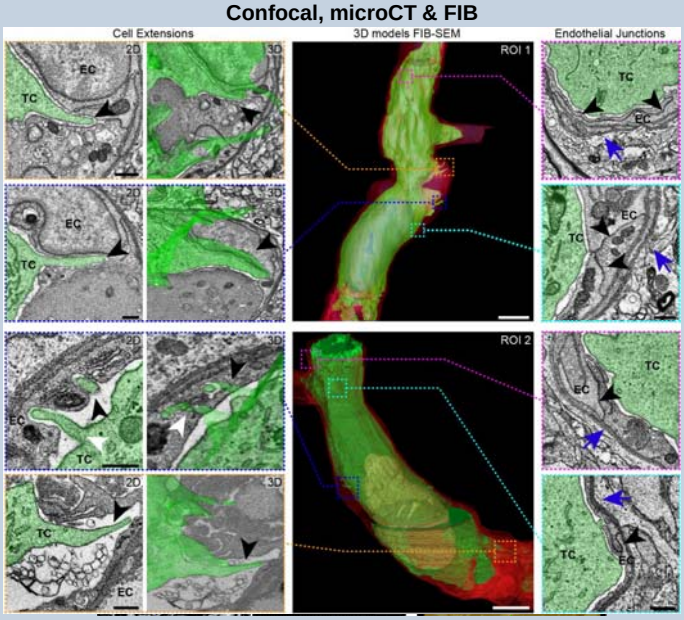
3D CLEM

Confocal & 3View



Booth et al (2016). Molecular Cell, 64:70-802

Confocal, microCT & FIB



Karreman et al (2016) Journal of Cell Science, 129:444-456

Questions?

TEM of Leishmania (E Johnson)