University of Nottingham
Nanoscale and Microscale Research Centre

nmRC Facilities

Facilities in this brochure:

- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)
- Raman spectroscopy
- X-ray photoelectron spectroscopy (XPS)
- Secondary ion mass spectrometry (ToF-SIMS & 3D OrbiSIMS)
- Atomic force microscopy (AFM)
- Nanofabrication suite
- Biophysical analysis
- Cryogenic materials characterisation
- Particle sizing suite

University of Nottingham
Nanoscale and Microscale Research Centre

Scanning electron microscopy (SEM)

Scanning electron microscopy uses a beam of electrons to characterise the topography and structure of a sample. High depth of field images with a 3D perspective and lateral resolutions down to 1-nm can be supplemented with simultaneous chemical and mechanical data.

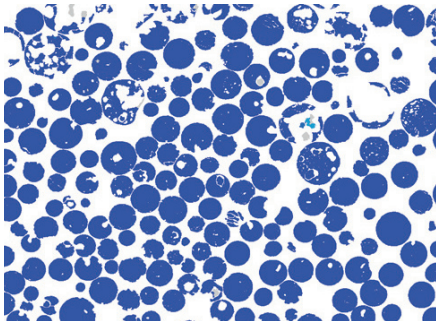
Variations of SEM allow for analysis options and sample manipulations that can accommodate a variety of sample types and formats.

Capabilities

- High magnification imaging of conducting and non-conducting materials
- Microscale topography and morphology analysis
- Elemental and compositional analysis
- Surface feature identification and measurement
- Sample sectioning, cryo-handling and micro-manipulation (FIB-SEM)
- Crystallographic analysis, micro-electron diffraction
- Wet, uncoated or dynamic environmental analysis (ESEM)

Typical applications

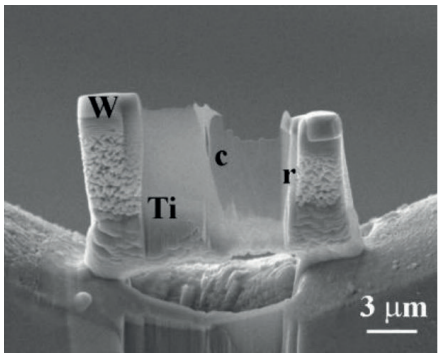
- Manufacturing analytics (stress and strain artefacts, wear and failure analysis and others)
- Micro- and nano-structural imaging and component identification
- Sample sectioning and high magnification full-structure visualisation
- Micro- and nano-particle/system sizing and localisation
- Grain sizing and qualitative/quantitative elemental mapping



Mineral mapping of compositionally uniform, magnetic microspheres

Mineral liberation analysis (MLA) enables automated large area analysis of sectioned samples in order to identify and quantify mineral distribution and composition. Image adjacent reveals high homogeneity for dense and porous, magnetic microspheres. A total of 1501 particles (99.7 wt%) were quantified and classified as $Ca_2Fe_2O_5$. Notably, this technique can be used to obtain a statistically significant set of data.

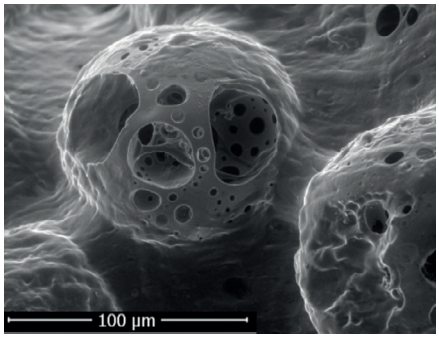
J Molinar Díaz, SA Samad, E Steer, N Neate, H Constantin, MT Islam, PD Brown, I Ahmed. *Materials Advances* (2020), DOI: 10.1039/D0MA00564A.



FIB sectioning of a biological Cryo-SEM sample

FIB-SEM manipulation allows delicate sample processing, such as cross sectional milling and 'lift-outs' which can in turn be imaged, or removed for analysis with further techniques (such as Raman spectroscopy, ToF-SIMS and TEM). This figure shows a lift-out section of a biomaterial interface between a Human Osteoblast (HOb) cell and a Titanium (Ti) foil. The lift-out section from a cryogenically frozen sample can be seen to contain a cell (c), resin (r), Ti and Tungsten (W) and is attached to a TEM support grid ready for further higher resolution analysis.

Microscopy at the life sciences/physical sciences interface. Paul D Brown, Hannah K Edwards and Mike W Fay. *Journal of Physics: Conference Series* 241 (2010), 012019



ESEM of phosphate-based porous microspheres interacting with mesenchymal cells

Environmental SEM (ESEM) was utilised to image phosphate-based glasses, porous microspheres (with Mg and Ti modifications, in this case M24T0) interacting with human mesenchymal cells. The technique allows the investigation of these materials in a hydrated or uncoated state. This is necessary to visualise the cellular interactions of the microspheres to assess growth and osteogenic potential. The image to the right shows cells migrating inside the pores of the microspheres in cell cultures on day 21.

MT Islam, L Macri-Pellizzeri, KMZ Hossain, V Sottile, I Ahmed. *Materials Science and Engineering: C* 120 (2021), 111668.

Our facilities

Zeiss Crossbeam 550 (HR-CAT-SEM): The high resolution cryogenic analytical and transfer (HR-CAT-SEM) instrument is a cryo-FIBSEM system with Field Emission Gun (FEG) optics that enables the highest levels of spatial resolution with cutting edge in-situ preservation and processing of a large variety of materials including biological, wet and magnetic samples. Extensive peripherals including a gas injection system (GIS), STEM detector and electron backscatter detector (EBSD) enable holistic interrogation of materials.

JEOL 7100F FEG-SEM: FEG-SEM for nanometre resolution imaging, with energy dispersive X-ray spectroscopy (EDS), wavelength dispersive X-ray spectroscopy (WDS) and electron backscatter diffraction (EBSD) capabilities in addition to a heating stage for exhaustive characterisation.

ThermoFisher (FEI) Quanta200 3D DualBeam FIB/SEM: FIB-SEM with cryogenic capability especially suited to biological samples and complex sample manipulations or lift out preparation.

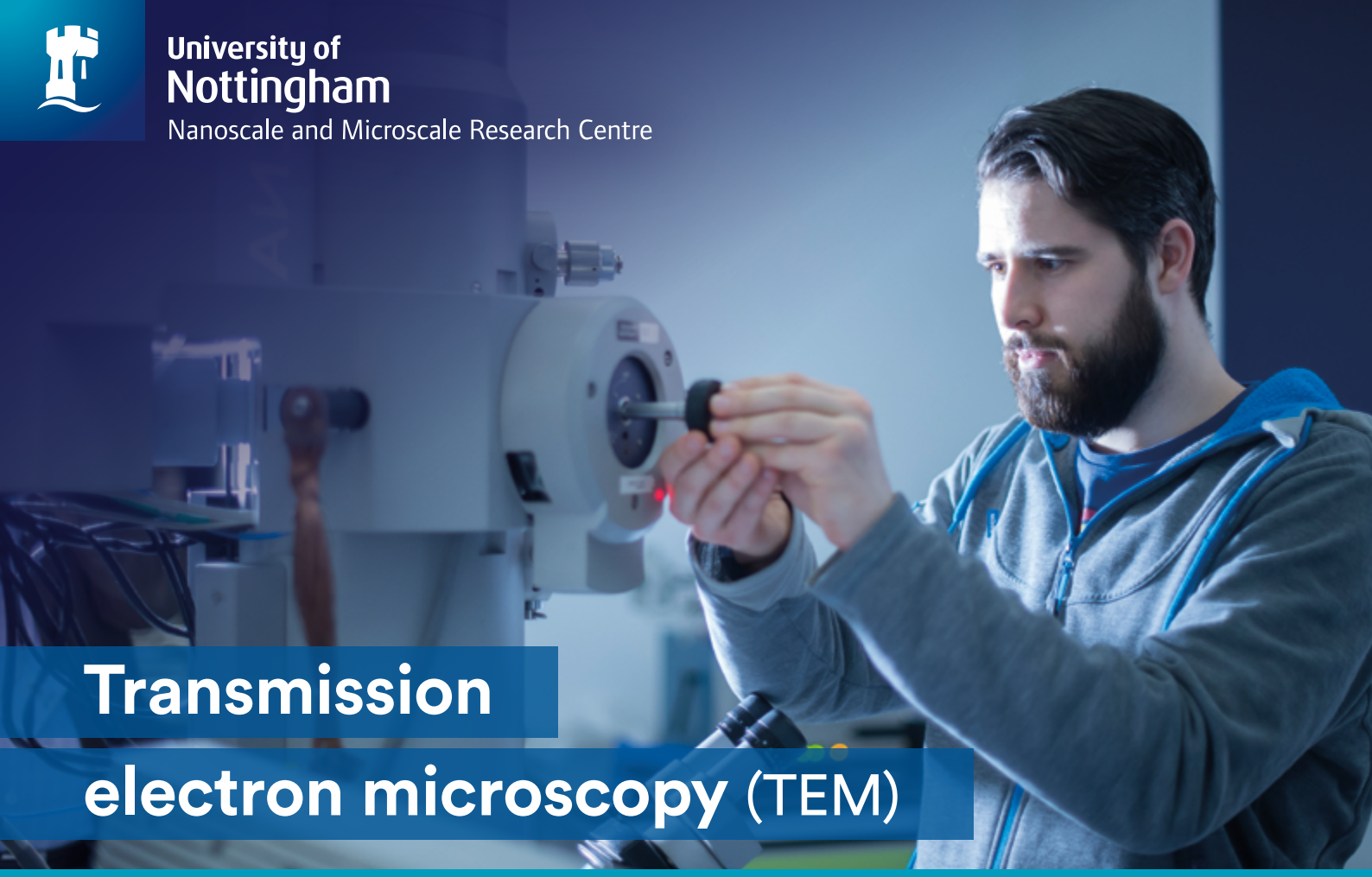
ThermoFisher (FEI) Quanta 650 ESEM: State of the art ESEM for the imaging of uncoated or wet samples in an air, water vapour or nitrogen environment with a high sensitivity EDS detector. A Peltier cooling stage allows for dynamic analysis with temperature and relative humidity control.

ThermoFisher (FEI) Quanta 600: Performs fully automated, large area and high-resolution analyses of polished sample specimens. Used to identify and quantify mineral composition and distribution (Mineral Liberation Analysis) with the provision of complex statistical analytics.

Also available... JEOL 7000F, ThermoFisher (FEI) XL30 SEM, JEOL JSM IT-200 SEM, JEOL 6490LV SEM.

Find out how SEM could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre



Transmission electron microscopy (TEM)

Transmission electron microscopy is an electron microscopy technique capable of imaging with a resolution down to an Ångstrom scale (~0.19 nm). It uses the spatial contrast generated by variations in electron transmission as they pass through specially prepared ultra-thin specimens to generate an image.

TEM can also provide advanced structural, crystallographic and chemical characterisation of samples on the nanoscale.

Capabilities

- Ultra-high magnification and resolution imaging.
- Micro- and nano-structural characterisation.
- Simultaneous elemental and compositional analysis.
- Thickness, pressure and process measurements.
- Nanotomograhy (3D profiling).

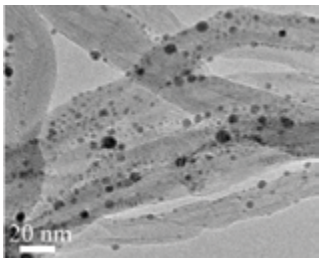
Typical applications

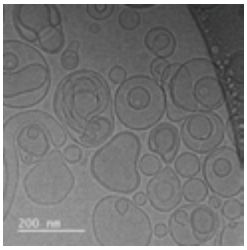
- Nanostructural analysis and component identification.
- Interrogation of coating, multi-phase alloy, fibre (and other) ultrastructures.
- Tissue and cellular imaging of with full-structure visualisation.
- Cryogenic visualisation of solution or suspension based nano-structures, such as liposomes.
- Small structure electron crystallography.

Imaging molecular assemblies

TEM offers nanoscale, high magnification imaging and chemical analysis. The smallest structural systems down to the atomic scale can be imaged and characterised in real time. Here silver nanoparticles can be visualised as having been successfully encapsulated in multi-walled carbon nanotube. Such capability ensures complex physicochemical processes to produce novel materials with bespoke characteristics can be validated for example size control, electron transport, heat transfer and others.

JA Watts, MW Fay, GA Rance, PD Brown, AN Khlobystovbc. *Carbon* 139 (2018), 538-544.





Electron transfer across biological membranes

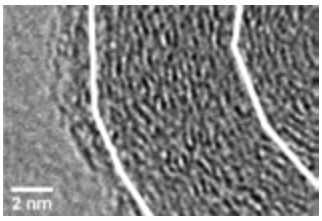
TEM was used to investigate the use of carbon nanotube porins (CNTPs) as wireless bipolar electrodes and artificial voltage-dependent anion-selective channels (switchable porins within the membrane) within biological systems. CNTPs were self-inserted into giant unilamellar vesicles and the fine-details of subsequent CNTP presence within the membrane was visualised with use of a cryogenic-TEM method.

JM Hicks, YC Yao, S Barber, N Neate, JA Watts, A Noy, FJ Rawson. *Small* 17 (2021), 2102517.

Quantifying soot nanostructures

TEM offers unique system diagnostics by combining molecular level structural and chemical information, such as particle morphology, size distribution, elemental presence/absence and others. One such example is soot-nanostructures. TEM analysis revealed the atomic structuring of the nano-sized soot-in-oil particulates and agglomerates from a gasoline direct injection deposit. This morphological information was used to evaluate image processing parameters, relevant for lattice fringe analysis, a common method to quantify soot nanostructures. White lines indicate the region of interest in this investigation.

SA Pfau, A La Rocca, MW Fay. *Combustion and Flame* 211 (2020), 430-444.



Our facilities

JEOL 2100F FEG-TEM

- Field emission electron gun (FEG) instrument, for use at 100kV and 200kV.
- A point resolution of 0.19nm.
- Bright field STEM detector.
- High angle annular dark field (HAADF) STEM detector.
- Gatan K3 IS 23.6 megapixel, electron counting direct detection (DDE) camera. Capable of 150 frames per second at full view, or >3500fps at 256×256 pixels.
- Gatan Tridiem Filter Spectrometer and 2K x 2K CCD camera, configured for use at 100kV and 200kV. Enables elemental mapping via electron energy loss spectroscopy (EELS) and energy filtered TEM (EFTEM).
- Oxford Instruments 80mm X-Max system for energy dispersive X-ray spectroscopy (EDS) analysis.
- Room temperature tomography: Gatan 916 room temperature tomography holder with up to 80 degrees tilt.
- Cryo-tomography and cryo transfer: Gatan 914 and Gatan Elsa Cryo-tomography holders including cold controller/cryo-workstation.
- Electrical holder. Gatan 936 DT analytical LN2 holder with temperature controller with EBIC stage option (four electrical connections) plus Smart EBIC.
- Gatan 4004 heating and gas exchange holder. Allows samples to be heated up to 800 °C, or air sensitive sample analysis.

JEOL 2100+ TEM

- LaB6 TEM for high throughput, high versatility analysis at 80kV or 160kV.
- Gatan OneView camera. High-resolution, 16-megapixel CMOS camera. Capable of 25 full frames per second or 300fps at 512×512 pixels.
- Bright field STEM detector.
- Oxford Instruments X-MaxN 80 TLE EDS detector.
- Gatan Enfinium EELS detector.
- HADDF detector.
- Range of specialised sample rods including heating and cryogenic stages.
- MEMS Heating holder. DENSsolutions Wildfire S3 capable of analyses up to 1300°C with millisecond heat and quench speed, and nanoscale sample drift with step changes of hundreds of degrees. Enables EELS and EDS mapping at elevated temperatures.

FEI Tecnai G2 12 Biotwin

- 120 kV LaB6 TEM for high contrast imaging.
- Gatan SIS Megaview IV digital camera.
- Tomography compustage for tomographic characterisation.
- Cryo-stage for low temperature observation of temperature dependent or hydrated samples.
- Ideal for low-contrast, beam-sensitive biological specimens, or other soft materials such as polymers.
- Gatan Orius (4k x 2.6k) Camera with digital streaming video for high-resolution and TV rate imaging.

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

nmrcenquiries@nottingham.ac.uk +44 (0)115 951 5046

nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre

Raman spectroscopy

Raman spectroscopy is a powerful vibrational spectroscopy technique that can operate in ambient and non-invasive conditions to achieve chemical identification and 2D/3D mapping.

It measures the inelastic scattering of laser light by a sample, to produce spectra of distinct Raman shifts that enable the assignment of sample chemistry and physical state.

Capabilities

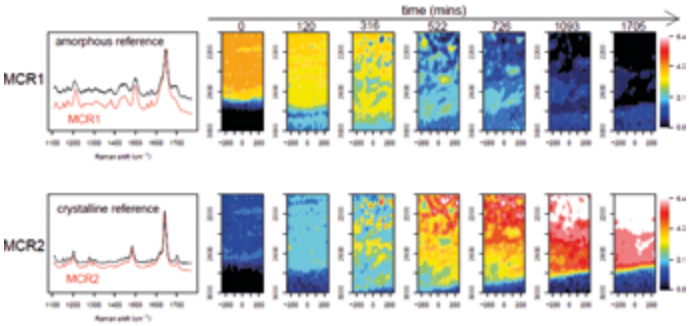
- Ambient chemical and physical state analysis.
- Real-time material transitions.
- Confocal analysis for 3D mapping.
- UV-Visible-IR excitation analysis.
- Temperature and time dependent chemical and physical state change identification.

Typical applications

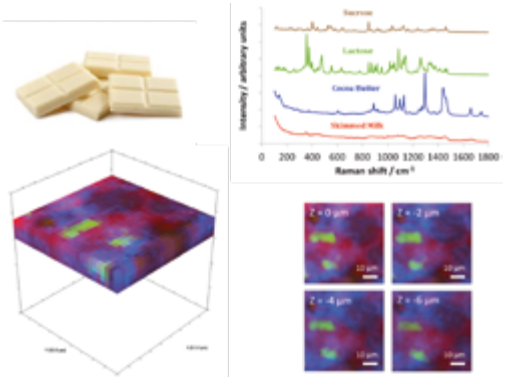
- Polymorph and ingredient analysis.
- Organic and inorganic material forensics.
- Microstructural composition (such as semiconductors, microparticles and others)
- Real time chemical event characterisation, for example swelling and dissolution.
- Mineralogical identification.

Real time 'in-situ' mapping of the re-crystallisation of a poorly soluble drug during dissolution

Raman has the capacity to perform real time, ambient, 'in-situ' analysis and mapping of the genesis or degradation of very subtle chemical signatures. One such example is of the amorphous or crystalline forms of the same drug. This figure details a re-crystallisation process over time by the intensity of the distinct Raman spectra of the two forms. As the amorphous signal decreases with time, the crystalline signal increases concurrently as seen by mapping. The capacity for such delicate distinction in ambient conditions makes Raman a powerful tool for chemical system analytics.



F Tres, K Treacher, J Booth, LP, Hughes, SAC Wren, JW Aylott, JC Burley. *Journal of Controlled Release* 188 (2014), 53-60.



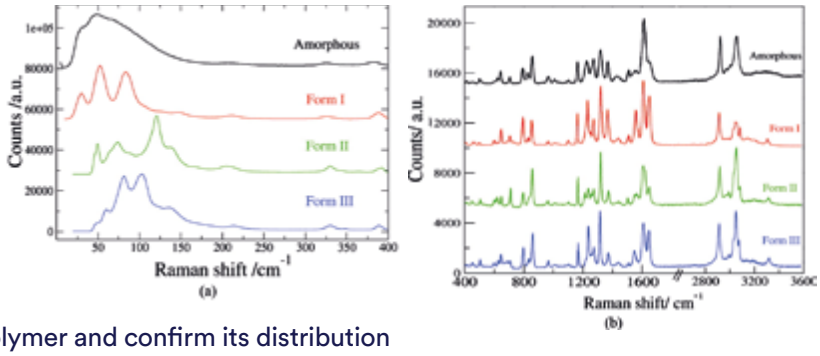
3D phase distribution of chocolate

Confocal Raman spectroscopy enables the characterisation of the local distribution of different sample components in 3D. This can be achieved with a z-resolution approaching the optical diffraction limit. The analysis can be performed at ambient conditions, without special sample preparation or doping of marker molecules. This figure shows the phase distribution of cocoa butter (blue), sucrose (brown) milk powder (red) and lactose (green) at and below the surface of a chocolate product. Such analysis is used to assess the homogeneity of dispersion of small domain-sized components that generate attractive textural properties.

Point spectra used with permission from Dr Qi He and Dr Bettina Wolf, School of Biosciences, University of Nottingham (2016).

Mapping the distribution of components of ionic liquids

Ionic liquids are being explored for a range of applications. They consist of a cationic and anionic component and exist as a liquid at room temperature and under vacuum. Here researchers used confocal Raman microscopy to map the spatial distribution of polymeric ionic liquids in a gel-polymer electrolyte. They map the intensity of ionic liquid functional group vibrational modes as a function of depth in the polymer and confirm its distribution throughout. 2-dimensional mapping also shows its distribution across the surface. The high sensitivity of the technique can be used as a diagnostic tool for testing new polymeric electrolyte membranes and methods of their manufacture, such as with 3D printing.



S Sen, SE Goodwin, PV Barbará, GA Rance, D Wales, JM Cameron, V Sans, M Mamlouk, K Scott, DA Walsh. *ACS Applied Polymer Materials* 3 (2021), 200–208.

Our facilities

Horiba LabRAM HR

- A powerful Raman spectrometer with four excitation wavelengths from UV through to Near Infra-Red (325nm, 532nm, 660nm, 785nm).
- Automated sample stage rastering for spectroscopic imaging.
- Olympus microscope stage with x10, x40, x50 and x100 objective lenses.
- Confocal capability allows z-axis sample slicing to produce 3D images.
- Thermal stage capabilities allow analysis of samples between -196 and 350˚C.
- SWIFT® and DuoScan® modes offer bespoke Raman analysis and/or mapping.
- Choice of gratings for standard or high-resolution spectroscopies.

Horiba XploRA INV Raman Microscope

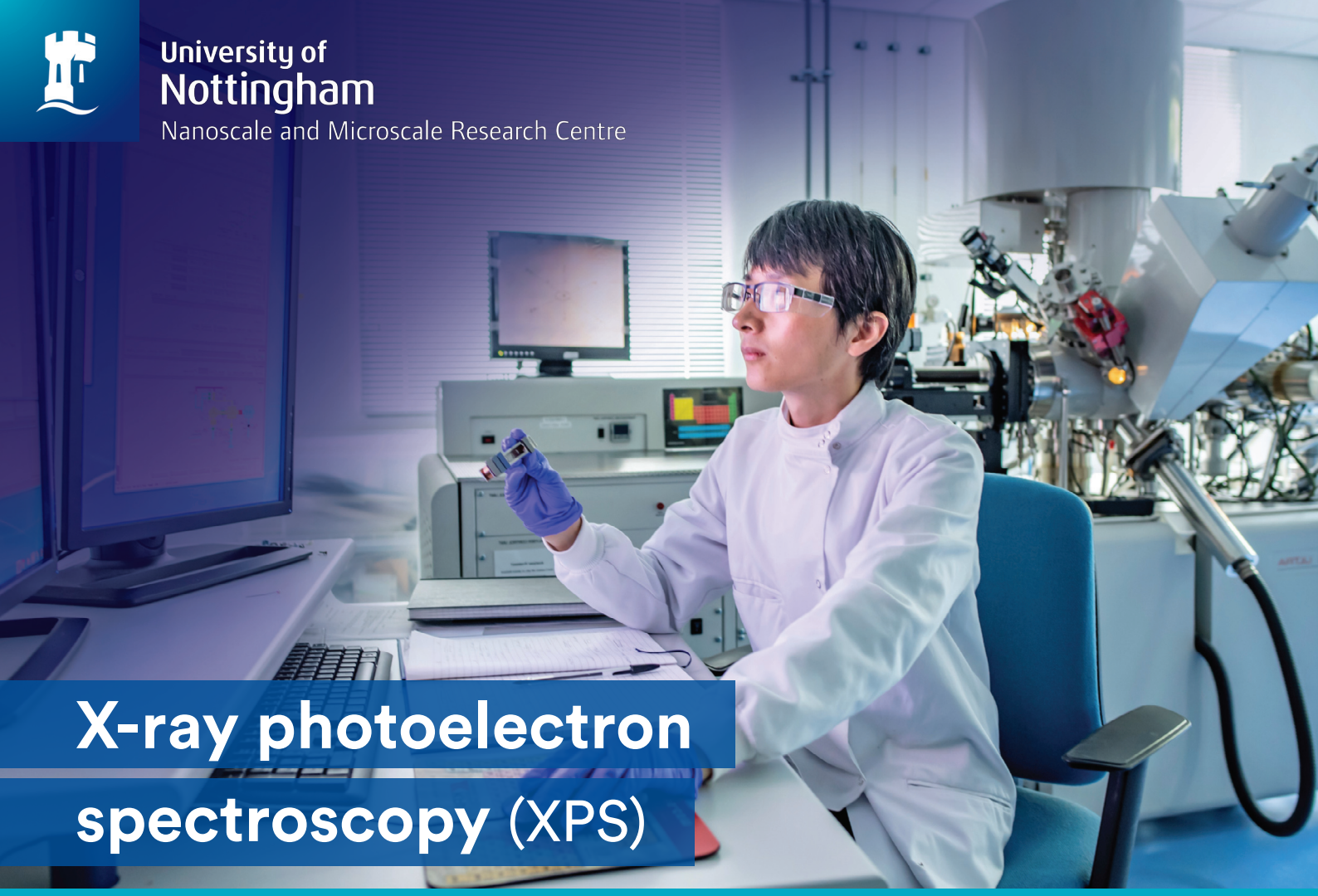
- Inverted microscope geometry, suitable for 'in-situ' analysis and biological samples.
- Excitation wavelength available: 785nm.
- Gratings available: 600, 1200, 1800 and 2400 lines/mm (depending on configuration.)
- Objectives available: 10x, 20x and 100x.

Find out how Raman spectroscopy could help with your applications, designs or solutions:

nmrcenquiries@nottingham.ac.uk +44 (0)115 951 5046

nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre



X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy provides a quantitative measurement of surface (5-10 nm) elemental composition and chemical state using X-ray stimulated photoelectron emission.

Characteristic electron binding energy 'fingerprints' for a sample can be produced, allowing determination of both atomic composition and subtle chemical state variations. Elemental imaging can be formed from XPS data, and depth profiling is a powerful adjunct to quantify progressive near-surface chemistry.

Capabilities

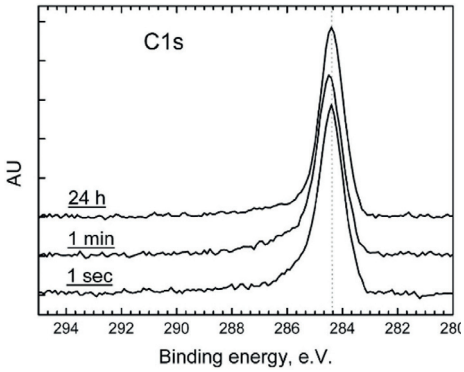
- Quantification of surface elemental composition
- Quantification of surface chemical state, such as Fe^{2+}/Fe^{3+} or carboxyl/alcohol
- Depth profiling using an Ar gas cluster ion source
- Elemental mapping (parallel XPS imaging)

Typical applications

- Contaminant identification and quantification (stains, corrosion, residues, phase separations)
- Oxidation state analysis
- Quantification of surface chemical modifications
- Thin film thickness (up to 15 nm) and composition quantification
- Catalyst surface characterisation

Quantification and qualification of self-assembled monolayer thickness

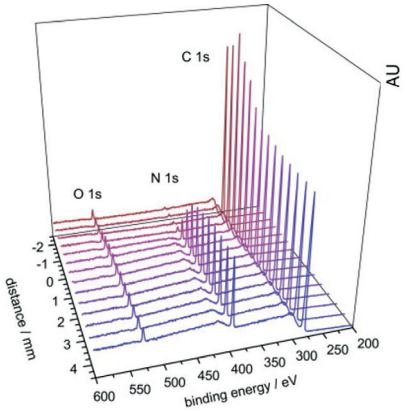
XPS can be used to qualify and quantify surface modifications. For example high resolution XPS carbon (C1s) region scans can be used to assess alkane thiol binding to gold (Au) surfaces when prepared at different adsorption times. The quality is assessed from the C1s region, which lacks any noticeable peaks related to oxygen-containing groups or any other possible contaminations. Au Layer thickness can also be quantified from the intensity of the C-C peak compared to the Au 4f core level (not shown).



Vladimir V Korolkov, Stephanie Allen, Clive J Roberts, and Saul JB Tendler. *Journal of Physical Chemistry* 115 (2011), 14899–14906.

Quantification of chemical gradients

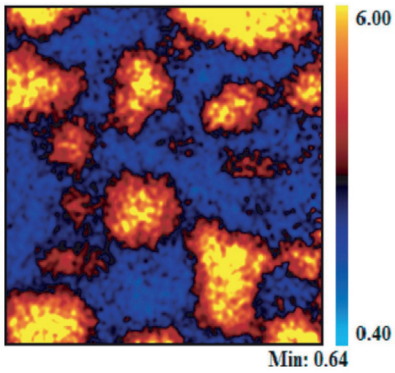
XPS can be used to provide characterisation of subtle differences in spatial chemical character. Here progressive (0.5mm increments) XPS widescans along a graded chemical interface are used to quantify the elemental concentration. This system is a thin hydrocarbon polymer deposited with a gradually changing thickness on a nitrogen containing polymer with uniform thickness. The gradient can be fully characterised from this information which allows the performance of this functional surface to be understood.



Mischa Zelzer, Ruby Majani, James W Bradley, Felicity RAJ Rose, Martyn C Davies, Morgan R Alexander. *Biomaterials* 29 (2008), 172–184.

XPS Imaging of the elemental corrosion in an optical filter

Spatially sequential XPS spectra can be processed into images to visualise elemental and chemical state distributions. This figure shows the corrosion of germanium to germanium oxide on an optical filter, as seen by XPS. An intensity map for germanium oxide (Ge 3d and O 2s) over a 400µm x 400µm area indicates significant variability incurred by moisture induced corrosion. The scale bar represents atomic %.



Emily F Smith, David Briggs, Neal Fairley. *Surface and Interface Analysis* 38 (2006), 69–75.

Our facilities

Kratos Liquid Phase Photoelectron Spectroscopy Machine (LiPPS)

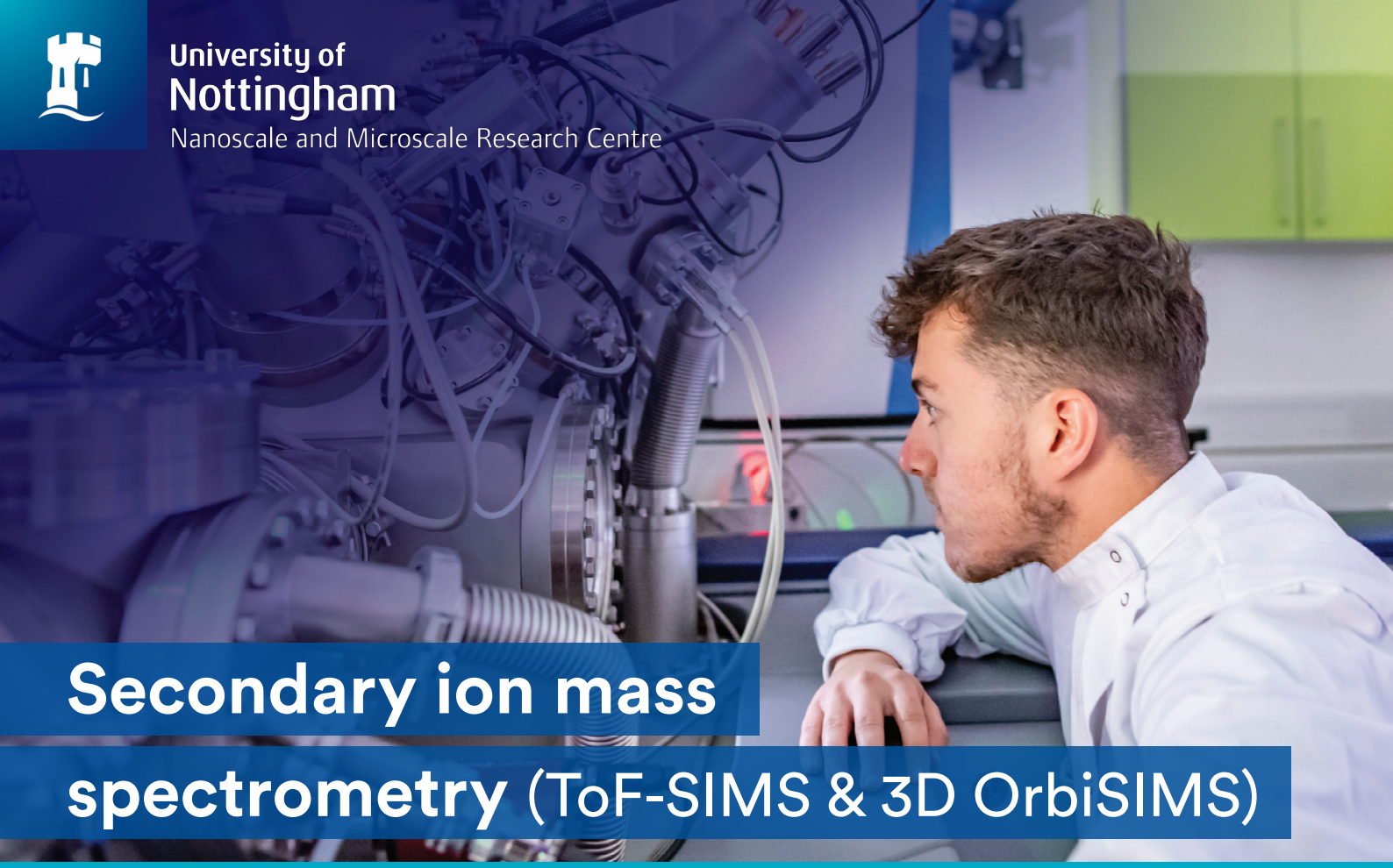
- Multiple monochromated X-ray sources including Al Kα emission at 1486.6 eV, Mg, or a high energy Ag source at 2984 eV.
- Argon gas cluster source for high resolution depth profiling of organic materials (biological samples and polymers).
- High throughput multi position programmable stage allows multiple samples in one experiment including a tilt stage for topographic samples.
- Magnetic immersion lens system allows the area of analysis to be defined by apertures.
- Electrostatic/magnetic lens system (hybrid lens) and a hemispherical analyser (CHA) to sort photoelectrons according to kinetic energy.
- Electron detection and counting with a triple channel plate and delay line detector (DLD).
- Heating and cooling capabilities.
- Electrochemistry stage for in situ work on ionic liquids.

Thermo Fisher K-Alpha Photoelectron Spectrometer

- Al Kα emission X-ray source at 1486.8 eV
- Ion gun with energy range of 200-4000 eV
- 180o double focussing hemispherical analyser with 128-channel detector
- Dual beam source charge compensation
- 4-axis sample stage, 60 × 60 mm sample area, 20 mm maximum sample thickness

Find out how XPS could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre



Secondary ion mass spectrometry (ToF-SIMS & 3D OrbiSIMS)

Secondary ion mass spectrometry is used to characterise the surface chemistry of a material. A beam of primary ions impacts the surface and liberates secondary ions from the sample which are then analysed in turn to produce mass spectra. The lateral distribution of chemical species (mapping) or their intensity with depth (depth profiling) can then be carried out.

The combination of a time-of-flight secondary ion mass spectrometer (ToF-SIMS) with hybrid OrbiTrap™ functionality provides an array of analytical options to provide 3D chemical analysis with exceptional surface sensitivity (1-3 nm), high mass and spatial resolutions.

Capabilities

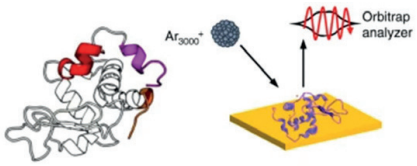
- Label-free large molecule identification (> m/z 1000)
- Sensitive trace element identification (ppm)
- Chemical mapping (down to a nm scale)
- Depth profiling of inorganics and organics

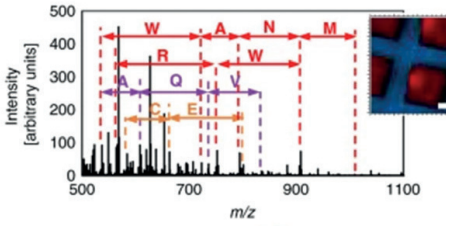
Typical applications

- Identification of unknown organic species in solids
- Contaminant identification and distribution
- Surface (such as coatings, films, deposits) composition and integrity
- 3D permeation assessment of active pharmaceutical ingredients
- Spatial resolution of chemical components.
- High throughput screening of polymers

In-situ protein identification for next generation biomaterials and tissue analysis

Using the ballistic fragmentation and high accuracy of the 3D OrbiSIMS, 16 undigested proteins were identified in-situ (in their native state). This was achieved without a chemical label, enzymatic digestion or use of a specific matrix. Using the ballistic approach the concentration of key proteins was tracked into human skin. The ability to directly measure proteins in their native state using a surface analysis technique has significant potential application in furthering the understanding of diseases and the development of new bio-materials.

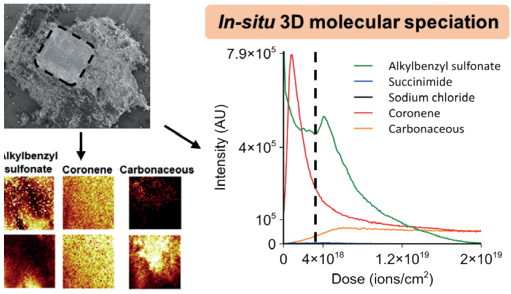




AM Kotowska, GF Trindade, PM Mendes et al. Protein identification by 3D OrbiSIMS to facilitate in situ imaging and depth profiling. *Nature Communications* 11 (2020), 5832. doi.org/10.1038/s41467-020-19445-x

3D insight into the molecular composition and formation of polluting engine deposits

Formation of deposits in internal combustion engines causes increased emissions and lower engine efficiency. Using the 3D OrbiSIMS technique it was possible to depth profile and image these complex layered materials and, coupled with sophisticated chemical filtering using molecular formula prediction for data processing, a comprehensive molecular characterisation of petroleum deposits was performed. Argon gas cluster depth profiles tracked the fate of molecules once deposited on the engine surface and unveiled plausible formation pathways of deposits for the first time. The combined insight into the composition, origin and formation of deposits will help mitigate the use of solubilising fuel additives and help reduce worldwide vehicle emissions.

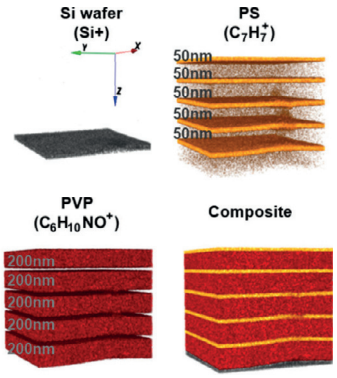


M Edney, J Lamb, M Spanu, E Smith, E Steer, E Wilmot, J Reid, J Barker, M Alexander, C Snape and D Scurr. ACS *Applied Materials and Interfaces* 12 (2020), 51026–51035.

MK Edney, AM Kotowska, M Spanu, GF Trindade, E Wilmot, J Reid, J Barker, JW Aylott, AG Shard, MR Alexander, CE Snape, DJ Scurr. *Analytical Chemistry* 94 (2022), 4703–4711.

3D ToF-SIMS imaging of polymer multi-layer films

ToF-SIMS imaging with argon cluster sputter depth profiling can provide a detailed insight into the three-dimensional chemical composition of organic material structures. 3D chemical images can provide information regarding the structure of multi-layer systems which can be used to inform future manufacturing and development. Outputs include sample layer chemistry, homogeneity, thickness and interface widths. Here we can see the analysis of spin-cast multi-layers comprising alternating polystyrene (PS) and polyvinylpyrrolidone (PVP) layers. The quality of the data allows a detailed analysis of the chemical structure of these systems, revealing minor imperfections within the polymer layers.



J Bailey, R Havelund, JS Sharp, AG Shard, I Gilmore, MR Alexander, DJ Scurr. ACS *Applied Matererials and Interfaces* 7 (2015), 2654–2659.

Our facilities

ION-TOF (GmBH) ToF SIMS V

- Liquid metal (Bin+n) ion gun (LMIG) for spectroscopy and imaging at a spatial resolution of ~ 200 nm.
- Argon gas cluster source for high-resolution depth. profiling of organic materials (polymers and biological samples) and 3D chemical characterisation.
- Mass sensitivity down to ppm (femtomole).
- A 5-axis multi-sample stage is fully automated and provides rotation for high-resolution (nm) depth profiling (Cs+ or Ar GCIB sources).
- Reflectron ToF mass analyser gives mass resolution > 13000 at m/z = 29.
- Chemical imaging of surface areas from the µm to cm scale.
- 3D elemental mapping possible.
- Sample size accommodation from a few mm up to ~ 10 cm.

Ion-TOF (GmbH) HybridSIMS

- ToF or OrbitrapTM analysis of organic and inorganic samples including petroleum deposits, biological materials (protein identification, skin, hair, leaves and others), polymers, semi-conductors, insulators, powders, foils and microarrays.
- High mass resolution spectrometry (>240,000 and 11,000 amu for the OrbiTrap and the ToF, respectively).
- High spatial resolution chemical imaging (<70 nm).
- Mass sensitivity down to ppm (femtomole).
- Gas cluster ion beam sputtering for controlled depth profile analysis of organics.
- Biosafety Level 2 (BSL2) preparation and analysis environment for cell/tissue analysis including cryogenic sample preparation facility, including high pressure freezing, freeze drying, cryo-ultramicrotomy and more.
- Chemical filtering and multivariate analysis software and expertise for complex chemometric data analysis.

Find out how ToF/3D OrbiSIMS could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre

Atomic force microscopy (AFM)

Atomic force microscopy is a high-resolution scanning probe technique that allows visualisation of material surfaces and their topographical features with nanometre resolution.

The interaction between a probe tip and the surface is measured via its deflection in response to surface properties. Variations of the technique allow you to calculate surface physicochemical and mechanical properties, measure interaction forces between material surfaces and map the thermal or electrical properties of a sample.

Capabilities

- Micro- and nano-structural visualisation.
- Texture analysis (roughness, topography, morphology).
- Surface forces quantification (adhesion, surface free energy and others).
- Surface mechanical properties assessment (hardness, Young's Modulus, friction and others).
- Mapping of different surface components and phases.
- Biomolecular interactions.

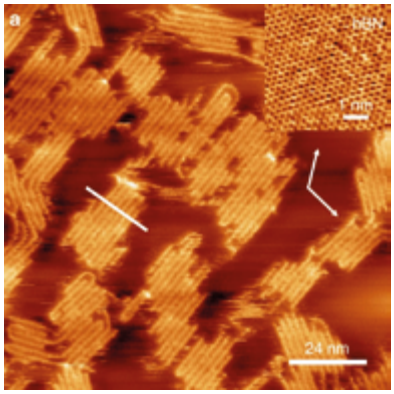
Typical applications

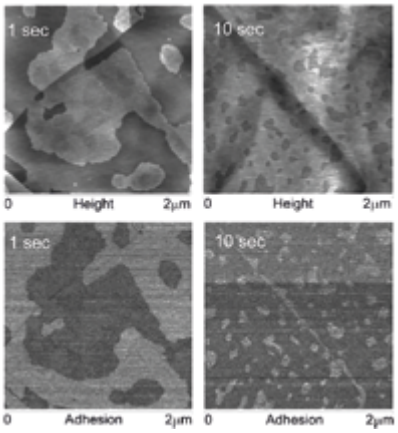
- Identification and localisation of phases in formulations.
- Material coating assessment.
- Visualisation of cell ultrastructure and molecular dynamics.
- Quantification of ligand-receptor interactions.
- Component integrity investigation.

High-resolution AFM images of polythiophene strands adsorbed on the surface of hBN

AFM can image material surfaces with nanometre resolution. In addition to nanostructures (such as peptides, nanoparticles and nanotubes) the technique can resolve molecular/atomic structures. This figure shows individual thiophene units and where a lattice of semicrystalline spin coated films of polythiophenes (PTs) may be resolved using AFM. Real-space images of polymers with sub-molecular resolution could provide valuable insights into the relationship between morphology and functionality of novel polymer based electronic devices.

Vladimir V Korolkov, A Summerfield, A Murphy et al. Ultra-high resolution imaging of thin films and single strands of polythiophene using atomic force microscopy. *Nature Communications* 10 (2019), 1537.





Topography and adhesion characterisation of self-assembled monolayer formation

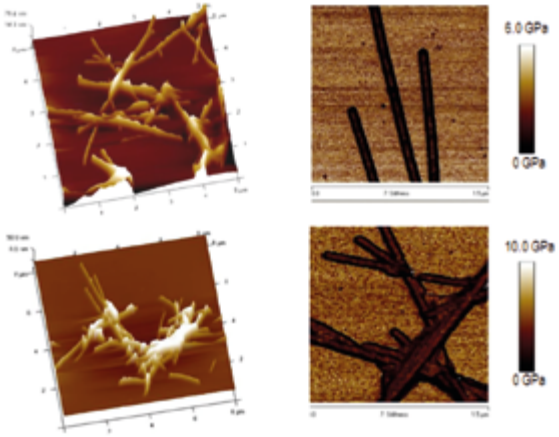
AFM allows the mapping of the adhesive character of a surface or its components. Adhesion forces can be used to calculate the interactivity of a surface and the data can be mapped to complement a topographical assessment. This figure shows height (upper row) and adhesion (lower row) AFM images (2 μm × 2 μm) detailing a real-time surface evolution due to the self-assembly of trimesic acid on a highly ordered pyrolytic graphite (HOPG) surface. The dark regions on the height and adhesion maps correspond to the areas with lower height and adhesion respectively.

Vladimir V Korolkov, Stephanie Allen, Clive J Roberts, and Saul JB Tendler. *Journal of Physical Chemistry C* 116 (2012), 11519–11525.

Mechanical mapping of peptide nanotubes

AFM can provide a mechanical assessment of a surface and its features. Here topography (3D) (left) and mechanical stiffness maps (right) are shown of nanotube structures which form in samples comprising 80% (top) and 60% (bottom) dinapthylalanine (di-Nal) peptides to diphenylalanine peptides (FF). This combination of data channels allows functional, structural, and mechanical analyses where systems with a uniform topography but different material phases can be distinguished.

Victoria L Sedman, Xinyong Chen, Stephanie Allen, Clive J Roberts and Saul JB Tendler. *Journal of Microscopy* 249 (2013), 165–172.



Our facilities

Dimension FastScan Bio™ (Bruker): Capable of scan speeds 100 times faster than traditional AFMs, this AFM offers high-resolution, live-sample observation of interacting molecules, membrane proteins, DNA-protein binding, inter-cellular signalling and other dynamic biological processes. Includes Bruker's PeakForce™ QNM™ (Quantitative Nanomechanical Measurement) capability.

Multimode® 8 Scanning Probe Microscope (Bruker): Nanoscale imaging capabilities supplemented by simultaneous high-resolution force mapping with Bruker's PeakForce™ QNM™.

EnviroScope™ AFM (Bruker): AFM analysis within an environmental chamber capable of precise temperature and humidity control.

Many more... Including Dimension® 3100 and 3000 AFMs (Bruker), ForceRobot® 300 (JPK Instruments), MFP-1D and MFP-3D™ (Asylum Research).

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

nmrcenquiries@nottingham.ac.uk +44 (0)115 951 5046

nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre

Nanofabrication suite

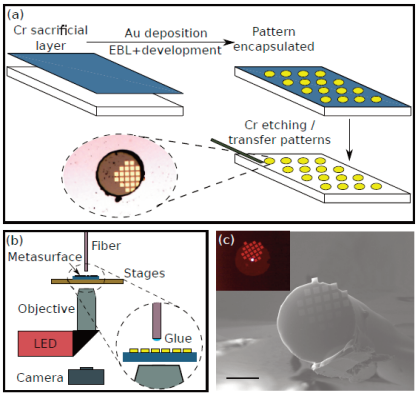
Nanofabrication is the process of creating nanometre sized features on a range of substrates. Electron beam lithography (EBL) can be used to create patterns as small as 40 nm. These patterns can then be transferred to a substrate via deposition of metals and/or dielectrics, or by selective etching of material. Our Nottingham Nanofabrication Suite can fabricate custom devices for a range of applications, and then validate them via a range of optical or thickness measurements such as ellipsometry.

Capabilities

- High resolution electron beam lithography (40 nm resolution)
- Photolithography (2 µm resolution)
- Thin film deposition of metals and dielectrics
- Wet or dry etching of substrates
- Thermal processing of materials
- Imaging ellipsometry
- Cell patterning

Typical applications

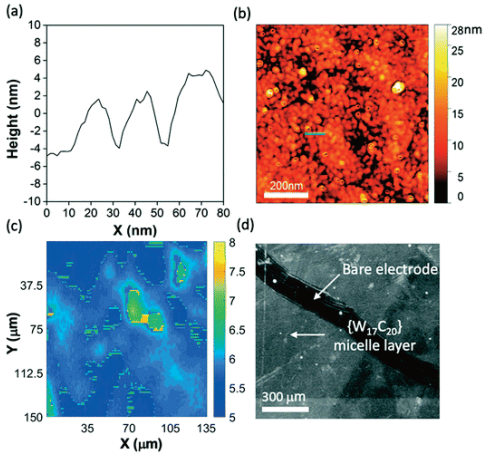
- Precise deposition of metals onto substrates such as glass
- Etching of substrates to create features such as gratings or waveguides
- Fabrication of bespoke designs for microfluidic chips
- Measurement of thickness and optical properties of 2D materials



Nanofabrication of ultrathin metallic metasurfaces for advanced imaging applications

Nanofabrication can produce precise nanostructures on a surface for a range of applications. Here, a three step procedure was performed to produce ultrathin metallic metasurfaces on an optical fibre surface. Electron beam lithography was used to pattern a chrome coated silicon substrate, photolithography to pattern a layer of resist material into discs (to encapsulate the metasurfaces), and wet etching to remove the encapsulated discs. These discs were then glued onto the tip of an optical fibre for use as endoscopic devices.

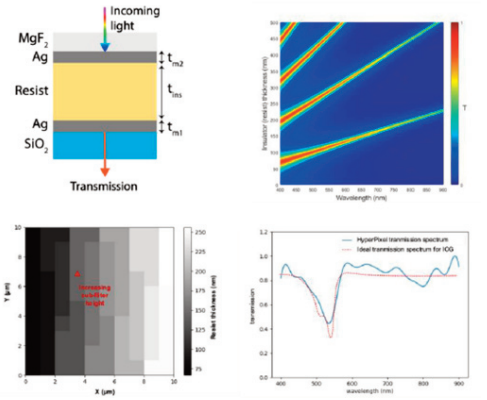
Rafael Fuentes-Dominguez, Fei He, Richard B. Cousins, Christopher J. Mellor, and George S. D. Gordon. Proceedings Volume 11953, *Optical Fibers and Sensors for Medical Diagnostics, Treatment and Environmental Applications XXII* (2022), 119530F.



Imaging ellipsometry for thickness measurements of self-assembled hybrid polyoxometalate nanostructures

Using imaging ellipsometry it was possible to determine the thickness and optical properties of monolayers formed on glassy carbon electrodes by self-assembly of hybrid polyoxometalate micelles. The combination of imaging ellipsometry with complementary techniques such as atomic force microscopy (AFM) and scanning electron microscopy (SEM) allowed the 6-8 nm monolayer thickness to be determined as well as full structural characterisation of the monolayers formed that have applications in electrocatalysis and sensing.

Sharad S. Amin, Jamie M. Cameron, Richard B. Cousins, James Wrigley, Letizia Liirò-Peluso, Victor Sans, Darren A. Walsh, and Graham N. Newton. *Inorganic Chemistry Frontiers* 9 (2022), 1777-1784.



Electron beam lithography for production of hyperpixel filter arrays

Electron beam lithography allows for the manufacture of unique materials that have tailored optical and electronic properties. Glass cover slips coated with a 22 nm silver layer were spin-coated with a greyscale polymer resist and then subjected to electron beam lithography to produce pixels of 0.5 – 10 µm size. The height of the pixels was also varied by controlling the electron dose. Once coated with a further silver layer this produced a filter array capable of customised spectral transmission properties.

Michaela Taylor-Williams, Richard B. Cousins, Calum Williams, Sarah E. Bohndiek, Christopher J. Mellor, and George S. D. Gordon. *Proceedings Volume 11954, Optical Biopsy XX: Toward Real-Time Spectroscopic Imaging and Diagnosis* (2022), 1195406.

Our facilities

Class 5 cleanrooms allowing for fabrication of a range of devices without the risk of contamination

Nanobeam nB5 instrument utilising an 80 kV electron beam with variable current allowing not only quick write speeds but also high resolution. Substrates from 5 to 76 mm can be used.

MJB3 and MA-6 Gen 3 mask aligners for repeated designs.

Corail 200II plasma etcher capable of reactive ion etching (RIE) and inductively coupled plasma (ICP).

Chlorinated and fluorinated gas chemistries allow for etching of Si, SiO2, GaAs, Al, photoresist and many more materials.

Range of deposition tools allowing for deposition of metals and dielectrics

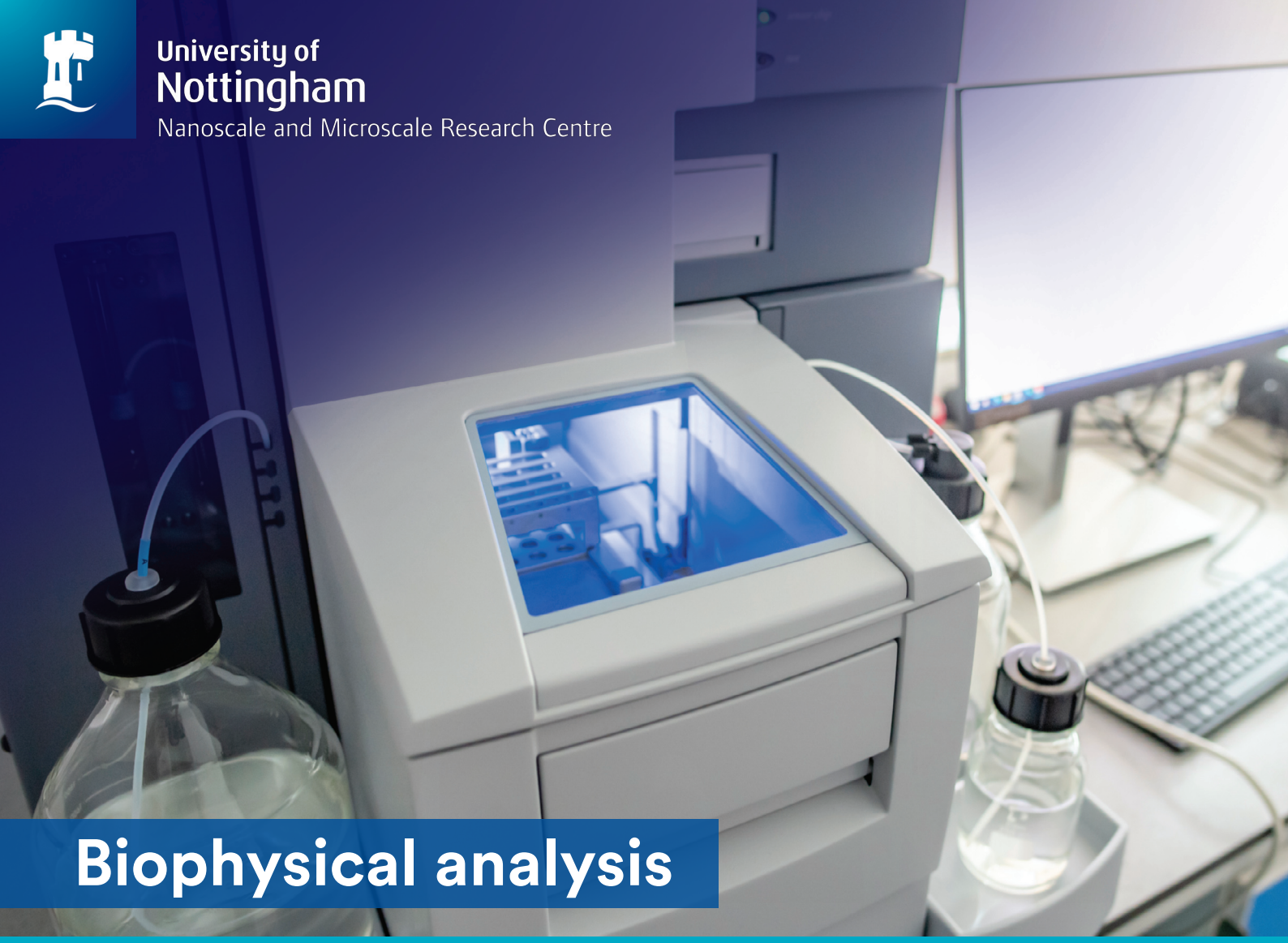
Accurion EP4 imaging ellipsometer capable of measuring optical properties and thickness of thin films with a lateral resolution of <5 µm.

Woollam M2000 variable angle spectroscopic ellipsometer (VASE) capable of making optical measurements between wavelengths of 190-1700 nm.

Alvéole Primo system attached to a **Leica DCiM inverted microscope** for cell patterning.

Find out how nanofabrication could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc or nottingham.ac.uk/nanofabrication

University of Nottingham
Nanoscale and Microscale Research Centre



Biophysical analysis

Biophysical analysis encompasses a range of techniques including Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR). These techniques study the binding interactions of a range of biomolecules such as proteins, peptides, nucleic acids (DNA, RNA), carbohydrates, lipids as well as interactions with small molecules, polymers, ions, or drug compounds. They also obtain data on the affinity, kinetics, stoichiometry, and thermodynamics of such interactions.

Capabilities

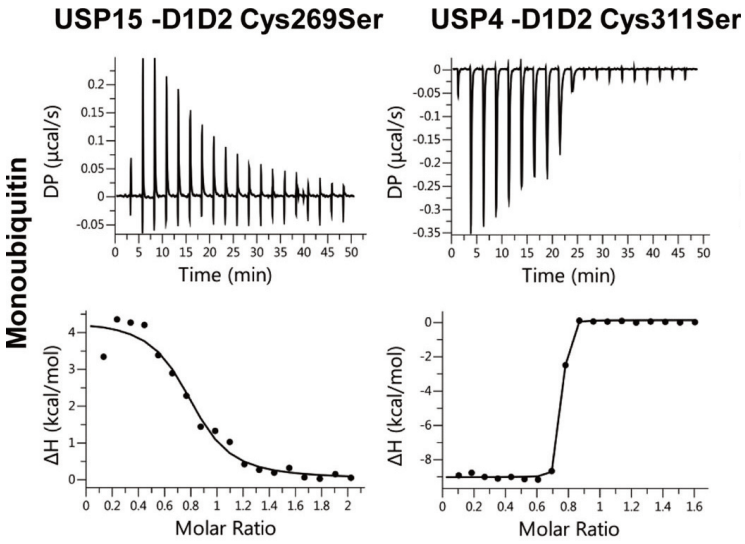
- Determination of thermodynamic parameters including binding affinity and stoichiometry
- Measurement of enzyme kinetics
- Molecular variant comparisons, e.g., mutant versus wild types
- Protein activity and stability analysis
- Epitope mapping

Typical applications

- Pharmaceutical drug discovery
- Identification of binding partners to targets
- Quality control for pharmaceuticals
- Detect and characterise molecular interactions

Enzyme-protein substrate and product interaction probed using MicroCal PEAQ ITC

ITC can enhance our understanding of the specificity of enzymes for substrates and thus was used to measure the interaction of USP15 and USP4 mutants with monoubiquitin (product) and linear diubiquitin (substrate) revealing insights on the interaction mechanism. Significant differences were observed in their thermodynamic isothermal profiles: an endothermic process was observed for the binding of USP15 mutant to both monoubiquitin and diubiquitin whereas an exothermic process was observed for USP4 mutant binding to the two proteins at 25 °C. The affinity of USP15 mutant for monoubiquitin was determined to be lower than that of USP4 mutant indicating that product inhibition plays a larger role for USP4 than USP15. Both mutant proteases had similar affinity for diubiquitin, but the interaction was associated with different enthalpy and entropy parameters.



Stephanie J. Ward, Hayley E. Gratton, Peni Indrayudha, Camille Michavila, Rishov Mukhopadhyay, Sigrun K. Maurer, Simon G. Caulton, Jonas Emsley, and Ingrid Dreveny. J. Biol. Chem. (2018) 293(45) 17362–17374.

SPR theory and setup

Surface Plasmon Resonance (SPR) uses optical biosensing for real-time monitoring of macromolecular interactions. In a standard SPR set up, one interacting partner (the ligand) is immobilized onto a gold sensor chip surface and an unbound interactant (the analyte) is then flowed over the surface. A change in the refraction index at the surface of the sensor (e.g., due to analyte binding or dissociation occurring near the surface) may be monitored as a shift in the resonance angle and is recorded as a sensorgram.

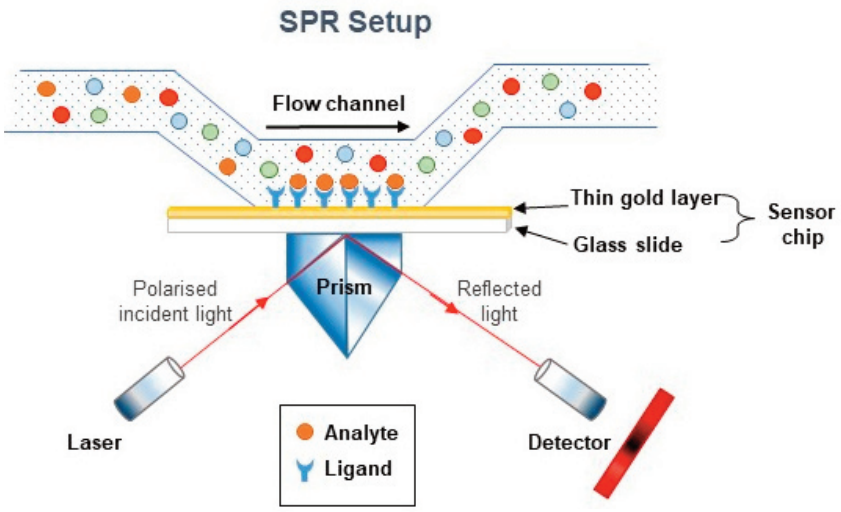


Figure courtesy of Marion J. Limo, nmRC, University of Nottingham

Our facilities

MicroCal PEAQ-ITC (Malvern)
A highly sensitive instrument designed for ease-of-use, requires as little as 10 µg of sample, and has a temperature range of 2 to 80 °C. It features user-friendly guided workflows with videos on running of experiments and performs automated cleaning of the sample cell and the titration syringe.

MicroCal PEAQ-ITC analysis software
Experimental design simulation software to guide users in selecting measurement parameters and simplify analyses with batch evaluation of large data sets and automated assessment of data quality. Final figures and graphs can also be generated quickly and easily.

Biacore T200 (Cytiva)
The Biacore T200 is a highly sensitive SPR instrument with high-throughput capabilities (up to 384 samples per run). Analysis temperature range from 4 to 45 °C, injection volume of 2 to 350 µL, affinity range from fM to mM, and concentration range > 1 pM.

Find out how biophysical analysis could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc-commercial

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

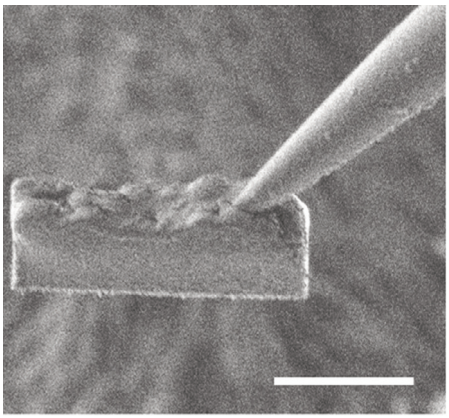
- Imaging and spectroscopy of frozen and soft matter materials
- Tomographic measurements of frozen and soft matter materials
- Cryo-focussed ion beam scanning electron microscopy (Cryo-FIB-SEM)
- Cryo lift-out of thin sections
- Detection, imaging or distribution mapping of volatile compounds
- Analysis 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

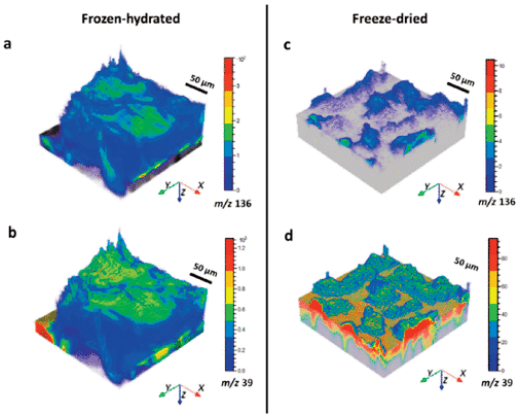
Cryo lift-out allows the preparation of lamella structures from a bulk sample by initially cutting through a sample using focussed ion beam (FIB) SEM and then using a cooled micromanipulator to secure the thin specimen. These lamellae can then be transferred for Cryo transmission electron microscopy 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:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc

University of Nottingham
Nanoscale and Microscale Research Centre

Particle sizing suite

Particle sizing is the characterisation of the size distribution (size range and/or mean size) and number of particles in a sample. It can be applied to solid materials, suspensions, emulsions and aerosols. Techniques include laser diffraction (LD), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), Taylor dispersion analysis (TDA), differential sedimentation (disc centrifuge (DC)) and simpler sieving and separation methods. Method selection will depend on the size range of the particles, the nature of the sample, the capabilities and limitations of the analytical method, the information and the sample throughput desired.

Capabilities

- Particle size determination
- Solution / suspension concentration
- Aggregate detection
- Assessment of colloidal stability
- Zeta potential analysis

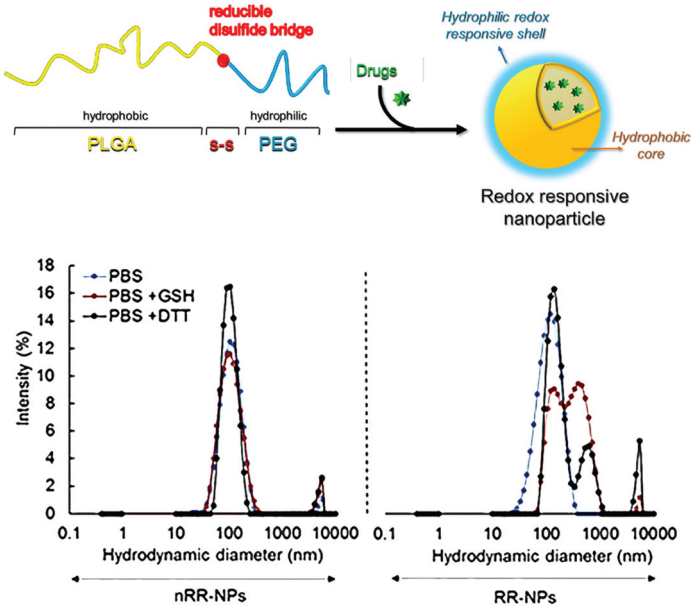
Typical applications

- Product performance; Quality control in different industries (i.e. pharmaceutical, chemical, food and energy)
- Process performance; Determination of efficiency of manufacturing process (i.e. where milling or grinding is used)
- Research and development (i.e. nanoparticle characterisation studies, study of surface modifications)

Size and zeta potential characterisation of nanoparticles designed for drug delivery

An important step in the design of drug carrier nanoparticles is characterisation of particle size and surface change in appropriate environments. Redox-responsive nanoparticles (RR-NPs) were synthesised for drug delivery into lung cancer tumour cells. The RR-NPs were designed to change surface properties when entering tumour microenvironments, which would in turn enhance their cell internalisation and delivery of drug cargo. Characterisation using a Zetasizer Nano ZS instrument of both RR-NPs and non-RR-NPs showed similar properties including hydrodynamic diameter (120 nm), low polydispersity, and high negative zeta potential values. However, size distribution curves showed lower colloidal stability of RR-NPs under in vitro reducing conditions.

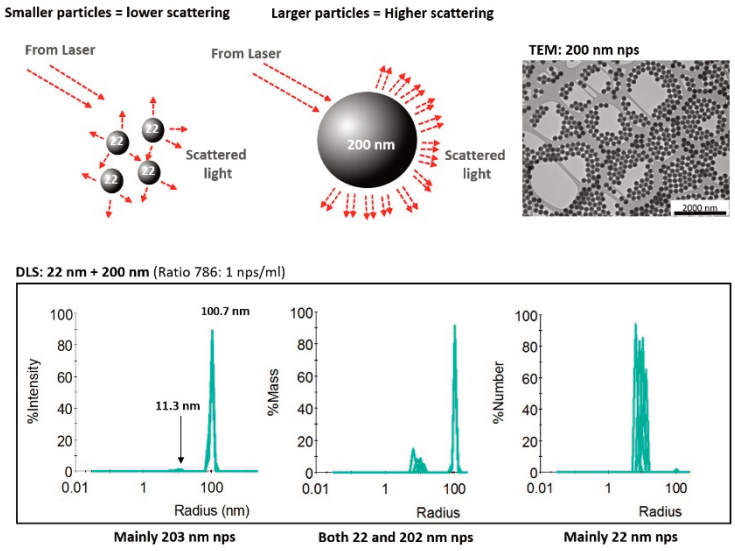
Claudia Conte, Francesca Mastrotto, Vincenzo Taresco, Aleksandra Tchoryk, Fabiana Quaglia, Snjezana Stolnik, and Cameron Alexander. *Journal of Controlled Release*, 277 (2018), 35-45.



Size analysis of a mixture of polystyrene nanoparticles

DLS measurements provide an intensity distribution of particle sizes that can also be converted to volume and number distributions. Comparing these distributions helps understand mixed populations, where larger particles scatter more light and cause variations in the distributions. A DynaPro Plate Reader II instrument was used to characterize a mixture of 22 and 200 nm polystyrene nanoparticles and found from the number distribution the 22 nm to be the major population in the mixture.

Data courtesy of Dr Marion Limo, nmRC, Nottingham.



Our facilities

DynaPro Plate Reader II dynamic light scattering instrument capable of measuring between 1 nm to 2 um hydrodynamic diameter and providing information of polydispersity.

Zetasizer Nano ZS for electrophoretic mobility of proteins, zeta potential of nanoparticles colloids.

Viscotek 802 dynamic light scattering which can be operated between 4 and 60 °C.

LA-960 Laser Particle Size Analyser that measures sizes between 10 nm and 5000 µm from both wet and dry samples such as powders, gels, and creams.

Zetaview NTA capable of measuring hydrodynamic particle size, zeta potential, concentration, and fluorescence.

Electron Microscopy suite including scanning and transmission electron microscopy for microscale and nanoscale particle sizing down to 1-2 nm sized features.

Find out how particle analysis could help with your applications, designs or solutions:
nmrcenquiries@nottingham.ac.uk | +44 (0)115 951 5046
nottingham.ac.uk/nmrc-commercial

University of Nottingham
Nanoscale and Microscale Research Centre

For internal enquiries:

www.nottingham.ac.uk/nmrc

nmrcenquiries@nottingham.ac.uk

For commercial enquiries:

www.nottingham.ac.uk/nmrc-commercial

nmcs@nottingham.ac.uk

Follow us on social media

@UoNnmRC

Nanoscale and Microscale Research Centre