Imaging of endocrine organs

Helen Christian

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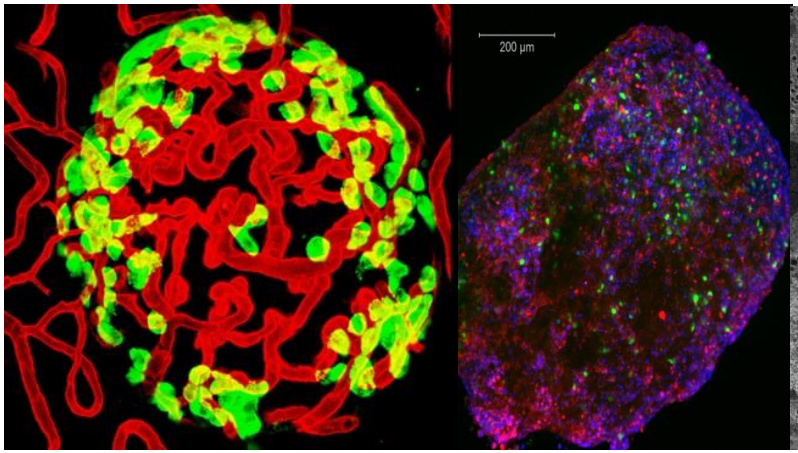
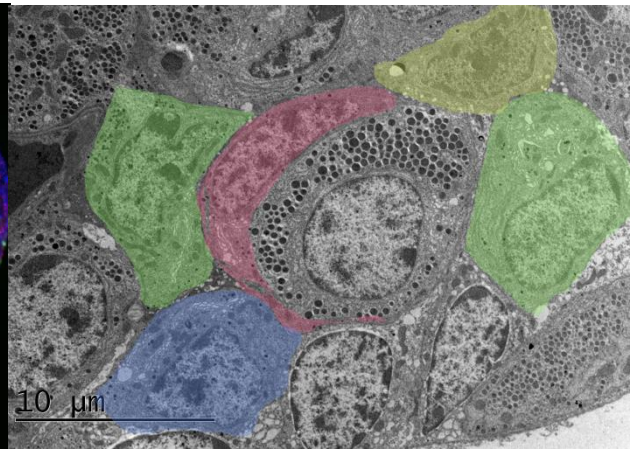
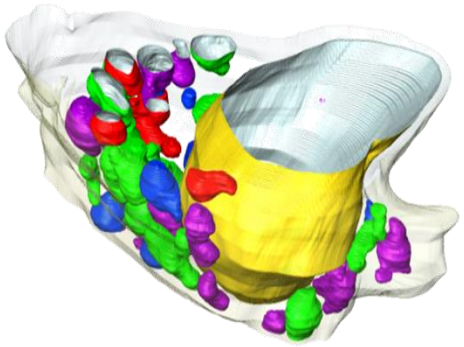
Diabetesforum, Stockholm 2017



Islets of Langerhan

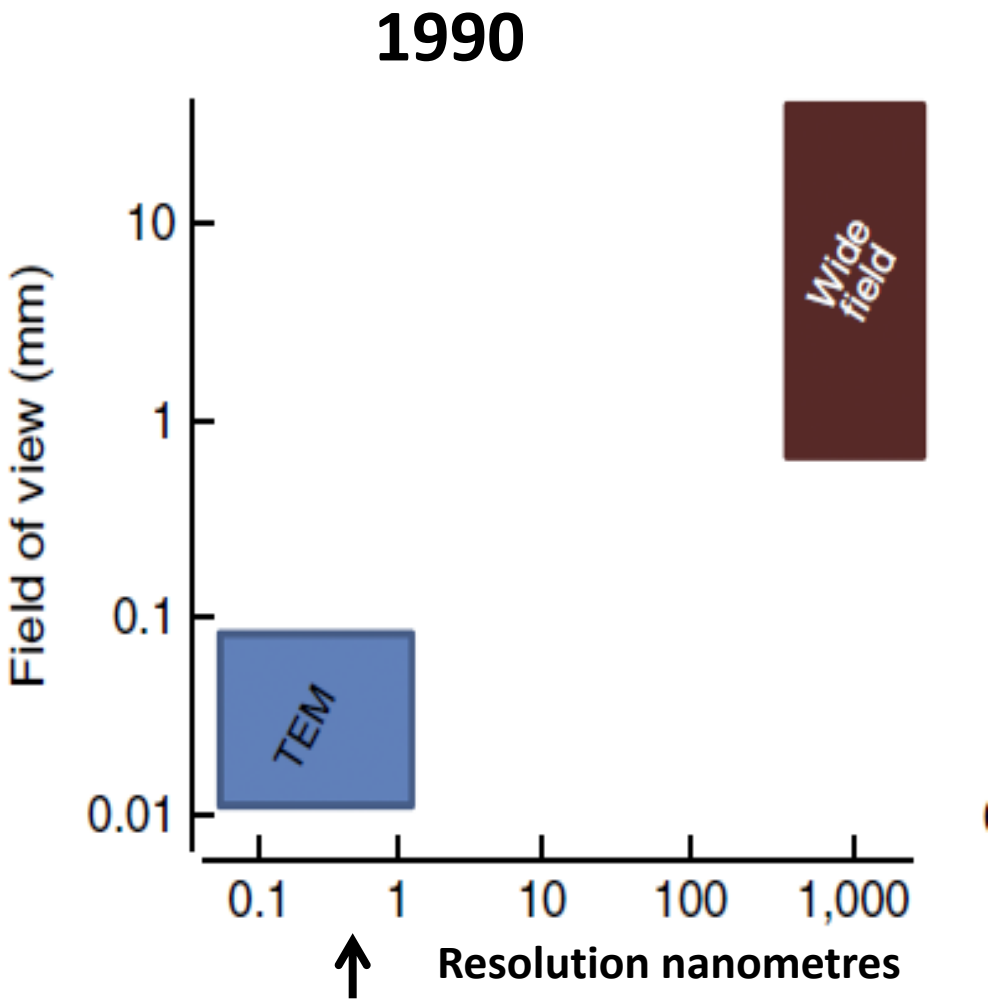


Pituitary gland



Renin cells in kidney

Imaging has made huge advances in recent years



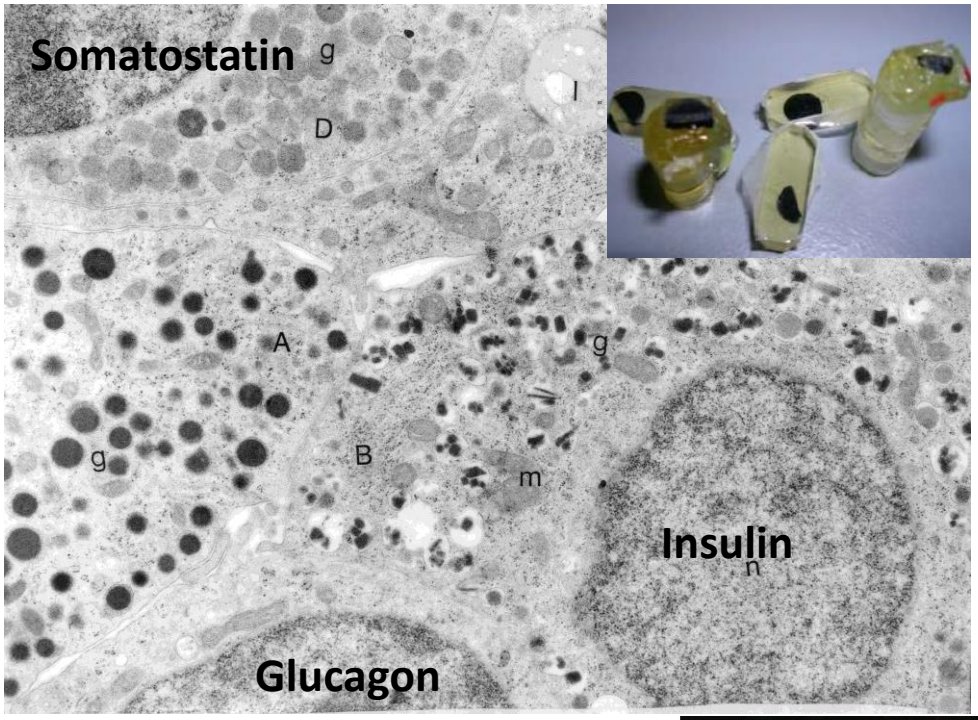
transmission electron microsocopy (TEM) and light microscopy: field of view vs resolution

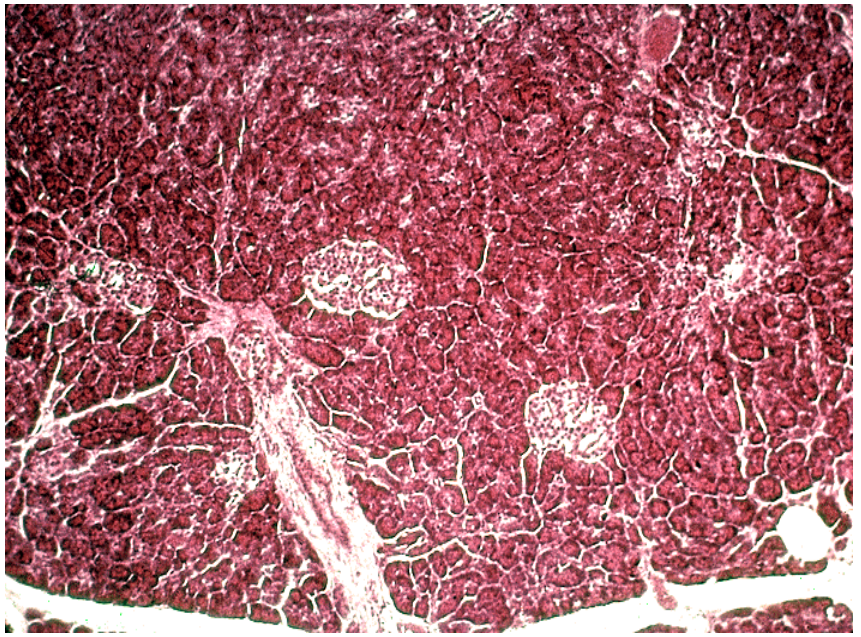
Fixed post-mortem cells/tissues, 2 D

De Boer et al 2015 Nature Methods

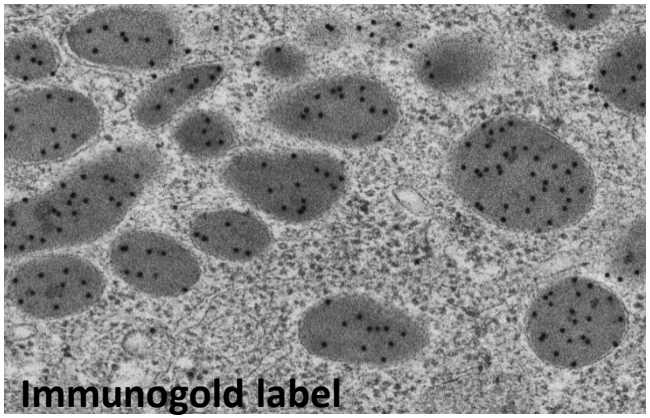
Imaging of the endocrine pancreas

Formaldehyde –fixed cells/tissues in 2D

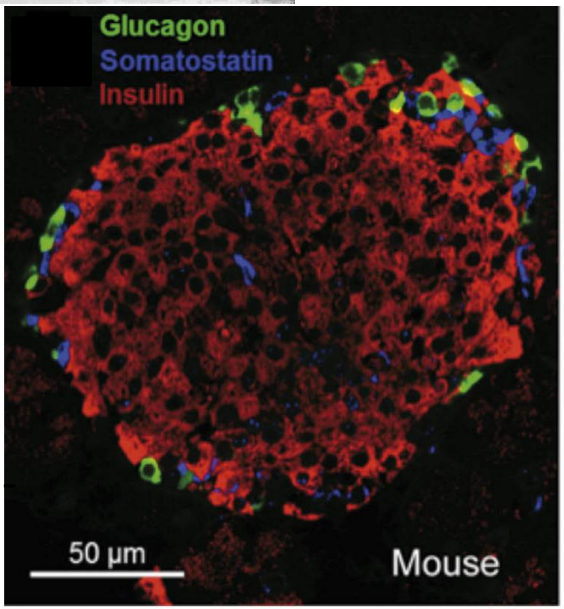


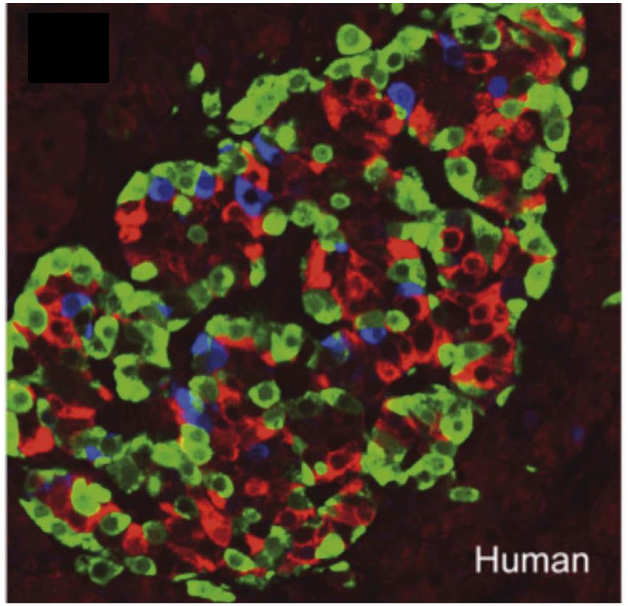


Light microscopy -low detail, large area



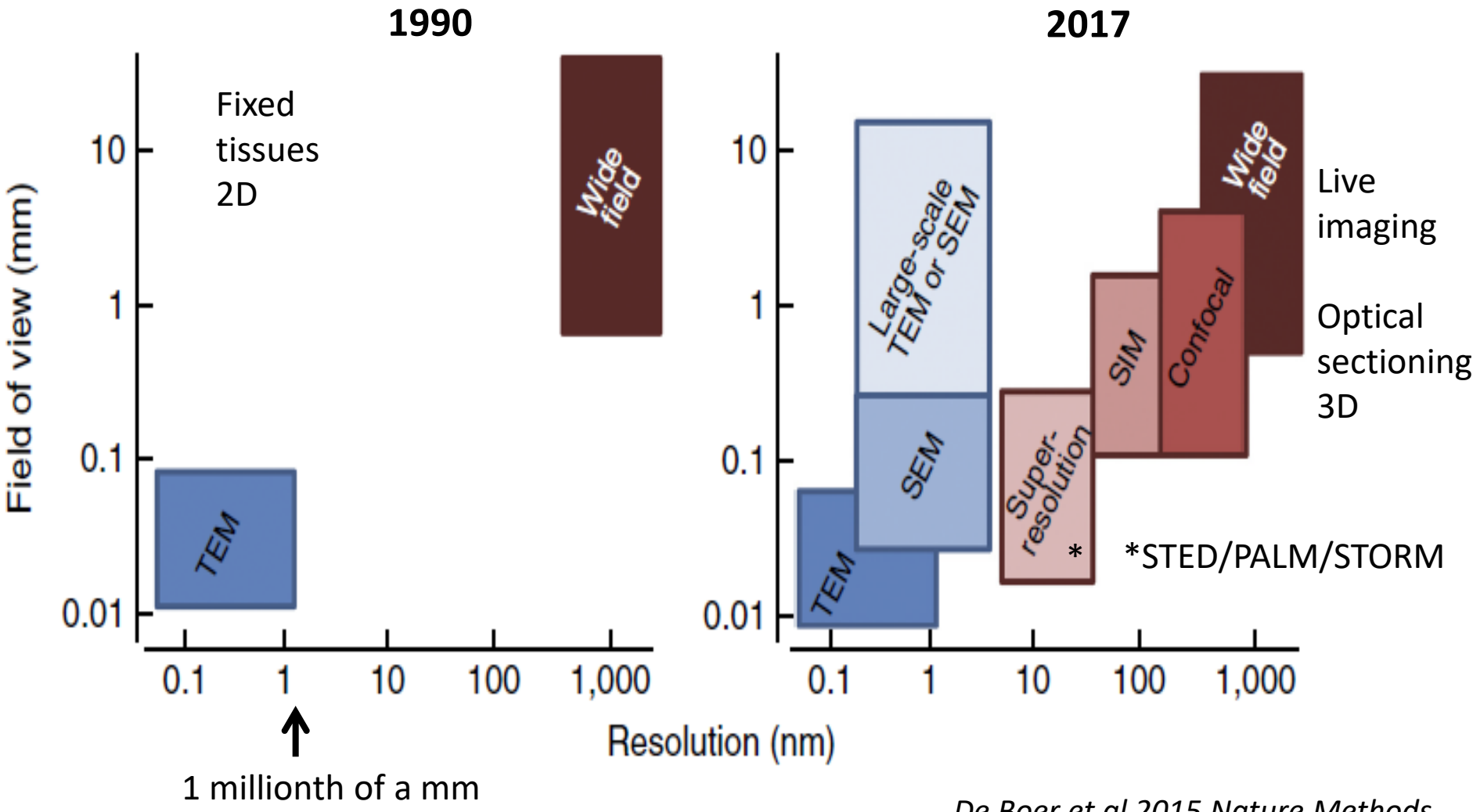
Transmission electron microscopy
high detail, small area





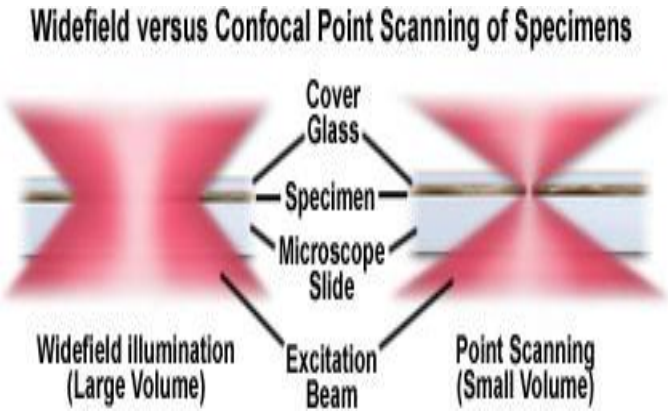
Imaging has made huge advances in recent years

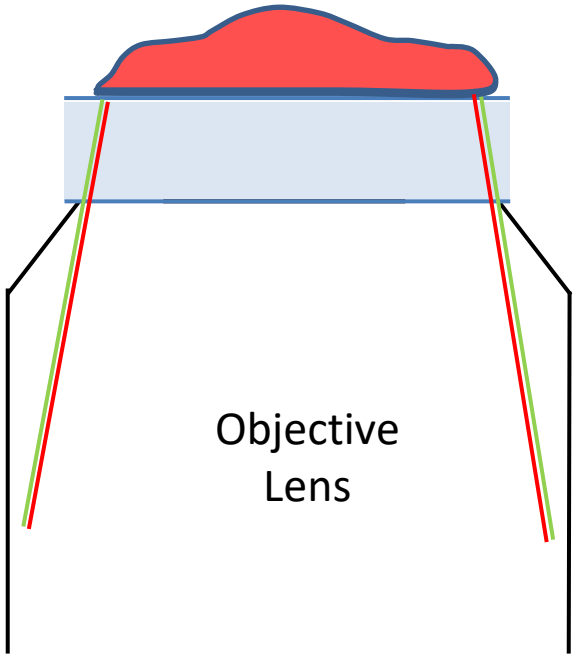
'from dead cells to live cell movies'



De Boer et al 2015 Nature Methods

Widefield microscopy of live endocrine cells in culture





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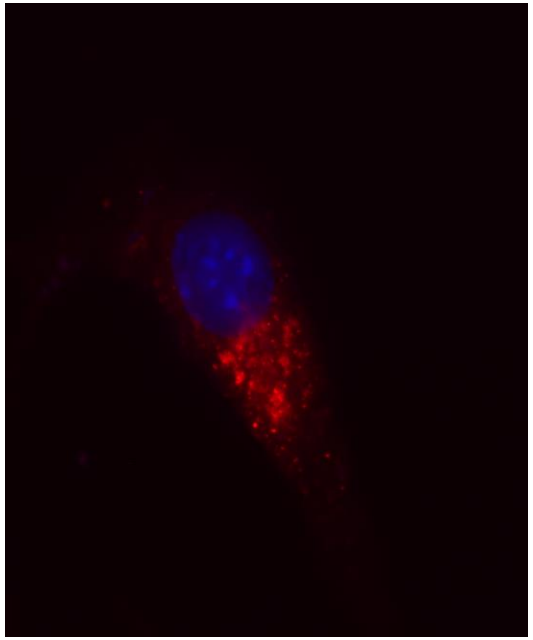
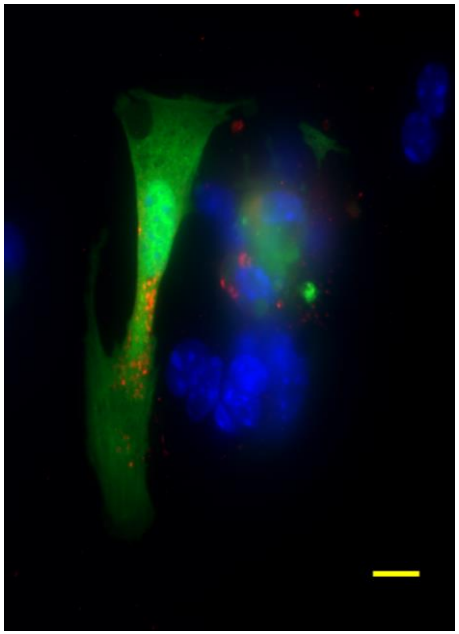
Live cell *in vitro* imaging in cell culture

- **Whole sample illuminated, all light collected**
- **can get high resolution from a single cell layer**



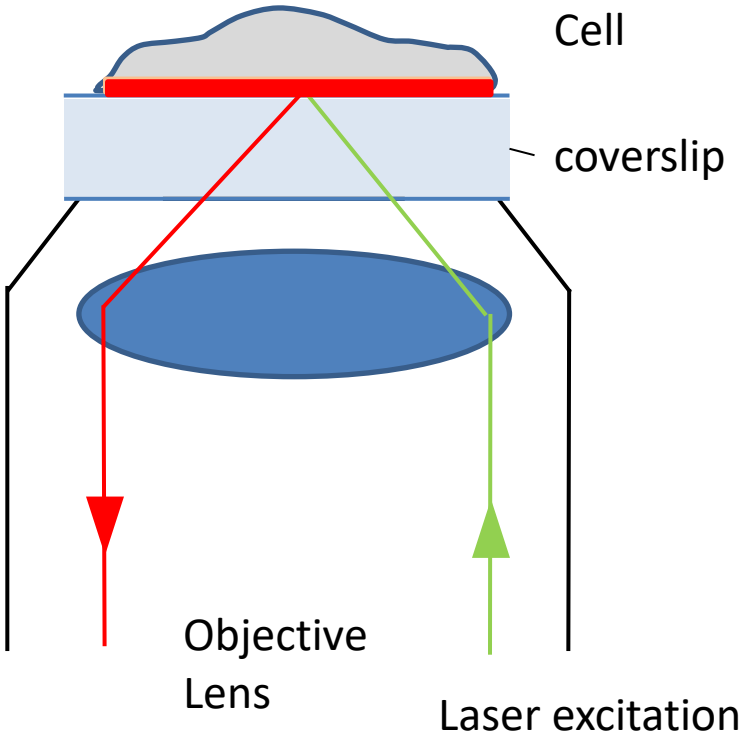
mCherry Golgi marker construct expressed in Ins1 cells; DAPI (blue) nucleus

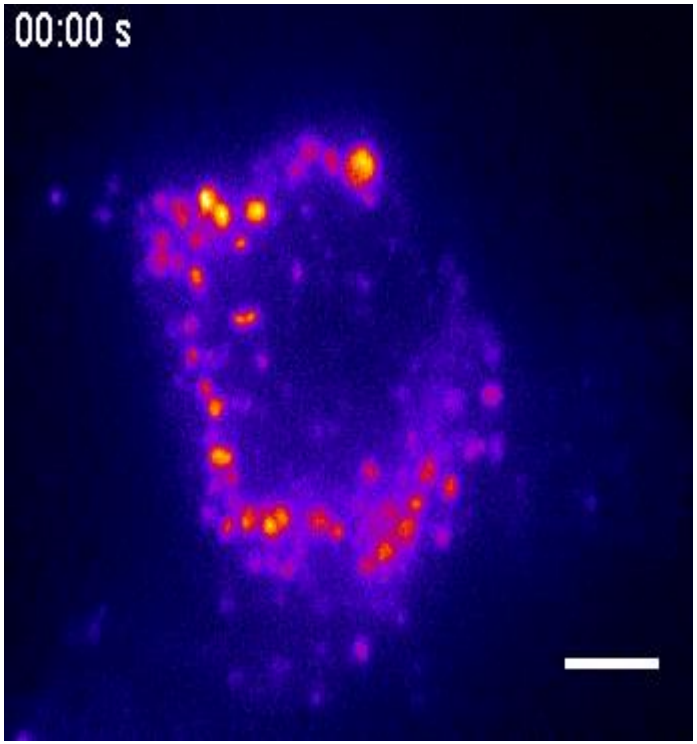


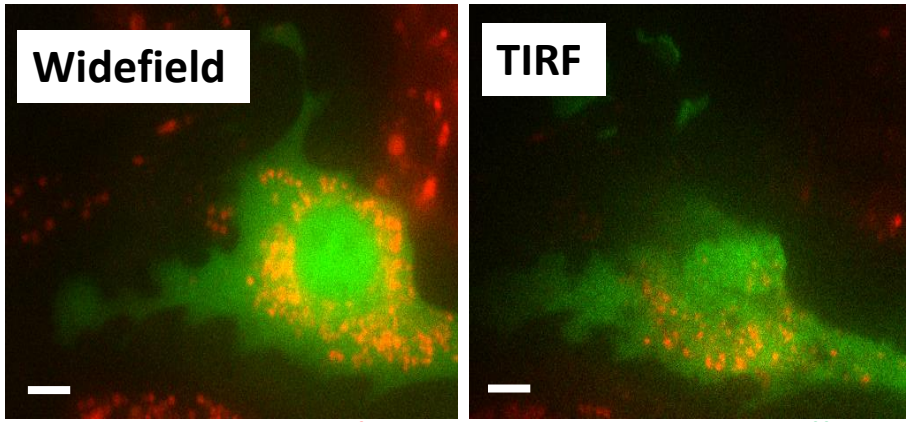
Renin cells GFP-labelled; RFP:Renin

Live *in vitro* imaging Total Internal Reflection Microscopy

- **valuable for studying secretory granule movement**
- **laser light only illuminates approx 200nm into sample so better resolution**







Scale bar: 5um Lysotracker Renin expressing cells

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ESRIC Edinburgh Super-Resolution Imaging Consortium

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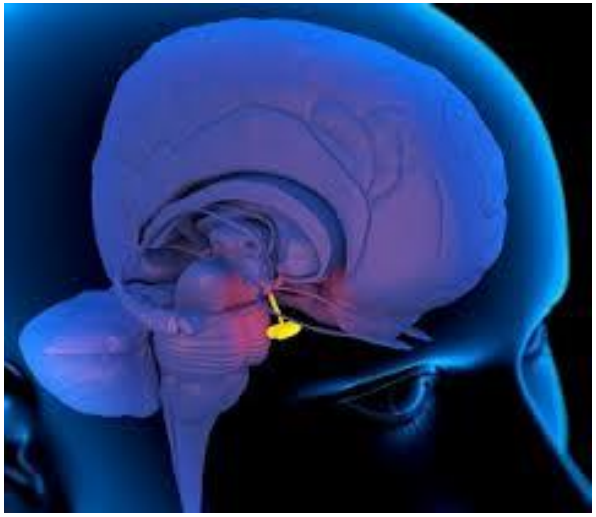
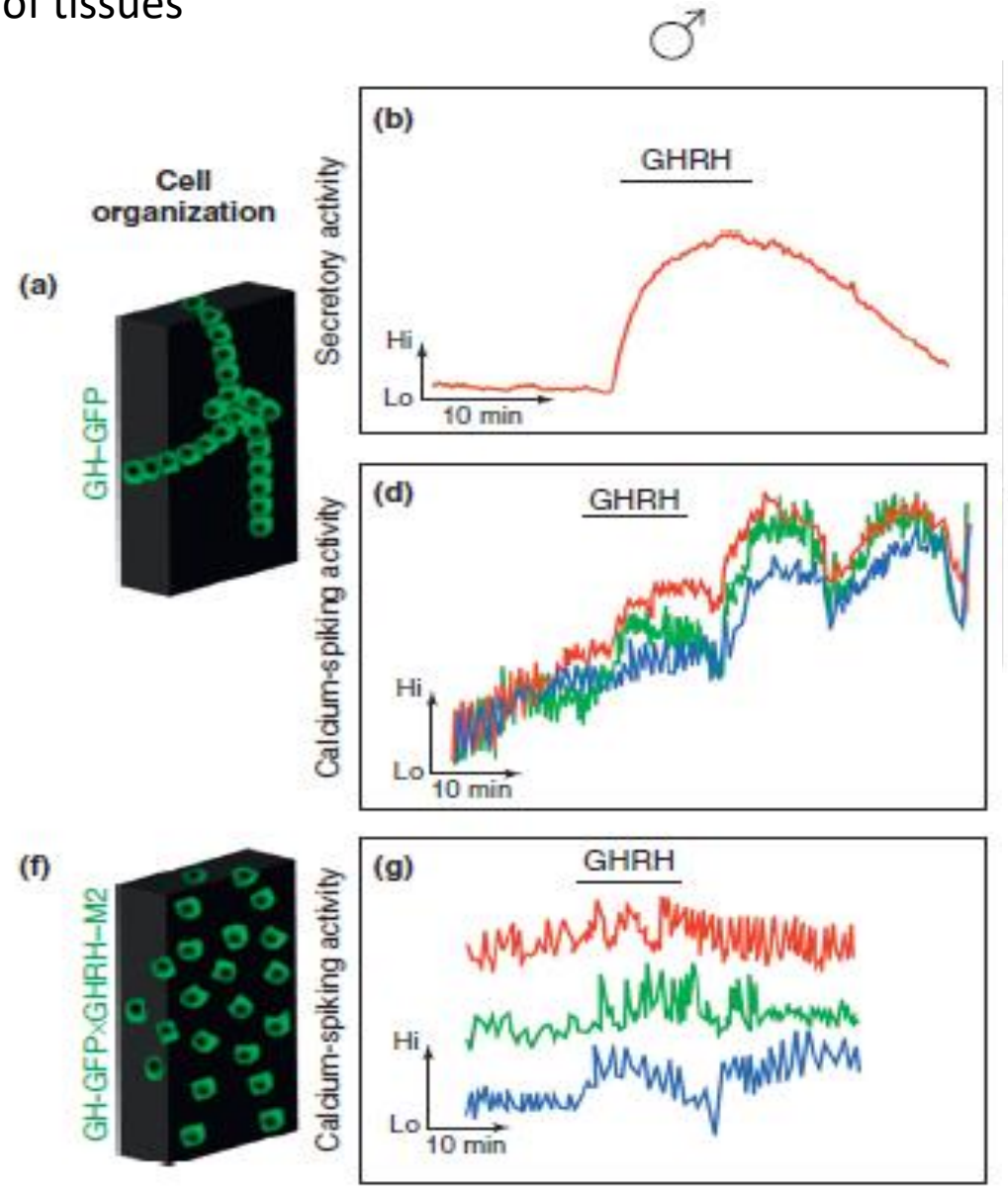
Pituitary cell networks- Two-photon excitation microscopy

Advantage - greater depth of 3D imaging of tissues



- **3D imaging revealed that cells of each hormone type form extensive and structured networks**

- **Networks contribute to the coordination of the cell response to stimuli**

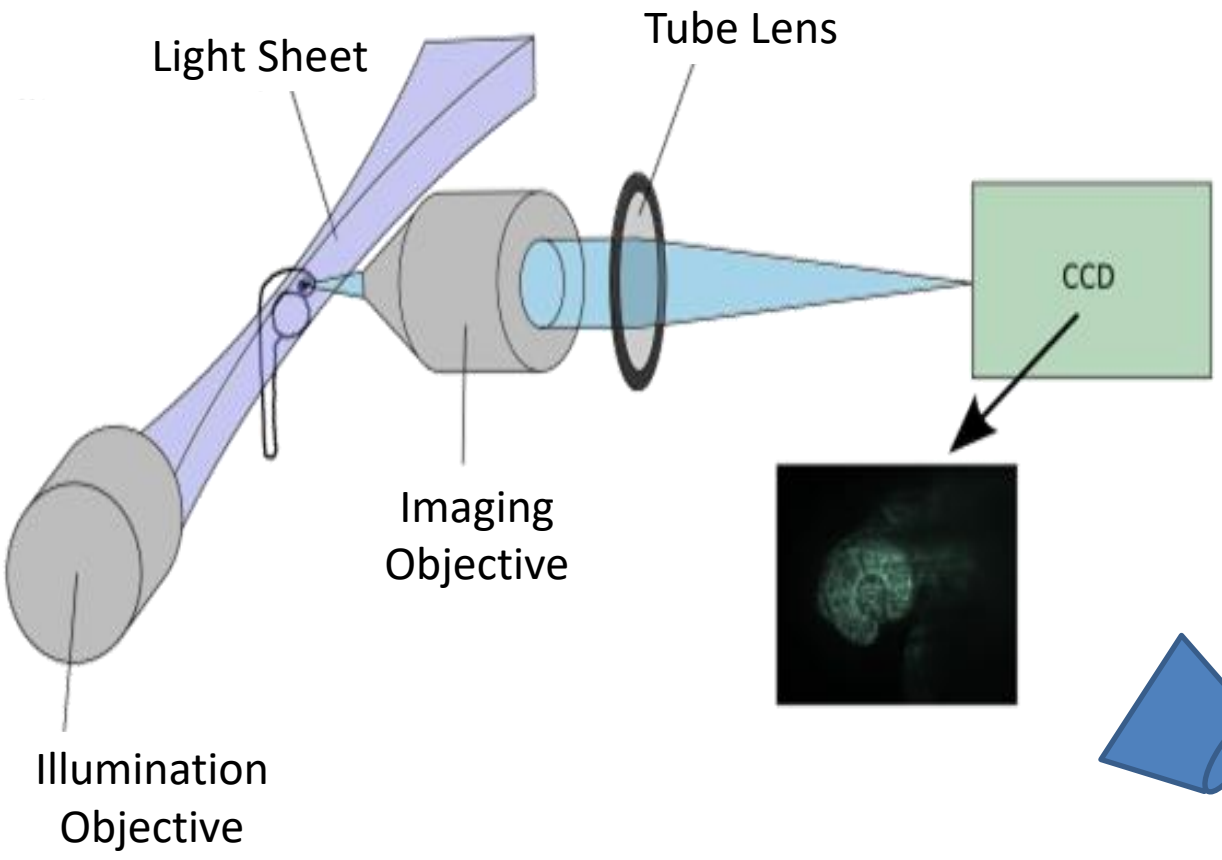


Light sheet: Selective Plane Illumination Microscopy (SPIM)

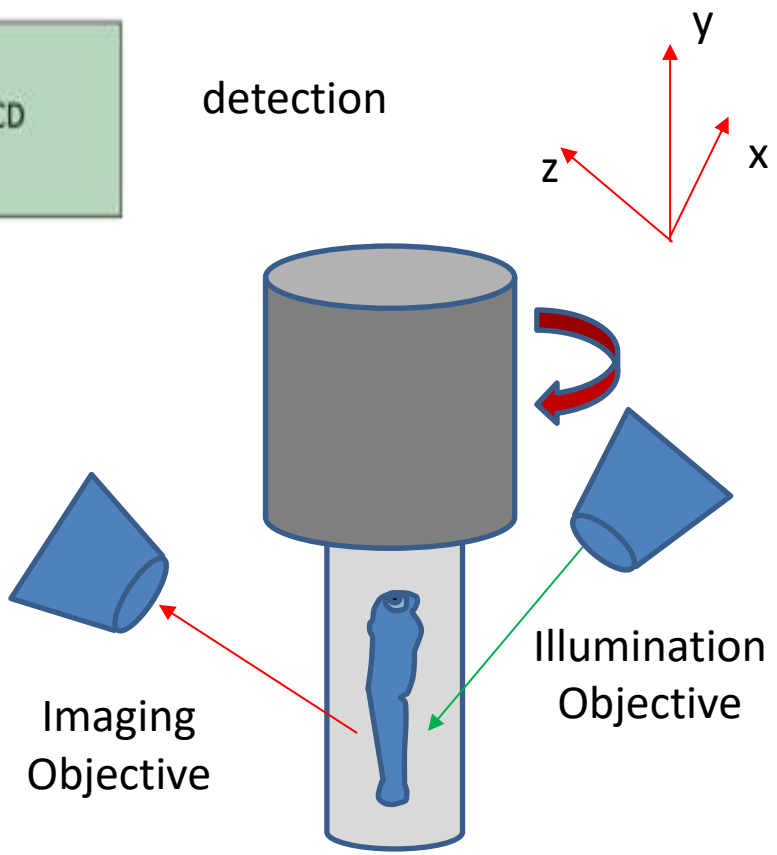
Live in vivo imaging

Reduced photobleaching,
Low phototoxicity
Good resolution
Rapid acquisition speed

Optical Setup



detection

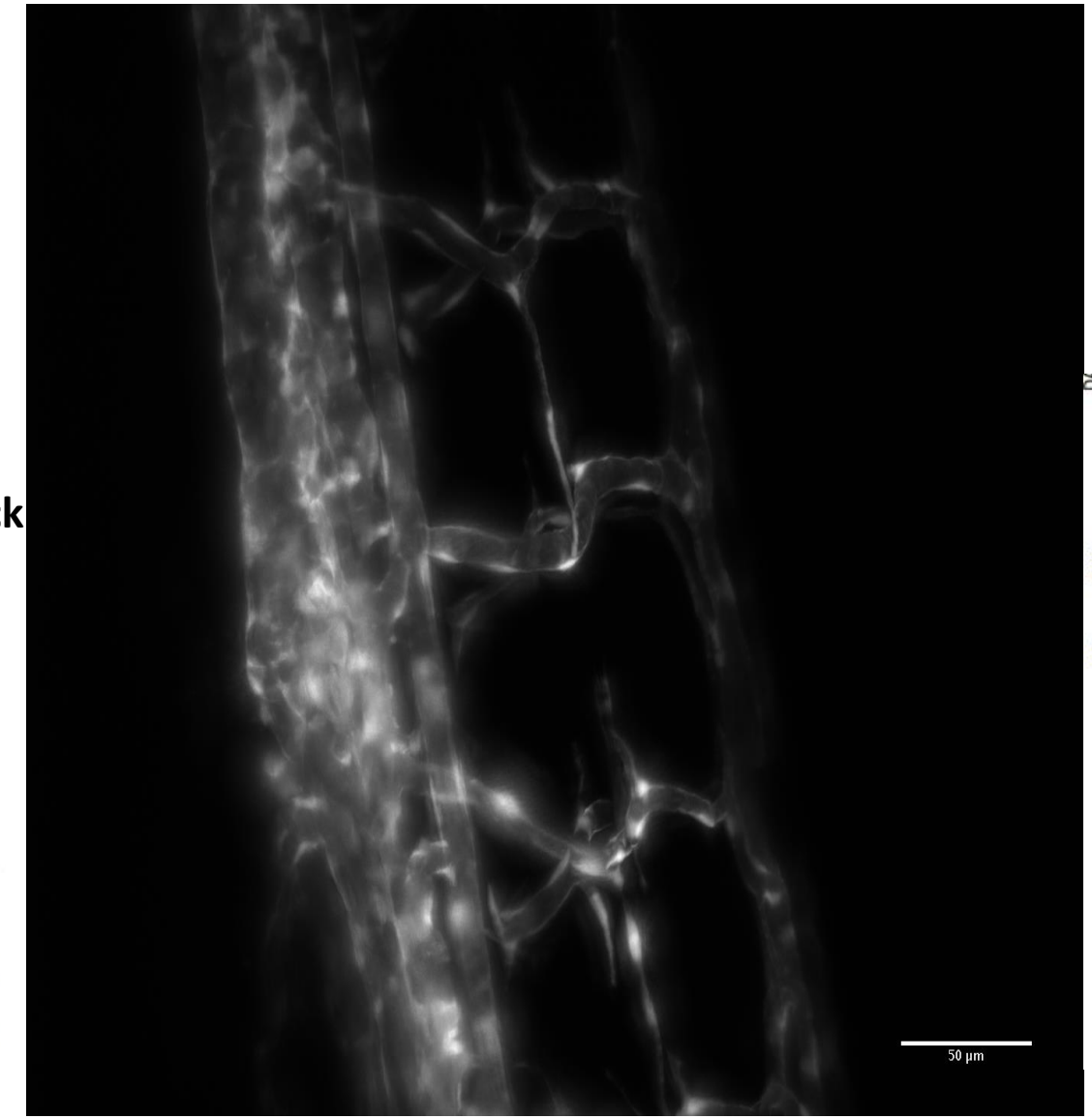


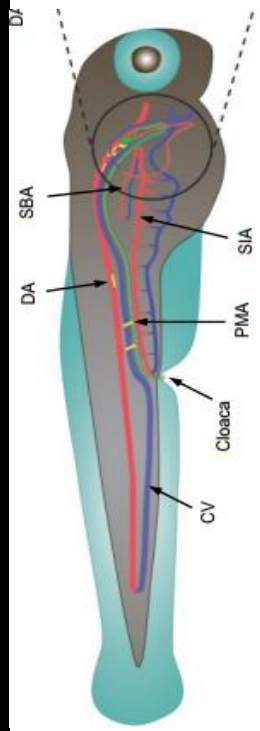
Specimens must be opaque for imaging

Light sheet: Selective Plane Illumination Microscopy (SPIM)

Example of resolution and imaging quality of vasculature

Live
4dpf Flk:GFP zebrafish
PTU Treated
Embedded 1% Agar
Projection of 100um stack





Durham University

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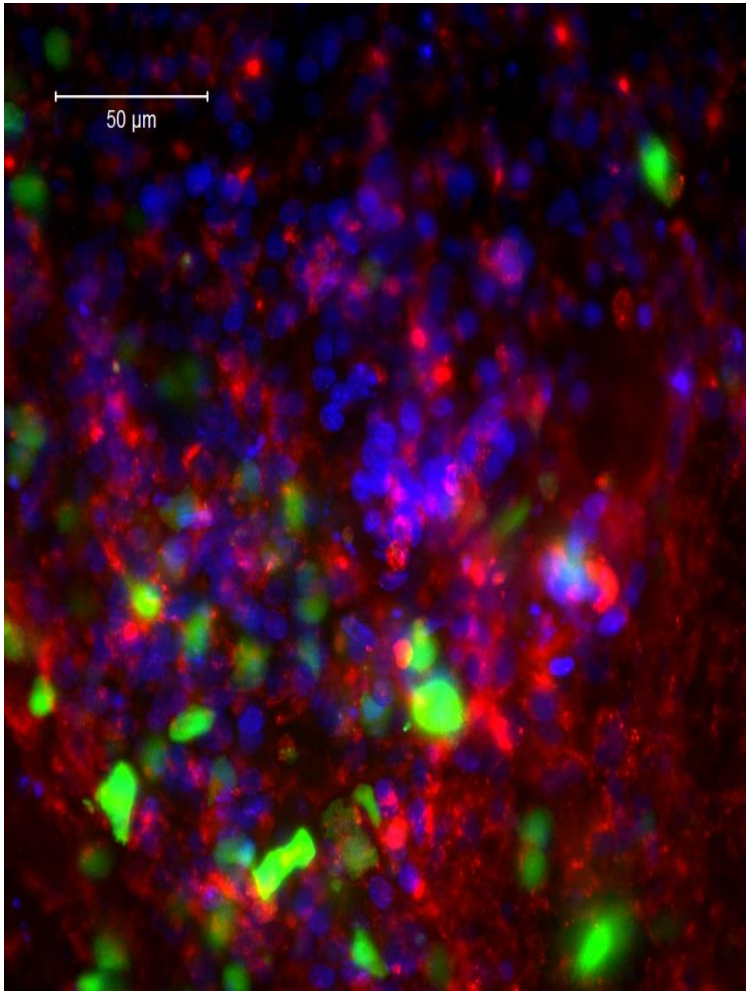
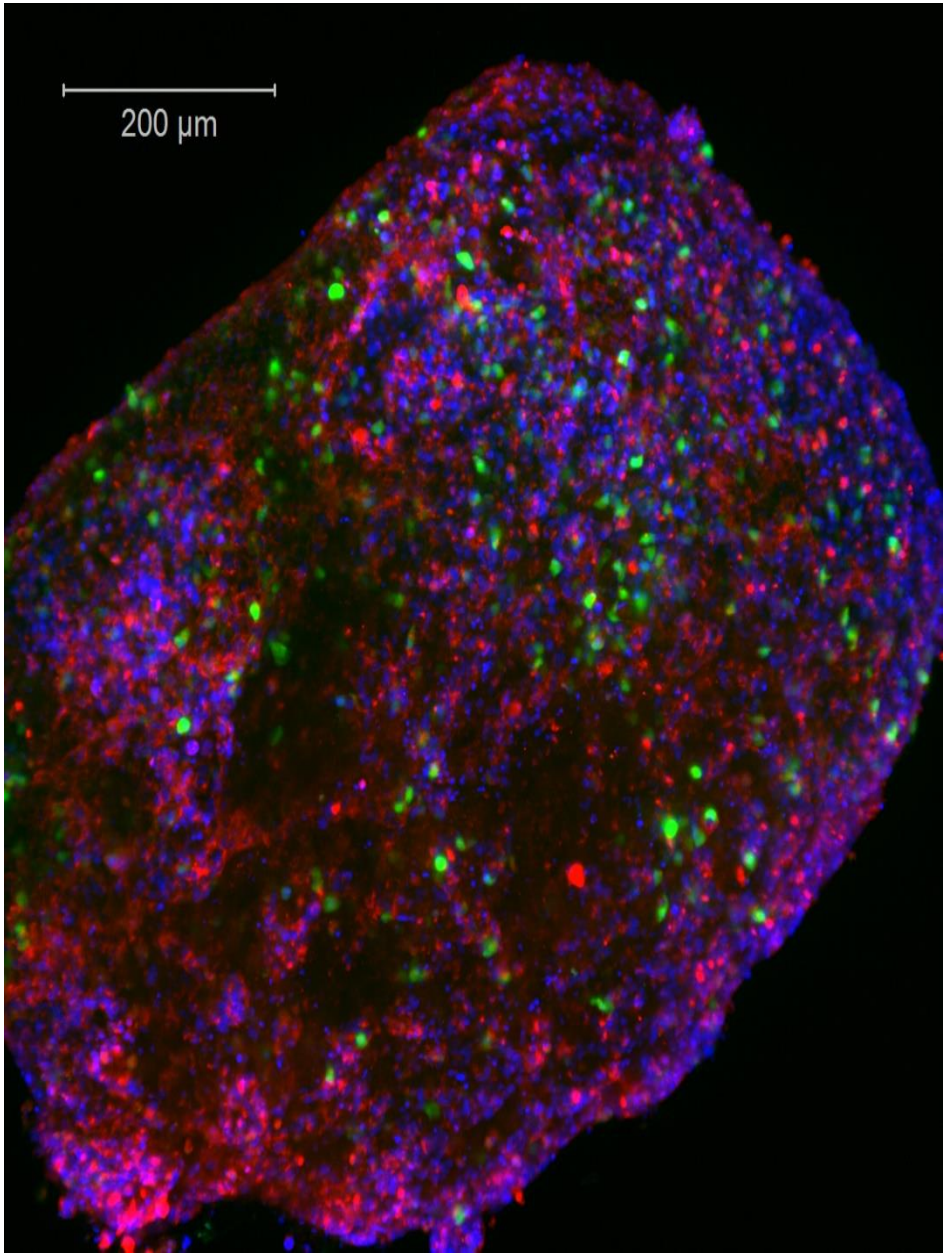
Light sheet imaging of pituitary





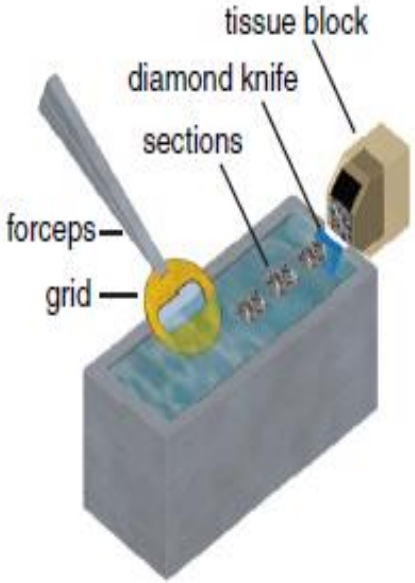
Red-peanut agluttinin-Alexa 647
Green- EGFP reporter of PRL promotor activity
Blue-Hoescht nuclear stain

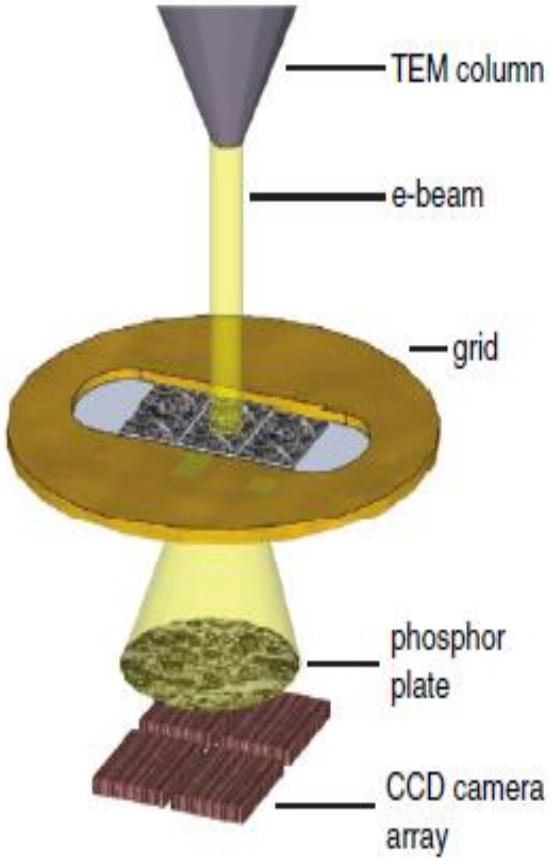
Anne McNamara, Dave Spiller, Mike White, Julian Davis

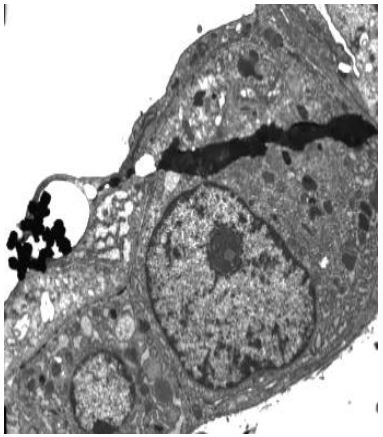
Volume Electron microscopy

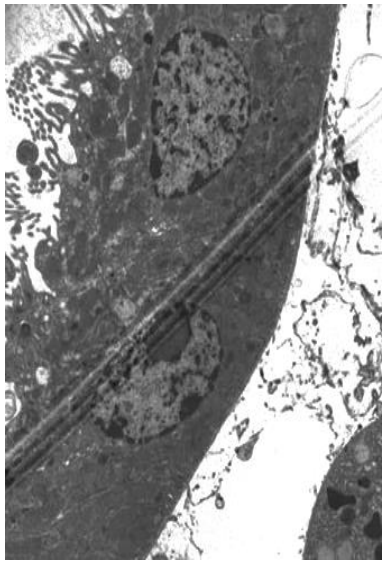
Challenge of analysis of large volumes of tissue

Manual 3D serial sectioning and reconstruction TEM





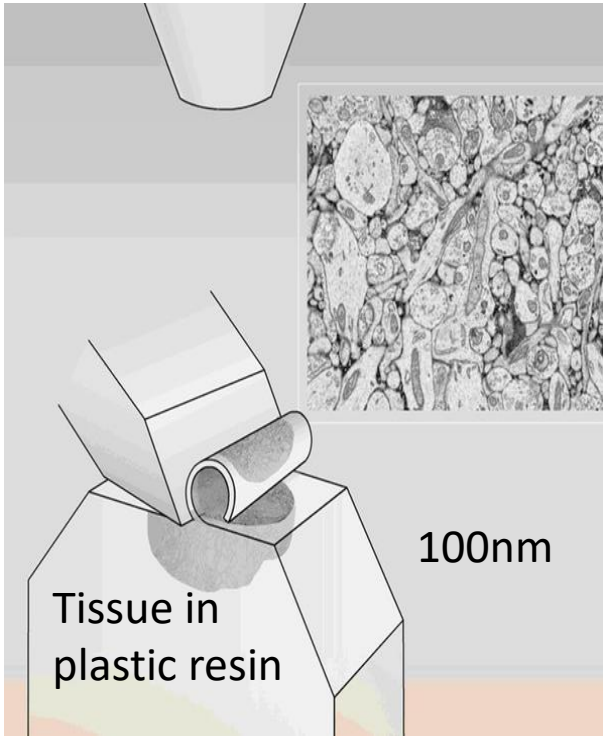


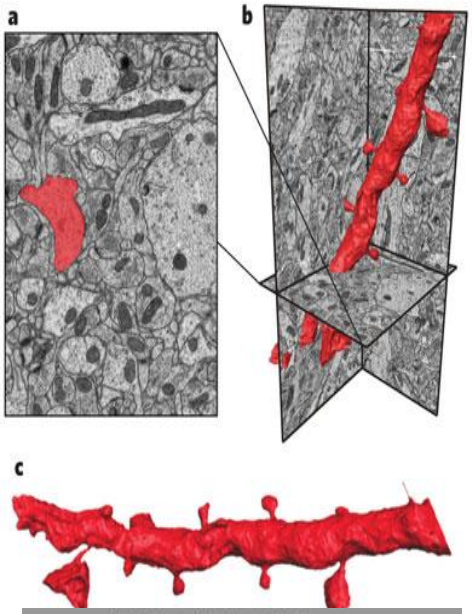


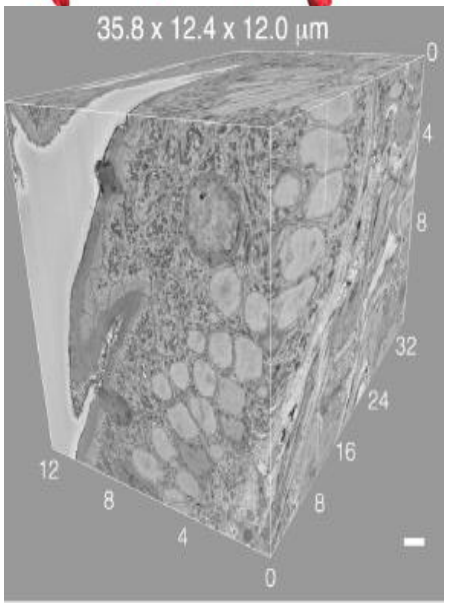
Advantages: Inexpensive, can immunolabel sections

Disadvantages: very labour intensive, easy to damage sections

Serial block face '3view' scanning electron microscopy







Peddie & Collinson (2014) Micron

Optimal imaging achieved with maximum contrast and conductivity of the specimen by impregnantion with multiple layers of heavy metals: osmium, lead and uranium

Volumes of 1mm^3 to 1000mm^3 published ; 500 sections 30GB

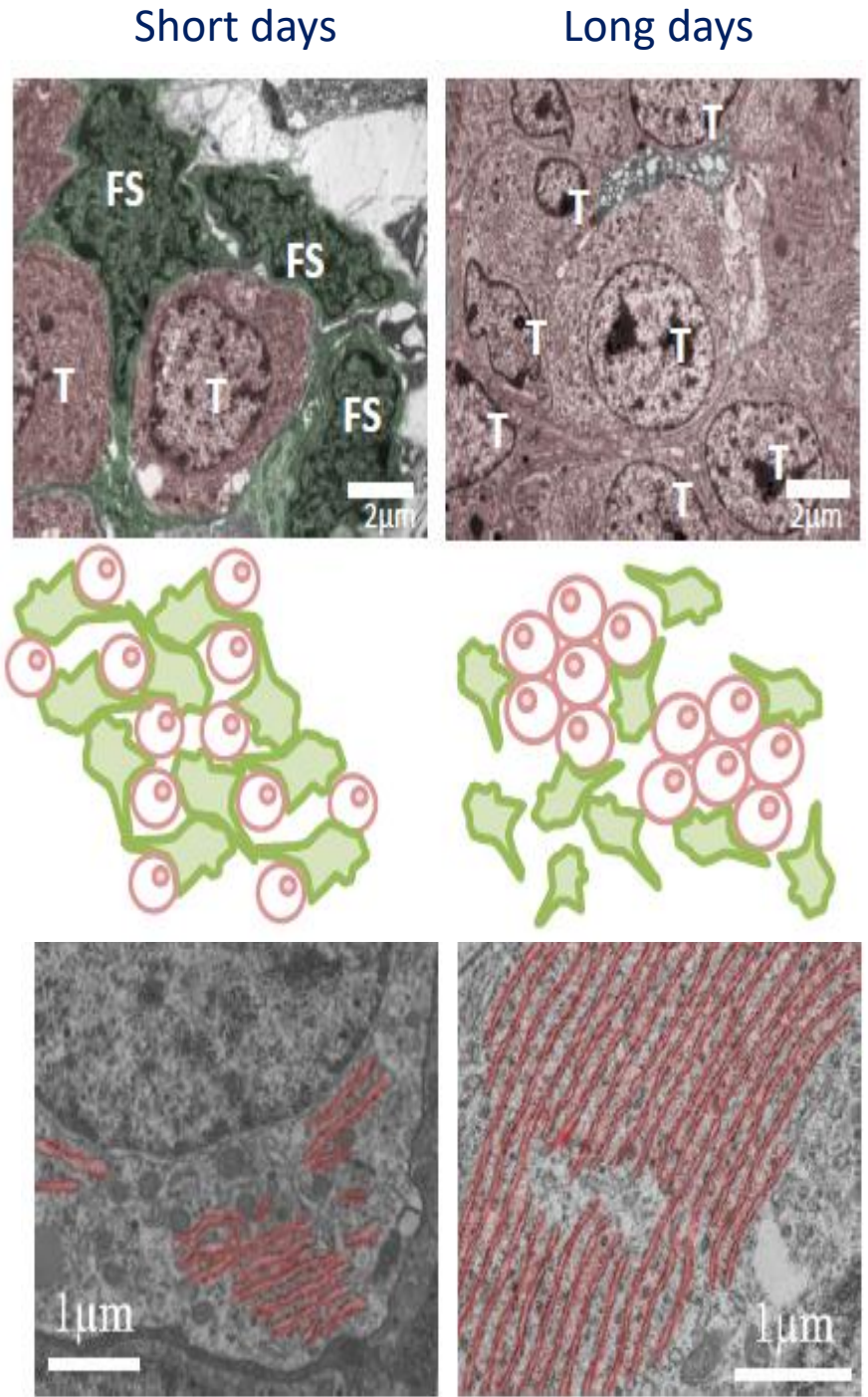
Cellular remodelling of pituitary cells with daylength

Folliculostellate (FS) and thyrotroph (T, melatonin sensitive) cells

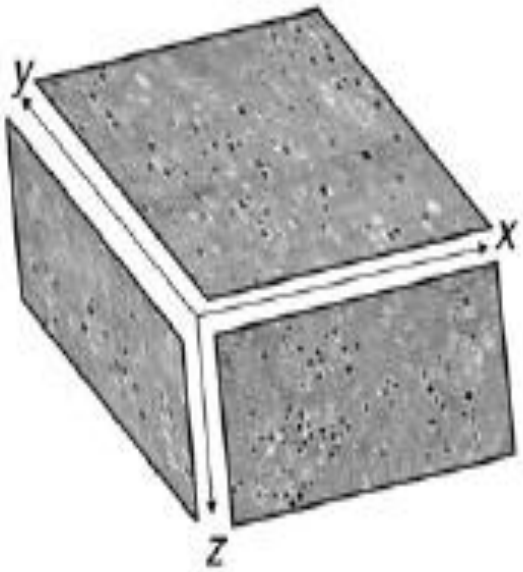
Circannual control of fertility (short days) and prolactin (high in long days)

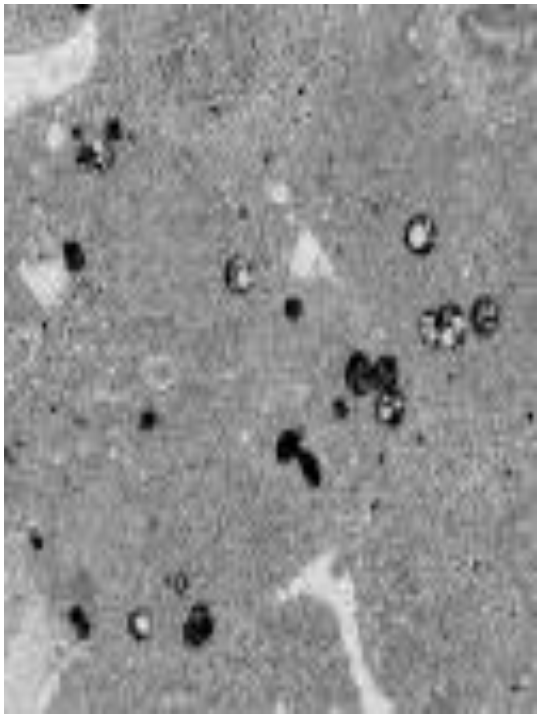
Increase in hormone production in long days

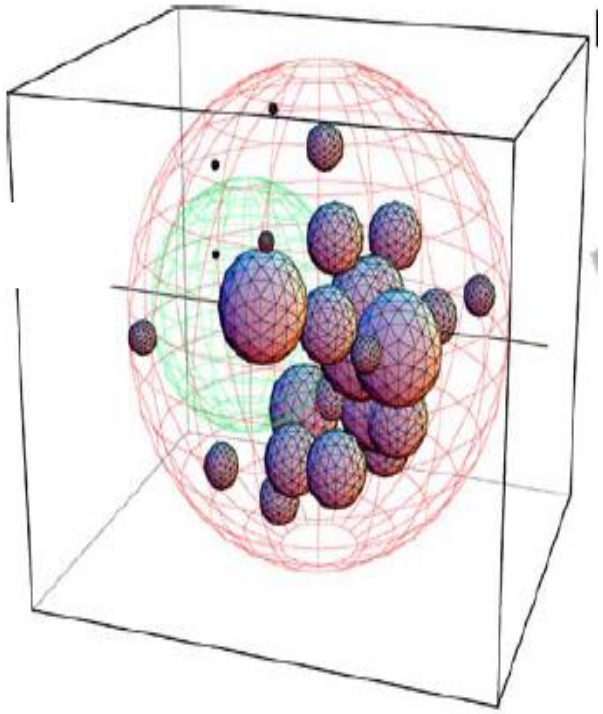
Wood, Christian et al 2015 Current Biology

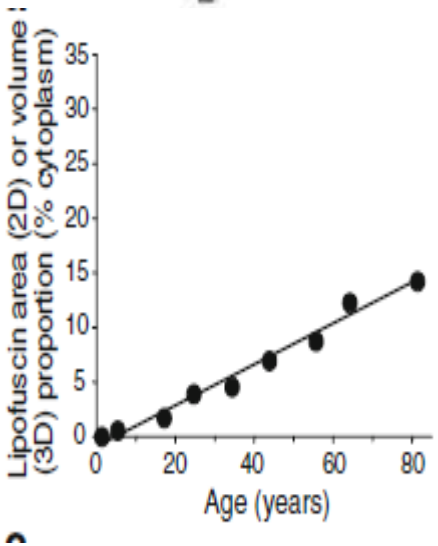


3view investigation of pancreatic beta cell lysosome dense bodies









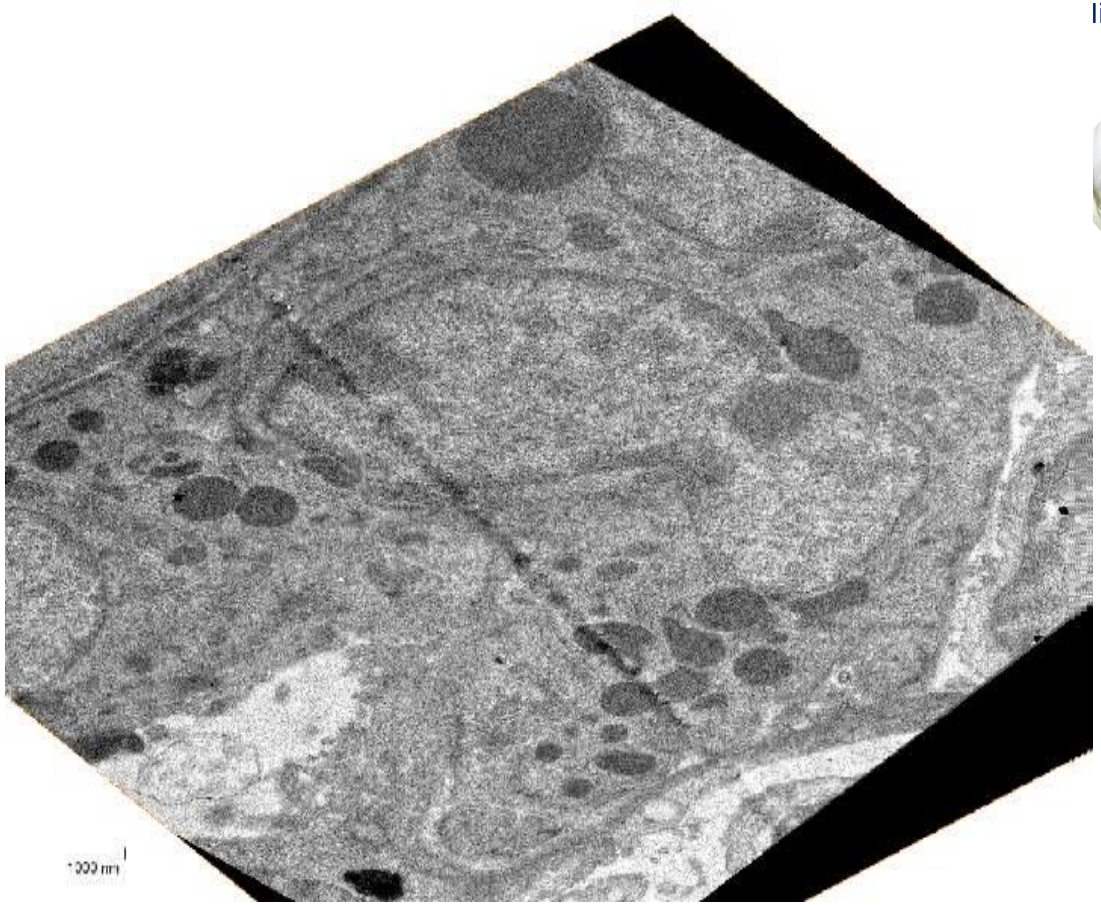
Automatic densitometric measurements possible due to electron-density of lipofuscin

3D reconstruction

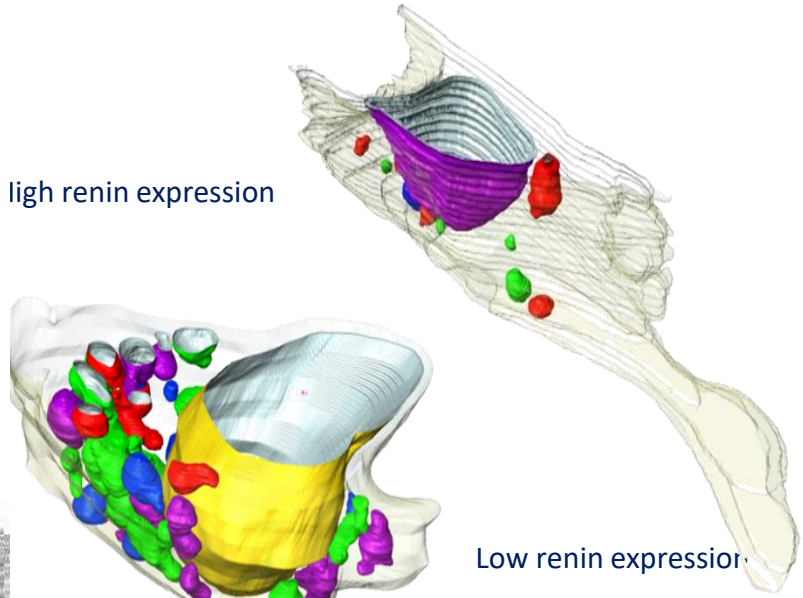
Cnop et al Diabetologia 2010

3view investigation of renin secretory granules in kidney

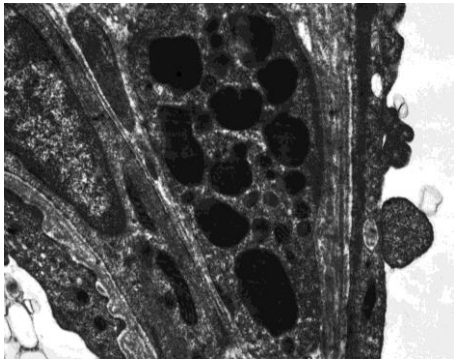
Granule volume and number in juxtaglomerular cells



ligh renin expression



Low renin expression



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Correlative light and electron microscopy (CLEM)

Enteroendocrine cells

Prepare fixed section for confocal

↓

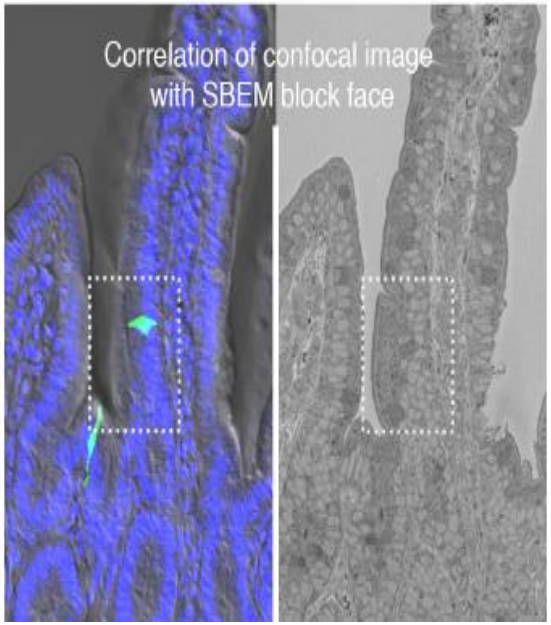
Image with confocal microscope

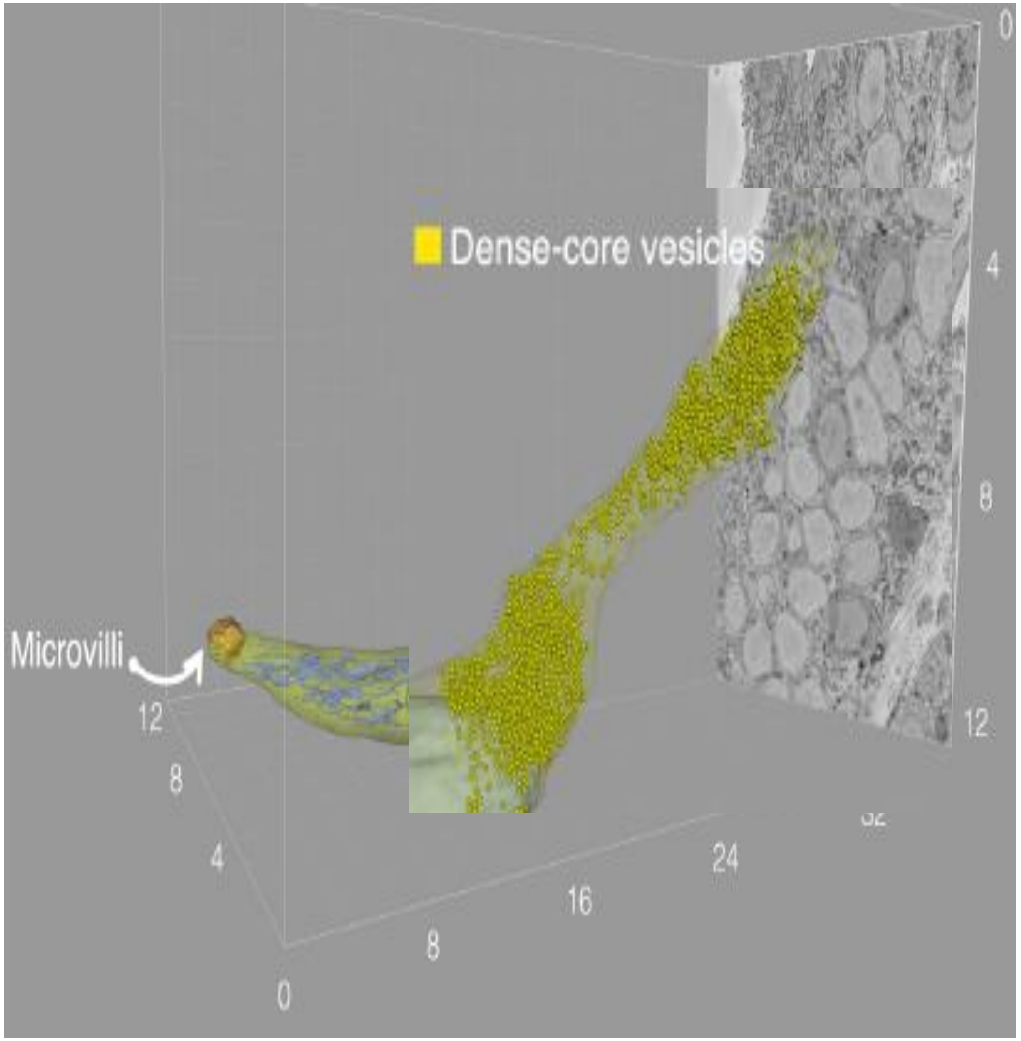
↓

Retrieve section and prepare for 3D EM

↓

Image with 3view EM



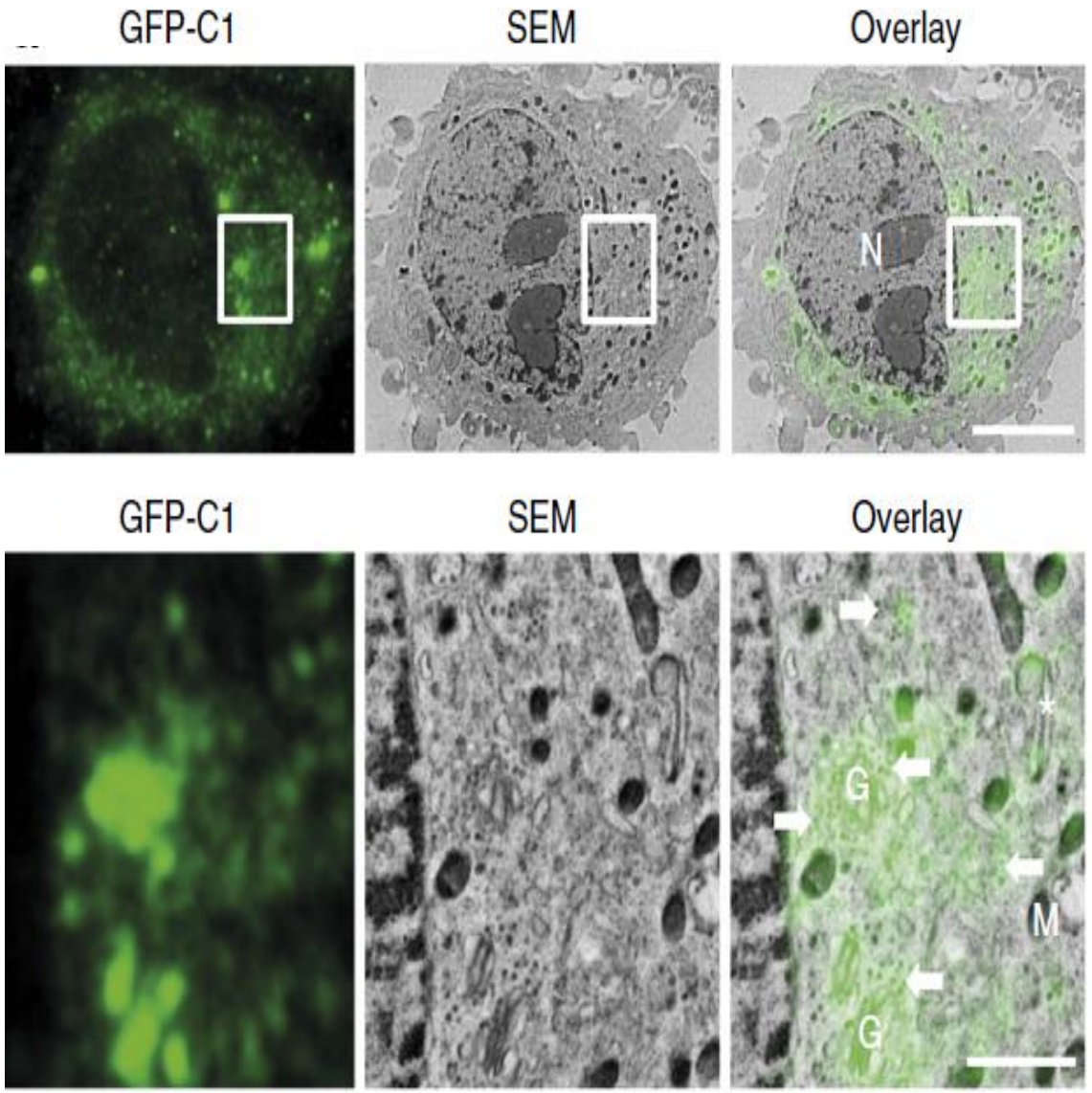


Bohorquez, PLoS one 2014

Simultaneous Correlative Light and Electron microscopy

Integrated light and electron microscopes

GFP is stable and active in resin-embedded cells



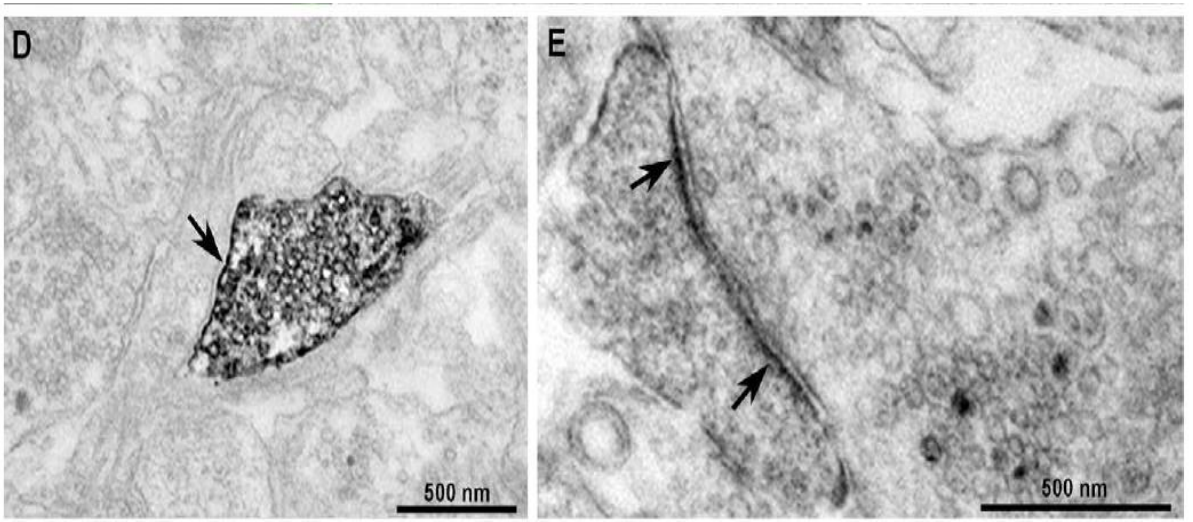
Peddie et al 2014

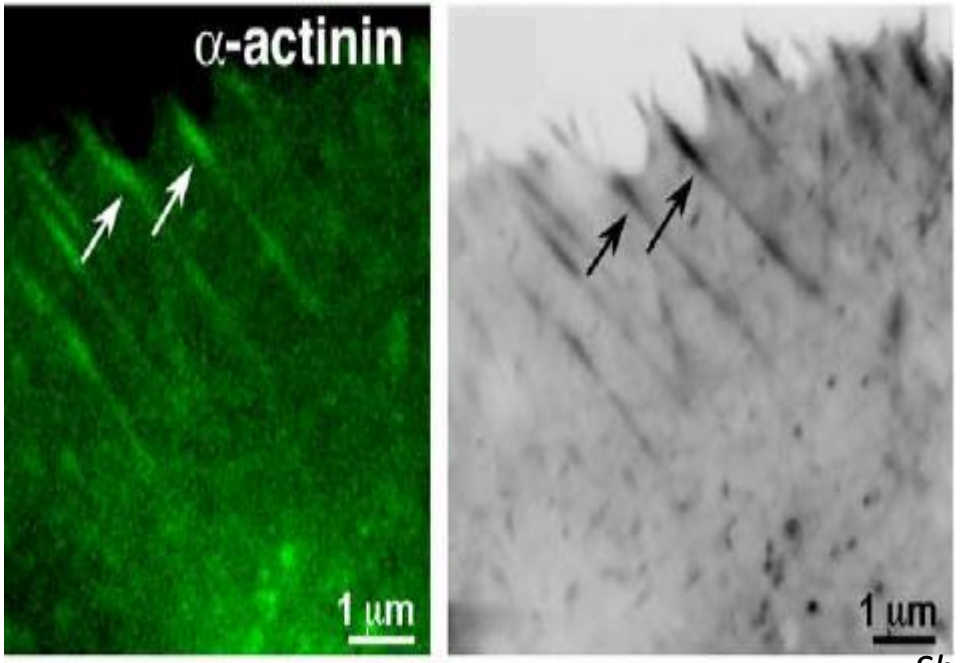
Genetically encoded markers for protein EM labelling

Minisog
-a fluorescent protein able to create electron dense reaction product on diaminobenzidine (DAB) treatment

APEX
-an ascorbate peroxidase tag that chemically converts DAB and can be fused to a GFP tag for localisation

Synaptic cell-adhesion protein 2 in mouse brain





HeLa cells

Shu, Tsien 2011

Future developments

- **New generation probes for light and EM ‘spaghetti monster FP’s’ which combine advantages of FP and peptide epitopes. Superfolded GFP with HA, myc, FLAG etc attached (Janelia labs; Viswanathan et al 2015 Nature Methods)**

- **Generation of vast quantities of structural information - challenges of data analysis and storage for volume EM**

- **Further innovations in microscope design and imaging protocols**