Multi-scale - correlative SEM imaging

Fluorescence-topography SEM, RT
Live imaging and Fluorescence-vEM, RT
Cryofluorescence-cryoSEM
Cryofluorescence-cryoFIB
Cryo-cathodoluminescence cryoSEM
cryoSEM-EDS
Raman-cryoSEM

Marie Vancová
Laboratory of Electron Microscopy
Biology Centre CAS, České Budějovice
Why multiscale.... (multimodal, multidimensional)

- all materials are hierarchical in some sense

Rich multi-dimensional correlative imaging

Philip J Withers and Timothy L Burnett
The Henry Royce Institute, School of Materials, University of Manchester, M13 9PL, United Kingdom
Corresponding author: Philip@manchester.ac.uk

a) an optical micrograph showing the cortical and trabecular bone, b) SEM of the trabecular network, c) micro CT of the Haversian system, d) Lamellar structure in polarized light, e) TEM of collagen fibrils
Why correlative....

**Imaging that analyses the same object by at least two different techniques**

also at different **scales**, often from biological tissue to the subcellular level with the aim to add information to the selected ROI or to find it.

**Fluorescence microscopy**
rare or transient phenotypes or specific subpopulations of cells within a complex tissues, dynamics
But: the impossibility of precisely identifying unlabeled structures, resolution

**Electron microscopy:** add subsequent ultrastructural context but not for screening larger sample areas and is not able to provide any data about cell dynamics.
Influence of viral replication on BBB integrity

Cells grown on the fibrinogen-coated glass slides
4% formaldehyde 15-30 min
Immunofluorescence
2.5% glutaraldehyde
OsO4, dehydration, CPD
Volume changes

Fiducial markers
Lipid droplets (intracellular)

Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood-brain barrier integrity


FLUORESCENCE-TOPOGRAPHY SEM, RT
Cells were fixed and attached to poly-L-lysine coated coverslides

Fluorescence-topography SEM, RT

To find rare events

FluoroNanogold (Nanoprobes)

Silver enhancement
EM sample prep.

BSE 6-10 kV.

Antigen combining region
Conjugation via hinge thiol

Silver/gold enhancement-different dimensions of particles-vs multiple labeling
Fluorescence-topography SEM, RT
Indium–tin oxide (ITO)-coated glass

- No adverse effects on the physiological behaviour (fibroblasts, cancer cell lines), can be further coated with e.g. type-I collagen to mimic the ECM
- For SEM imaging of uncoated biological samples without charging artefacts in high vacuum SEM
- Compatible with FL

Advantages of indium–tin oxide-coated glass slides in correlative scanning electron microscopy applications of uncoated cultured cells

H. PLUK*, D.J. STOKES†, B. LICH†, B. WIERINGA*
& J. FRANSEN*
*Department of Cell Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
†FEI Company, P.O. Box 80066, 5600 KA Eindhoven, The Netherlands
Shuttle & Find for ZEN Imaging Software

Repositioning Accuracy • < 25 μm (initial accuracy, depending on stage specification) • < 5 μm (using software option for fine calibration)

<table>
<thead>
<tr>
<th>Sample Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fixation</td>
</tr>
<tr>
<td>• Embedding</td>
</tr>
<tr>
<td>• Labeling</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mounting into Correlative Holder</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Specimen holder for TEM grids</td>
</tr>
<tr>
<td>• Specimen holder for cover glasses</td>
</tr>
<tr>
<td>• Or use any holder with 3 calibration markers</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Light Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Widefield</td>
</tr>
<tr>
<td>• LSM</td>
</tr>
<tr>
<td>• Superresolution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Optional: Sample preparation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electron Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SEM</td>
</tr>
<tr>
<td>• FIB-SEM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evaluation &amp; Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Correlation</td>
</tr>
<tr>
<td>• Image processing</td>
</tr>
</tbody>
</table>
Horiba: nanoGPS navYX™

<table>
<thead>
<tr>
<th>HORIBA Raman microscope</th>
<th>Optical microscope with high precision XY stage</th>
<th>SEM with regular XY stage</th>
<th>SEM with high precision XY stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - 15 μm</td>
<td>5 - 10 μm</td>
<td>15 - 30 μm</td>
<td>5 - 15 μm</td>
</tr>
</tbody>
</table>
Correlia: an ImageJ plug-in to co-register and visualise multimodal correlative micrographs

LORENS ROHDE, ULF-DIETRICH BRAUMANN, MATTHIAS SCHMIDT

First published: 03 June 2020 | https://doi.org/10.1111/jmi.12928 | Citations: 11

Present address: Florens Rohde, Institute of Computer Science, Faculty of Mathematics and Computer Science, Leipzig University, Leipzig 04109, Germany.

Correlia is open source software and available from www.ufz.de/correlia
To find ROI

Pixel size: 3 x 3 x 50 nm

Fluorescence-vEM, RT

Borrelia

250 x 250 µm

1,5 µm

18 x 12 µm

1071.4 µm

683.1 µm

609.1 µm
Pablo Suárez Cortes, Max Planck Institute for Infection Biology, Berlin
Malaria parasites in the mosquito: Imaging the needle in the haystack
Laboratory of Electron Microscopy, Biology Centre CAS, Budweis, M. Vancová, J. Týč

GOAL: Understand role of secretory organelles in mosquito stages for transmission and characterization of ookinete traversal of the midgut
... but at first, we need to find parasites within mosquito tissues

How:

Mosquito intestine → Finding ROI using IF → Map the ROI onto the original sample → Specimen preparation: staining, resin embedding, targeted trimming → Image serial sections in SBF-SEM → Process and analyse the data
Dynamics: Visualization of ER contact sites with early and late endosomes

Epifluorescence time-lapse movie (dynamic behavior) and FIB-SEM (ultrastructural context), RT. HeLa cell transfected with LAMP-1-GFP (Lysosomes) and treated with dextran-Alexa568 (endosome-lysosomes)

Images were captured in the GFP channel for a period of 2 minutes at a rate of 0.4 seconds per frame. Images were deconvolved using Softworx 6.5.2 and manually traced using MTrackJ (colored traces). The movie is played back at a rate of 15 frames per second (×6 real time). 3D EM reconstruction and segmentation was performed using IMOD 4.9. Scale bar: 2 μm.

Single organelle dynamics linked to 3D structure by correlative live-cell imaging and 3D electron microscopy

Job Fermie, Nalan Liv, Corlinda ten Brink, Elly G. van Donselaar, Wally H. Müller, Nicole L. Schieber, Yannick Schwab, Hans C. Gerritsen, Judith Klumperman

First published: 16 February 2018 | https://doi.org/10.1111/tra.12557 | Citations: 51
The Coral Life workflow combines dynamic data with the best possible sample fixation by then washing in ethanol and dried at 60°C for 48 h.

How Putting Dynamic Live Cell Data into the Ultrastructural Context - Oxygenie + HPF ICE

then washing in ethanol and dried at 60°C for 48 h
How Putting Dynamic Live Cell Data into the Ultrastructural Context-
HPM010, HPM100, HPF compact 02, From Live to HPF: 1.26 seconds

Live imaging - vEM, RT

Extra Long Working Distance Objectives

locatize your cell of interest

Removing the CryoCapsule to reach the sample
Chapter 6 - HPM live $\mu$ for a full CLEM workflow

Xavier Heiligenstein $^{a}$, Marit de Beer $^{b,c,d}$, Jérôme Heiligenstein $^{a}$, Frédérique Eyraud $^{e}$, Laurent Manet $^{a}$, Fabrice Schmitt $^{f}$, Edwin Lamers $^{g}$, Joerg Lindau $^{h}$, Mariska Kea-te Lindert $^{b,c}$, Jean Salamero $^{i}$, Graça Raposo $^{j,k}$, Nico Sommerdijk $^{b,d}$, Martin Belle $^{a}$, Anat Akiva $^{b,c}$

https://doi.org/10.1016/bs.mcb.2020.10.022
How Putting Dynamic Live Cell Data into the Ultrastructural Context-
Live-dead probes

Pleomorphism and Viability of the Lyme Disease Pathogen *Borrelia burgdorferi* Exposed to Physiological Stress Conditions: A Correlative Cryo-Fluorescence and Cryo-Scanning Electron Microscopy Study

Ice sublimation was carried out for 10-20 min at a range from -98°C to -95°C under high vacuum. Samples were sputter coated for 10-20 sec with gold.
Correlative cryo-fluorescence (A,B) and cryo-scanning electron microscopy (C,D) of *Borrelia burgdorferi*-GFP on the surface of human neuroblastoma cells grown on carbon-coated sapphire discs. A series of images of one particular GFP-tagged spirochete (green) interacting with the cell counterstained with Hoechst 33342 (blue). Images of region of interest from low magnification FM to high magnification SEM. Scale bars: (A) 50 μm, (B) 25 μm, (C,D) 1 μm.
Cryofluorescence-cryoFIB

FL integrated in FIB-SEM, to be sure that target is contained in the final lamella

Check-back option during and after lamella milling. Correlation with the iFLM Correlative System allows the lamella milling process to be monitored step-by-step to ensure that the target is contained in the final lamella.

Cryo-lamella prepared with the Aquilos 2 Cryo-FIB (left). Overlay of SEM image with fluorescence image obtained with the iFLM Correlative System (right).
CL-SEM applications in biology

– easy integration into simultaneous multimodal imaging

– applications in geosciences, photonic nanostructures,…

– applications in biology: (biofunctionalized) CL nanolabels e.g. nanodiamonds or rare-earth element-doped nanocrystals.
CL-SEM applications in biology

Ultrabright and Stable Luminescent Labels for Correlative Cathodoluminescence Electron Microscopy Bioimaging


Rare-earth-doped nanophosphors for multicolor cathodoluminescence nanobiomaging using scanning transmission electron microscopy

Taichi Furukawa, Shoichiro Fukushina, Hirohiko Niioka, Naoki Yamamoto, Jun Miyake, Hashimoto

CL-cryoSEM

LaF$_3$:Tb$^{3+}$ particles
Not published
Luminescent transition metal complexes as promising CL organelle-specific probes in cryo-SEM

In vivo FL

HPF freeze fracturing opt. etching and Au-coating

SEM -135°C

fac-{Re(CO)₃(1,10-phenanthroline)(4-pyridyltetrazolate

iridium complexed with cyclometalated 2-phenylpyridine and the 5-(5-(4-cyanophen-1-yl)pyrid-2-yl)tetrazolate ligand

The availability of the probes:

Prof. Sally Plush, University of South Australia
Prof. Max Massi, Curtin University, Perth, Australia

Bader CA, et al. DOI: 10.1002/1873-3468.12365
Sorvina A, et al. DOI: 10.1038/s41598-018-24672-w
FL a CL imaging of endoplasmic reticulum

Cryo CL-SEM

Holographic microscope 3D Cell Explorer (Nanolive)

Cryo-cathodoluminescence cryoSEM

Intact cell

Freeze-fractured cell

Fluorescence

SE

z-slice RI signal

20 μm

10 μm
Elemental Mapping
Energy Dispersive X-Ray Spectroscopy

Massive Accumulation of Strontium and Barium in Diplonemid Protists

Jana Pišťová, Daria Tashyrevá, Jiří Týč, Marie Vancová, Syed Nadeem Hussain Bokhari, Radim Skoupý, Mariana Klementová, Hendrik Küpper, Peter Možíš, Julius Lukes

strontium sulfate (SrSO₄)
chemical mapping, differentiate between modifications and conformations of the same molecule, hyperspectral imaging, staining not needed
(A) Schematics showing the nitrogen-doped multiwall CNTs device encapsulated in polydimethylsiloxane used to enrich viruses (Top Left). The viruses are enriched between CNTs where the Au nanoparticles are predeposited. Raman spectra are then collected from the virus-enriched samples (Top Right). A scanning electron microscope image (Bottom Left) of a sample shows CNTs, Au nanoparticles, and trapped viruses (purple colored). Raman spectra from different virus samples are shown (Bottom Right) (FLUB in red, FLUA H1N1 in green, and FLUA H3N2 in blue).
Leukocyte subpopulations can be identified and differentiated via Raman spectroscopy. Further Raman spectroscopy detected immune cell activation and apoptosis.

Fig. 4 Comparison of Raman spectra of leukocytes and SKBR-3 cells. The presented spectra are averaged over the measured cells shown in Fig. 3, normalized to one and smoothed using a Savitzky–Goaly 7-point filter. Vertical lines are drawn to highlight Raman bands showing specificity for different cell types and their corresponding Raman frequency values are indicated. The 679, 727, and 781 cm⁻¹ bands are pronounced for lymphocytes, while SKBR-3 cells show clear peaks at 743 and 1157 cm⁻¹.
Raman imaging

Chemical information can be acquired with a resolution down to 300 nm
Detection Limits: ≥1 wt%; Depth Resolution: Confocal mode 1 – 5 µm

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:15</td>
<td>1</td>
</tr>
<tr>
<td>3:00</td>
<td>1</td>
</tr>
<tr>
<td>6:00</td>
<td>1</td>
</tr>
<tr>
<td>9:00</td>
<td>1</td>
</tr>
<tr>
<td>12:15</td>
<td>2</td>
</tr>
<tr>
<td>13:00</td>
<td>4</td>
</tr>
<tr>
<td>16:00</td>
<td>cell division</td>
</tr>
<tr>
<td>19:45</td>
<td>1</td>
</tr>
</tbody>
</table>

Lipid
Starch
PolyP

Figure 7. Raman maps showing the distribution of lipid droplets (red), starch bodies (green) and polyP granules (blue) in the same two innermost cells of eight-celled coenobia as presented in Figure 1C. The time counted from the beginning of the cell cycle and the number of nuclei determined by DAPI staining of the culture are indicated in the top row. For a given biomolecule, the color scale is the same for all maps. The white bars correspond to 2 µm.

Cells 2021, 10(1), 62; https://doi.org/10.3390/cells10010062

SEM further provides structural information with high spatial resolution
Freeze fracture -160°C
Freeze etching -98°C
Pt/Pd 30 sec

Hemistasia-clade
genera
Diplonema
biflagellated
unicellular protist

Galina Prokopchuk
Jiří Vaněček
• Laboratory of Electron Microscopy
• Biology Centre CAS, České Budějovice