

Cryogenic Scanning Electron Microscopy—Introduction

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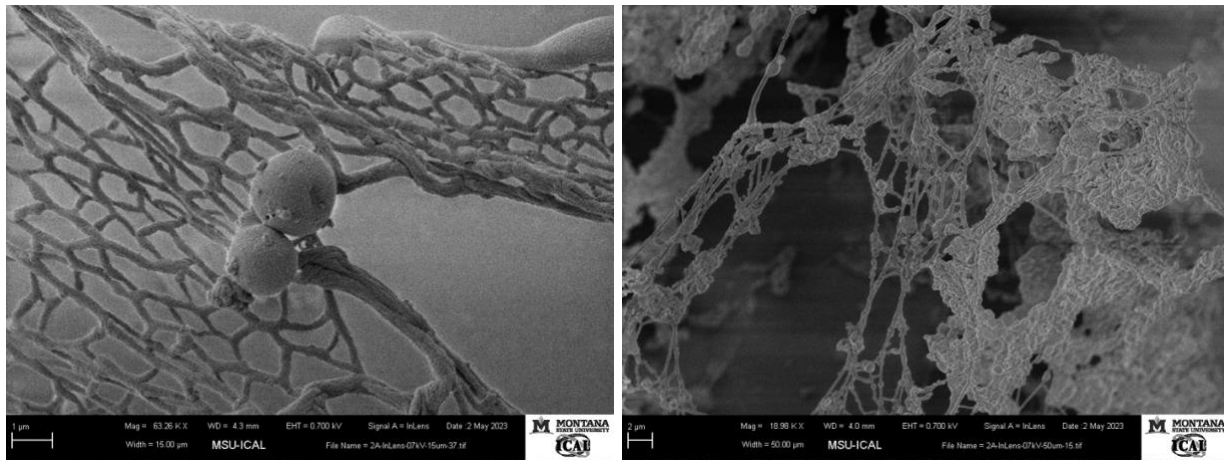
Cryogenic Scanning Electron Microscopy (Cryo-SEM) extends the capability of conventional SEM imaging and analysis to hydrated biological samples, biofilms, polymers, food products, pharmaceuticals, ice structures and other materials that may be sensitive to damage under the electron beam. Using Cryo-SEM, samples are flash frozen by immersion in liquid nitrogen (LN₂).

- Cryo-SEM avoids issues related to changes of material morphology as a function of sample preparation methods (e.g., desiccation from air drying, glutaraldehyde treatments, ethanol dehydration, critical point drying; see examples from Fratesi, S.E., Lynch, F.L., Kirkland, B.L. and Brown, L.R., 2004. Effects of SEM preparation techniques on the appearance of bacteria and biofilms in the Carter Sandstone. *Journal of Sedimentary Research*, 74(6), pp.858-867).
- Use of a low beam voltage (1-2 keV) provides for high-resolution imaging and also decreases charging problems for insulating samples.
- Low temperature sublimation is usually needed to remove ice crystals that may have formed on the sample surface.
- Samples may be purposefully fractured after freezing to reveal delicate interior cross sections and features such as cellular structure.
- EDS elemental analysis can be done (if the sample can withstand >15keV beam voltage). Freezing minimizes the potential for loss of material due to volatilization or diffusion. Elemental maps, e.g., for plant tissues, can be quite useful and revealing.
- A novel use of Cryo-SEM allows use of EBSD to analyze crystallographic orientation of ice crystals from ice core recovered by deep drilling of glaciers.
- Cryo-SEM provides an important complement to other micro-imaging methods such as confocal microscopy (see resources at the Center for Biofilm Engineering Bio-Imaging and Analytical Core Labs, <https://biofilm.montana.edu/biofilm-labs/index.html>) and Transmission Electron Microscopy (see Cryo-EM facility, <https://www.montana.edu/cryo-electron-microscopy/>).

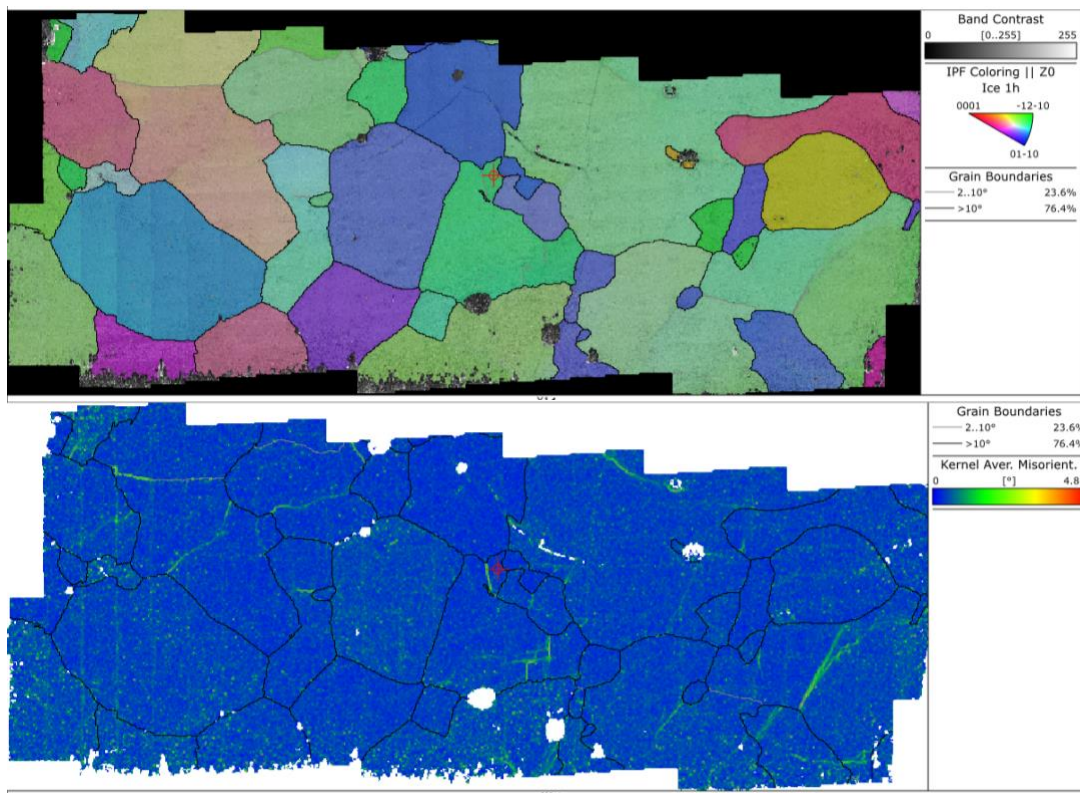
Excellent training in Cryo-Electron Microscopy can be found at MYSCOPE Microscope Training from Microscopy Australia with modules on theory, sample preparation, instrument operation, microanalysis and artefacts: https://myscope.training/CRYO_Introducing_cryo_SEM

Refer to the attached Standard Operating Procedures for Cryo-SEM use of the ICAL Zeiss Supra Field Emission SEM. ICAL staff will need to schedule dedicated time on this instrument as there is significant effort needed to set up the instrumentation, LN₂ must be ordered and available, and there is extended time needed to cool down and warm up the sample chamber prior to and after the experiment.

Examples of Cryo-SEM Imaging and Analysis



Cryo-SEM images of biofilms formed on microbially altered pyrite (photo: D. Mogk, Eric Boyd lab)



EBSD grain map (top) and dislocation map (bottom) of ice samples from ice core provided by COLDEX project Oregon State University

References: These references provide good examples of numerous applications of Cryo-SEM:

Plant Tissues

Wightman, R., 2022. An overview of cryo-scanning electron microscopy techniques for plant imaging. *Plants*, 11(9), p.1113.

Energy Materials

Ren, X.C., Zhang, X.Q., Xu, R., Huang, J.Q. and Zhang, Q., 2020. Analyzing energy materials by cryogenic electron microscopy. *Advanced Materials*, 32(24), p.1908293.

Zhang, Z., Cui, Y., Vila, R., Li, Y., Zhang, W., Zhou, W., Chiu, W. and Cui, Y., 2021. Cryogenic electron microscopy for energy materials. *Accounts of Chemical Research*, 54(18), pp.3505-3517.

Food Products

Ong, L., Dagastine, R.R., Kentish, S.E. and Gras, S.L., 2011. Microstructure of milk gel and cheese curd observed using cryo scanning electron microscopy and confocal microscopy. *LWT-Food Science and Technology*, 44(5), pp.1291-1302.

Hickey, C.D., Sheehan, J.J., Wilkinson, M.G. and Auty, M.A., 2015. Growth and location of bacterial colonies within dairy foods using microscopy techniques: a review. *Frontiers in microbiology*, 6, p.99.

Hydrated Polymers and Microgels

Liang, J., Xiao, X., Chou, T.M. and Libera, M., 2021. Analytical cryo-scanning electron microscopy of hydrated polymers and microgels. *Accounts of Chemical Research*, 54(10), pp.2386-2396.

Microbial Sediments

DeFarge, C., Trichet, J., Jaunet, A.M., Robert, M., Tribble, J. and Sansone, F.J., 1996. Texture of microbial sediments revealed by cryo-scanning electron microscopy. *Journal of Sedimentary Research*, 66(5), pp.935-947.

Biofilms

Hrubanova, K., Nebesarova, J., Ruzicka, F. and Krzyzanek, V., 2018. The innovation of cryo-SEM freeze-fracturing methodology demonstrated on high pressure frozen biofilm. *Micron*, 110, pp.28-35.

Erlandsen, S.L., Ottenwaelter, C., Frethem, C. and Chen, Y., 2001. Cryo field emission scanning electron microscopy. *BioTechniques*, 31(2), pp.300-305.

Hassan, A.N., Frank, J.F. and Elsoda, M., 2003. Observation of bacterial exopolysaccharide in dairy products using cryo-scanning electron microscopy. *International Dairy Journal*, 13(9), pp.755-762.

Pharmaceuticals

Liang, J., Koo, B., Wu, Y., Manna, S., Noble, J.M., Patel, M., Park, J.H., Kozak, D., Wang, Y. and Zheng, J., 2022. Characterization of Complex Drug Formulations Using Cryogenic Scanning Electron Microscopy (Cryo-SEM). *Current Protocols*, 2(4), p.e406.

Rock Fractures

An, Q., Hong, C. and Wen, H., 2023. Fracture patterns of rocks observed under cryogenic conditions using cryo-scanning electron microscopy. *Processes*, 11(7), p.2038.