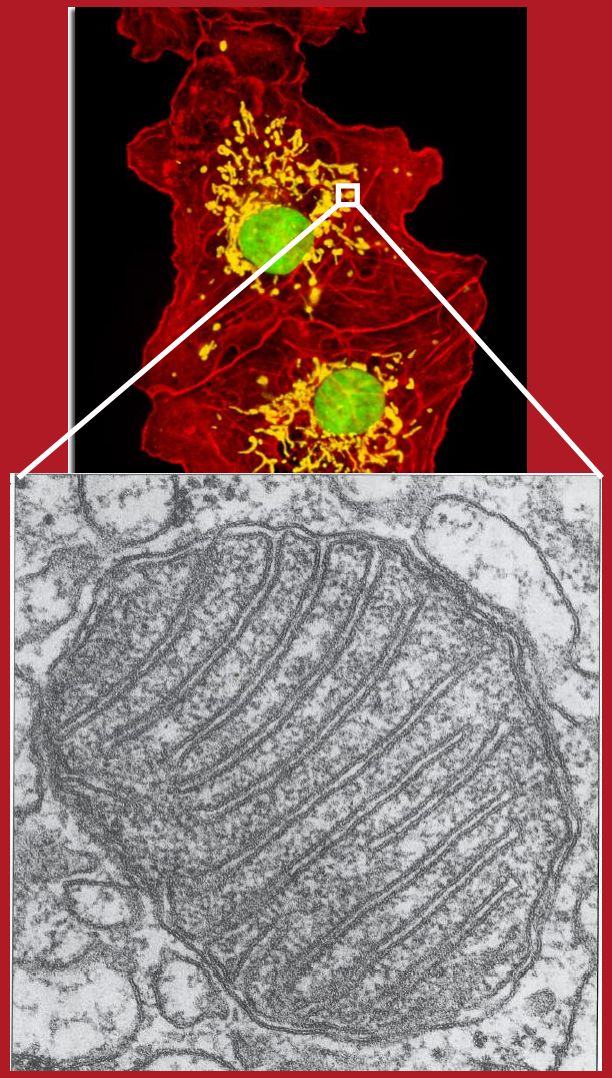


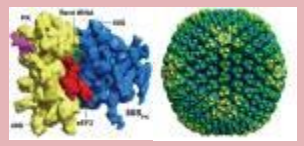
Correlative Microscopy: Bridging the Gap Between Light Microscopy and Electron Microscopy

Wim Voorhout

Advanced Technologies for Life Sciences
Institut Pasteur
14-15 September, 2010

Life Sciences Landscape

Structural Biology

Nucleic Acid/Protein/Virus Structure

Sequencing
NMR, XRD
AP/SP

Cell Biology



Molecular Localization, Function and Dynamics in Cells and Tissues

Optical Imaging, CLSM
Flow Cytometry, Molecular Biology
MicroArray

Healthcare

Diagnostics / Drug Delivery

MRI, CT, Chemical Assays
Optical Pathology
Nanomedicine

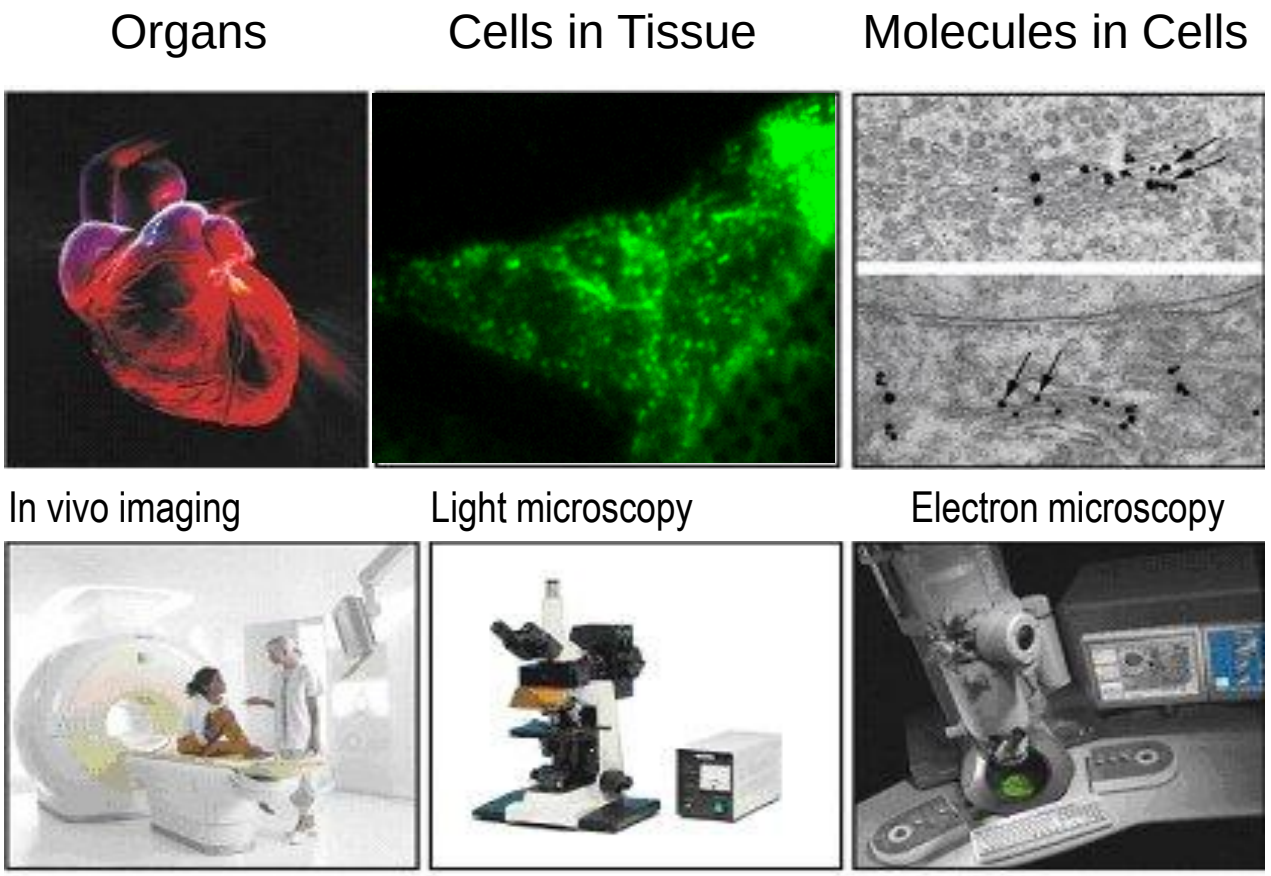
Discovery → **Integration** → **Validate and Standardize**

Research Lab

Clinical/Translational Medicine

FEI

Imaging landscape

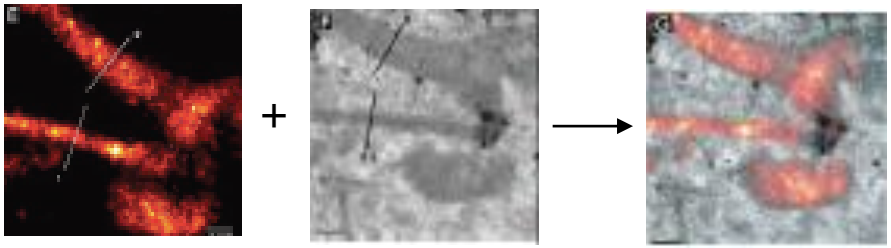


Correlative Microscopy - Motivation

LM and EM are complementary techniques:

- LM for identifying locations of interest and dynamic events using fluorescent tags
- TEM for zooming in to nm resolution to provide cellular context

As recently reported in Nature Methods, Jennifer Lippincott-Schwartz states: "Unorthodox super-resolution microscopy discoveries will also need support from electron microscopy. The latter is especially important as it provides the needed nanometer-scale resolution of cell ultra structure to correlate with super resolution images."



Correlative Microscopy

From macromolecular structure to it's cellular context and v.v.

Available technologies

- Light Microscopy
- Scanning Electron Microscopy
- Transmission Electron Microscopy
- Scanning Probe Microscopy
- XRD
- NMR

Correlative SEM – Fluorescent microscopy

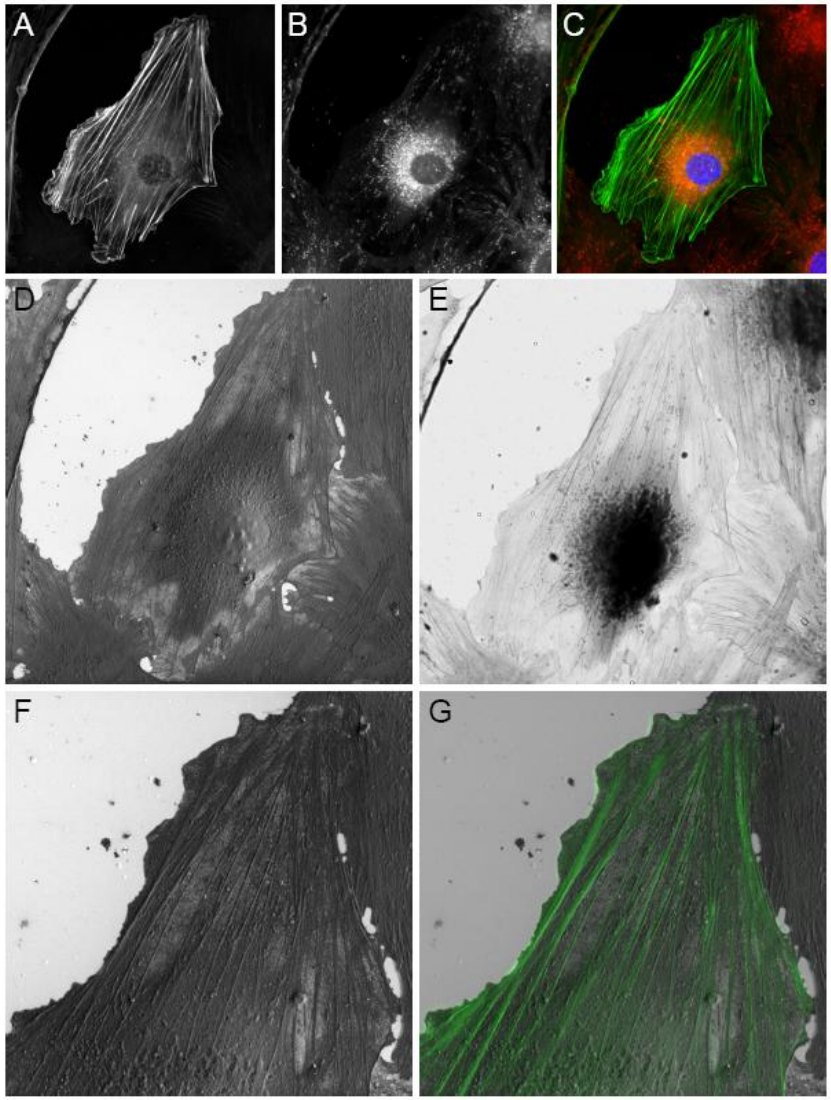
Mouse embryonic fibroblasts expressing YFP-ß-actin, grown on Indium-Tin Oxide slides

Correlate

- Fluorescent Light Microscopy
- Secondary Electron
- Backscatter Electron

H. Pluk, J. Fransen University

Nijmegen, Netherlands

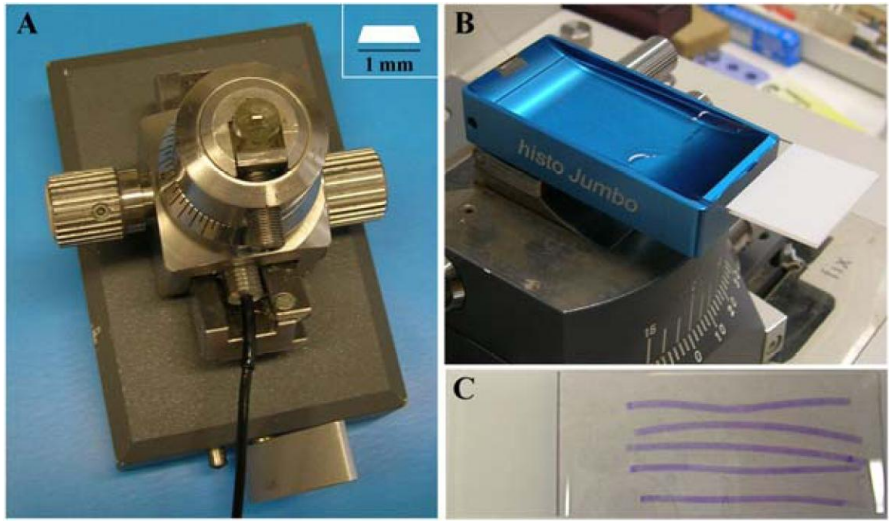


Pluk et al. J Microsc. 2009; 233(3): 353-63

Array Tomography

Array Tomography: A New Tool for Imaging the Molecular Architecture and Ultrastructure of Neural Circuits

Kristina D. Micheva[1,*] and Stephen J Smith[1,*]
[1] Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305, USA
*Correspondence: kmicheva@stanford.edu (K.D.M.), sjsmith@stanford.edu (S.J S.)
DOI 10.1016/j.neuron.2007.06.014



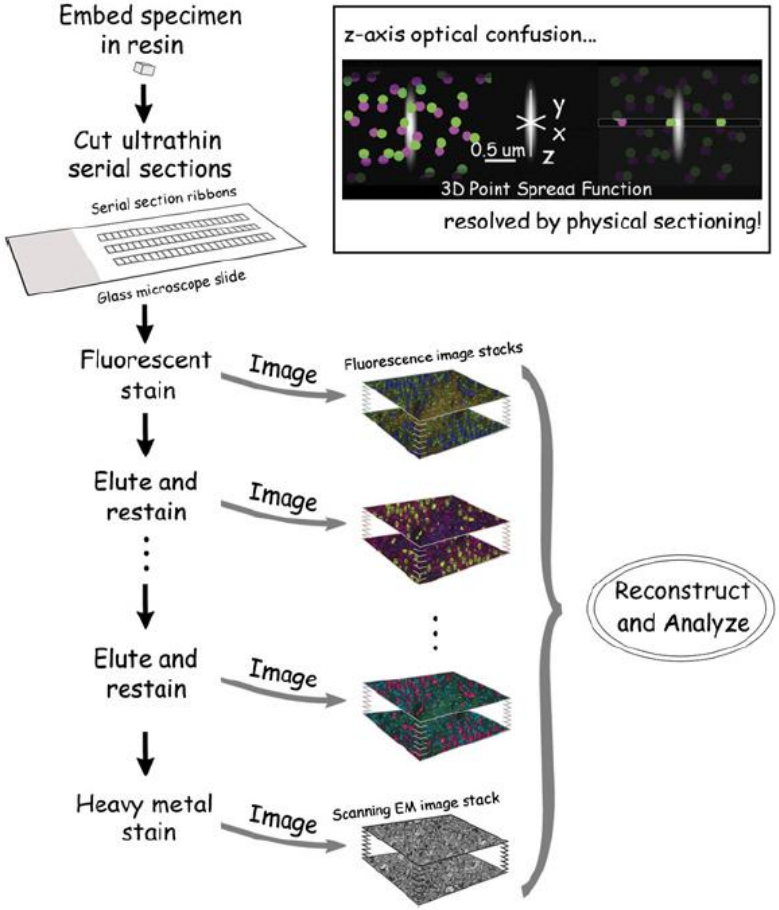


Figure 1. Schematic Representation of the Array Tomography Method

Reprinted from: Neuron 55, 25 – 36, 2007

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Array Tomography

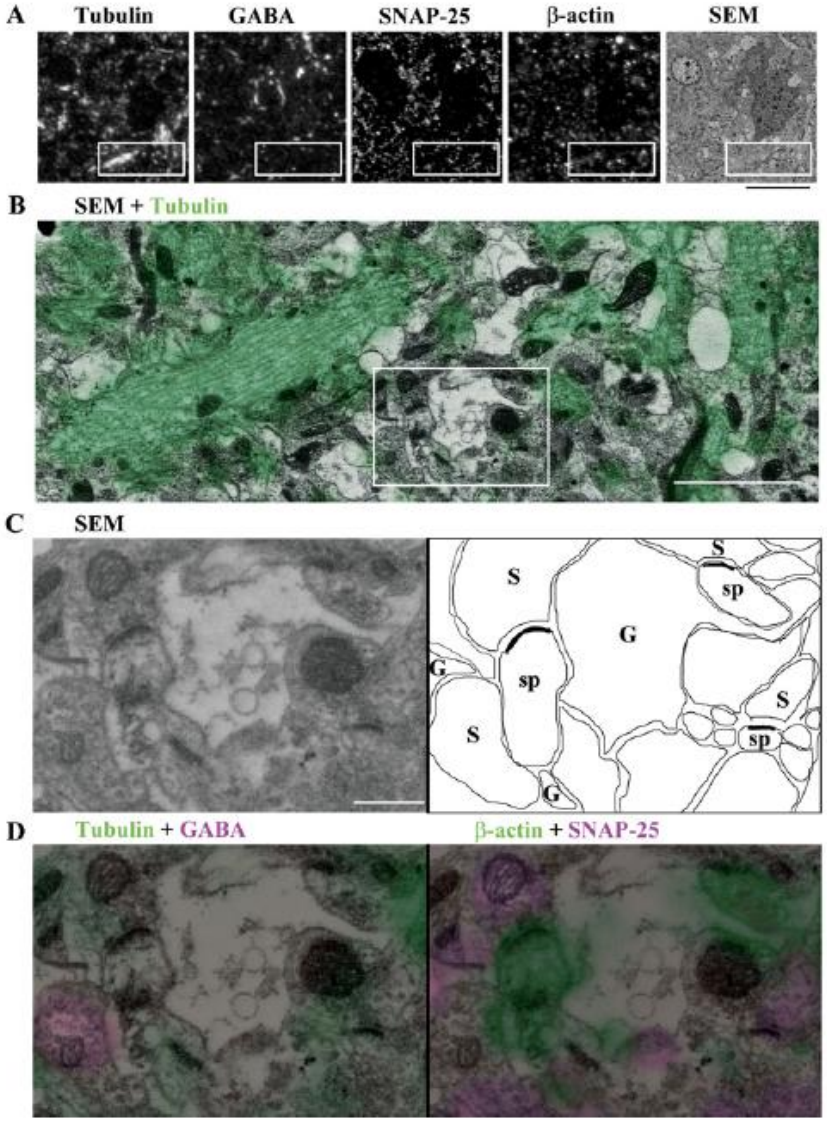

Figure 7. Demonstration of Naturally Excellent Registration of Light and Electron Microscopic Imaging of an Individual Specimen by Array Tomography

(A) The same region of a 70 nm section from the mouse cerebral cortex is shown as immunostained for tubulin, GABA, SNAP-25, and β-actin and imaged in the SEM. Scale bar, 10 μm.

(B) The boxed region in (A) is imaged at a higher magnification in the SEM, and the corresponding immunofluorescent labeling for tubulin (green) is overlayed. Scale bar, 2 μm.

(C) A higher-magnification SEM image of the boxed region in (B) and a schematic map of the same region: G—glia, S—presynaptic bouton, sp—spine. Scale bar, 0.5 μm.

(D) Immunofluorescence for tubulin and GABA, and β-actin and SNAP-25 superimposed on the SEM image in (C). Scale bar, 0.5 μm.

Reprinted from: Neuron 55, 25 – 36, 2007

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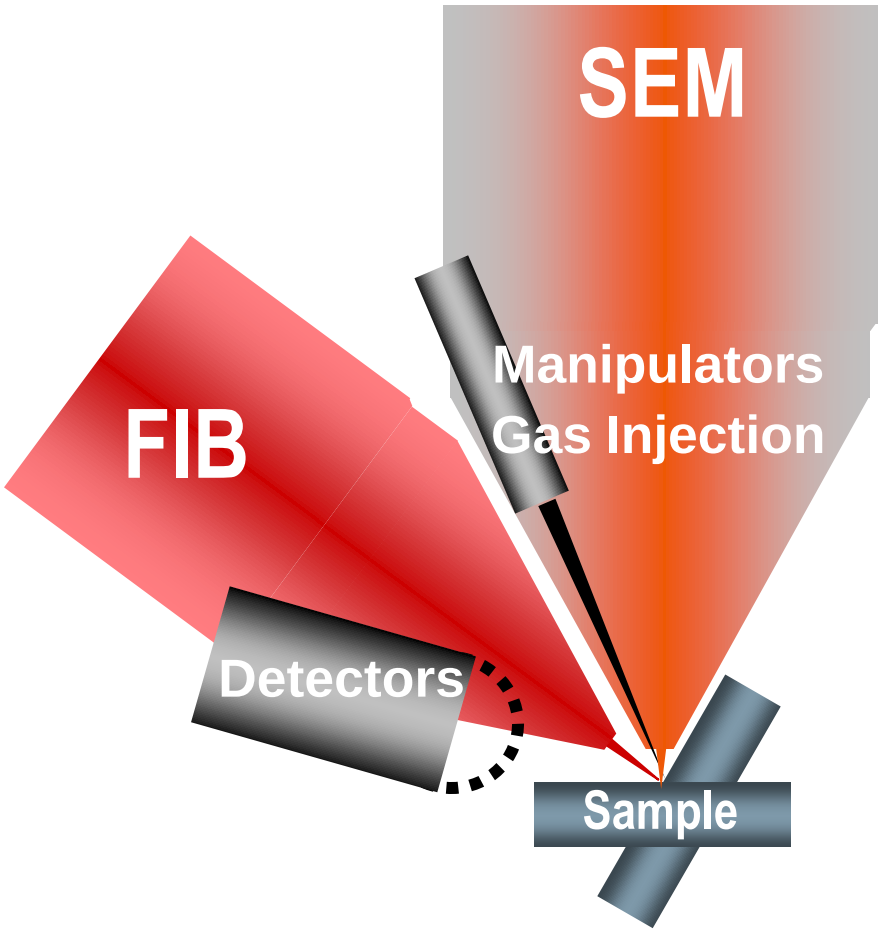
Correlative Microscopy

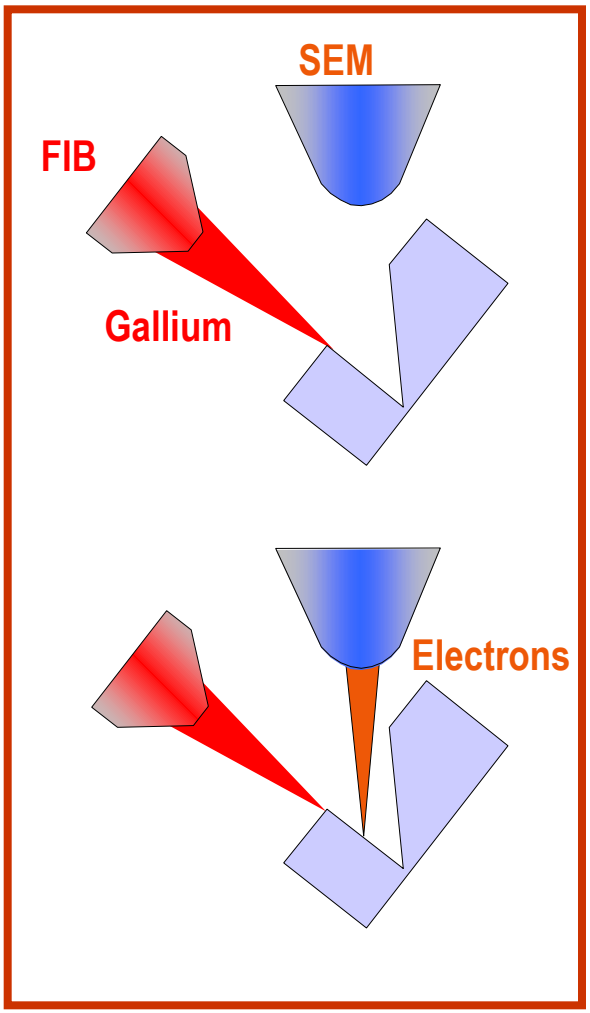
From macromolecular structure to it's cellular context and v.v.

Available technologies

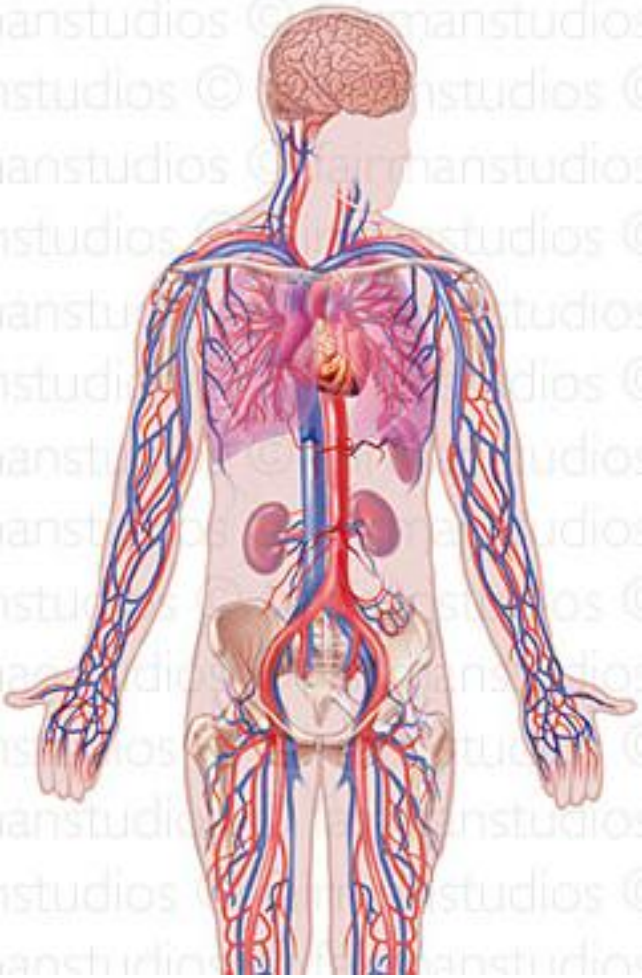
- Light Microscopy
- Scanning Electron Microscopy
- Transmission Electron Microscopy
- Scanning Probe Microscopy
- XRD
- NMR

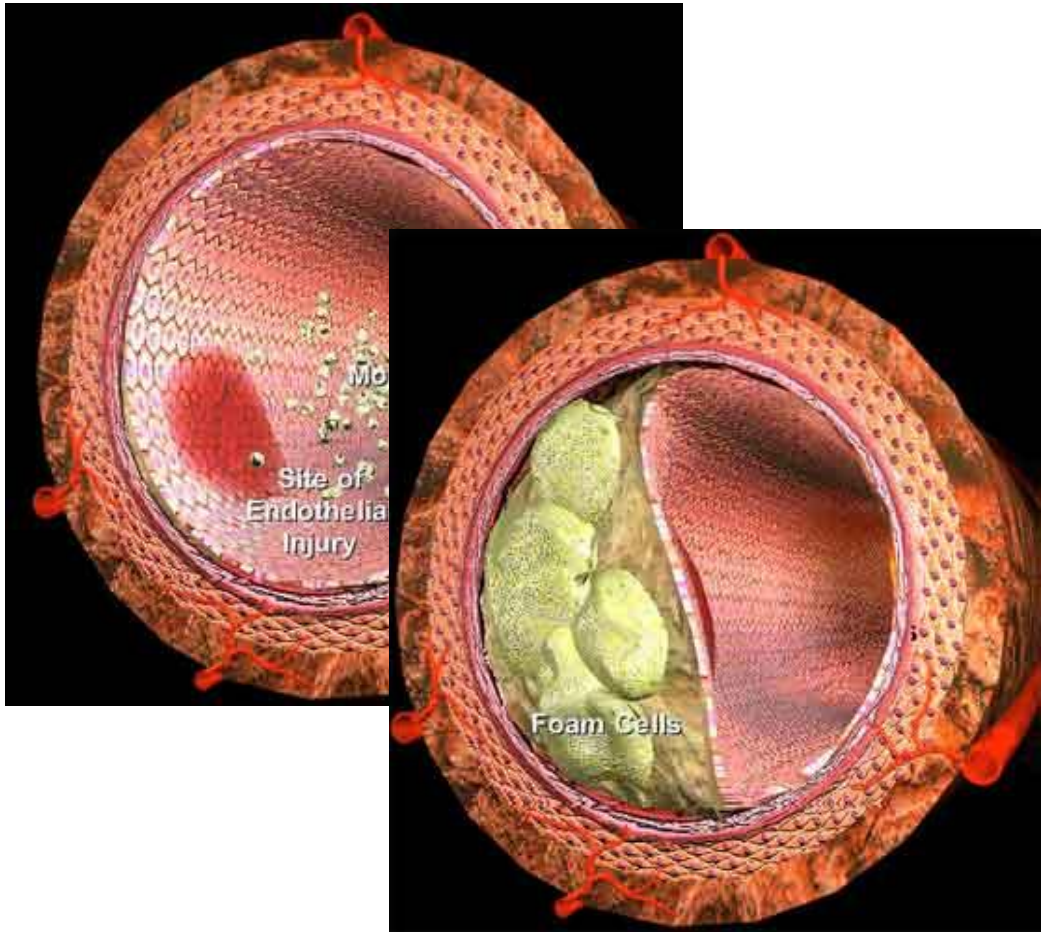
What is a DualBeam?





Atherosclerosis : Needle in the haystack problem



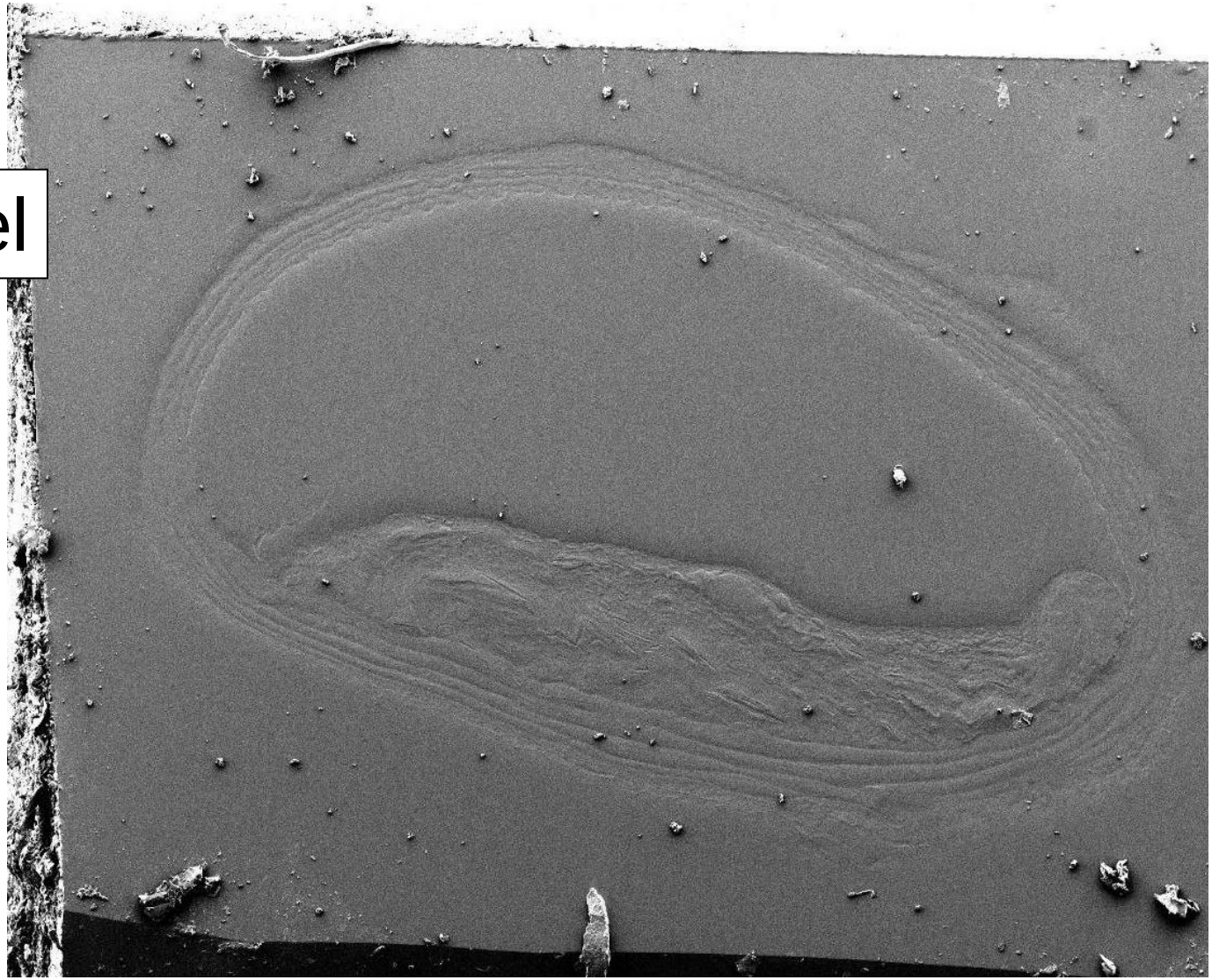


Courtesy : Prof Verkley, University Utrecht

www.strokecenter.org/

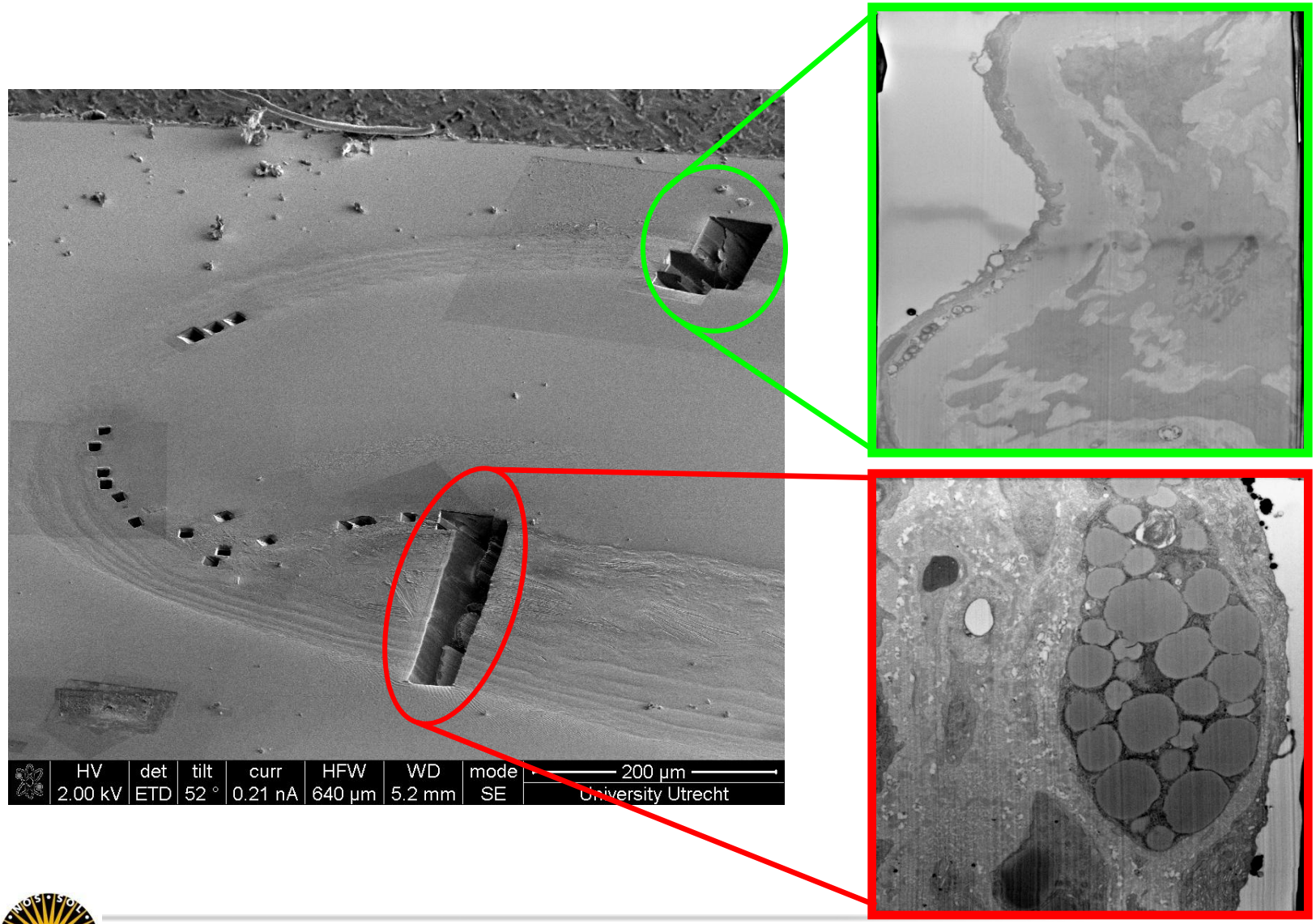
Atherosclerosis : Needle in the haystack problem

The vessel



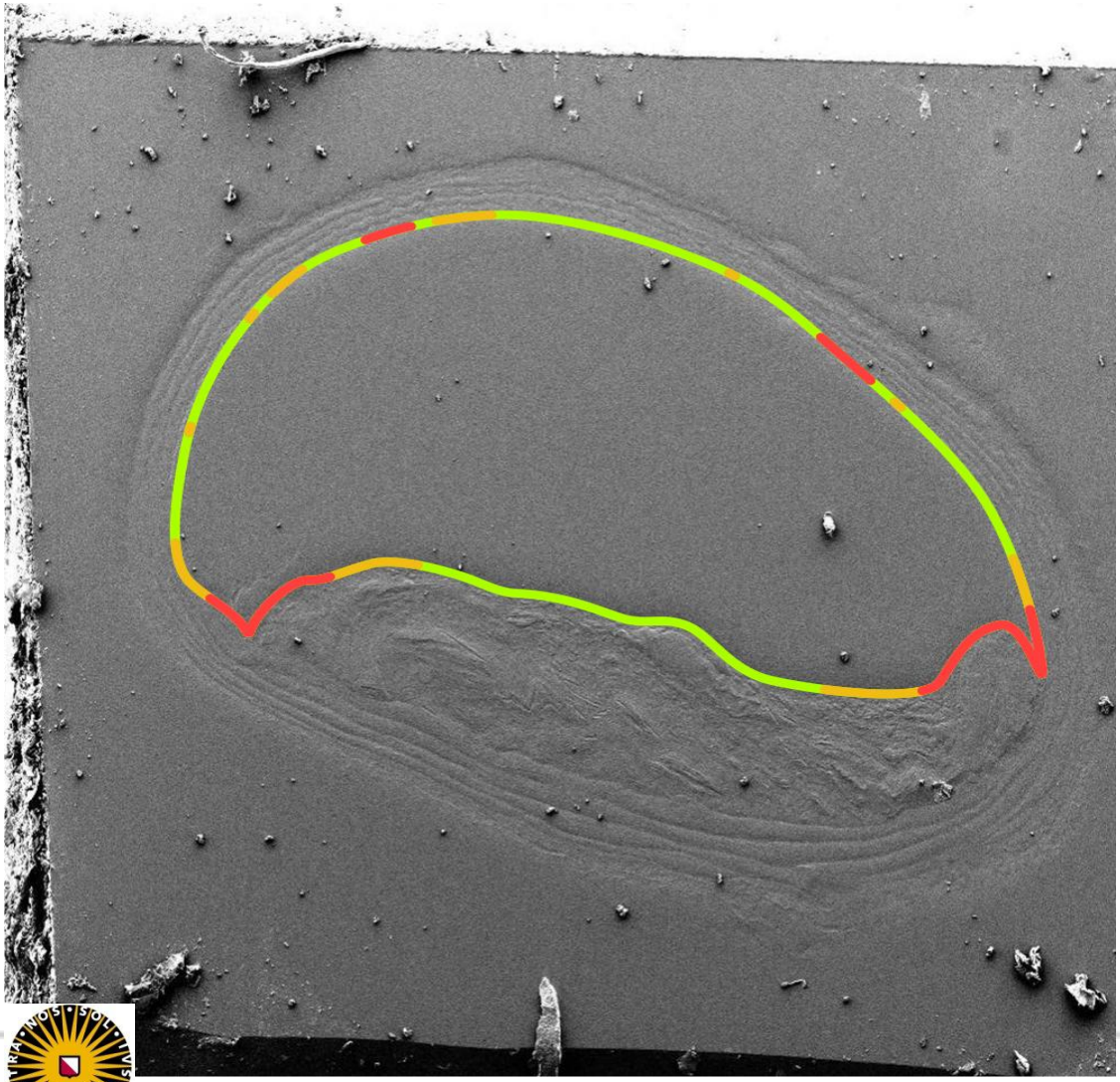
Courtesy : Prof Verkley, University Utrecht

Atherosclerosis : Needle in the haystack problem



Courtesy : Prof Verkley, University Utrecht

Spatial Density of ICAM specific label at inner blood vessel



high number of gold particles could be indicative for early stage plaque development

low number indicates healthy tissue

Journal of Microscopy, Vol. 235, Pt 3 2009, pp. 336-347

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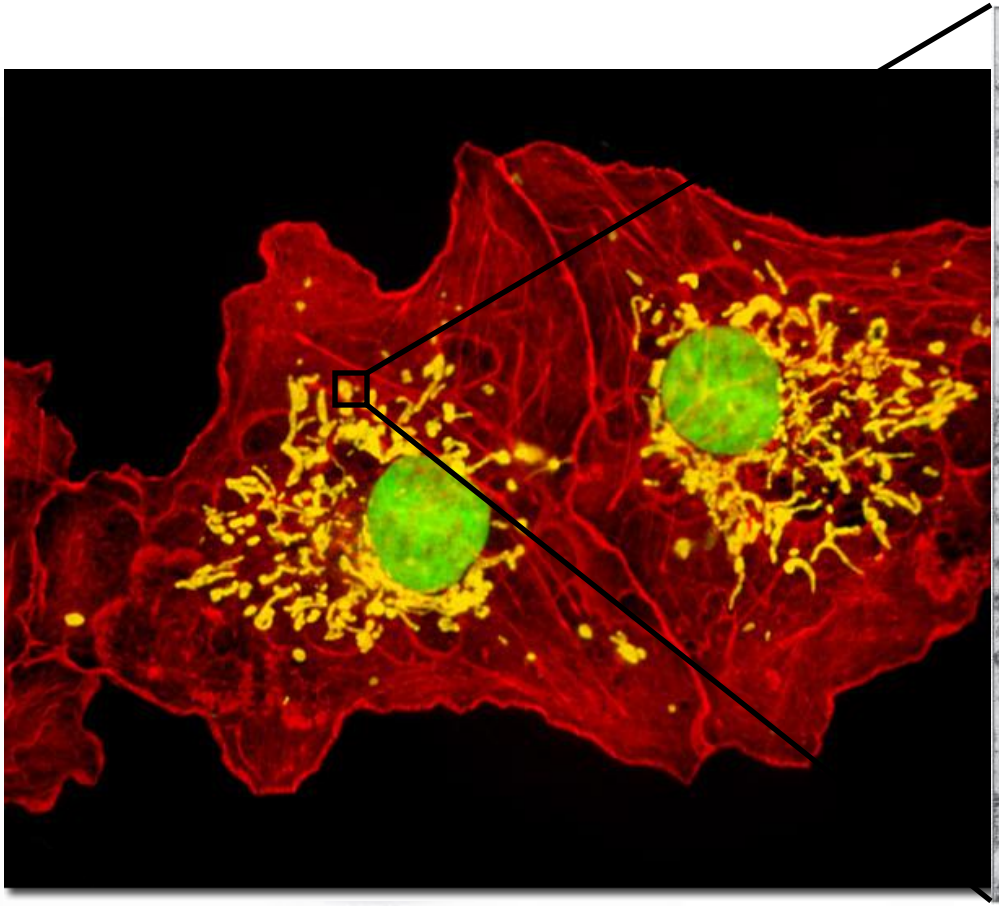
Correlative Microscopy

From macromolecular structure to it's cellular context and v.v.

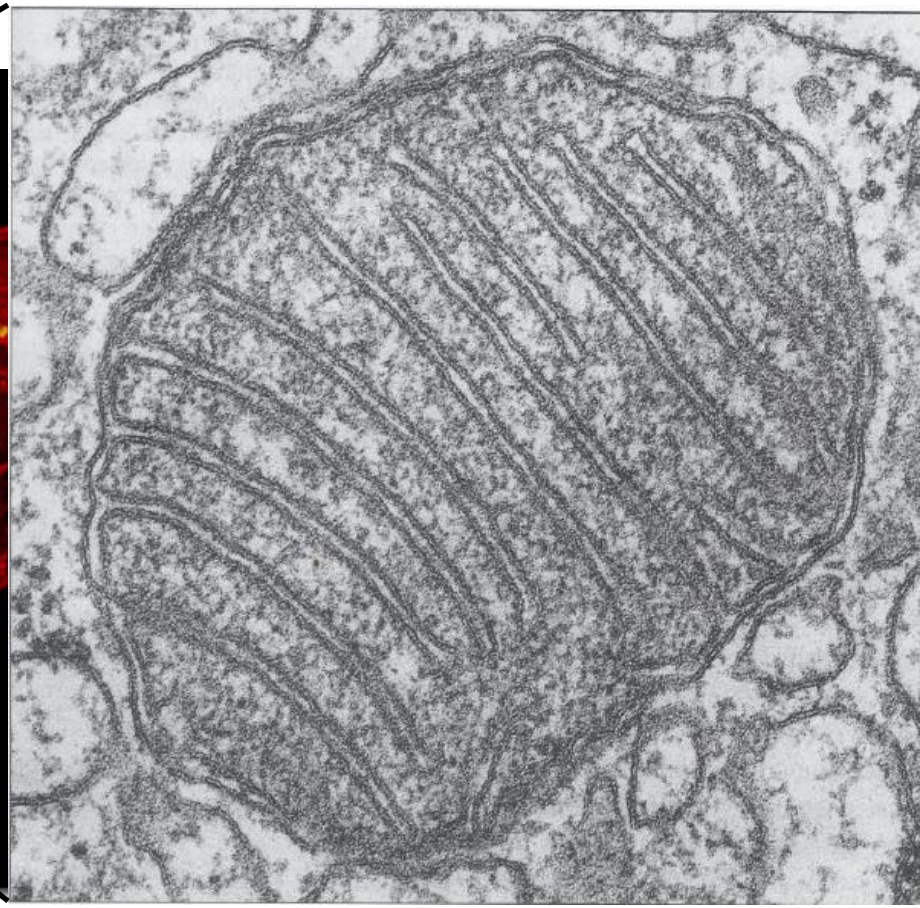
Available technologies

- Light Microscopy
- Scanning Electron Microscopy
- Transmission Electron Microscopy
- Scanning Probe Microscopy
- XRD
- NMR

Correlative Microscopy – Leveraging the Strengths of Both LM and EM



Olympus Inverted Light Microscope



FEI Tecnai Spirit TEM

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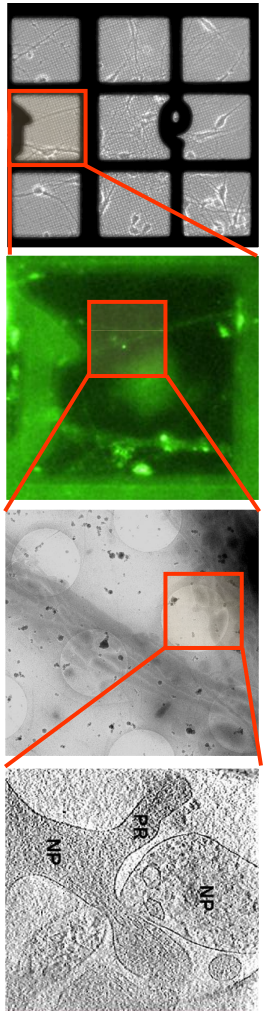
Correlative Microscopy

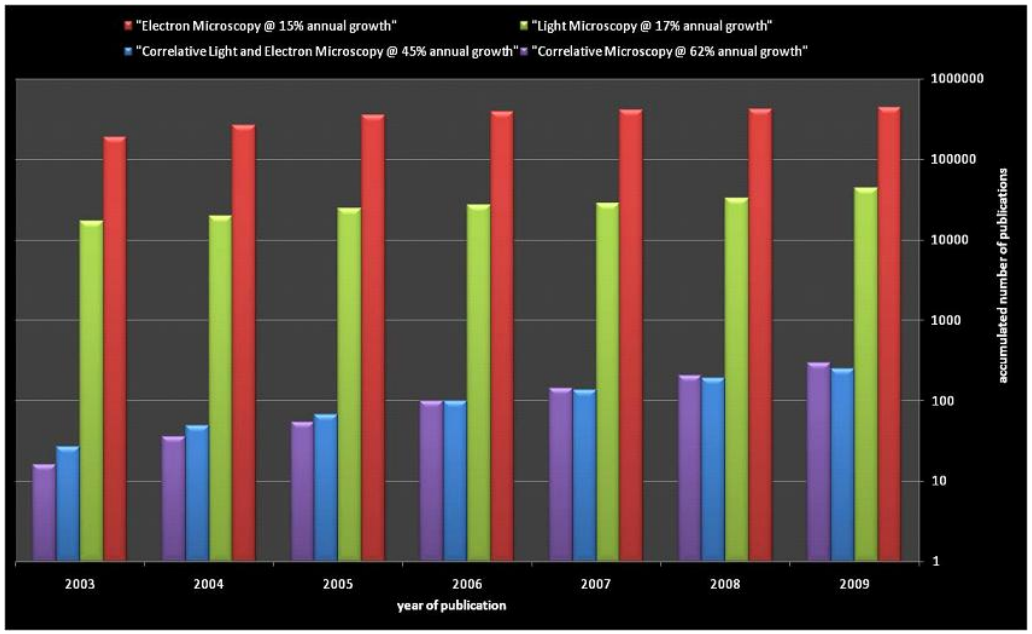
Light Microscopy (widely used in biology)

- Large field of view / overview
- Live cell imaging
- Limited or no sample preparation
- Combination with fluorescent labels (GFP..)
- Resolution ~ 200 nm

Electron Microscopy

- Excellent spatial resolution with small field of view
- Provides the cellular context
- Extensive specimen preparation, especially for labeling techniques





Growth rates for correlative papers are highest. EM=15%, LM=17%, CLEM=45%, CM=62%. ***A clear trend towards correlative microscopy.***

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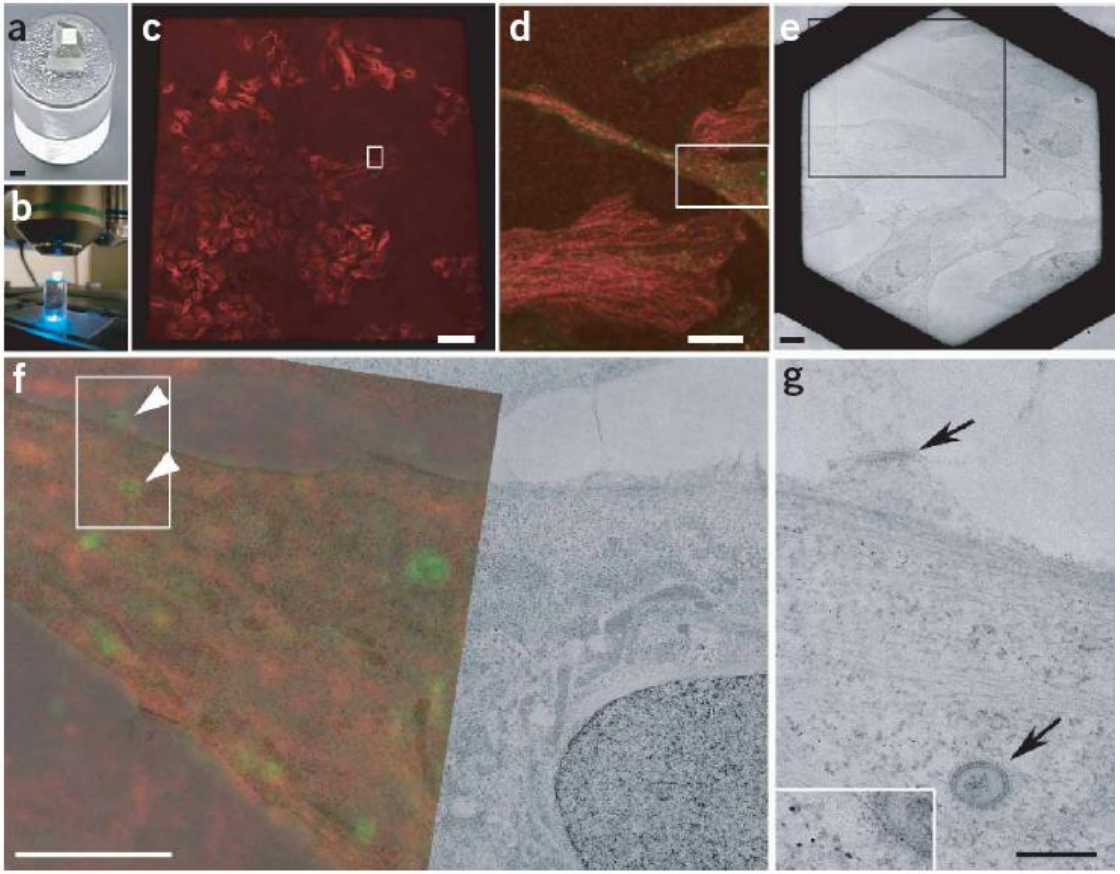
Correlative Microscopy (manual)



Confocal block-face imaging

Ultrathin sectioning

TEM

Correlated light and electron microscopic imaging of multiple endogenous proteins using Quantum dots

Ben N G Giepmans, Thomas J Deerinck, Benjamin L Smarr, Ying Z Jones & Mark H Ellisman

NATURE METHODS | VOL.2 NO.10 | OCTOBER 2005 | 743

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Correlative Microscopy

Correlated light and electron microscopic imaging of multiple endogenous proteins using Quantum dots

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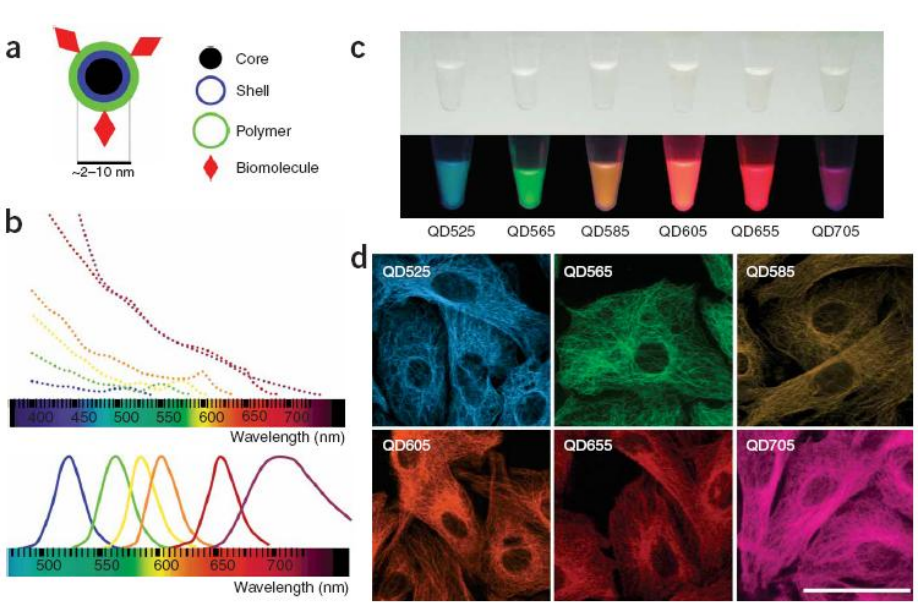
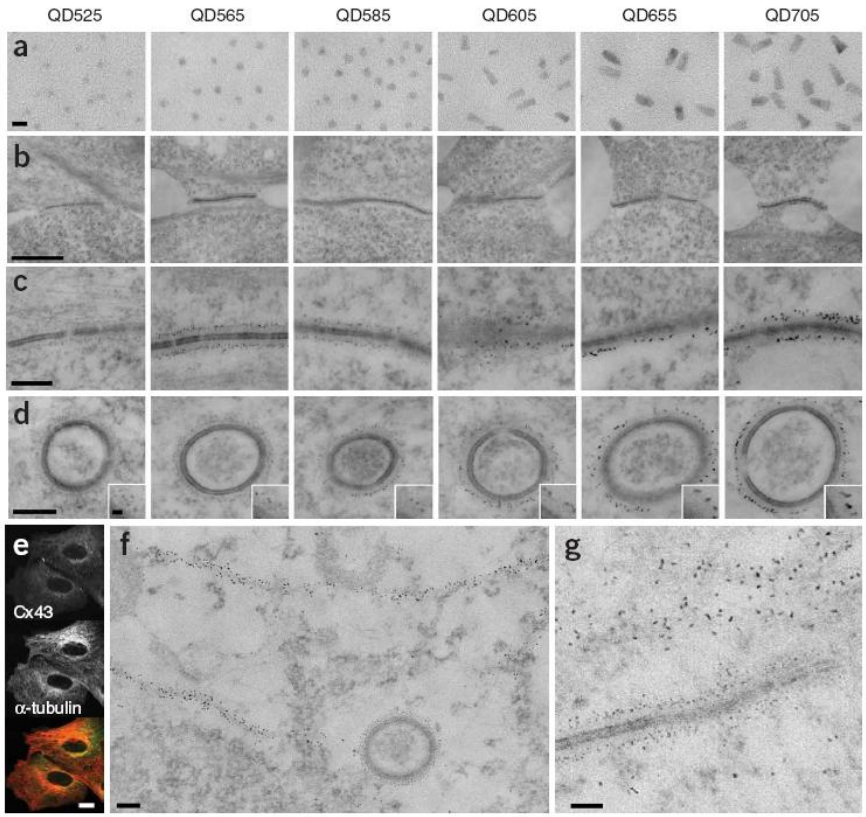
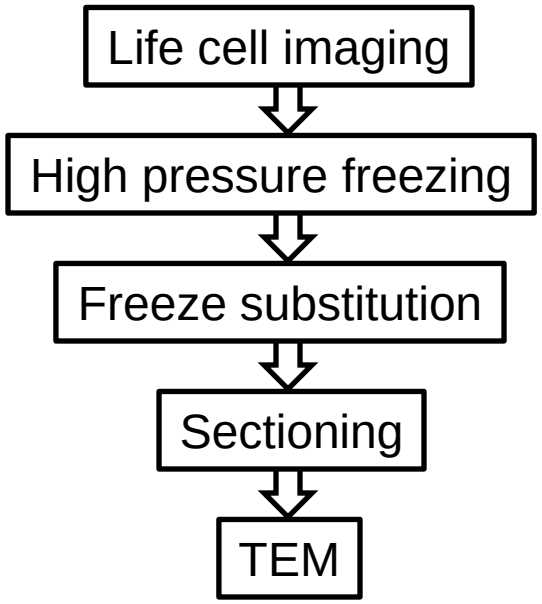
NATURE METHODS | VOL.2 NO.10 | OCTOBER 2005 | 743

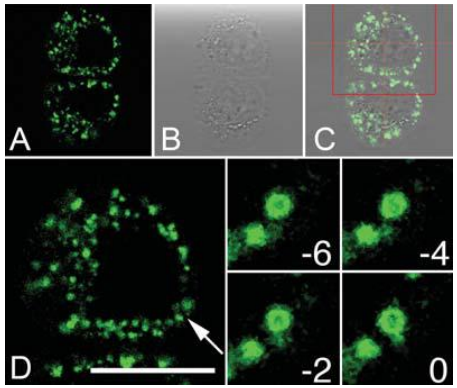


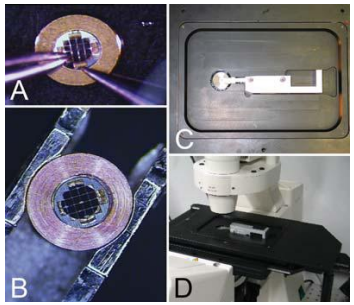


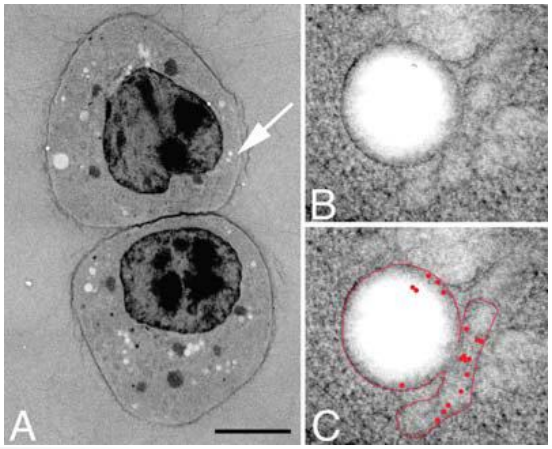
Correlative workflow

- Example Bristol (Paul Verkade)







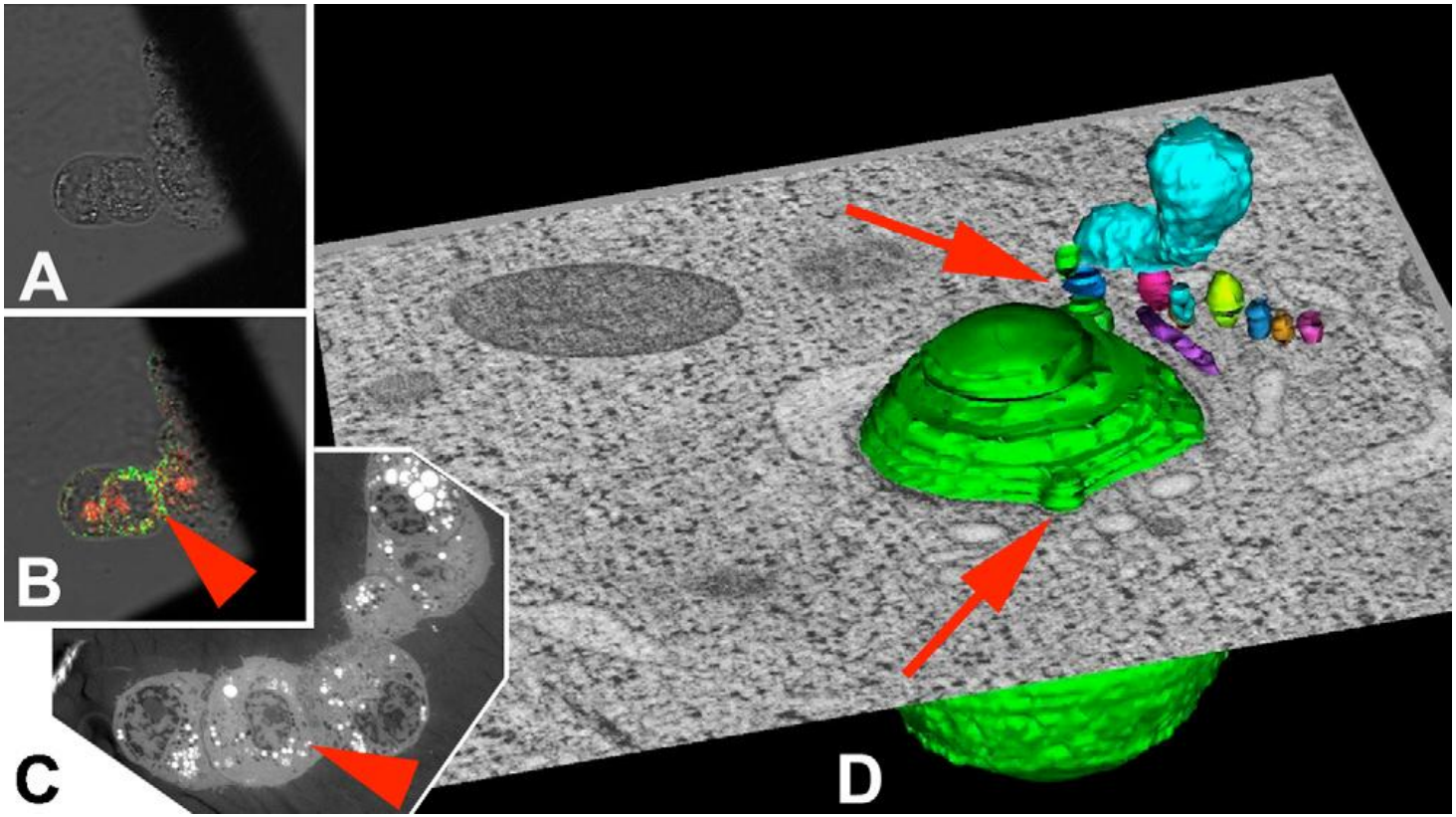


P. VERKADE
Moving EM: the Rapid Transfer System as a new tool for correlative light and electron microscopy and high throughput for high-pressure freezing

Journal of Microscopy, Vol. 230, Pt 2 2008, pp. 317–328

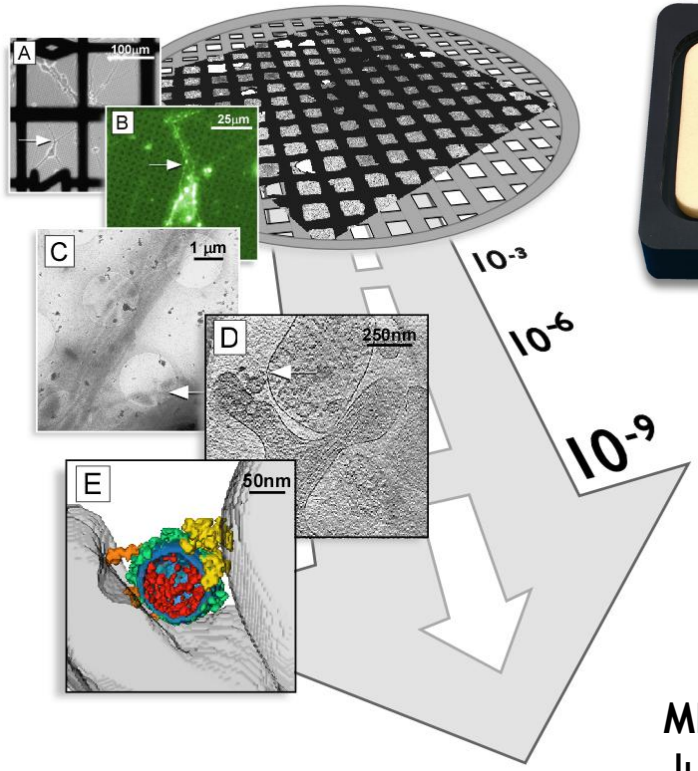
Correlative workflow

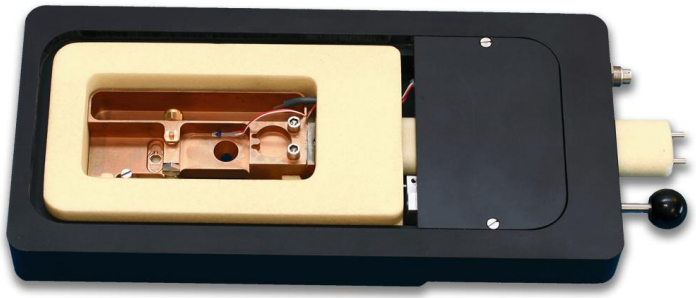
- Example Bristol (Paul Verkade)



E. Brown et al. / Seminars in Cell & Developmental Biology *20 (2009) 910-919*

Cryo-correlative workflow



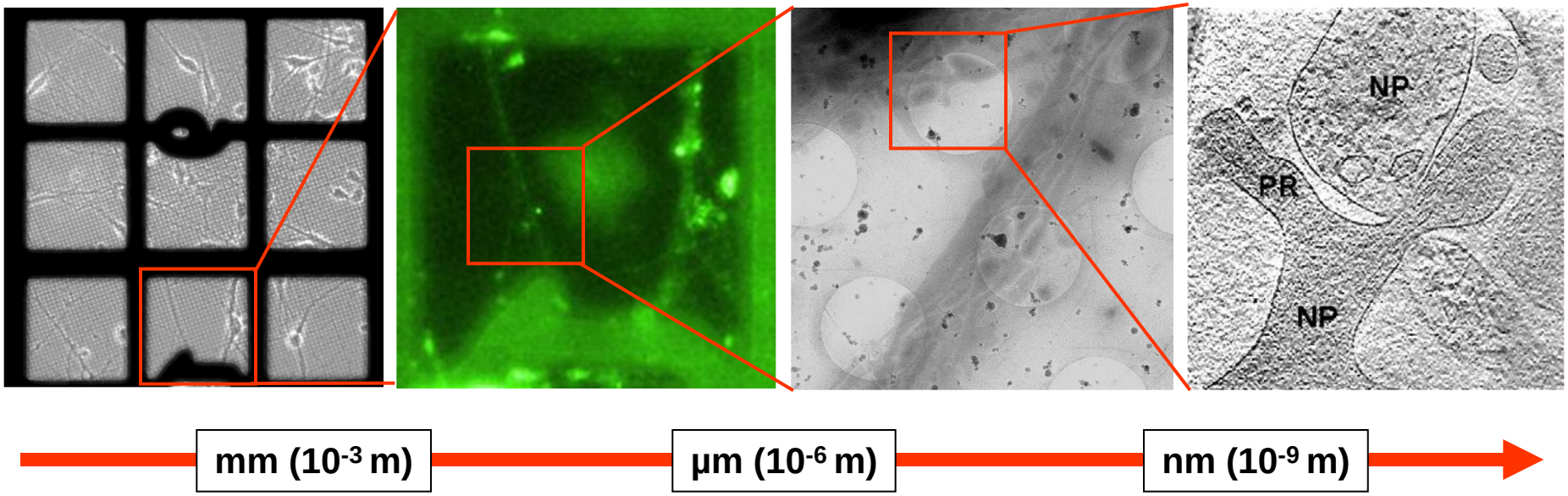


MPI of Biochemistry:
Juergen Plitzko
Alexander Rigort
Andrew Leis
Anna Sartori (Pasteur)

Cryo-correlative Microscopy

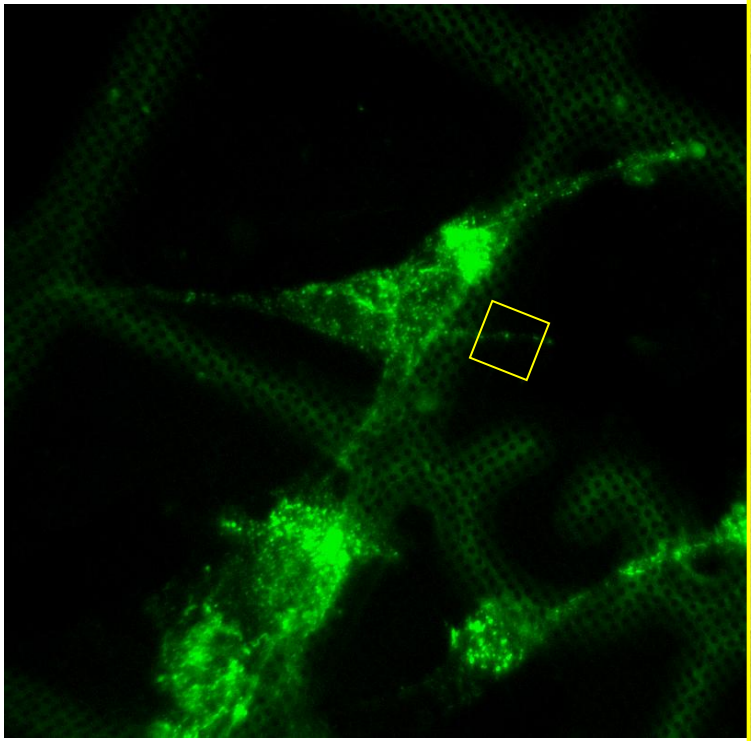
Correlative microscopy - characteristics

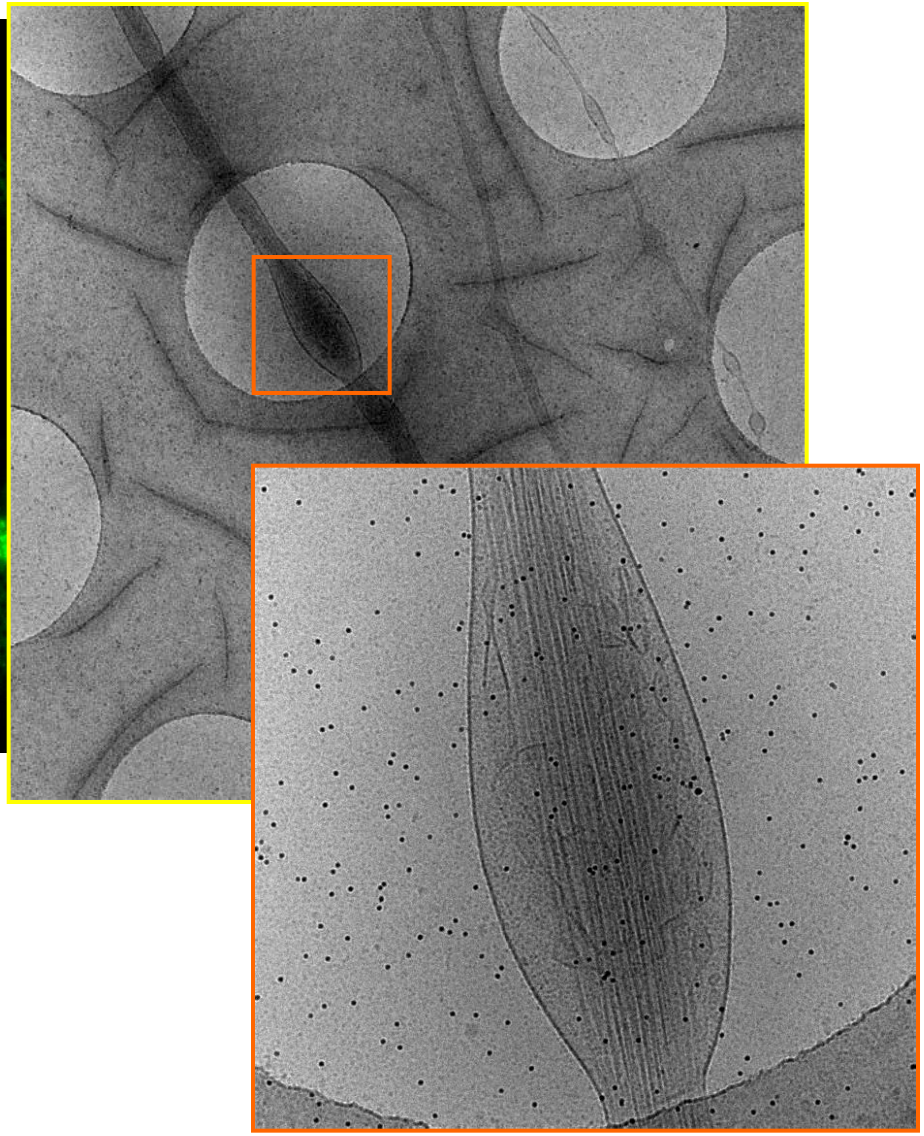
- Examining one and the same sample by both light and electron microscopy
- LM provides a survey over large cellular landscapes
- FM allows positive identification of features of interest
- EM and ET permits zooming in on such features at much higher resolution



Example given: Cultured neurons grown on EM gold finder grids

Correlating FM and cryo-ET: Cryo-FM features





HFF (Human Foreskin Fibroblasts) live immunolabelling with Ab conjugated to Alexa488

Integrated Correlative Microscopy

= *i*LEM

Hans C. Gerritsen, Sasha Agronskaia, Abraham J. Koster, Arie J.Verkleij

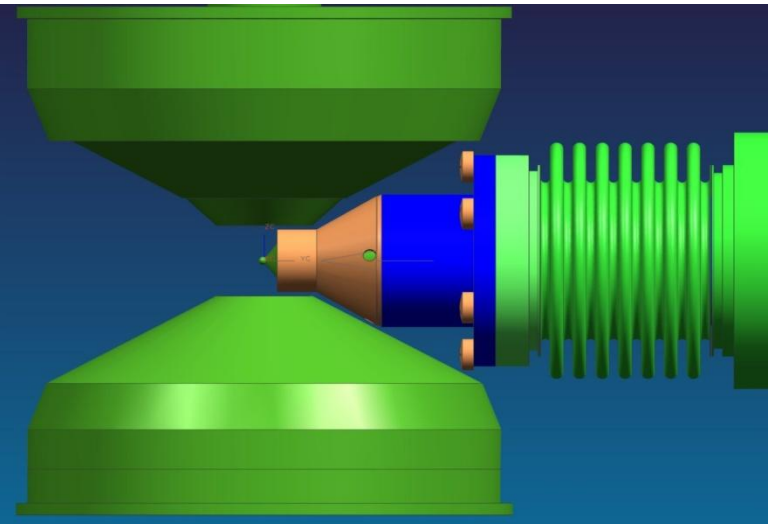
Universiteit Utrecht

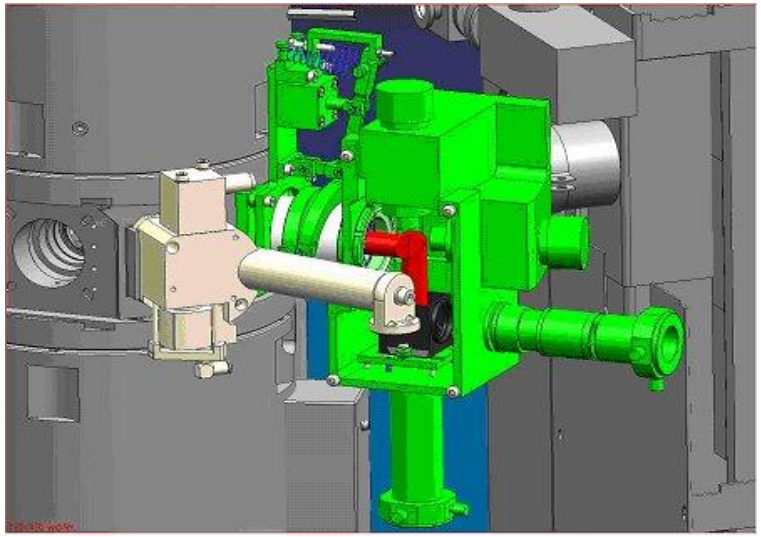
FEI™

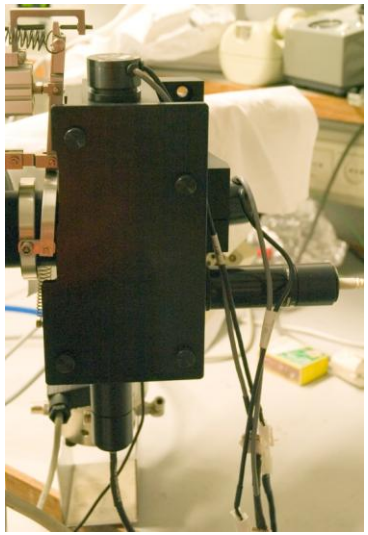
FEI contributed to the design and construction of a prototype

Correlative Microscopy

Integrated Correlative Light Electron Microscopy (ILEM)



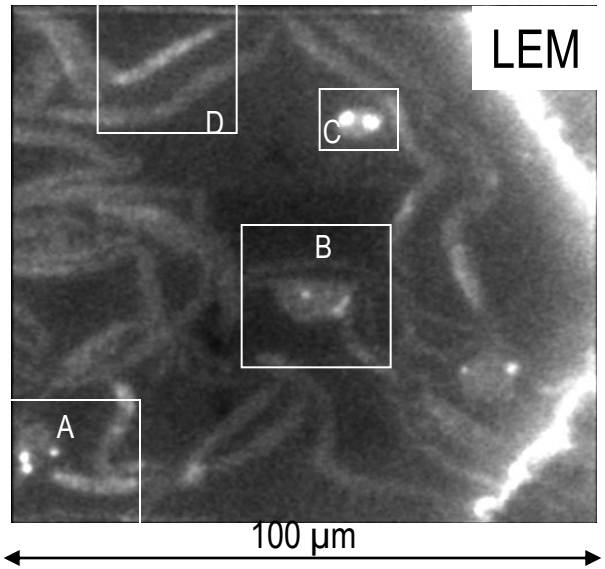


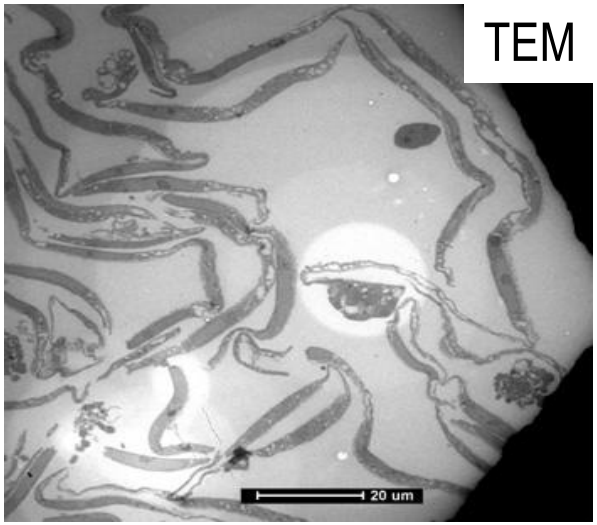


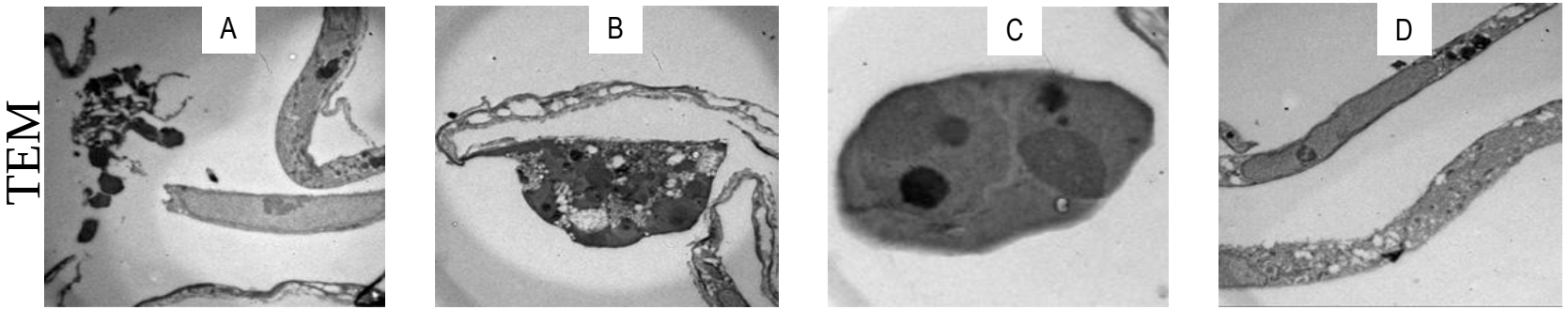
- Optimal Navigation Tool for correlative microscopy
  - Faster correlation between LM and EM
  - Less potential contamination (cryo)
  - Less photo-bleaching of fluorescent labels (vacuum)
  - No compromise on EM performance, slight decline in LM resolution
- Prototype ILEM up and running on Tecnai 12 at Utrecht University
  - Paper published in J. Structural Biology (***Volume 164, 2008, 183-189***)
- Second prototype was recently installed at Leiden University
- First application results have been obtained (next slides)
- Funded by STW

Correlative Microscopy – ILEM

UVC stressed HUVEC cells, staining: γ-H2AX with Alexa488 & gold

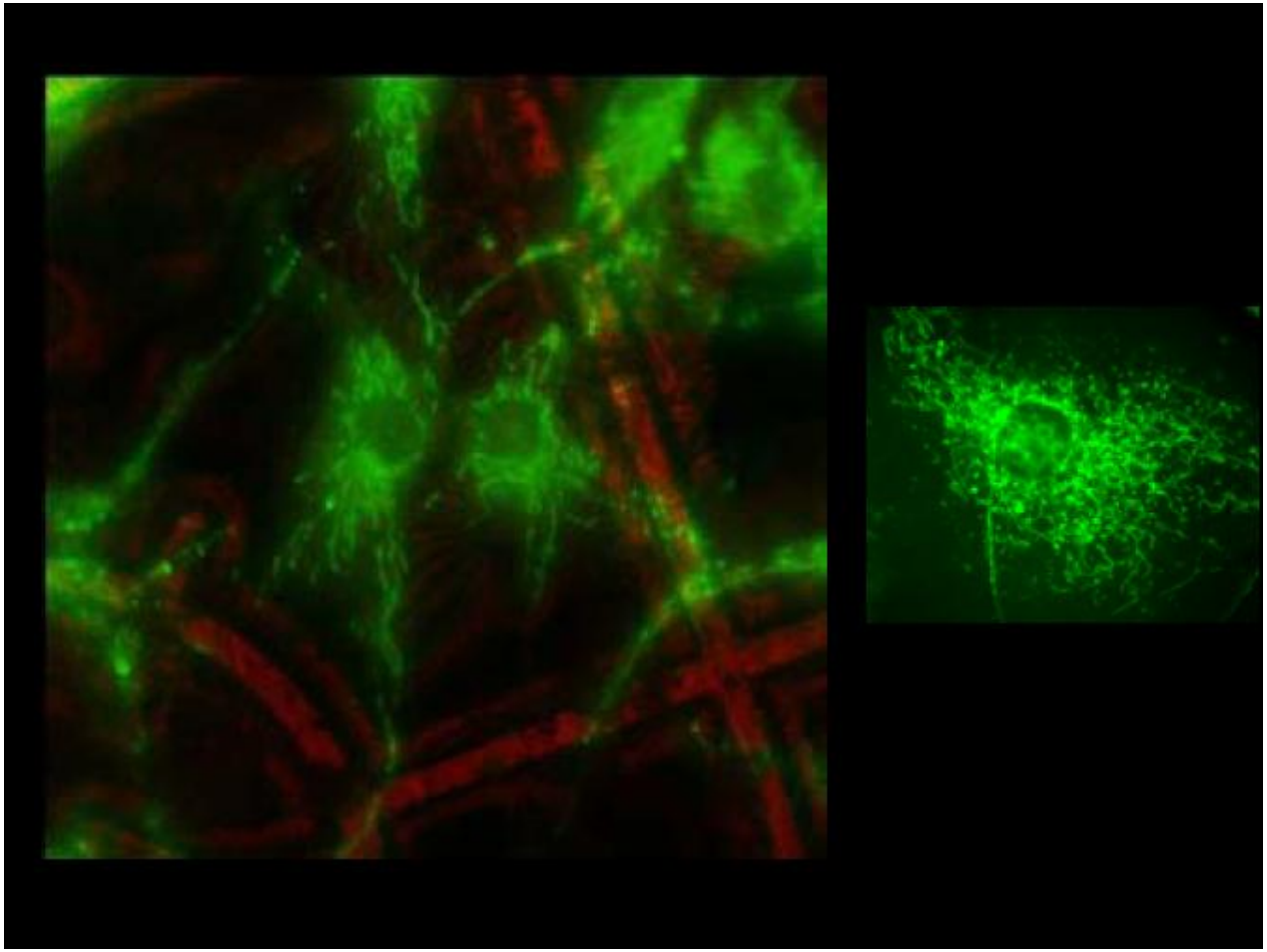






M.A. Karreman et al., Biology of the Cell, (2009) 101, 287-299

Cryo-correlative microscopy

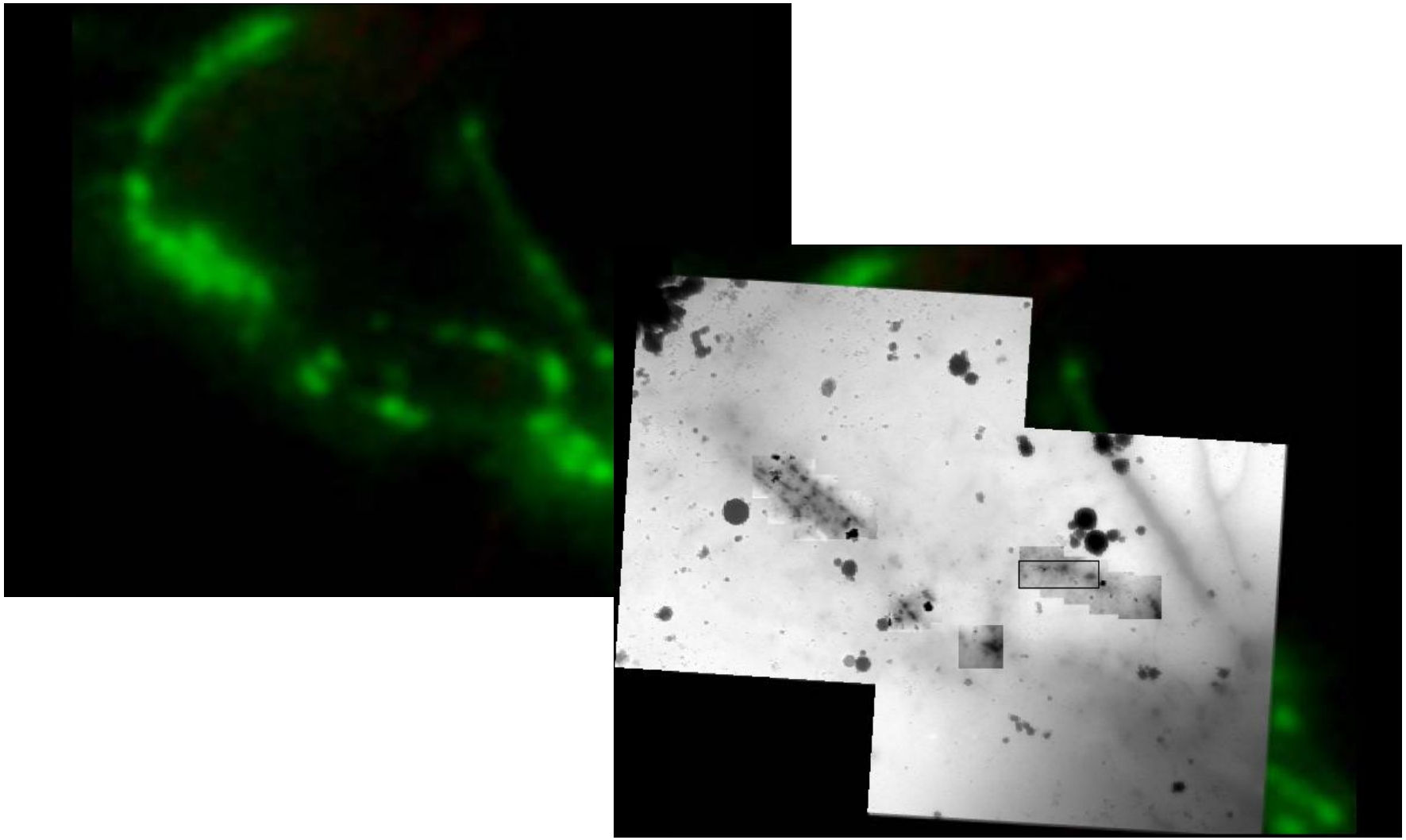


Courtesy: Linda van Driel and Bram Koster, Leiden University, The Netherlands

LUMC

FEI

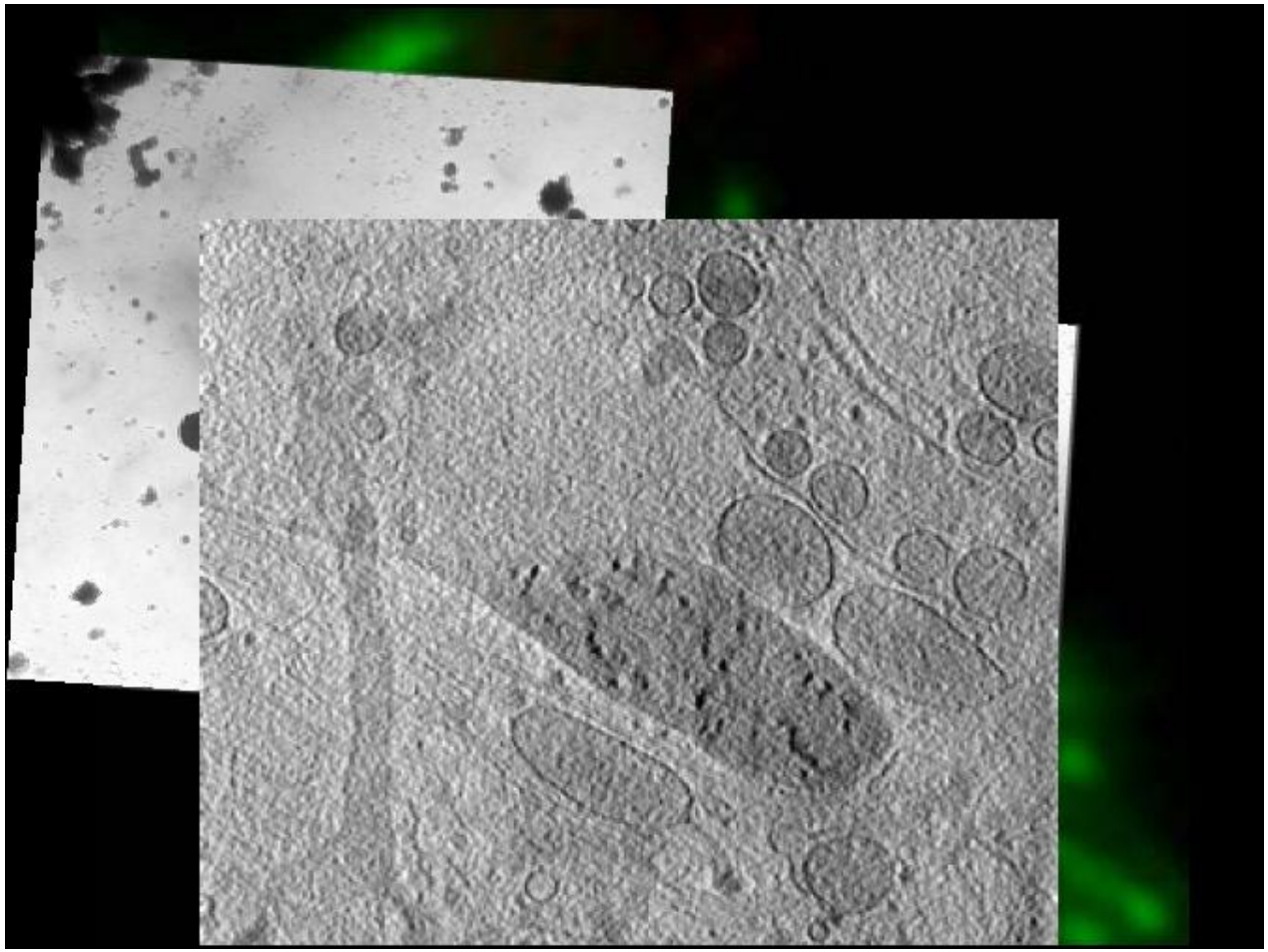
Cryo-correlative microscopy



LUMC

FEI

Cryo-correlative microscopy



LUMC

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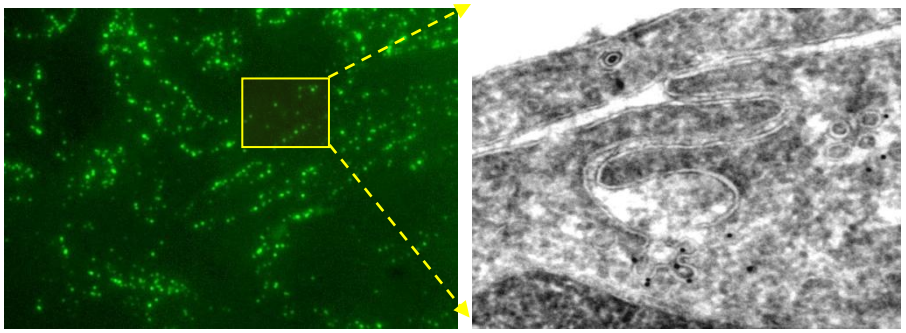
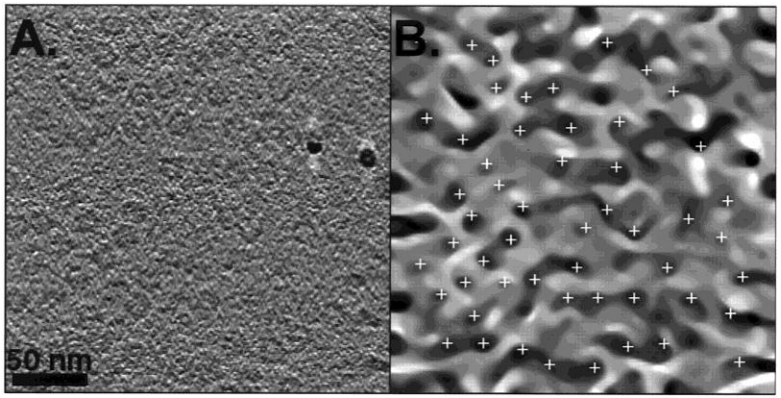
Biology Challenges

- Link MRI/CT Medical Data to ultra-structural data
- Link Light Microscopy data to EM Data
- Link Cellular Architecture context to localization of specific Macromolecules
- Link X-ray Structures to EM Data
- With minimal Artifacts (Close to living state)
- In a significant volume

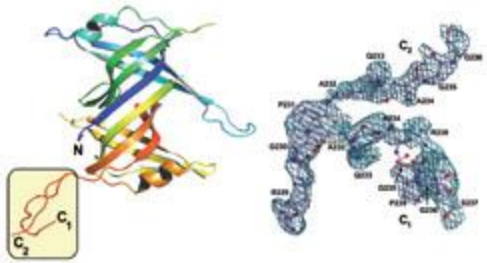
The key is correlation!

Biomarker Localization: LM **Biomarker Validation: EM**





Böhm J et al. PNAS 2000;97:14245-14250



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Acknowledgements

Molecular Biophysics UU
Hans Gerritsen
Sasha Agronskia

Cell Biology UU
Arie Verkleij
Theo Verrips
Jan Andries Post
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