UK 3View Users Group Meeting

Tips, tricks & troubleshooting

A tiny saw to cut your blocks

Details of supplier

Modellingtools.co.uk

http://www.modellingtools.co.uk/jlc-saw-anniversary-set-9975-p.asp

"Great for cutting resin pieces off blocks, plastic parts off the runner, soft metal, clear plastic canopies in half.

A must have item for every modeller."

£17.00 comes with two spare blades

Supplementary information

- Alternative – EXACTO saw/knife, has a fine blade and handle, available from Amazon

David Johnston

daj1u06@soton.ac.uk

Contact adhesive to stop section flaking

OPEN ACCESS Freely available online

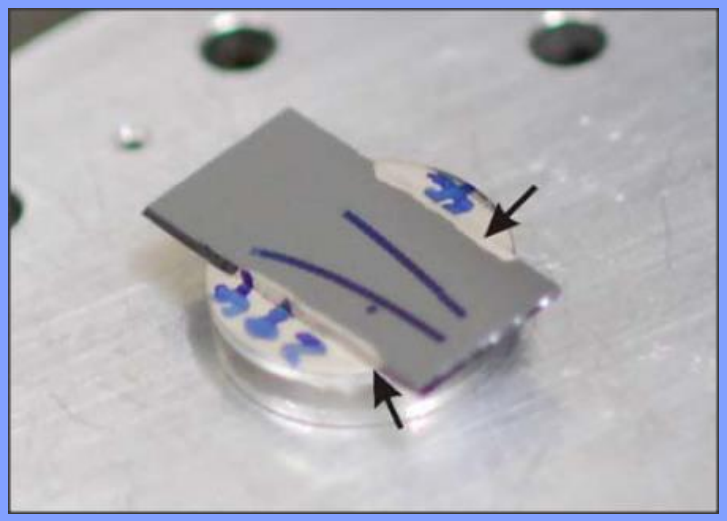
PLoS one

Serial Section Scanning Electron Microscopy (S^3EM) on Silicon Wafers for Ultra-Structural Volume Imaging of Cells and Tissues

Heinz Horstmann[1], Christoph Körber[1], Kurt Sätzler[2], Daniel Aydin[3], Thomas Kuner[1]*

1 Institute of Anatomy and Cell Biology, Heidelberg University, Heidelberg, Germany, **2** Biomedical Sciences Research Institute, University of Ulster, Coleraine, Northern Ireland, **3** Department of Biophysical Chemistry, Heidelberg University, Heidelberg, Germany

PLOS One - April 2012 **7**(4), e35172



Details of supplier

- Search online on your favourite online shopping or auction site –£4 / 35g tube
- NOT the stuff with a shoe on the package
- NOT the transparent stuff

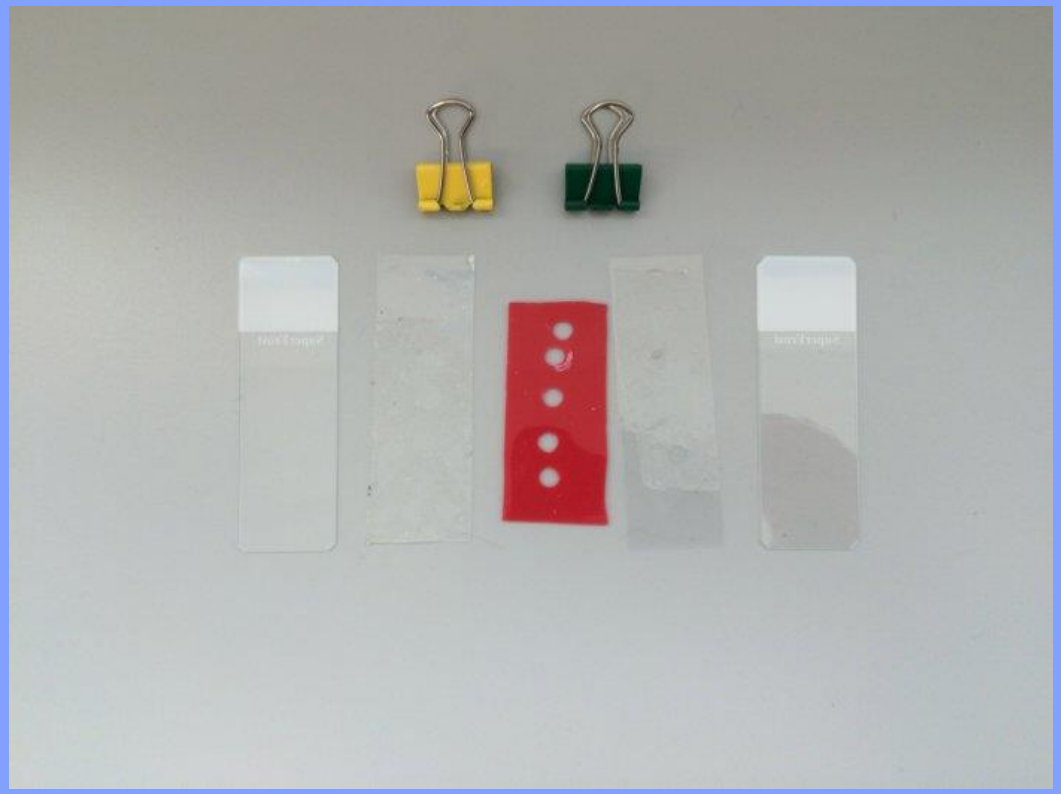
Supplementary information

- If using Durcupan ACM as resin of choice, it is advisable to extend infiltration stages i.e. 50:50 overnight, 75:25 few hours, 100% overnight, 100% few hours.

Saskia Lippens

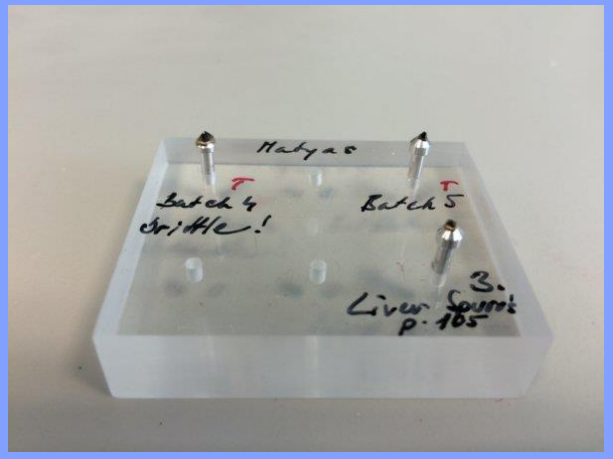
Saskia.Lippens@irc.vib-UGent.be

Tools for flat embedding



Conductive epoxy for glueing resin blocks on pins – dry in the oven overnight

Home-made equipment for transporting and storing pins



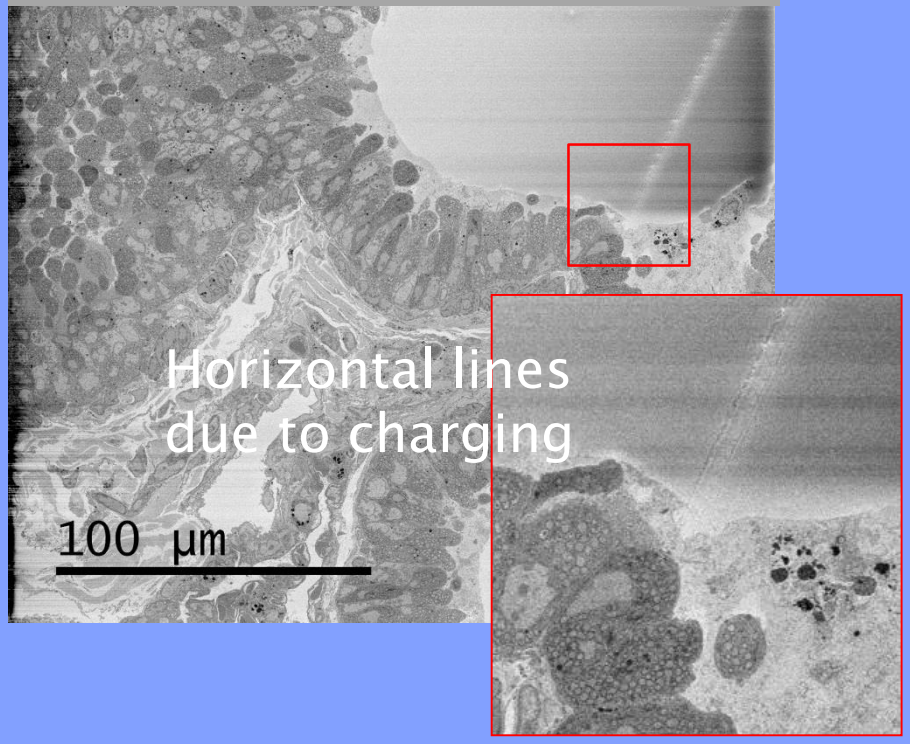
Toby Starborg

tobias.starborg@manchester.ac.uk

Knife mark troubleshooting tip

Rotate the image scan and use secondary imaging in order to differentiate cutting problems from lines caused by imaging problems.

Lung sample imaged at higher vacuum than optimal



Secondary electron image scan rotated 25°

Supplementary information

- Switching on secondary electron detector may help reduce charging
- Reduce magnification and/or dwell time to reduce chatter, may take 10-20 sections to see effect.
- Wavy lines in sections are chatter to do with the hardness of the resin. You can influence chatter by changing the speed and angle of the knife.

Anton Page

a.page@soton.ac.uk

How do you clean the SEM to get rid of the resin sections?

- What do you use to clean the chamber?
- Do you clean it after each run?
- Do you stop part way through a run, clean it and restart it?

Supplementary information

- Use an airline and blow around chamber
- Use dust-off, sometimes mid run on a very long run (several days). Pause run, clean, join image series afterwards.

David Dinsdale

dd5@le.ac.uk

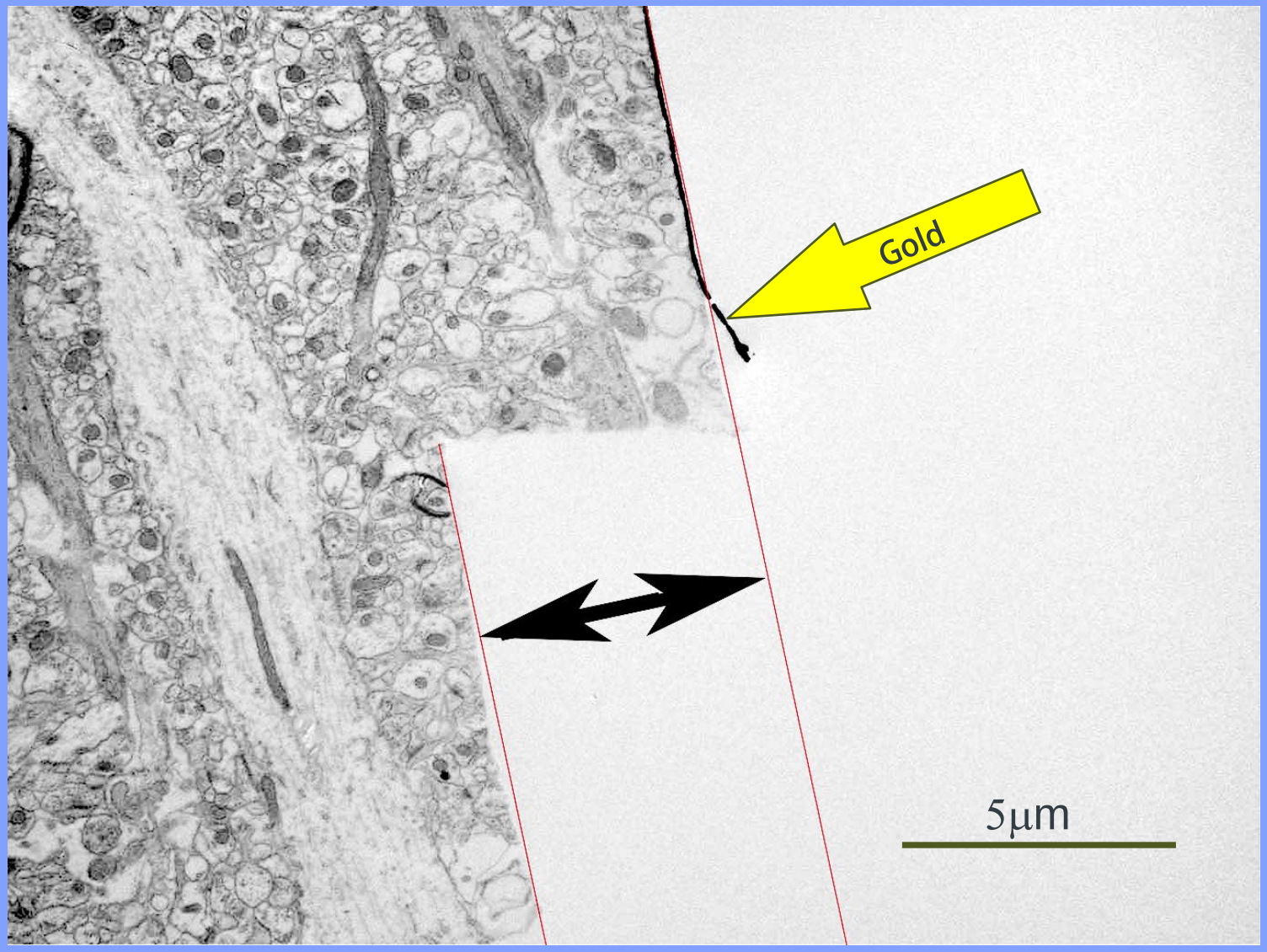
MRC | Toxicology Unit

How thick are your slices?

The stereological analysis of tissues requires an accurate estimation of both the area examined and the thickness of the section. We needed to check the accuracy of the slice thickness indicated by the '3View' software but clearly our usual technique, of re-embedding sections and sectioning them orthogonally, was not appropriate.

The mean section thickness was estimated by gold-coating the sample and sectioning only half of the block face with '3View'. The number of slices cut, after removal of the gold, was recorded before the whole block was re-embedded and sectioned orthogonally, with an ultramicrotome. Ultrathin sections were mounted on grids and the depth removed by '3View' was then measured by transmission electron microscopy.

The mean thickness of slices cut by our instrument, when set @ 80nm, was 86nm.



With thanks to Maria Guerra-Martin, Tim Smith & Diego Peretti

Ref: Peretti D, et al. Nature. 2015 Feb 12;518(7538):236-9.

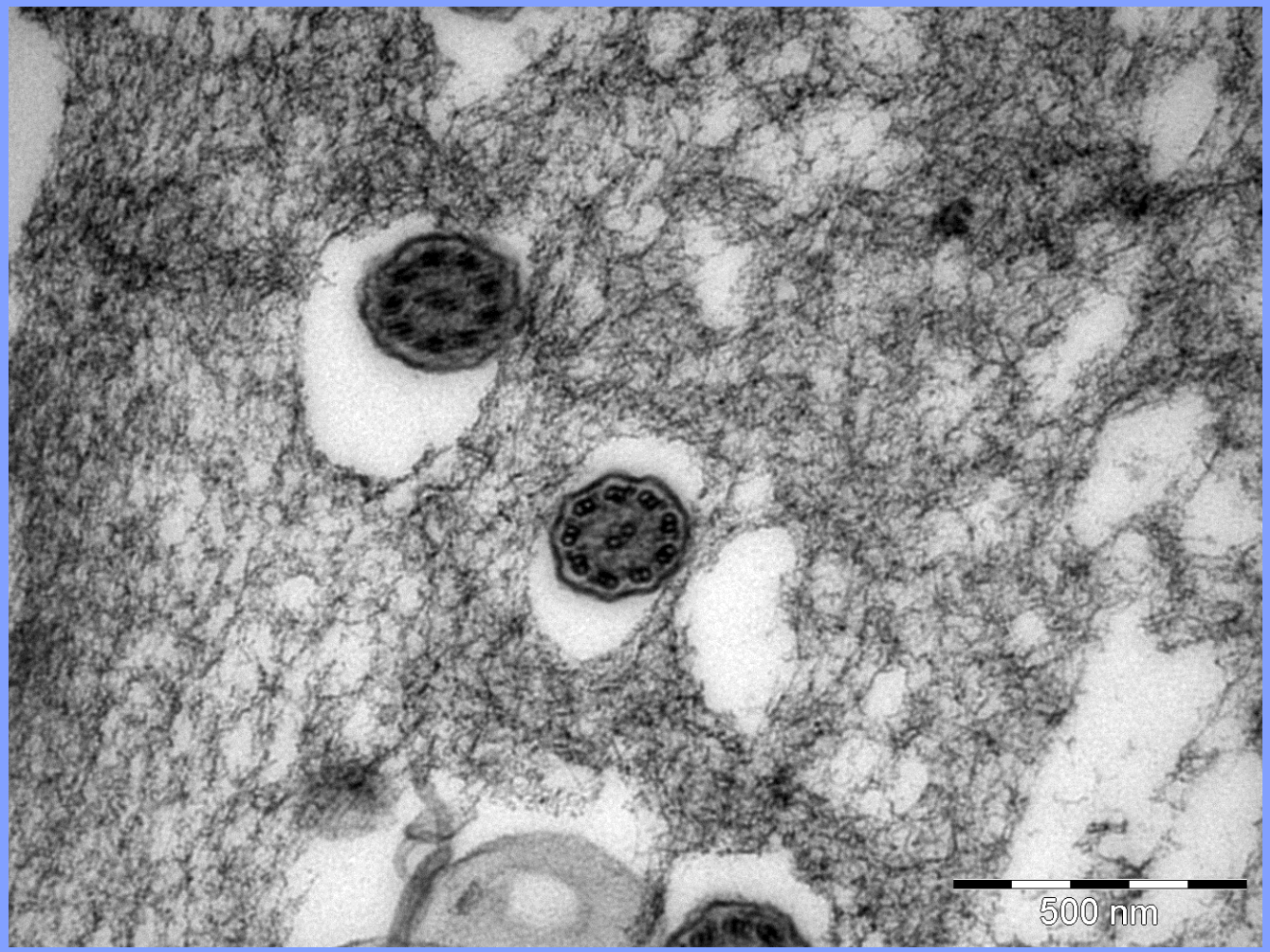
Supplementary information

- Another way to test accuracy of cutting is to use beads of known size.

Anton Page

a.page@soton.ac.uk

Alginate embedding



Journal of Microscopy, Vol. 175, Pt 2, August 1994, pp. 166–170.
Received 14 March 1994; accepted 1 June 1994

SHORT TECHNICAL NOTE

Calcium alginate encapsulation of small specimens for transmission electron microscopy

A. M. PAGE, J. R. LAGNADO, T. W. FORD & G. PLACE*
Royal Holloway, University of London, Egham, Surrey TW20 0EX, U.K.
**Bayer plc, Stoke Court, Stoke Poges, Slough SL2 44Y, U.K.*

Key words. Alginate, encapsulation, cell culture, immunocytochemistry, TEM.

Summary

A technique of encapsulating small objects in calcium alginate for further processing for transmission electron microscopy is described. Five methods are outlined which enable a variety of specimens including single cells (in suspension and on agar plates), small organisms and

can then be frozen down in 1-ml volumes and individual aliquots can be defrosted just prior to use.

Method 1

A 10-ml suspension of *Trypanosoma brucei brucei* strain

Supplementary information

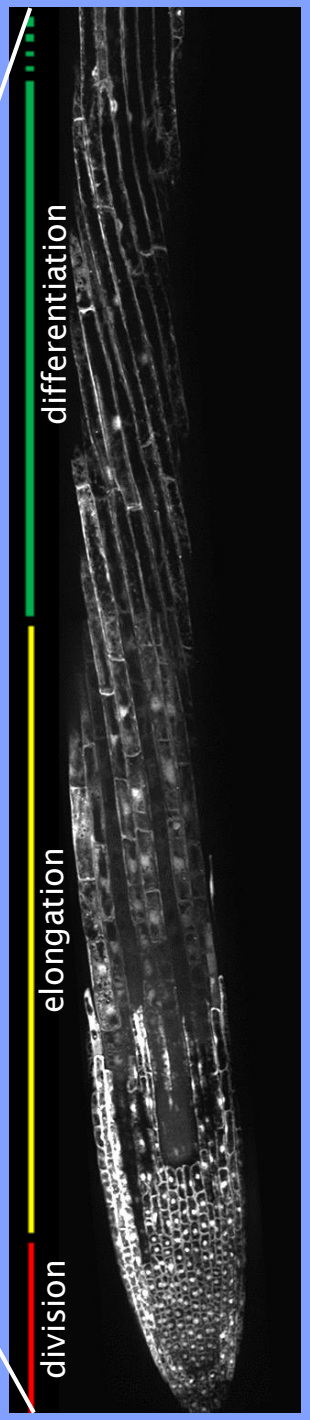
- Can also use 4% agarose +4% gelatin. Bare resin is susceptible to charging. To minimise bare resin around cells, embed in 4% LMP agarose impregnated with 4% gelatin after primary fix.
- Agarose can be cut into squares which retain their shape well.
- ? Use of Ca in washing buffers.

Claire Boulogne

boulogne@isv.cnrs-gif.fr

FRANCE-BIOIMAGING

“What we dream to do”: exploring *Arabidopsis* root in 3D



All major cell states are visible in roots :

- Quiescence
- Proliferation
- Growth (elongation)
- Differentiation

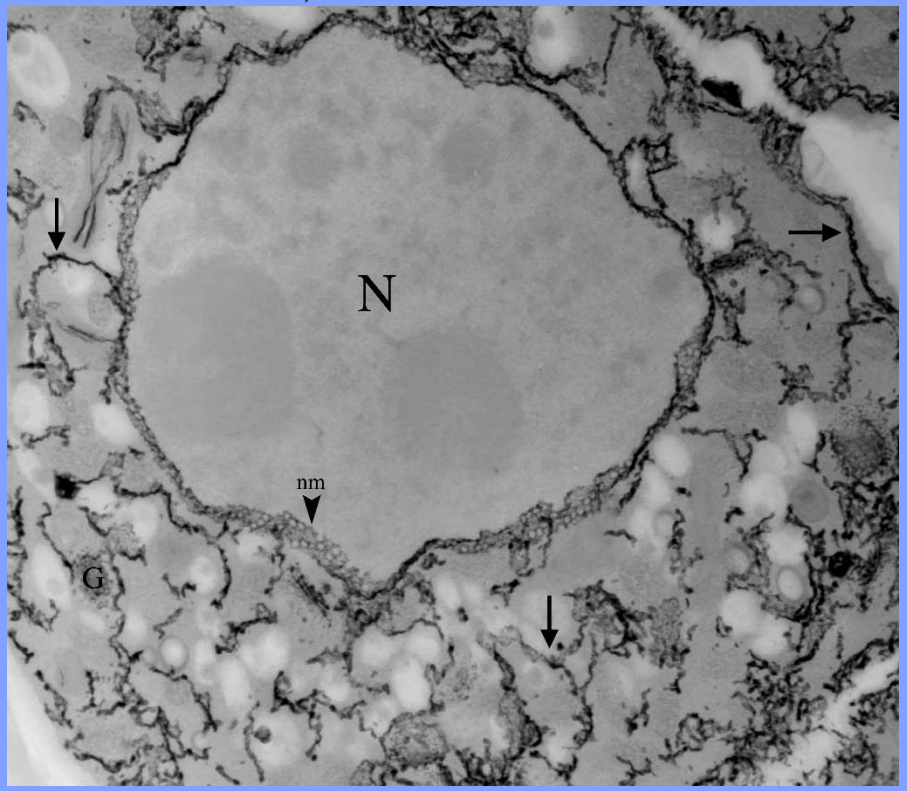
Many questions about endomembranes

- Evolution of the interface Golgi/reticulum along the cell cycle
- Membrane repartition during cell division
- Vacuole morphogenesis during differentiation
- Secretion for cell wall formation
- Regulation of autophagy (autophagosomes formation)

image : Romain Le Bars

Many questions :

- Can we reconstruct a whole root (2 mm in length)?
- Specific preparation for plant samples?
- Zinc Iodide Osmium (ZIO) fixation? (good contrast for reticulum and Golgi, but what about other membranes?)



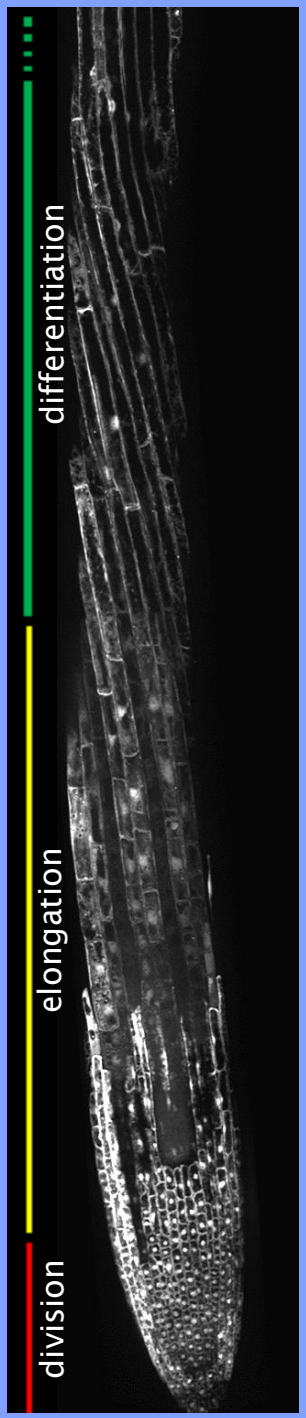


image : Romain Le Bars

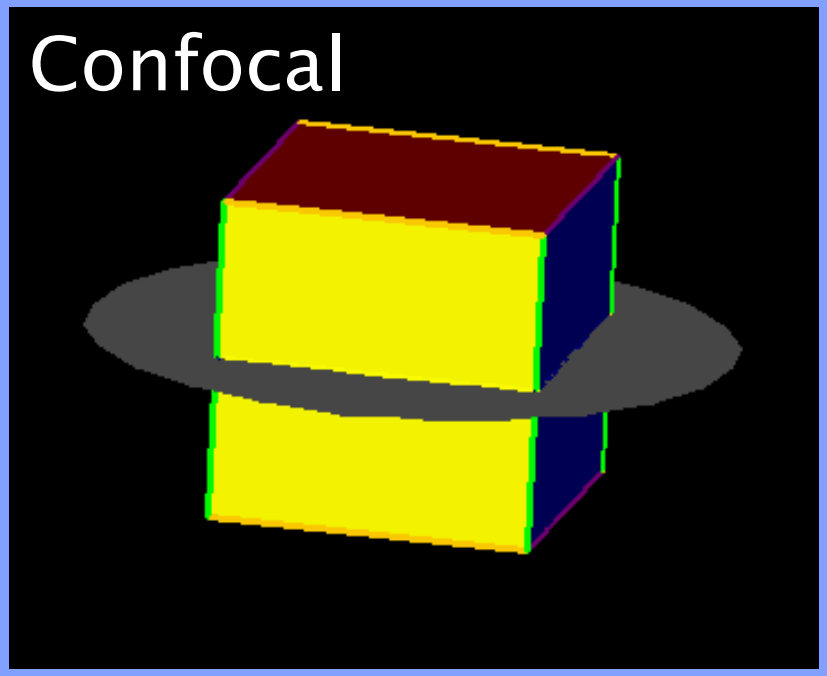
Martin Jones

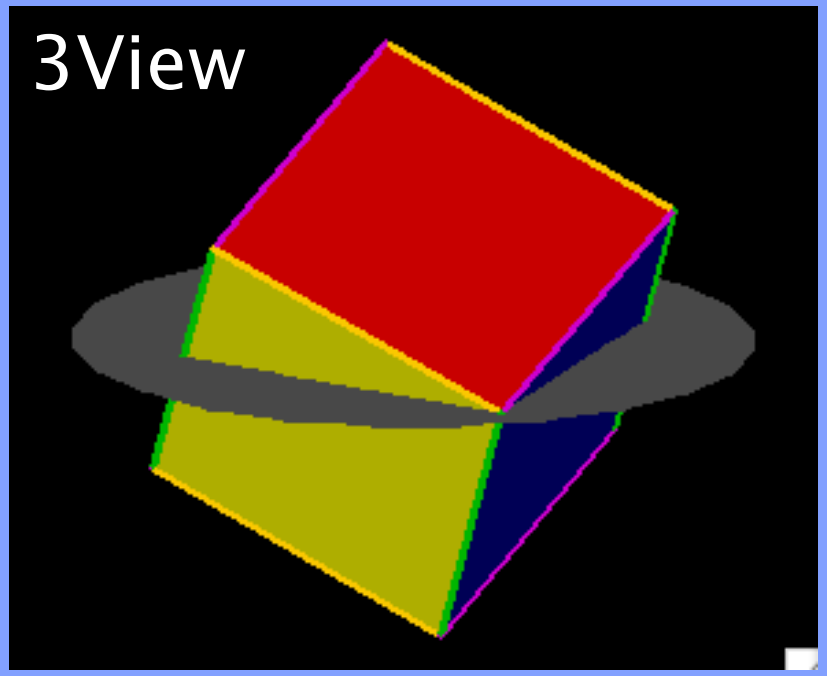
martin.jones@cancer.org.uk

martin.jones@crick.ac.uk

Stack alignment in CLEM

- Extract angle data from 3View
- Make LM voxels isotropic
- Use affine transformation to realign LM data (with interpolation)
- Use features to register EM-LM
- Selecting EM slice returns LM data for that slice





Software used

FIJI plugins

- Erik Meijering's TransformJ plugin for general Affine transforms
- Bioformats for handling proprietary microscope formats *(work with raw data where possible to preserve metadata and reduce duplication)*
- BigDataViewer plugin for visualising large virtual stacks of EM data

MATLAB for phantom data generation

Coming soon as an integrated downloadable FIJI plugin

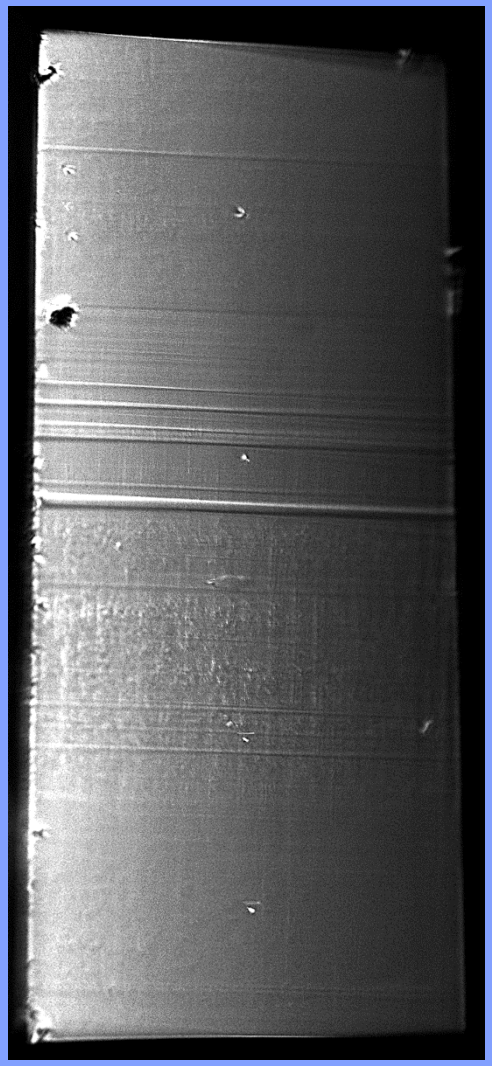
- Including absolute 3D registration
- Interpolated reslicing of LM data to provide EM overlays

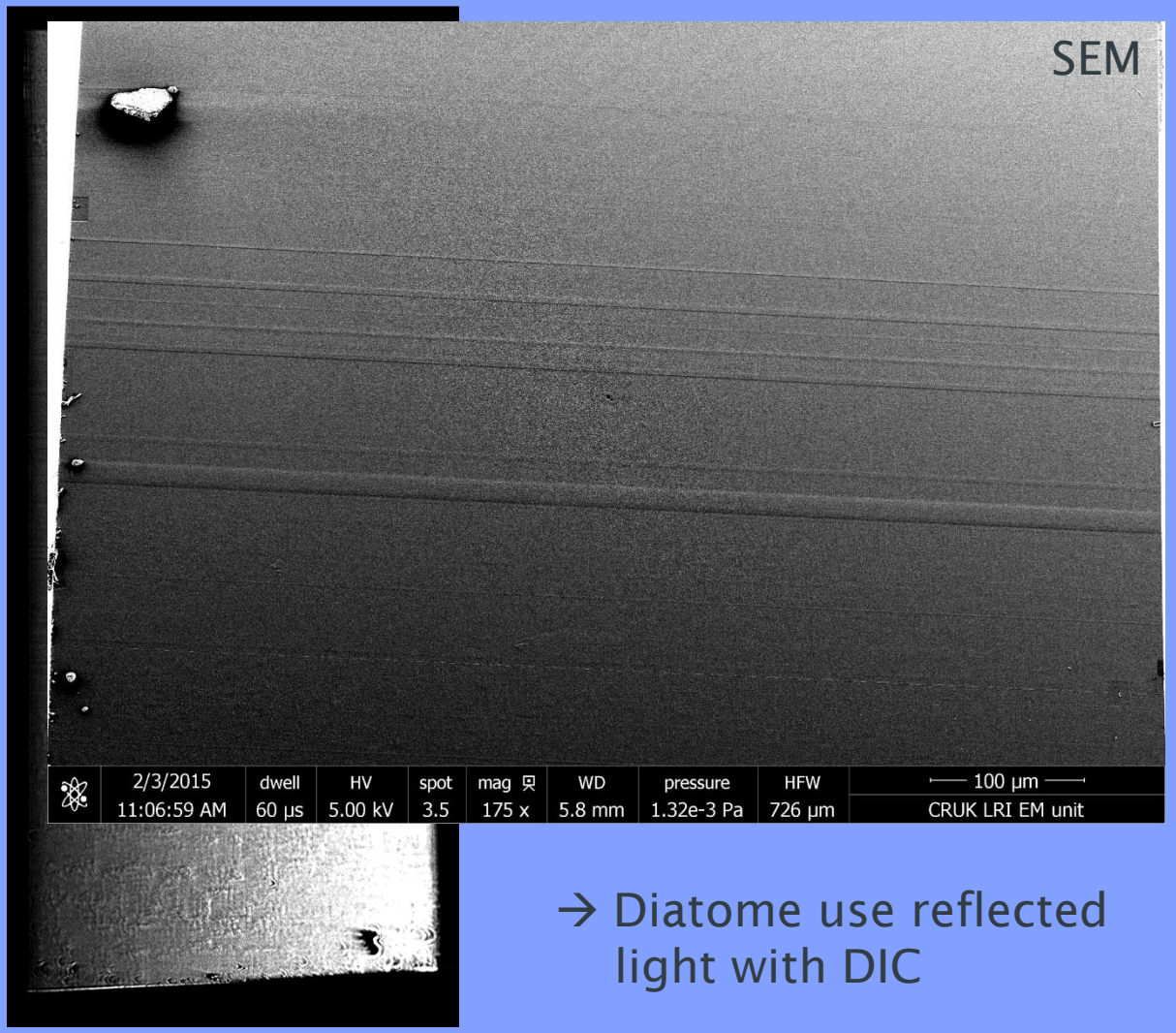
Chris Peddie

christopher.peddie@cancer.org.uk

(christopher.peddie@crick.ac.uk)

Assessment of knife damage





→ Diatome use reflected light with DIC

3View reporting metrics

Details of volume	Imaging conditions
x-, y-, z- resolution	Instrument
Number of slices	Dwell time
HFW	Pressure
Volume dimensions	Voltage
Total volume	Aperture

Supplementary information

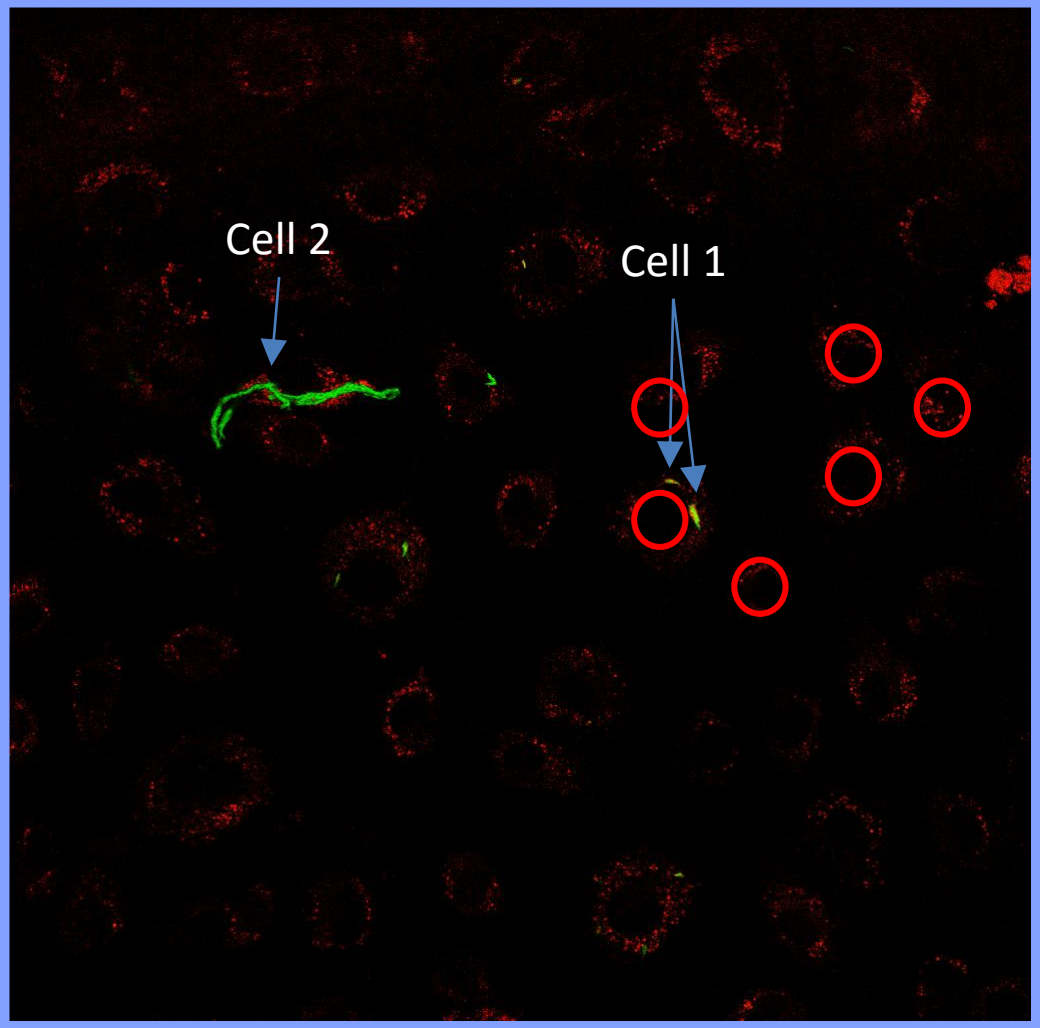
- Use hairspray on the block to ribbon sections.
- Diatome use reflected light and DIC microscopy to assess knife quality.
- Clean the knife as seldom as possible.
- Use knife of different angle for materials.

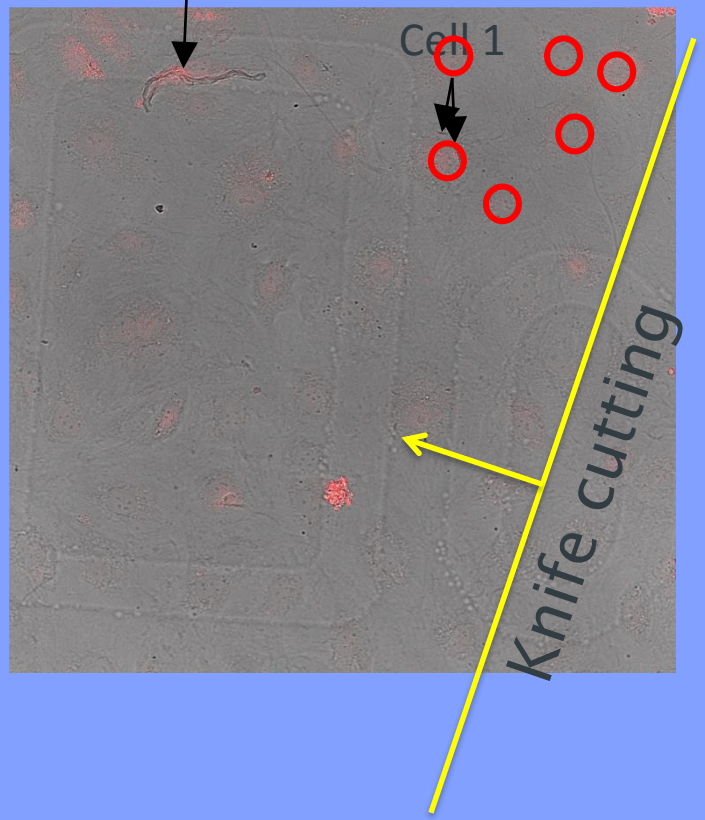
Matt Russell

matt.russell@cancer.org.uk

3View CLEM

Flip images to correspond to block face -

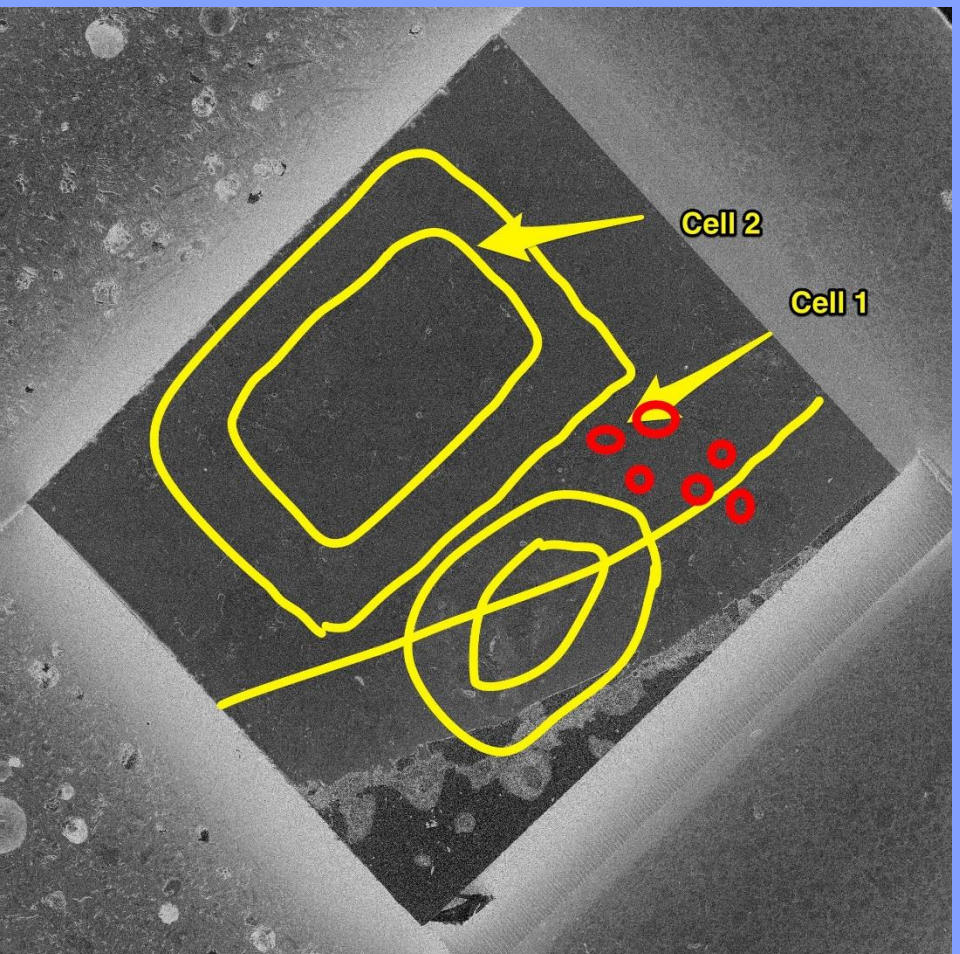


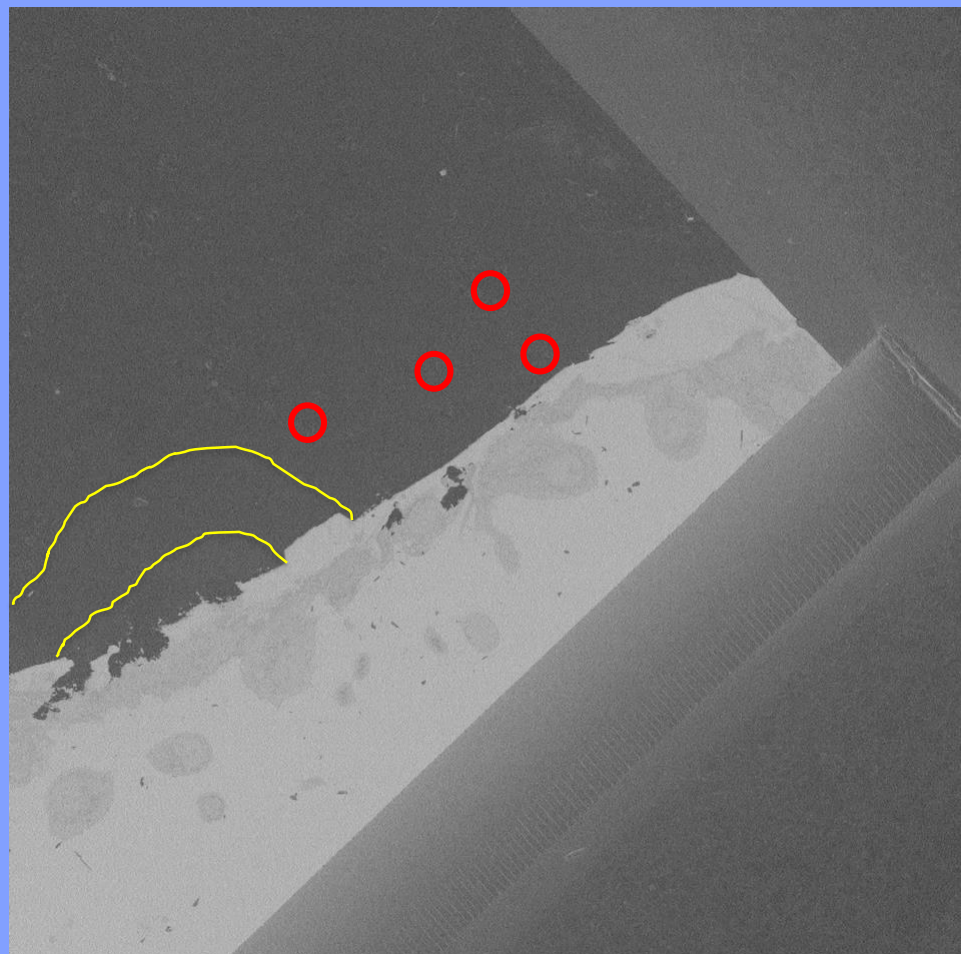


3View CLEM

Track your progress by comparing images as you go along

Note feature locations and check off as the knife cuts through them





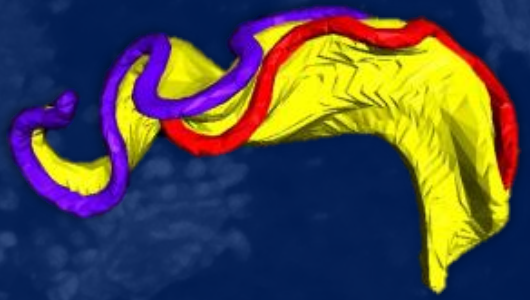
Supplementary information

- 3-view CLEM. Look at cells of interest ahead of the cell you're looking at. Compare images as you go along. Use tool in DM.

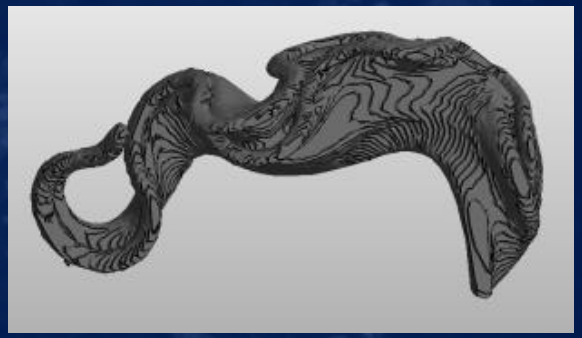
Louise Hughes

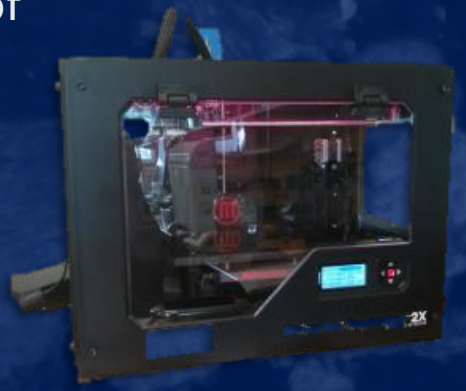
lhughes@brookes.ac.uk

Transform 3View data into physical models



- Segmentation
  - (currently Amira, possibly Imaris?)
- Simplfying
  - Aim for ~60k triangles
- Check for inverted triangles
  - Netfabb studio (free)
- Load onto printer softward
- Select print parameters
  - Orientation of print, amount of scaffold, speed of print etc.
- Print!





Details of supplier

Online resources

www.shapeways.com

www.imaterialise.com

3D printer – Makerbot replicator 2x experimental

lhughes@brookes.ac.uk

Supplementary information

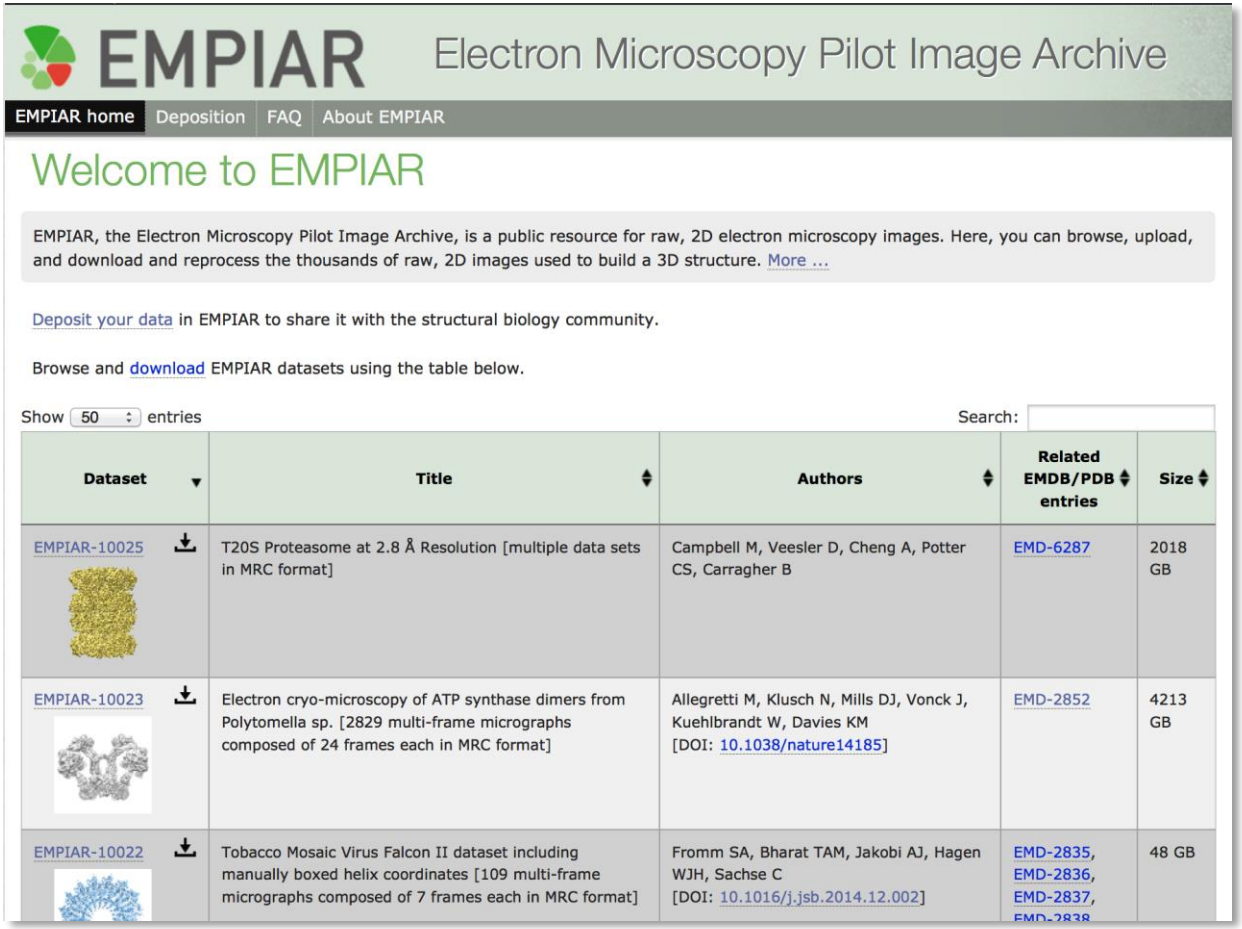
- Simplify the model to about 600,000 triangles. Save as .stl file.
- Amira A*(B>0), attach Volren. Doesn't produce surface or volume data. 5 or 6 slices and interpolate.

Lucy Collinson

Lucy.Collinson@cancer.org.uk

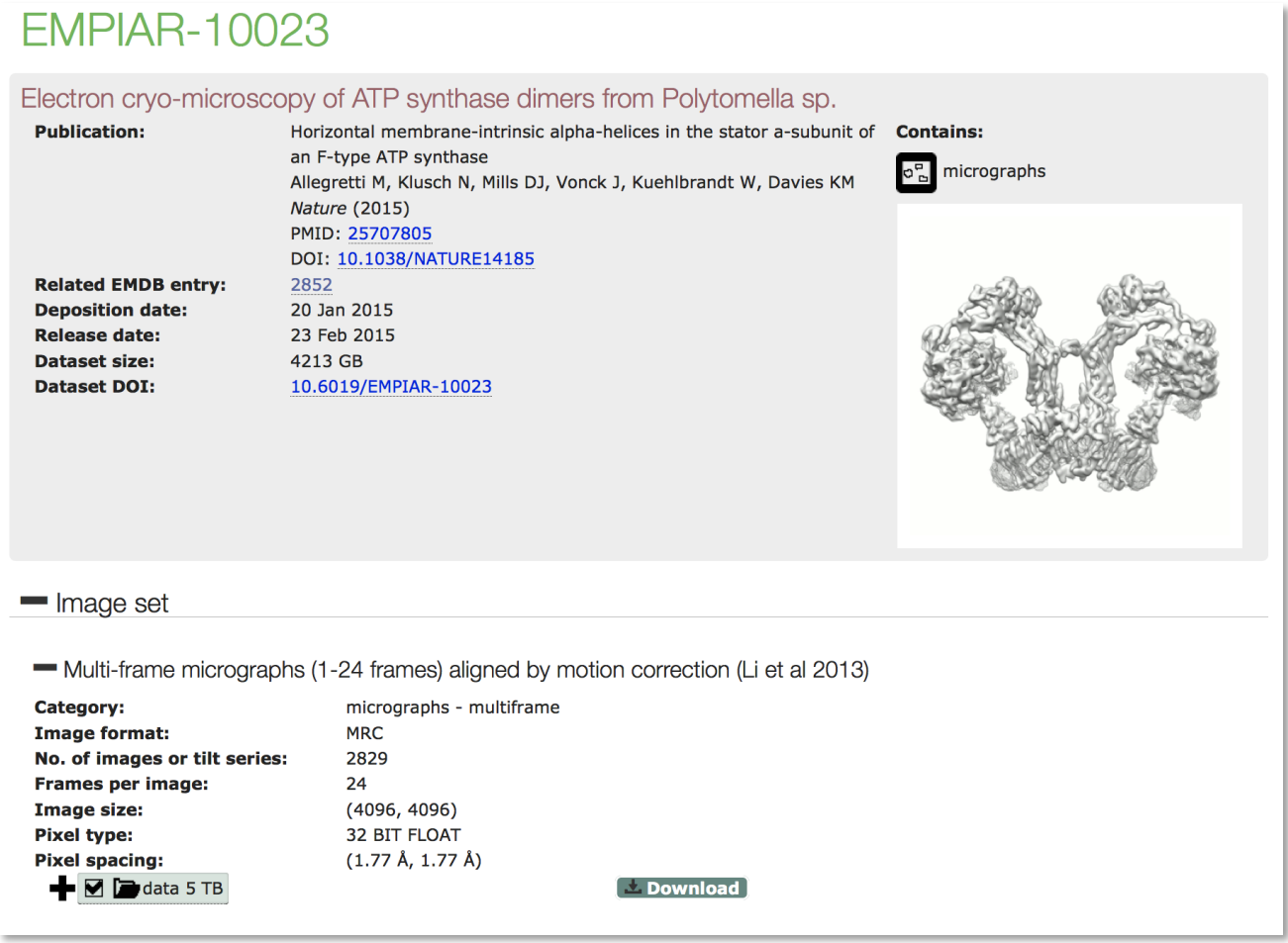
EMPIAR (pronounced ‘empire’)

- EMPIAR – Electron Microscopy Pilot Image Archive
- Stores raw image data related to 3D reconstructions deposited to EMDB
- Single-particle and electron tomography data
- MRC/BBSRC funded project for 3 years
- We now have additional funding to consider how EMPIAR could be used to store 3View, FIB-SEM, SXT and correlative LM data
- Uses include validation, develoment of techniques, remining of data



EMPIAR – technological underpinnings

- Currently 23 data sets ranging from a few GB to 4TB
- Capacity to scale to at least PB range but
  - Growth needs to be gradual
  - Business model works for ‘reference data’ but not as a dump for any image data
- Uses Aspera for data transfers – has worked fine for TB uploads and downloads (EBI has used it for PB transfers)
- We also support Globus (GridFTP) and have tested this option
- Currently developing a deposition system and better support for viewing image data on the web



EMPIAR – 3View

- Understand communities view on deposition/archiving of data
- What are good reference datasets - datasets corresponding to publications??
- Nitty gritty issues
  - Meta data to capture
  - Data formats

Patricia Goggin

P.Goggin@soton.ac.uk

Conductive resin

1. (WO2015009941) HIGHLY CONDUCTIVE NANOCOMPOSITE, BIOLOGICAL AND SMALL MOLECULE MATERIALS FOR ENHANCED RESIN CONDUCTIVITY .

PCT Biblio. Data | Description | Claims | National Phase | Notices | Drawings | Documents

...

Latest bibliographic data on file with the International Bureau ⇨ Submit observation

PermaLink

Pub. No.: WO/2015/009941 **International Application No.:** PCT/US2014/047046
Publication Date: 22.01.2015 **International Filing Date:** 17.07.2014
IPC: *H01B 1/24* (2006.01)

Applicants: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 North Franklin Street 5th Floor Oakland, California 94607 (US)

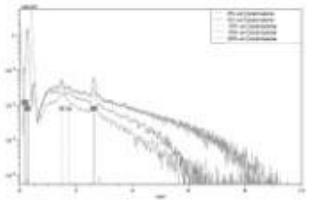
Inventors: **ELLISMAN, Mark, H.**; (US).
JOHNSON, JR., Donald; (US).
DEERNICK, Thomas, J.; (US).
BUSHONG, Eric, A.; (US).
BOUWER, James; (US).
RUMACHANDRA, Ranjan; (US).
SIEGEL, Jay, S.; (CH)

Agent: **FALLON, Steven, P.**; (US)

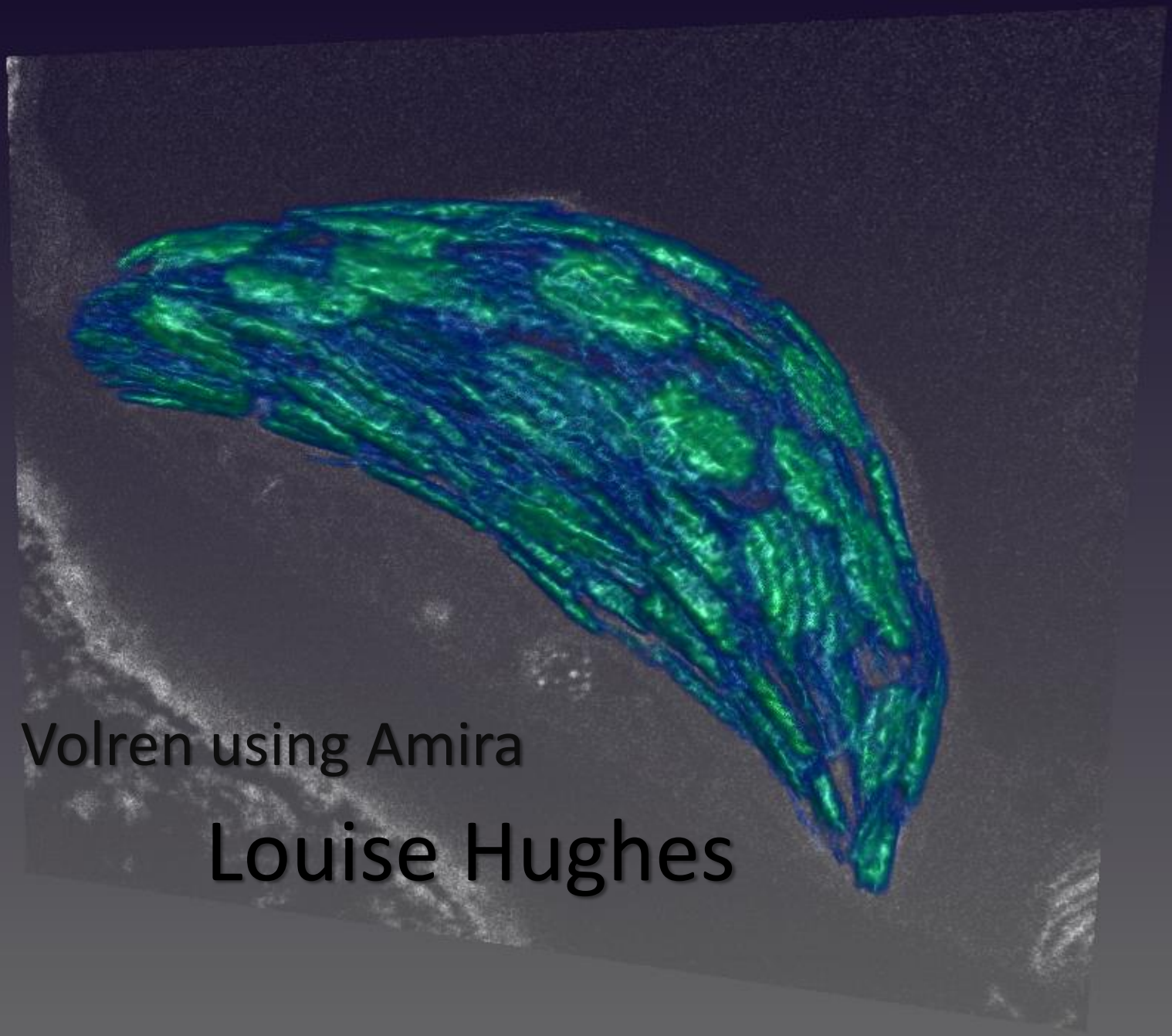
Priority Data: 61/847,402 17.07.2013 US

Title **(EN)** HIGHLY CONDUCTIVE NANOCOMPOSITE, BIOLOGICAL AND SMALL MOLECULE MATERIALS FOR ENHANCED RESIN CONDUCTIVITY
(FR) MATÉRIAUX NANOCOMPOSITES, BIOLOGIQUES ET À PETITES MOLÉCULES EXTRÊMEMENT CONDUCTEURS AMÉLIORANT LA CONDUCTIVITÉ DE RÉSINES

Abstract: **(EN)**A highly conductive nanocomposite material. The material is particularly useful for serial block-face scanning electron microscopy. A polymer resin of the invention is stabilized for conductivity with a conductivity stabilizer selected from one of multi-walled carbon nanotubes, Perylene dianhydride, Hemoglobin, Epoxy-Corannulene, and Bovine Serium Albumin (BSA). The conductivity stabilizer is monodisperse in preferred resins. A preferred nanocomposite material includes a base component of a curable resin, a curing agent or hardener and monomers of carbon containing networks of sp2 hybridized carbon atoms that are dispersed in the base resin. In preferred embodiment, tissue samples are within the resin. Highly effective serial block face scanning electroscopy techniques are provided.

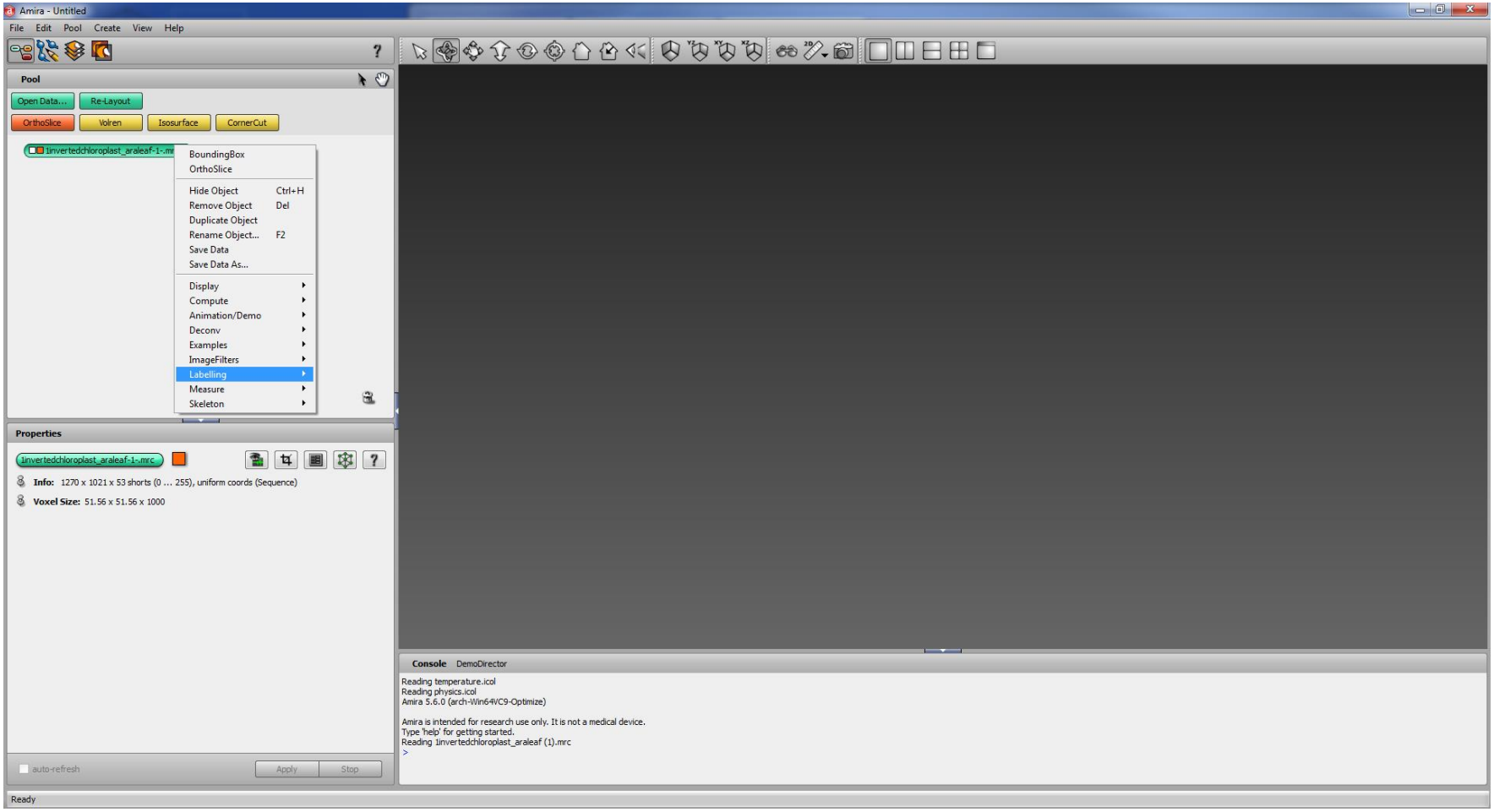


(FR)L'invention concerne un matériau nanocomposite extrêmement conducteur. Le matériau est particulièrement utile dans un processus de microscopie à balayage appelé SBF-SEM (Serial Block Face-Scanning Electron Microscopy) pour créer des séries d'images de face en bloc. Une résine polymère de l'invention présente une conductivité stabilisée au



Volren using Amira

Louise Hughes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Re-Layout
OrthoSlice
Volren
Isosurface
CornerCut
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BoundingBox
OrthoSlice
Hide Object Ctrl+H
Remove Object Del
Duplicate Object
Rename Object... F2
Save Data
Save Data As...
Display
Compute
Animation/Demo
Deconv
Examples
ImageFilters
Labelling
Measure
Skeleton
Properties
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Info: 1270 x 1021 x 53 shorts (0 ... 255), uniform coords (Sequence)
Voxel Size: 51.56 x 51.56 x 1000
auto-refresh
Apply
Stop
Console DemoDirector
Reading temperature.icol
Reading physics.icol
Amira 5.6.0 (arch-Win64VC9-Optimize)
Amira is intended for research use only. It is not a medical device.
Type 'help' for getting started.
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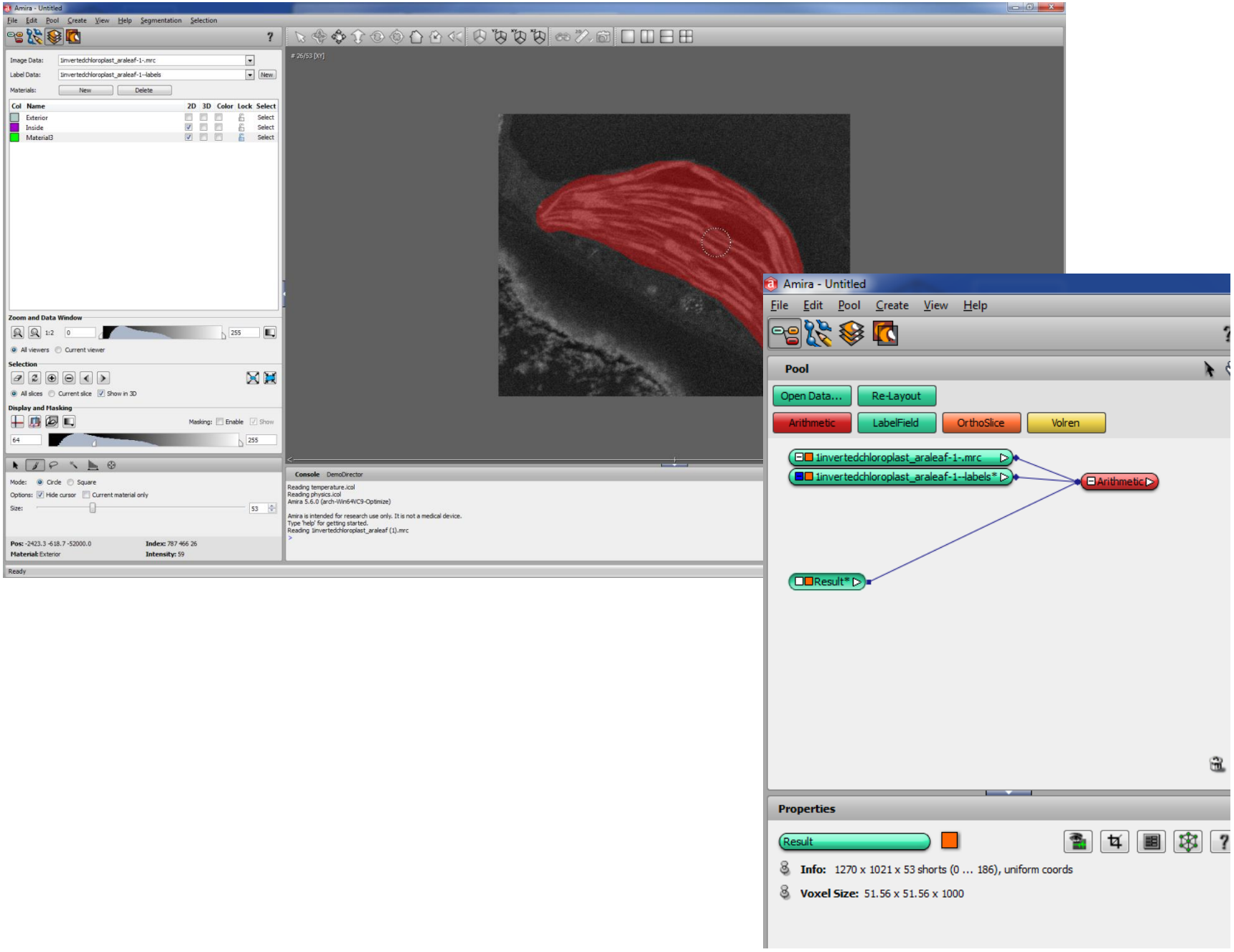
>
Ready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Exterior Select
Inside Select
Material3 Select
Zoom and Data Window
Selection
Display and Masking
Console DemoDirector
Reading temperature.icol
Reading physics.icol
Amira 5.6.0 (arch-Win64VC9-Optimize)
Amira is intended for research use only. It is not a medical device.
Type 'help' for getting started.
Reading 1invertedchloroplast_araleaf (1).mrc
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Open Data... Re-Layout
Arithmetic LabelField OrthoSlice Volren
1invertedchloroplast_araleaf-1-.mrc
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Result*
Properties
Result
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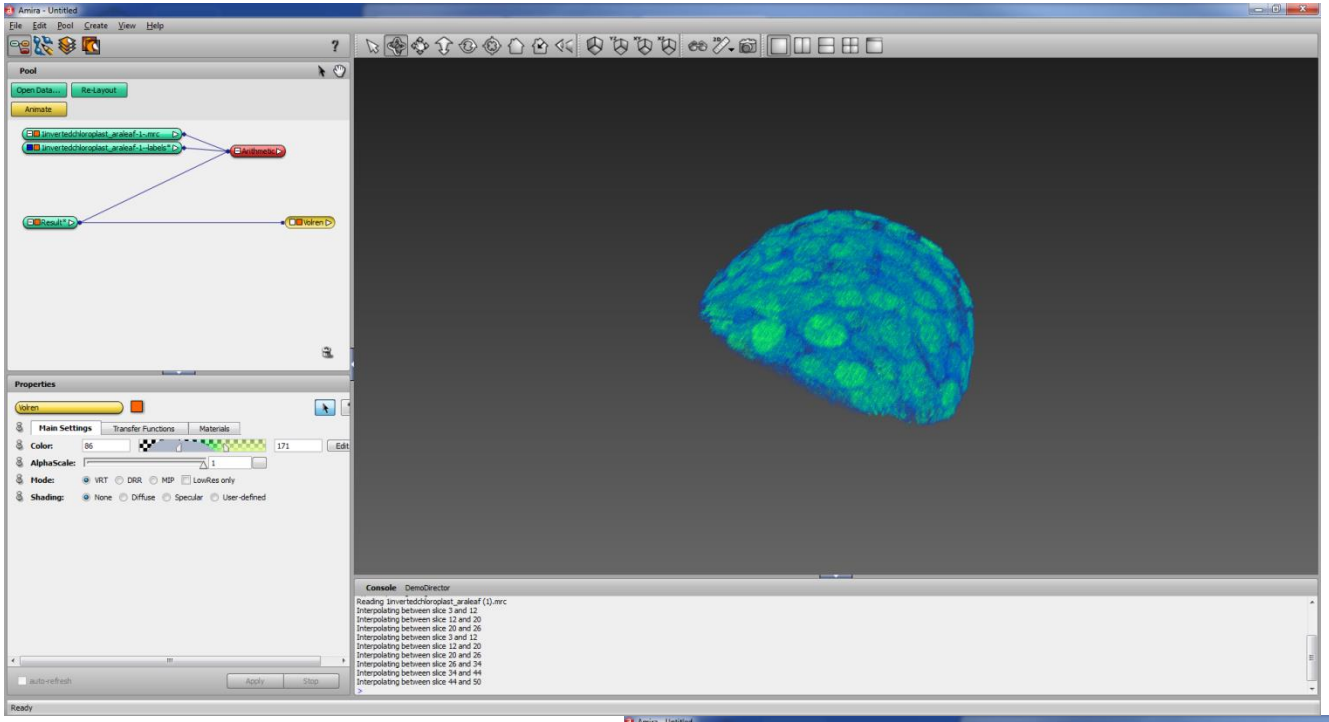
Amira - Untitled
File Edit Pool Create View Help
Pool
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Re-Layout
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Arithmetic
Result
Volren
Properties
Main Settings
Transfer Functions
Materials
Color:
AlphaScale:
Mode:
Shading:
Console
DemoDirector
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Interpolating between slice 3 and 12
Interpolating between slice 12 and 20
Interpolating between slice 20 and 26
Interpolating between slice 3 and 12
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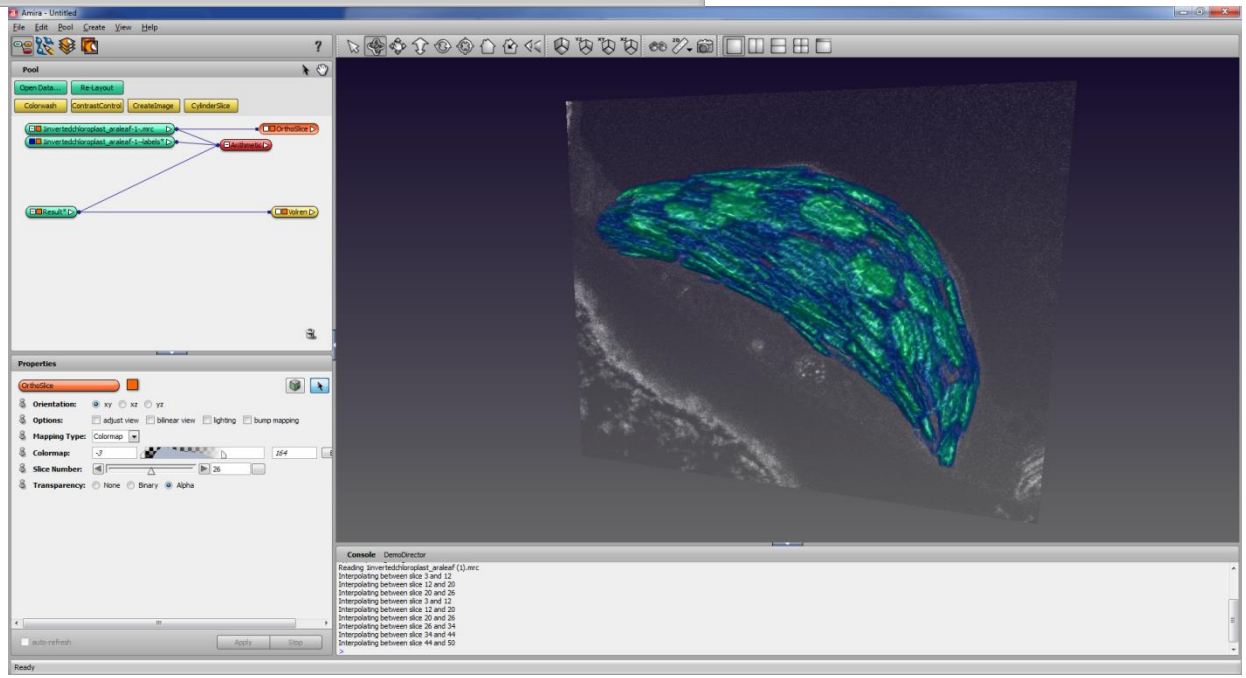
Interpolating between slice 20 and 26
Interpolating between slice 26 and 34
Interpolating between slice 34 and 44
Interpolating between slice 44 and 50
Ready


Amira - Untitled
File Edit Pool Create View Help
Pool
Open Data...
Re-Layout
Colorwash
ContrastControl
CreateImage
CylinderSlice
OrthoSlice
Arithmetic
Result
Volren
Properties
Orientation:
Options:
Mapping Type:
Colormap:
Slice Number:
Transparency:
Console
DemoDirector
Reading 1invertedchloroplast_araleaf (1).mrc
Interpolating between slice 3 and 12
Interpolating between slice 12 and 20
Interpolating between slice 20 and 26
Interpolating between slice 3 and 12
Interpolating between slice 12 and 20
Interpolating between slice 20 and 26
Interpolating between slice 26 and 34
Interpolating between slice 34 and 44
Interpolating between slice 44 and 50
Ready

Microscopy Image Browser

Ilya.Belevich@helsinki.fi
Eija.Jokitalo@helsinki.fi

A tool for

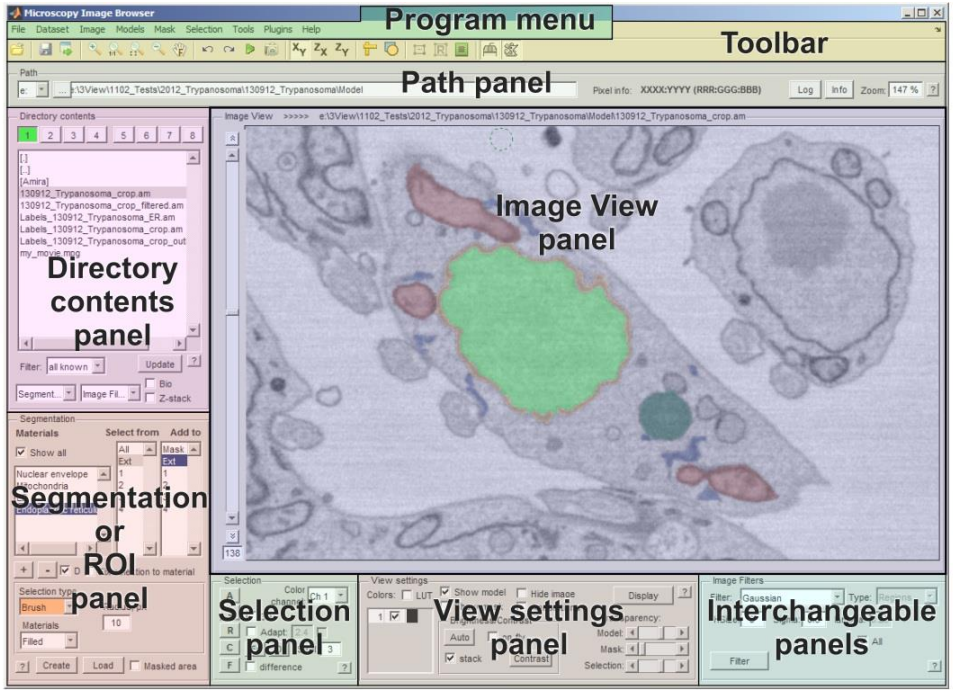
- image processing of microscopy images
- segmentation of objects out of them
- basic visualization of volumes and models

Program menu
- the main gateway to most tools and actions of MIB

Path panel
- selection of the working directory

Directory contents panel
- Directory navigation
- Selection of files to load and assemble in stacks

Segmentation/ROI panel
- list and selection of materials of models
- definition of region of interests



Toolbar
- quick access to universal actions

Image View panel
- evaluation of datasets
- interaction with datasets

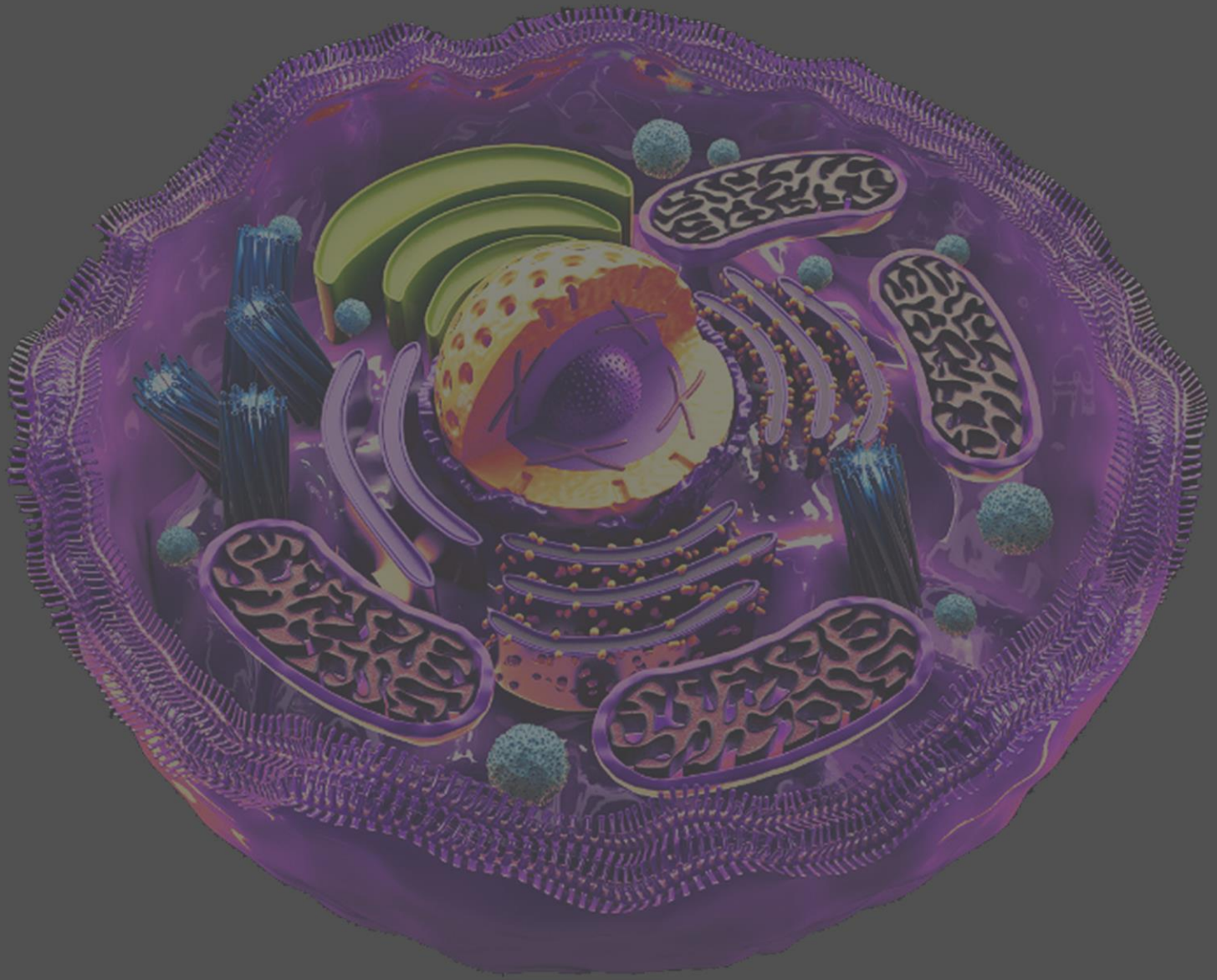
Selection panel
- tools for modification of the Selection layer

View Settings panel
- toggle and modify settings for image evaluation

Interchangeable panels
- image filters
- mask generators
- background removal
- Fiji connect

- Coming soon as a freely distributed open-source program
- For a test version: contact Ilya!
- http://mib.helsinki.fi

Supplementary information

- Other software to try – Voreen (for volume), Meshlab and PARAVIEW

- Contact Ilya Belevich to try beta version of Microscopy Image Browser