Ultrastructural analysis of biological and bio-composite samples by electron microscopy

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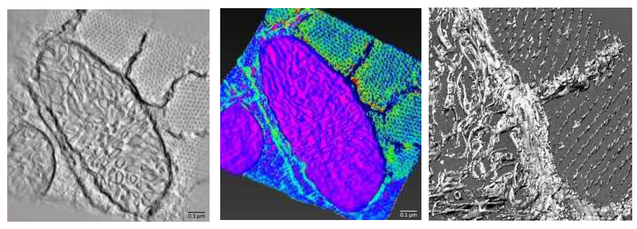
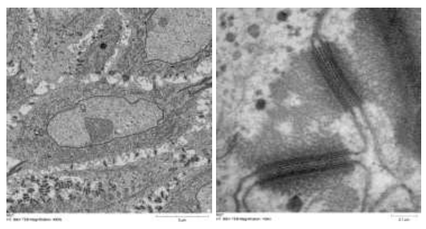
Electron microscopy (EM) is a powerful imaging tool for analysis of composition, ultrastructure and function of biological samples and bio-composite materials. From tissues and cells to subcellular structures and macromolecular complexes, Transmission and Scanning electron microscopy (TEM and SEM) provides 2D and 3D morphological analysis with optional detection and localization of specific antigens by immuno-gold labelling. As a core technology platform open to A*STAR institutes, Academia and industry, the IMB-IMCB Joint Electron Microscopy Suite provides instrumentation and expertise in use of ultrastructural imaging and complex services from sample processing to imaging, data analysis and training. Equipped with scanning and transmission electron microscopes and outstanding cryo-EM sample preparation equipment, our team collaborates on development and fine-tuning of technical approaches to support challenging imaging projects.

How to start ? ***What we support ?***

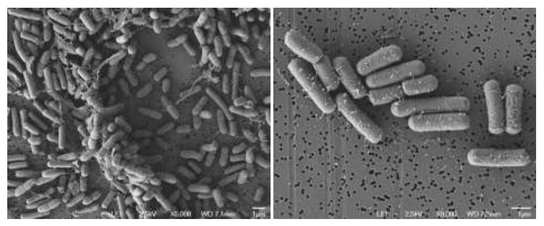
Objectives of analysis → Method selection for processing & imaging → Sampling and QC → Sample processing [RT/cryo] → Ultra-thin sectioning [TEM only] → Immunolabeling [optional] → TEM/SEM imaging [2D or 3D] → Data processing and analysis



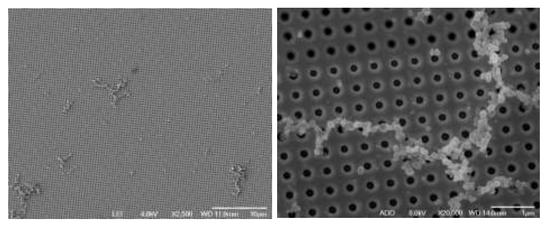
3D-reconstruction of Mitochondria cristae (EM tomography)



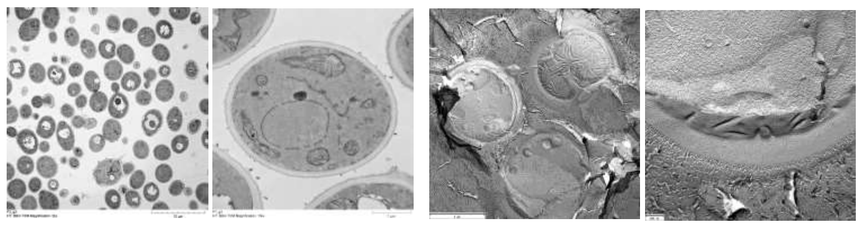
Keratinocyte desmosomes (TEM)



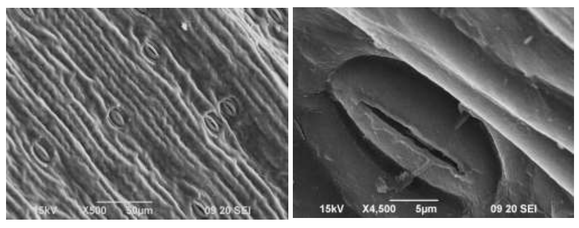
Airborne bacteria (critical-point drying, SEM)



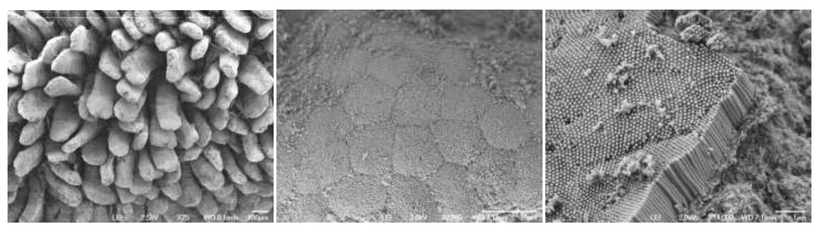
Nanoparticles on porous silicon chip (SEM)



Yeast cells wall by TEM: ultra-thin sections (left), freeze-fracture replica (right)



Plant leaf surface and stomata (freeze-drying, SEM)



Mouse intestine villi and microvilli (critical-point drying, SEM)

Aim?

Internal 2D/3D ultrastructure and composition and/or antigen detection and localisation

Superior ultrastructure and immunoreactivity preservation and/or ultra-high resolution

3D Surface morphology

Sample processing and imaging at IMB-IMCB Joint EM suite

RT-processing

Unfixed sample

Chemical fixation (mild) → Gelatin embedding → Cryo-protection → Snap freezing (LN2) → Cryo-sectioning (ultra-thin) → Immunolabeling [optional] → Grid embedding (MC film)

Unfixed sample → Immunolabeling [optional] → Negative staining

Chemical fixation → Post-fixation → Dehydration (Ethanol series) → Resin embedding (RT) → Sectioning (semi-thin) → ROI localization (Light microscope) → Section contrasting

Resin embedding (RT) → Sectioning (ultra-thin) → Immunolabeling [optional] → Section contrasting

Cryo-processing

High pressure freezing (*Sample > 0.5 µm*) → Automated freeze substitution → Resin embedding (Low Temp) → Sectioning (ultra-thin) → Immunolabeling [optional] → Section contrasting

High pressure freezing (*Sample > 0.5 µm*) → Freeze fracturing → Etching & metal shadowing → Carbon replica

Plunge freezing (*Sample < 0.5 µm*) → Cryo-sectioning (ultra-thin)

Plunge freezing → Freeze drying (under vacuum)

RT-processing

Chemical fixation → Post-fixation → Dehydration (Ethanol series) → Critical point drying or HMDS processing → Sputter coating (conductive metal)

Unfixed sample → Sputter coating (conductive metal)

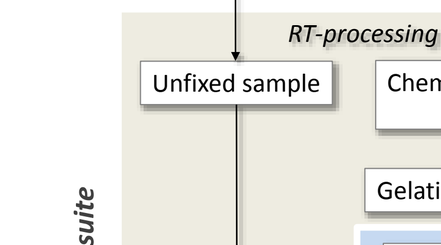
TEM (RT) → EM Tomography

TEM (RT) → CLEM ← Light Microscopy

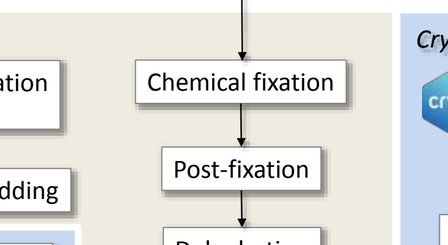
Cryo-TEM

SEM (RT)

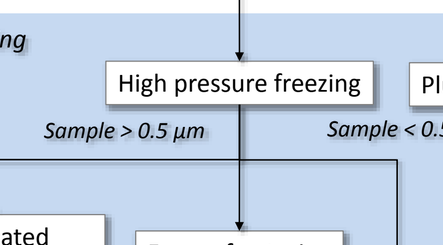
EM imaging



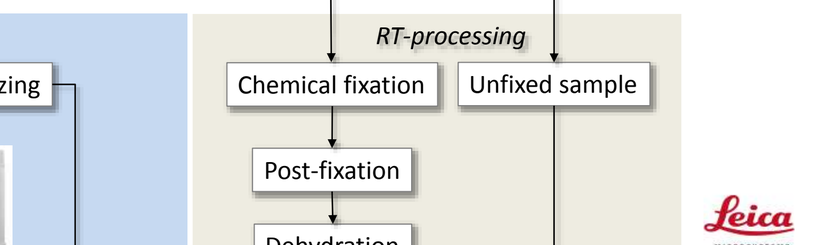
Purified exosome fraction (Negative staining, TEM)



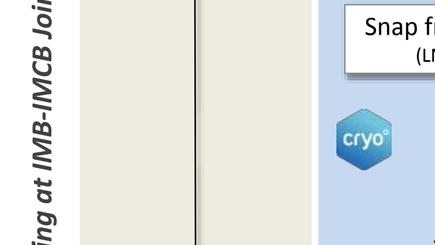
Iron oxide nanoparticles (15 nm) (Plunge-freezing and cryo-TEM)



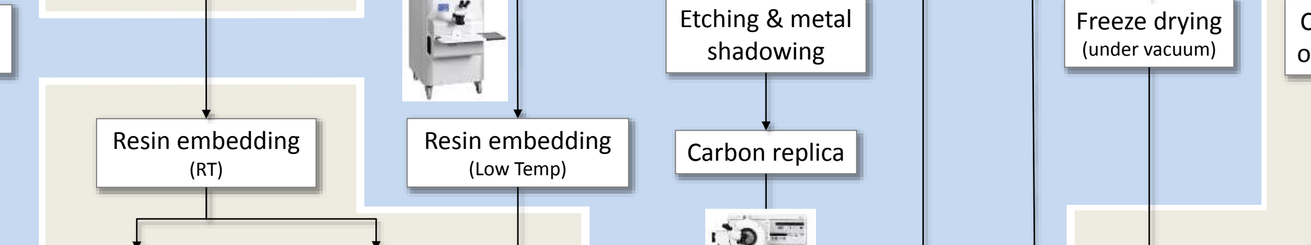
Caulobacter membrane ultrastructure (plunge-freezing, cryo-TEM)



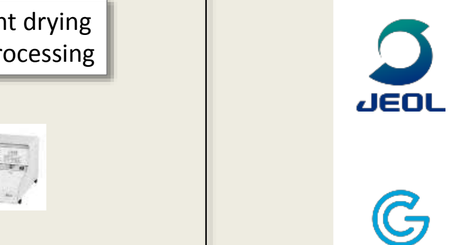
Xenopus skin and cilia ultrastructure (SEM)



Cardiac muscle sarcomere (ultra-thin sections, TEM)



Localization and ultrastructure of inter-cellular junction (single cell Correlative Light-Electron Microscopy, CLEM)



Hela cell Golgi complex ultrastructure (Thawed cryo-sections for immuno-EM)

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