University of Nottingham
Nanoscale and Microscale Research Centre

Cryogenic materials characterisation

Cryogenic materials characterisation refers to the use of low temperatures to enable the measurement and observation of the physical and chemical properties of materials. These techniques are particularly useful for studying hydrated material, biological specimens or volatile samples to retain them in their native state. These species can be difficult to study using conventional characterisation techniques as they are sensitive to damage by vacuum conditions or exposure to an electron or ion beam. Cryogenic techniques can also be used for time series studies or to stabilise samples during imaging.

Capabilities

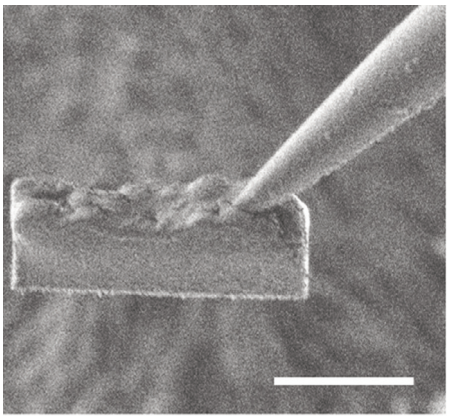
- Imaging and spectroscopy of frozen and soft matter materials
- Tomographic measurements of frozen and soft matter materials
- Cryo-focussed ion beam scanning electron microscopy (Cryo-FIB-SEM)
- Cryo lift-out of thin sections
- Detection, imaging or distribution mapping of volatile compounds
- Analysis of biomolecules and biological samples in their native state – maintaining hydrated state of samples such as hydrogels, tissues, biofilms

Typical applications

- Micro and nanoscale structural, compositional, and cross-sectional determination of biological and high-water content materials
- Sensitive trace element identification (ppm) and nanoscale chemical mapping
- Studies of frozen material using correlative techniques

Cryo-FIB-lift-out: practically impossible to practical reality

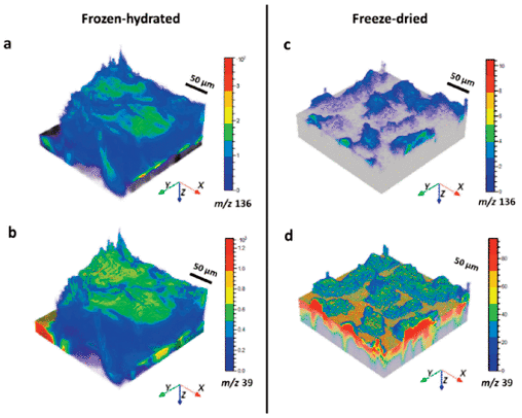
Cryo lift-out allows the preparation of lamella structures from a bulk sample by initially cutting through a sample using focussed ion beam (FIB) SEM and then using a cooled micromanipulator to secure the thin specimen. These lamellae can then be transferred for Cryo transmission electron microscopy imaging for further analysis. Shown is an overview of the Cryo lift-out procedure including; approach and positioning of the micromanipulator, lamella contact, attachment of the micromanipulator, milling of connection between lamella and the sample, and successful securement of the lamella to the tip.



C. D. Parmenter and Z. A. Nizamudeen. *Journal of Microscopy* 281 (2020), 157-174.

Cryo-OrbiSIMS for 3D molecular imaging of a bacterial biofilm in its native state

Cryogenic conditions allow analysis of bacterial biofilms in a native state and mapping of the chemistry for a highly hydrated sample in 3D. Samples composed mainly of water lose their 3D structure when drying. Shown below is a comparison of ToF-SIMS images of (a, b) the frozen hydrated biofilm and (c, d) freeze-dried biofilm. The samples were prepared using a Leica EM ICE high-pressure freezer and transported to the instrument using a Leica EM Vacuum Cryo Transfer system.



J. Zhang, J. Brown, D. J. Scurr, A. Bullen, K. MacLellan-Gibson, P. Williams, M. R. Alexander, K. R. Hardie, I. S. Gilmore, and P. D. Rakowska. *Analytical Chemistry* 92 (2020), 9008-9015.

Our facilities

Instrumentation	Sample preparation equipment
JEOL 2100F Transmission Electron Microscope (TEM) High resolution TEM with a direct detection electron camera, and EDS capabilities.	**Leica EM GP2 and Gatan CP3 Automatic Plunge Freezers** Plunge freezing of liquid or thin samples into liquid ethane with automatic blotting. **Gatan Cryo 626 single tilt holder** Conventional low temperature TEM imaging. **Gatan Cryo 914 high tilt holder** Low temperature transfer and tomographic TEM studies. **Gatan ELSA Cryo-transfer holder** Frost-free transfer into a TEM.
JEOL 2100+ TEM TEM with a high performance camera and EDS capabilities.	
Thermo Fisher (FEI) Tecnai G2 12 Biotwin TEM TEM suited towards biological samples.	
Thermo Fisher (FEI) Quanta2003D Dual Beam FIB-SEM Focussed ion beam SEM used for milling samples.	**Leica EM GP2 or Gatan CP3 Automatic Plunge Freezer** Plunge freezing of liquid or thin samples into liquid ethane with automatic blotting. **Leica EM ICE High pressure freezer** Immobilisation of aqueous samples at simultaneously low temperature and high pressure. **Quorum 3010 preparation system** Cryogenic preparation chamber allowing fracture, sublimation and coating (platinum) under vacuum. **Quorum 3010 Cryo rotate stage** Rotating stage maintaining sample at cryo-temperatures and permitting full range of movement in the FIB-SEM. **Linkam CMS196 v1 and v3 Cryo-Correlative Microscopy Stage** Cryogenic stage for light and fluorescence microscopy or confocal microscopy. Includes a transfer system for up to three frozen TEM grids, or three high pressure frozen 3mm planchettes to enable correlative light and electron microscopy (Cryo-CLEM). Can be used for larger samples, 6mm planchettes, 1cm coverslips, on request. Can be used to freeze samples in situ, if ultrafine structural preservation is not required. **Leica MM80 Metal Mirror Freezer** Suitable for vitreous freezing of (approx. 15um) thin preparation of gels, creams, pastes, tissue.
ZEISS Crossbeam 550 SEM Focussed ion beam SEM with EDS, EBSD, and STEM capabilities	
3D OrbiSIMS Label free large molecule identification.	**As above** **Leica EM VCT and VCM** Transfer of samples under cryogenic conditions.

Find out how cryogenic techniques could help with your applications, designs or solutions:
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