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In-situ multi-modal microscopy using finely focused ion and electron beams

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The development of new characterisation methodologies and instruments is a key enabler for solving questions in a wide range of scientific and technological fields. In this context, the structural characterisation and the chemical analysis at the nanometer scale are of particular relevance in a large variety of fields, ranging from the high-resolution imaging of dopant distributions in complex electronic devices to the generation of chemical maps of sub-cellular structures in biological samples to understand the underlying physiological processes. Key characteristics that are required are highest spatial resolution, excellent chemical sensitivity, high dynamic range (for the detection and mapping of elemental concentrations varying over several orders of magnitude) and isotopic selectivity.

In response to these requirements, we developed the npSCOPE instrument [1], which is based on the Gas Field Ion Source (GFIS), providing finely focussed He^+ and Ne^+ ion beams, as a unique key enabling technology and which enables a combination of three characterisation techniques in one single platform:

- **Secondary Electron (SE) imaging**, providing morphological and topographical information, imaging using a standard Everhart Thornley detector. The SE imaging resolution using the He^+ beam is 0.5 nm.
- **Secondary Ion Mass Spectrometry (SIMS)**, providing high-sensitivity chemical information, using a compact high-performance double-focussing magnetic sector mass spectrometer coupled to high-efficiency secondary ion extraction optics. This SIMS system developed by LIST is equipped with a novel low noise position sensitive focal plane detector, allowing a full mass spectrum to be recorded for each single pixel. We have demonstrated that our SIMS system is capable of producing (i) a full mass spectrum with mass resolution $M/\Delta M$ = 400-500 for each single pixel, (ii) very local depth profiles and (iii) elemental SIMS maps with a lateral resolution down to 12 nm.
- **Scanning Transmission Helium Ion Microscopy (STHIM)**, providing bright field and dark field imaging by detecting the transmitted He atoms and ions, enabled by a novel position sensitive detector based on a microchannel plate with a delay line readout structure placed after the sample [2].

The npSCOPE is furthermore equipped with a cryo-stage to allow the analysis of frozen-hydrated samples and a load-lock system compatible with a cryo-vacuum-suitcase.

To demonstrate the npSCOPE concept and the performance of the instrument, we selected the field of nanotoxicology and investigated the composition and localisation of metal nanoparticles after incorporation by living cells and organisms. This application is very relevant for answering nanotoxicological research questions in the context of pollution and resulting metabolic metal imbalances or the subcellular fate of nanomaterials as functional components of consumer products (e.g. titanium particles contained in food or paint). Until now, these kinds of analyses had to be performed on a number of instruments consecutively, often requiring different kinds of sample preparation. Here, we show how morphological and chemical investigation of tissues can be performed with the npSCOPE: three different imaging modalities (SE, STHIM, SIMS) are consecutively applied on the same sample yielding subcellular information on structural or metabolic markers as well as nanoparticle loading. Figure 1 shows a typical example where TiO_2 nanoparticles are clearly recognised within the gut of Daphnia magna, a small water organism prone to nanoparticle ingestion and associated death.

In parallel to the npSCOPE instrument, we also developed additional multi-modal characterisation platforms [3] that combine at least two of the aforementioned techniques:

- **TEM-SIMS**, consisting of an FEI Tecnai F20 Transmission Electron Microscope equipped with a Ga^+ FIB column and a dedicated SIMS system, allowing for the correlation of Transmission Electron Microscopy (TEM), Energy-Dispersive X-Ray Spectroscopy (EDX) and SIMS data [4].
- **HIM-SIMS**, consisting of a ZEISS ORION NanoFab Helium Ion Microscope (HIM) with a LIST magnetic sector SIMS system, allowing for the correlation of SE and SIMS data [5,6].

- **FIB-SIMS**, consisting of a Thermo Fisher DualBeam platform with a LIST magnetic sector SIMS system, allowing for the correlation of SE, EDX and SIMS data.
- **SIMS:ZERO**, which is a novel highest-resolution highest-sensitivity FIB-SIMS platform combining the latest generations of zeroK's high brightness Cs^+ ion source (based on the Low-Temperature Ion Source (LoTIS) technology) and LIST's high-performance magnetic sector SIMS system with continuous focal plane detector. The use of Cs^+ as a primary ion enhances the ionisation of sputtered particles and hence further improves the analytical sensitivity.

Here, we will review the performance of the different instruments with a focus on new developments such as cryo-capabilities and new detectors allowing parallel detection of all masses, showcase methodologies for high-resolution 3D chemical imaging, present a number of examples from various fields of applications in life sciences and beyond and give an outlook on new trends and prospects.

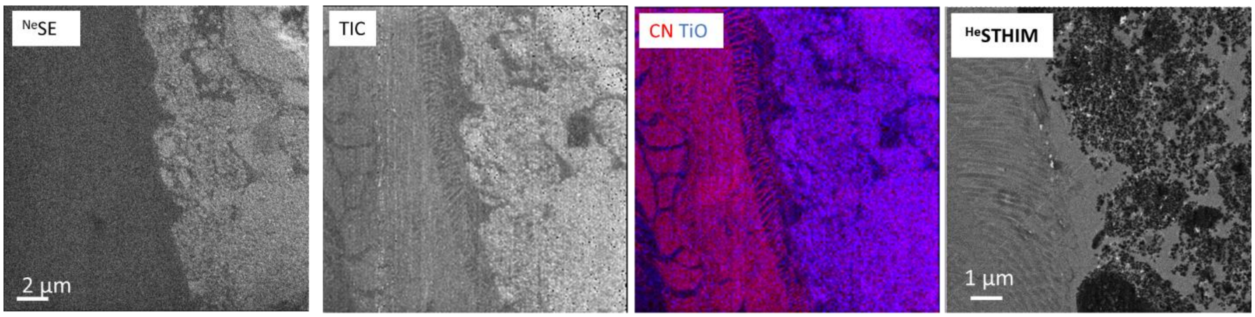

Figure 1. Multimodal imaging on the npSCOPE of Daphnia magna fed with TiO2 nanoparticles. The TiO2 nanoparticles accumulating in the gut lumen can directly be recognised in the SE image due to their rough surface even in flat sections. TIC (total secondary ion signal acquired on the focal plane detector in negative mode) as well as STHIM can be used to directly visualise the biological information missing in SE mode. The gut epithelium can be recognised by the villi structure on its apical surface facing the gut lumen. SIMS on the focal plane detector allows the simultaneous investigation of all relevant marker ions, here CN cluster ions (red false colour) highlighting e.g. proteins in biological tissue and TiO (blue false colour) from the nanoparticles.

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