*English:*

# Network presentation document

## ALL-MICRO

ALL-MICRO is a high-end optical and electron microscopy network that offers instrumentation and scientific and technical support to public or private entities approaching microscopy techniques for technological advancement and innovation. The network develops throughout the Italian-Slovenian macro-region. It includes two universities (Trieste and Nova Gorica), two research centers (Nanocenter in Ljubljana and CNR-IOM in Trieste), and two technology hubs (PTP in Nova Gorica and Tec4i in Udine) that promote accessibility and exchange with small, medium and large companies and public entities.

The network aims to raise awareness of the potential of recent scientific and technological innovations in optical and electron microscopy and their application, to increase the competitiveness of the macroregion and neighboring areas. The most innovative technical knowledge in optical and electron microscopy is often limited to specialized institutions and personnel. The high cost of instrumentation and continuing technological evolution make it difficult to move these methodologies from purely academic or basic research to their use in applied research, industry, and services. As a result, these technologies are not readily available to those seeking to improve their products or services through these techniques but do not possess specific expertise in the field. ALL-MICRO makes it possible to bridge the gap between academia and basic research on the one hand, and industry and services on the other.

ALL-MICRO offers a wide range of microscopy-related services by experienced and qualified personnel, among which applications of:

- **Scanning Electron Microscopy (SEM)**[1], including Environmental SEM (ESEM) and Focused Ion Beam (FIB)-SEM coupled techniques

[1] SEM creates an image by detecting electrons that are reflected or knocked off by the sample. The technique is suitable to get information regarding the sample surface. ESEM works at lower vacuum than SEM, allowing the imaging of non conductive samples, without coating. The ESEM technique is particularly suitable for biological samples. A FIB instrument is similar to a SEM instrument but the sample is scanned using a Focused Ion Beam rather than an electron beam. FIB-SEM instruments have a double mode of analysis, through both ions and electrons. The technique is suitable for material science samples.


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- **Transmission Electron Microscopy (TEM)**[2]
- **Scanning Probe Microscopies (AFM, STM)**[3]
- **Optical Microscopies**
- **Raman Microscopy**

Thanks to an Interreg Italy-Slovenia grant (ALL-MICRO funded project), the network provides the possibility to access services through pilot projects in which proof-of-concept experiments will be conducted using the instrumentation available to the network. In addition to pilot projects, the network offers opportunities for collaboration with one or more participating institutions.

This paper presents the instrumentation available to the network and the applications for which the network offers its scientific expertise.

[2] TEM creates an image by detecting electrons transmitted through the sample. The technique allows for the visualization of structures at atomic resolution of both material science and biological samples.

[3] Scanning Probe Microscopies detect the sample surface using an atomic-sized probe, with nanometric resolution.


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## SCANNING ELECTRON MICROSCOPY

SEM is a technique used to obtain details regarding the surface morphology of samples of any nature up to a nanometric resolution. In a scanning electron microscope, a nanometer-sized beam of electrons is focused on successive points, scanning point-by-point the sample to be analyzed. For each position, different detectors placed inside the microscope allow information to be collected from various phenomena caused by the impact of electrons on the sample. Among them, secondary electrons expelled from the surface layers of the sample show its morphology, down to details smaller than 1 nm ($10^{-9}$m, or a thousandth of a μm). Backscattered electrons are higher energy electrons that come from more internal layers and allow for the chemically different areas of the sample to be distinguished.

Due to the impact of electrons, X-rays are also ejected from the sample revealing the chemical composition present at each point of the sample. Energy Dispersive X-ray Spectroscopy (EDS) analysis performed with an SEM instrument can reveal the elemental composition using the X-rays emitted by the sample upon electron impact. The technique allows to obtain quantitative or semiquantitative determination of the chemical composition in precise locations of the area analyzed.

In general, specimens used in SEM microscopy must be able to conduct electrons. For this purpose, the samples are often covered with a metal layer. In addition, samples must be dry, and fresh biological samples must be fixed and dehydrated. To obtain the highest resolution and magnification the sample must be flat or very thin, e.g. a monolayer of nanomaterial or a polished section.

SEM works with a wide range of samples, including (but not limited to) composites, rocks, minerals, and biological samples. Analyzing the backscattered electrons it is possible to easily detect differences between elements with different atomic weights, e.g. organic contaminations on metal materials, or vice versa metal nanoparticles on organic materials.

The Low Vacuum and Environmental SEM techniques allow the imaging of non-conductive samples in low vacuum conditions, without the need for metal coating. This modality can be used to analyze samples that are wet or dry and not conductive and for which fixing and dehydration would cause structural damage, or for which the metal coating cannot be applied. For example, cell-seeded materials, samples derived from plants, and unicellular organisms can be analyzed with ESEM, albeit with a lower resolution than for the high vacuum conditions.


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FIB-SEM instruments couple the electron beam scanning of the sample of the SEM instrument with an additive ion beam, usually using gallium ions. Unlike electrons, the ion beam is destructive for the sample, allowing milling and cutting to expose the cross-section of the material or to prepare small sections of the material. The FIB column, coupled with the SEM column, can be used to analyze the microscopic structure of the bulk of the sample, by carving out different sections.

SEM instruments available through the network:

*ZEISS Supra 40 SEM Microscope*

The Supra 40 instrument is an SEM equipped with a Field Emission Source (FEG[4]) used for high-resolution imaging. The multi-detector equipment designed and installed for specific analyses, from morphological to phase contrast imaging allows fine and in-depth investigation of samples. The STEM detector enables imaging using transmitted electrons with improved final resolution (1 nm). The TEM grid holder that can accommodate up to 12 grids is also available.

In addition, special holders can be designed and constructed. The large chamber can accommodate sample sizes up to 10 cm. The system also includes a state-of-the-art EDS detector (from Oxford) for elemental microanalysis, including elemental mapping capability.

Hard materials are the typical samples investigated with the Supra 40 microscope. STEM is effective with nanoparticles, rods, or fibers for catalytic and biological samples (after a proper drying procedure). In Figure 1, examples of the characterization of Metal-organic frameworks (MOFs) by SEM-EDS using the Supra 40 microscope[5].

[4] Field Emission Guns produce smaller and more coherent electron beams, with up to three orders of magnitude greater brightness (i.e., current density) than conventional thermionic emitters.

[5] Brandner, Lea A., et al. "Ordered Transfer from 3D-oriented MOF Superstructures to Polymeric Films: Microfabrication, Enhanced Chemical Stability, and Anisotropic Fluorescent Patterns." Advanced Materials (2024): 2404384.


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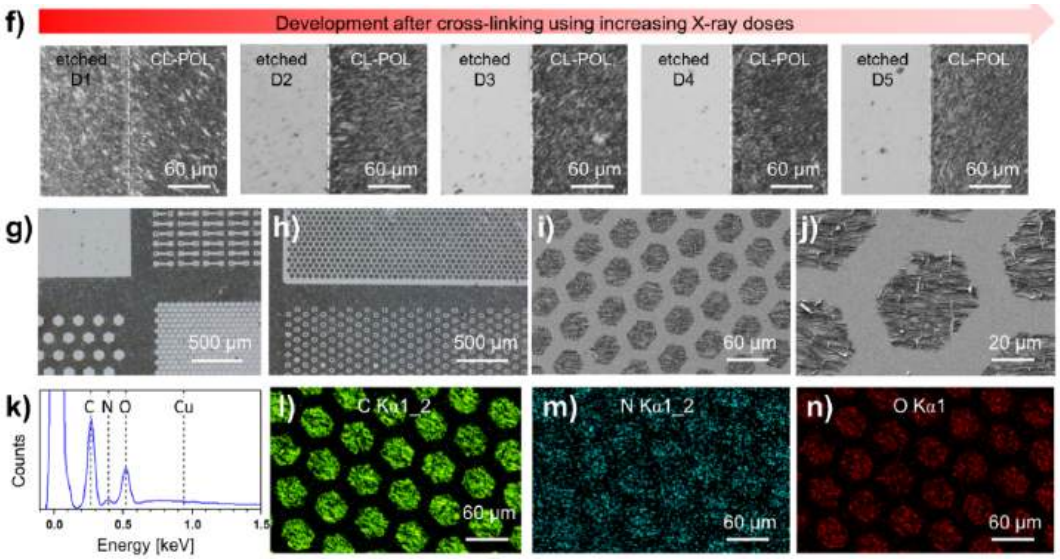


*Figure 1: SEM imaging of Metal-Organic Frameworks*

The Supra 40 microscope is located at the CNR Electron Microscopy Facility (Q2 Building - Area Science Park Strada Statale 14 km 163.5 - 34149 Basovizza - Trieste). In the facility, samples can be prepared by drying using CPD treatment, carbon coating, or plasma treatment. All instruments for sample handling and storage are available at the SEM laboratory.

Facility manager: Simone Dal Zilio (facility manager, technologist). Technical staff in charge: Behnaz Abbasgholi Nejad Asbaghi (post-doc, user support).

*Zeiss GEMINI 300 SEM/CLEM Microscope*

The Gemini 300 scanning electron microscope has a field emission source (up to 30 kV) and is equipped with an Everhart-Thornley secondary electron detector for morphological analysis and a 6-segment backscattered electron detector (BSD) for chemical contrast and TEM-like imaging. High-resolution imaging at high magnifications can be achieved using the InLens detector for secondary electrons and the Energy Selective Backscattered (EsB) detector. In addition, chemical microanalysis can be performed using the Bruker XFlahs 610M EDS probe, with a Silicon Drift Detector. The instrument is also equipped with a STEM detector to analyze TEM samples.


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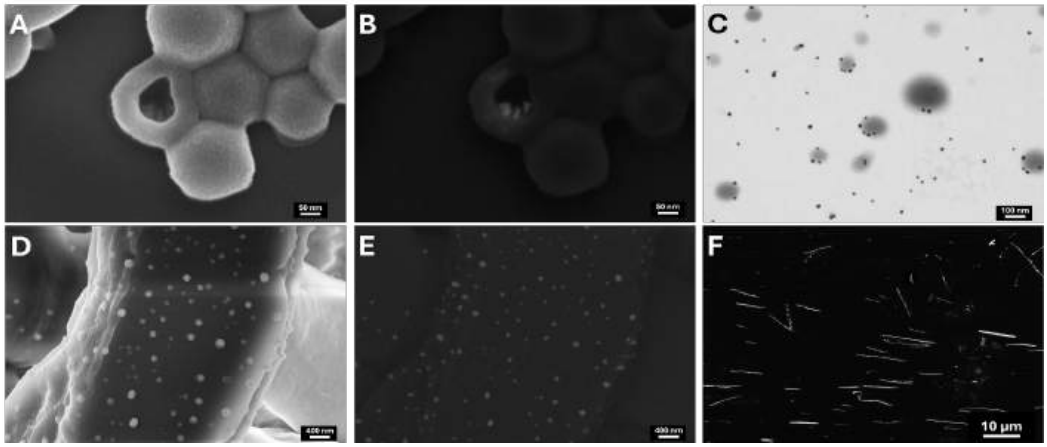


*Figure 2: Various examples of high-resolution imaging. Metal nanoparticles embedded in polymeric microparticles (courtesy of Prof. Macor) acquired with secondary (A) and backscattered (B) electrons, and in STEM mode (C). Polymeric nanofibers coated with silver nanoparticles acquired with secondary (D) and backscattered (E) electrons. Nanocomposite based on polymer enriched with metal flakes, acquired with backscattered electrons (F, courtesy of Prof. Lughi).*

The Gemini instrument performs SEM analyses on various samples, from nanomaterials to dehydrated and fixed biological samples (Figure 3). Details of a few nanometers can be obtained. The chemical composition of samples of any nature can be investigated using backscattered electrons. The analysis requires a polished section, which must be dry and conductive.

This instrument can also perform STEM imaging of ultrathin sections or nanomaterials (Figure 2). The sample must be prepared for TEM analysis and placed on TEM grids. However, the STEM mode is less versatile with respect to TEM analysis. Biological sample sections can be imaged after staining with contrast agents (e.g. $OsO_4$). TEM-like images can be also obtained working with the BSD on SEM samples (Figures 2 and 4).

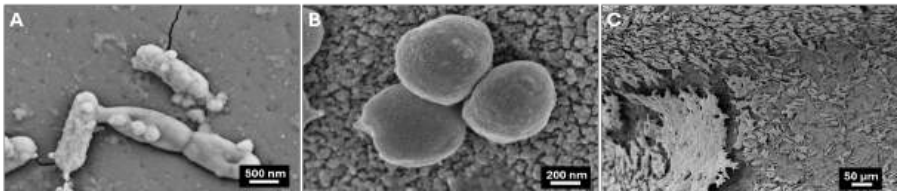


*Figure 3: Imaging of cell morphology: bacteria treated with an antibacterial compound (A, courtesy of prof. Bandiera), bacteria adhered on an implant (B), osteoblasts adhered on an implant (C).*

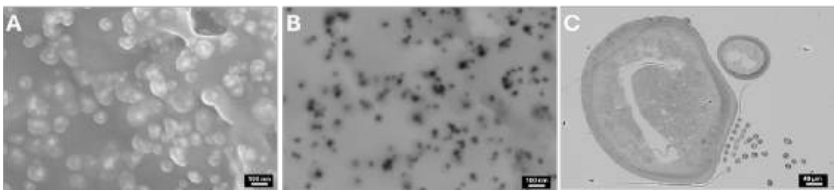


*Figure 4: Silver nanoparticles deposited on polymeric nanofibers acquired with secondary electrons (A) and backscattered electrons in TEM-like mode (B). Thin section of a micro-organism sample acquired with backscattered electrons in TEM-like mode (C, courtesy of Prof. Giulianini).*


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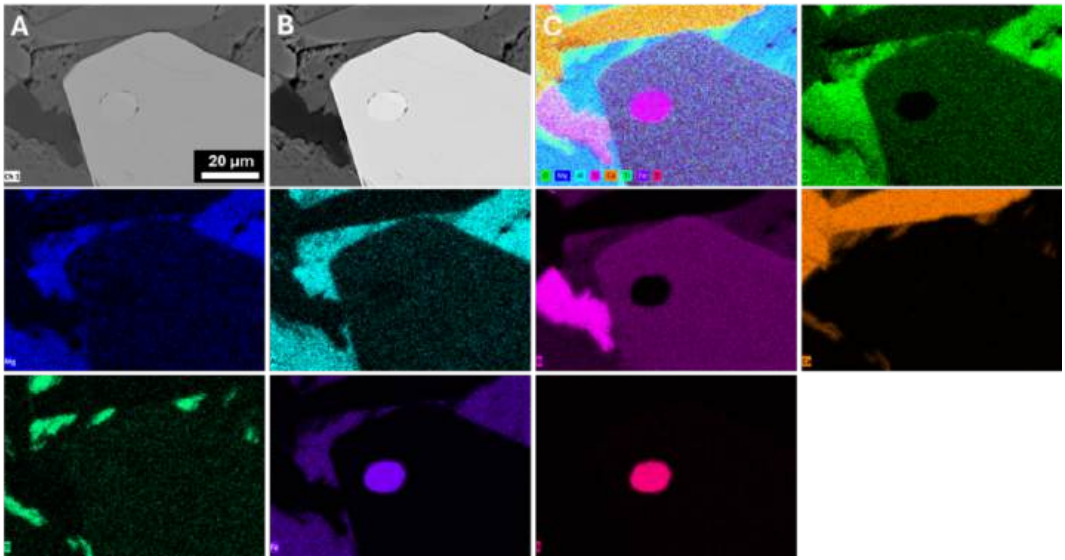


*Figure 5: Thin section of a rock sample acquired with secondary (A) and backscattered (B) electrons, together with the EDS map of the main elements, merged in C (courtesy of prof. Ziberna).*

The instrument is very versatile in terms of electron beam energy and intensity. It can be used on large samples for wide-field imaging at low magnifications and high-resolution nanoscale imaging at low energies. A huge variety of samples can be analyzed: nanomaterials, nanocomposites, fixed and dehydrated biological samples, thin sections, to name a few. The field emission source and EDS probe enable fast and accurate microanalysis in various setups, from spectra to spot, profile, and mapping analysis. The Gemini 300 SEM can be used with the Elyra 7 SIM (see below) microscope to perform correlative optical electron microscopy and combine the fluorescence image with the scanning electron microscope image.

The Gemini 300 microscope is hosted at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (room 2, via Fleming 31/A, Trieste). The instrument is available for 15 percent of machine time for network activities. In the Center, different sample preparation techniques are available: critical point drying, sample coating with Gold, Chromium or Carbon (for analyses in TEM-like and backscattered mode and for EDS). Specialized personnel will help with sample manipulation and preparation before the analysis, and will advise on image analysis.

Responsible for the instrument: Dr. Davide Porrelli (MSc in Medical Biotechnologies - Nanobiotechnologies, PhD in Nanotechnology).


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*JSM 7100f-TTL JEOL SEM Microscope*

This SEM has acceleration voltage in the range of 1-30 keV (HV<1 keV is possible, using the Gentle Beam technology). It is equipped with an EDS apparatus (X-MAX 80, Oxford instruments) and a Cathodoluminescence (CL) spectroscopy apparatus (MonoCL4, GATAN). It is also equipped with a heating stage for in-situ heating (range: RT-700 °C) and in-situ gas-dosing.

The microscope can be used for imaging of conducting and dry surfaces with a resolution of a few nm. Besides surface morphology (secondary electrons) and phase-sensitive imaging (backscattered electrons), the analysis can be extended to the composition (EDS, spectroscopy, or maps) and the optical emission (cathodoluminescence spectroscopy and mapping). Cathodoluminescence spectroscopy can be used to characterize some sample electronic and structural properties, such as, for example, the optical band gap or the presence of defects in the material (Figure 6). It is possible to heat the sample (and dose gas) *in situ* (i.e. during the SEM analysis).

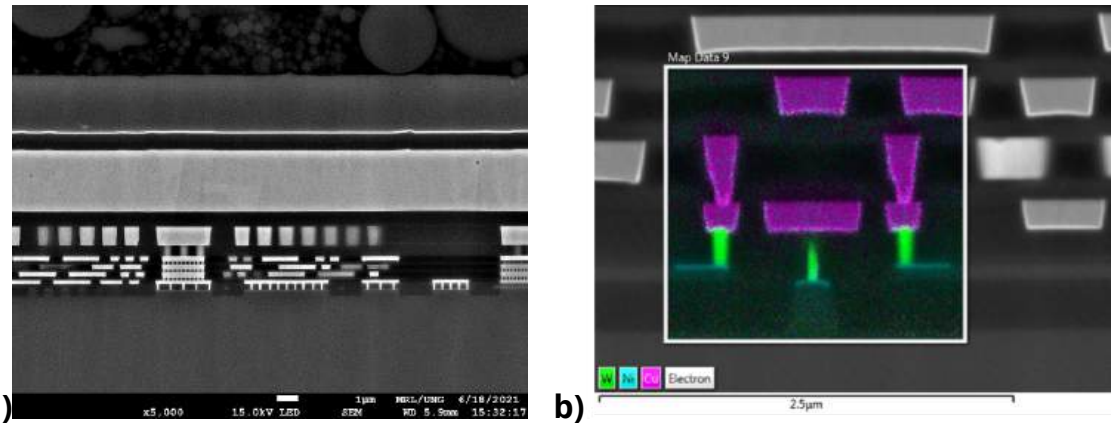


*Figure 6: Morphology of catalyst nanoparticles investigated by SEM. (a) The cross-section of an integrated circuit, obtained by Argon-ion polishing (available at UNG) and observed by SEM. (b) A detail of the integrated circuit cross-section where the concentration maps of some elements (W, Ni and Cu) acquired by EDX (available at UNG) are shown.*

This SEM is available at the Material Research Laboratory of the University of Nova Gorica (room P8, Univerza v Novi Gorici, Vipaska 11c, 5270 Ajdovščina, Slovenia), with an availability of 6 hours per week for ALL-MICRO network activities.

Instrumentation for sample preparation is also available in the laboratory, including diamond wire saw (Well), precision etching and coating system (PECS, GATAN), precision ion polishing system (PIPS, GATAN), ultrasonic disc cutter (GATAN), dimple grinder and disc grinder (GATAN), cross-section polisher (JEOL), rotary polisher (STRUERS), along with


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additional ancillary equipment (heating plates, vacuum box, ovens, water deionizer, stereomicroscope, etc. ).

In charge of the instrument: Assoc. Prof. Mattia Fanetti. Technical staff in charge: Dr. Blaž Belec.

ESEM instruments available through the network:

*QUANTA 250 FEI SEM Microscope*

The Quanta250 scanning electron microscope has a thermionic source (up to 30 kV) equipped with an Everhart-Thornley secondary electron detector for morphological analysis and a backscattered electron detector for chemical contrast imaging. It is also equipped with a wide field detector and a gaseous secondary electron detector for working in low vacuum and ambient conditions to analyze non-conductive, dry or wet samples.

This microscope is a versatile instrument that can perform imaging both in high vacuum (Figures 7 and 10), low vacuum, and environmental (humid) conditions (Figures 8, 9 and 11). The morphology of samples of any nature can be analyzed in high vacuum conditions using secondary electrons, up to a resolution of a few nanometers. In addition, the microscope can perform imaging of the morphology of samples in low vacuum conditions. This modality can be used to analyze dry samples that are not conductive, when coating might damage or alter the structure of the sample and the sample has to be preserved in its form. For example, ceramics, polymer nanocomposites, and plastics can be analyzed in low vacuum, albeit with a lower resolution than for high vacuum conditions.

The QUANTA 250 microscope is hosted at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (room 4, via Fleming 31/A, Trieste). The instrument is available for 15 percent of machine time for network activities.

The laboratory also allows sample preparation by sputtering with Gold, Chromium, or Carbon. The laboratory is also equipped with a Critical Point Dryer for dehydration of biological samples.

In charge of the instrument: Dr. Davide Porrelli (MSc in Medical Biotechnologies - Nanobiotechnologies, PhD in Nanotechnology).


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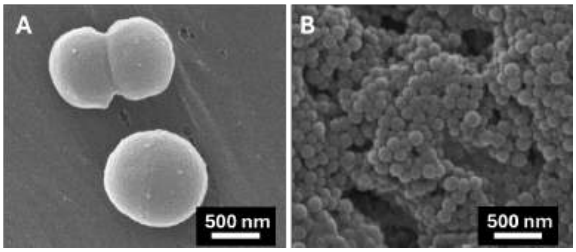


*Figure 7: High magnification images of bacterial cells (A) and polymeric nanoparticles (B).*

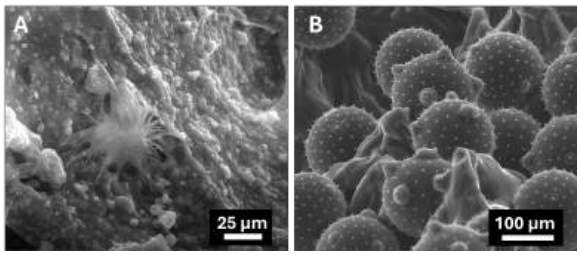


*Figure 8: Osteoblast cell cultured in a biomaterial (A), and pollen (B, courtesy of Prof. Candotto Carniel) analyzed in Environmental conditions.*

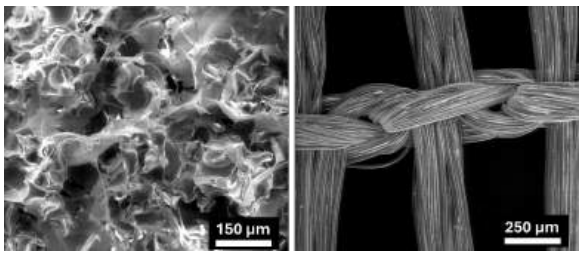


*Figure 9: Morphological analysis of a porous polymeric material (A) and a polymeric-based bandage (B) acquired in Low Vacuum conditions.*

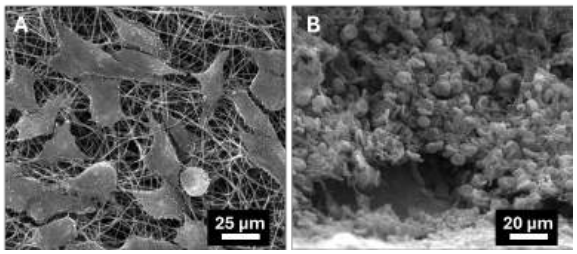


*Figure 10: Interactions of eukaryotic cells with biomaterials: osteoblasts on nanofibers (A), blood clot on titanium implant (B).*

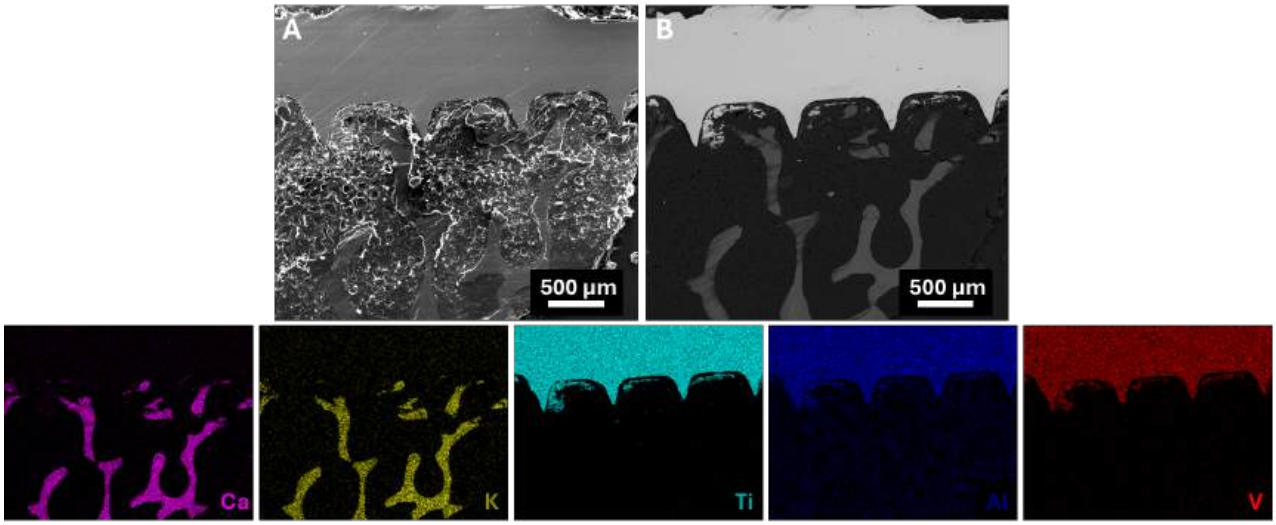


*Figure 11: Thin section of a bone biopsy with a titanium alloy implant, acquired with secondary (A) and backscattered (B) electrons, together with the EDS map of the main elements.*

<u>SEM-FIB instruments available through the network:</u>

*Zeiss Leo 1540XB SEM-FIB Microscope*

Zeiss Leo 1540XB microscope is a Cross Beam SEM instrument with a FEG emission gun and Zeiss electromagnetic optics that enable reaching a resolution down to 2 nm in SEM mode and 150 nm in FIB mode.


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The FIB emission gun is based on a Ga source, allowing the milling of the sample for cross-section inspection. The system is integrated with an EDS detector. In addition, a cryo-stage allows for inspecting frozen samples, a useful technique for samples containing water, such as biological samples, or for samples sensitive to heat damage. A load-lock is present for the cryo-sampling, allowing in situ sputter coating of the sample, without breaking the cooling during the entire process of freezing and imaging.

The instrument is designed for material analysis, from morphological to elemental inspection. The cryo-stage opens the possibility for biological samples, or samples with high water content, without invasive drying procedures (Figure 12). Cells, foods, biological species, and gels are some of the samples that can be easily imaged with this instrument. The presence of the FIB column allows sample milling for cross-section imaging to investigate the morphology beneath the sample surface.

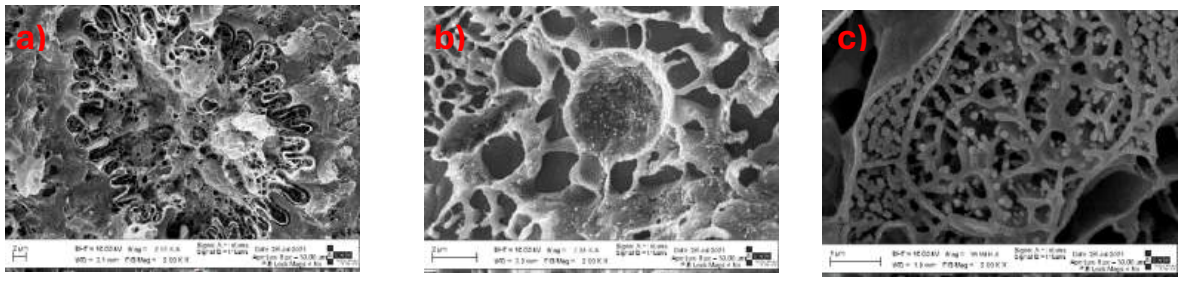


*Figure 12: Characterization of biological tissues—a) lung, b) liver, and c) kidney—by Cryo-SEM*

The Leo 1540XB microscope is located at the CNR Nano-Fabrication Facility (Building MM - Area Science Park Strada Statale 14 km 163.5 - 34149 Basovizza - Trieste). The instrument is available for network activities for 25% of the machine's time. Sample preparation and storage are also possible in the facility, with periodic access.

Facility manager: Simone Dal Zilio (FNF manager). Technical staff in charge: Martina Conti (Technologist).

*SEM-FIB Aquilos2 ThermoFisher microscope*

ThermoFisher's Aquilos2 microscope is a state-of-the-art high-resolution imaging SEM (source up to 30 kV) with a FIB column for milling (Ga ion source up to 30 kV). The system is equipped with a liquid nitrogen cryo-stage, which opens up the possibilities for biological sample analysis. The system can accommodate TEM grids and allows easy transfer during sample preparation, avoiding water contamination. The system also includes an in situ sputtering coating system. In addition, Aquilos2 is equipped with fluorescence correlative imaging for Scanning Electron-Light Microscopy. A


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micromanipulator is installed in the instrument for the extraction of lamellae suitable for TEM analysis.

Thanks to the cryo-stage, this microscope is suitable for biological samples, especially cells or tissues. Cells must be (at the moment) seeded and cultured in TEM grids to be inserted into the microscope. It is also possible to work with other heat-sensitive materials, such as polymers or lithium-based materials.

The Aquilos2 microscope is hosted at the CNR Cryo-EM Facility (Building MM - Area Science Park Strada Statale 14 km 163.5 - 34149 Basovizza - Trieste). The instrument is available for network activities for 15% of the machine's time.

The facility allows sample preparation with drying using CPD treatment and a plunge freezer for controlled freezing of samples (currently only for TEM grids). The laboratory also has a sputtering system for carbon coating or plasma treatment. A biochemical preparation laboratory is also available. Complementary instruments and characterization tools (optical microscope, chemical fume hood, ultrapure water source) are also present.

Facility manager: Simone Dal Zilio (Facility Manager, Technologist).

*DualBeam FIB Microscope*

The DualBeam FIB microscope is an advanced device that incorporates an ion beam with an electron microscope (FEG-SEM, reaching a resolution of 0.8 nm, which enables a magnification of million times) for attachment, nanostructuring, 3D tomography of materials and thin films, and cross-sectional analysis of devices.

The applications of this instrument range from industrial quality and defect analysis to the preparation of samples for TEM investigations through micromanipulation and production and analysis of cross-sections. The characterization of industrial materials using the DualBeam FIB microscope includes quality control of constituents, analysis of the homogeneity, detection of defects and unwanted phases, and phase and pore volume determination with nanometer resolution (Figure 13). The instrument is suitable also for the preparation of nano-objects and electron lithography.

The DualBeam FIB microscope is equipped for *in situ* EDS analysis, allowing the chemical analysis of surfaces and generated cross-sections (depth profiling). The EDS detector can perform qualitative and quantitative elemental analysis, elemental line scan, and elemental mapping.


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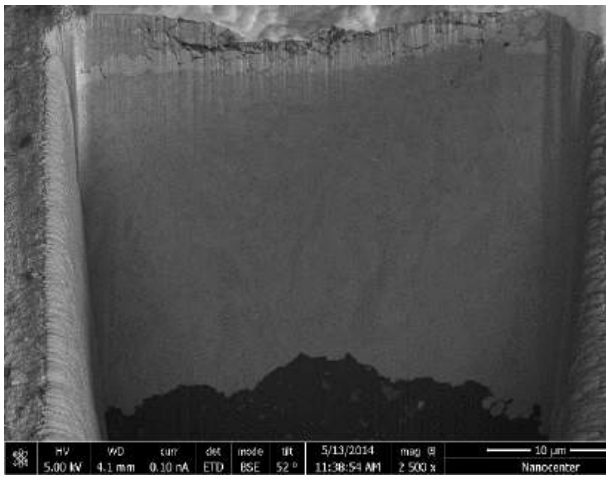


*Figure 13: Example of quality and defect analysis on a material.*

In academic studies, this microscope has been applied for surface analysis of materials, 3D tomography, production of TEM lamella in different orientations, nanometer-sized circuits (e.g., superconducting circuits such as nTron), investigations with high spatial and depth resolution, analysis of semiconductor elements on Si substrate, production and analysis of cross sections, production of nano-objects and lithography, characterization of heterostructures (graphene, h-BN, FET monolayers).

The DualBeam FIB microscope can perform micromanipulation, using a built-in micromanipulator. Small objects can be attached inside the microscope and transferred onto a substrate or mounted for further processing at a different location (e.g., using High-Angle Annular Dark Field STEM or High-Resolution TEM). The instrument can be applied, for example, in the micromanipulation of nanowires, the preparation of memory elements based on TaS2 (CCM), and the fabrication of nanosized devices onto E-Chip for TEM in situ experiments (electrical and thermal).

This microscope is also suitable for 3D tomography, an advanced technique that allows the construction of 3D models of the bulk of the sample with 10 nm resolution. Using this technique we can determine the three-dimensional constitution of specific phases within the bulk and quantitatively describe higher-order microstructural parameters such as specific surface area, specific interface area, connectivity of phases, triple phase boundary density, tortuosity, porosity, etc.

The DualBeam FIB microscope is housed at the Nanocenter in Ljubljana (Jamova 39, Building B, Jožef Stefan Institute). The instrument is available for 20% of machine time for network activities.


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In the same building, there is the AQDL Advanced Quantum Devices Laboratory (Figures 14-16), which offers rigorous contamination control measurements and provides a range of tools and processes for the design, creation, and analysis of micro- and nano-scale structures and devices. Typical services and equipment at the nanofabrication facility include nanolithography, metrology and inspection, oxygen-free processing, and heat treatment.

Facility managers:

- Dr. Bojan Ambrožič, a researcher with expertise in geology and material science, FIB sample preparation, FIB analysis, and FIB lamella preparation, a leading Slovenian expert in the field of meteoritics. Dr. Bojan Ambrožič is also the head of the AQDL laboratory.
- Dr. Gregor Kapun, a researcher with expertise in material science, nanotechnology, all-solid-state battery, fuel cells, FIB sample preparation and analysis, Advanced FIB processes, FIB Tomography, FIB lamella preparation, FIB sample preparation for In Situ TEM experiments, UHR-SEM analysis.


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*Figure 14: AQDL hosts a large air-free glove-box for processing the sensitive samples in a nitrogen atmosphere. The methods that are regularly available inside a protected atmosphere are optical microscopy, AFM microscopy, spin-coating, baking or heating on hot plates, and thin metal film deposition by e-beam evaporation.*

*Figure 15: AQDL can offer some lithographic services. Laser lithography with direct laser writing system (DWL) Midalix DaLI enables maskless patterning and illumination of UV-sensitive films (photoresists). It is mostly used for prototyping, circuit design testing, custom electronic device fabrication, and small-volume chip production. The standard lithographic processes as photoresist spin-coating, development, removal and lift-off can all be preformed in the AQDL clean room or even in glove-box.*

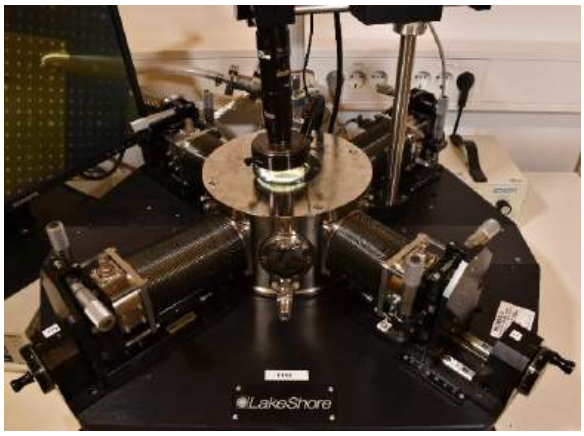


*Figure 16: In AQDL also some electrical measurements and characterization of the conductive samples can be preformed. For this purpose there are two 4-probe LakeShore measuring stations for DC and AC transport measurements in the temperature range from 4K-400K and in the vertical magnetic field 0T - 2.6T. The stations are equipped with all needed instruments, computer and software for routine acquisition of the measured data.*


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## TRANSMISSION ELECTRON MICROSCOPY

A transmission electron microscope is capable of analyzing thin samples of different materials, both biological (from cells to cellular organelles, up to single proteins) and inorganic (from nanotechnological materials to mineral samples). In the transmission microscope, an electron beam is produced in a high vacuum column, focused by the condenser lens system, and passed through the sample. Passing through the sample, the electrons are scattered in different directions and, in part, absorbed by the sample itself. The objective lens allows an image of the sample to be formed from the scattered electrons that have passed through the sample. The system of projective electromagnetic lenses allows the magnification of the image obtained. With the transmission electron microscope, images with a resolution up to approximately one-tenth of a nanometer ($10^{-10}$ m) can be obtained.

TEM instruments available through the network:

*EM208 TEM Microscope*

This transmission electron microscope operates at a voltage of 20-100 kV and is equipped with a Quemesa (Olympus Soft Imaging Solutions) camera. Image acquisition is achieved using RADIUS software. This electron microscope combines good magnification power and resolution with easy handling and is particularly suitable for organic and biological samples. The low accelerating voltage (100 kV) of this microscope is ideal for the observation of samples prone to structural damage due to the electron beam, avoiding the destruction of the specimen. The EM208 has a resolving power of about 2 nm for biological specimens, usually observed at low magnification (≤ 20,000 x) (Figure 17).

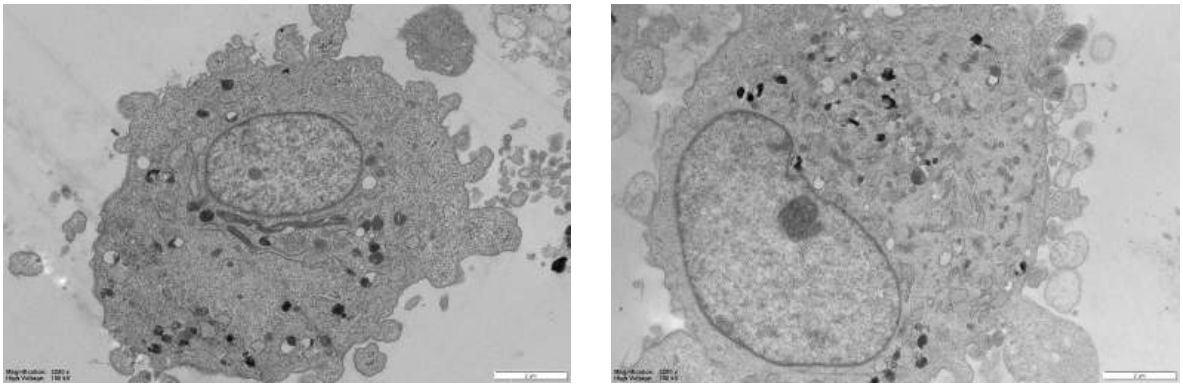

*Figure 17: Human fibroblasts (glutaraldehyde and osmium tetroxide fixations, resin embedding, and 90 nm section staining with UranyLess and lead citrate, EM Stain)*


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The EM208 microscope is suitable to investigate many fields of cell biology and medicine using a variety of preparatory methods. Analysis of samples of cells or tissues is routinely performed on this microscope. The resolution power of the microscope allows the imaging of cellular organelles, among which vesicles like exosomes and liposomes, and to investigate, for example, the effect of nanomaterials on cells. These samples are prepared by treatment with chemical stains.

The EM208 microscope is located at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (via Fleming 31/B, Trieste). The instrument is available for 15 percent of machine time for network activities.

The results of the analysis with the EM208 microscope depend on a series of crucial procedures, among which fixation procedures, resin inclusion, section preparation with ultra-microtome (60 -100 nm thickness), and observation. The CIMA has the facilities and the specialized personnel to prepare thin sections of the samples.

Instrument manager: Dr. Roberta Bortul (PhD in Human morphological sciences, responsible for the preparation of biological samples).

*CM200 TEM Microscope*

This transmission electron microscope operates with a voltage of 20-200 kV and is equipped with a fully motorized stage table, a high-performance super twin a-lens, and a LaB6 source. All these features plus an energy-dispersive X-ray spectrometer (EDAX, Oxford Instruments) make this microscope a versatile instrument in terms of analytical and imaging capabilities. It provides high-resolution images down to a line resolution of 0.10 nm and is equipped with a Quemesa camera (Olympus Soft Imaging Solutions). Image acquisition is performed by RADIUS software.

The CM200 is a very versatile transmission microscope, perfect for nanomaterial characterization at the atomic level, as well as appropriate for conventional morphology characterization. The EDS probe (Oxoford) accessory can provide qualitative point-by-point information about the surface composition of the sample in a fast and intuitive way. The analysis of nanomaterials is relatively straightforward and does not require specific sample preparation. The sample just needs to be deposited on a metallic grid and thoroughly dried before the insertion in the microscope. This TEM is particularly suitable for the characterization of metallic nanoparticles (Figure 18), crystals (via diffraction analysis), organic and inorganic (macro)molecules, composites, and polymers.


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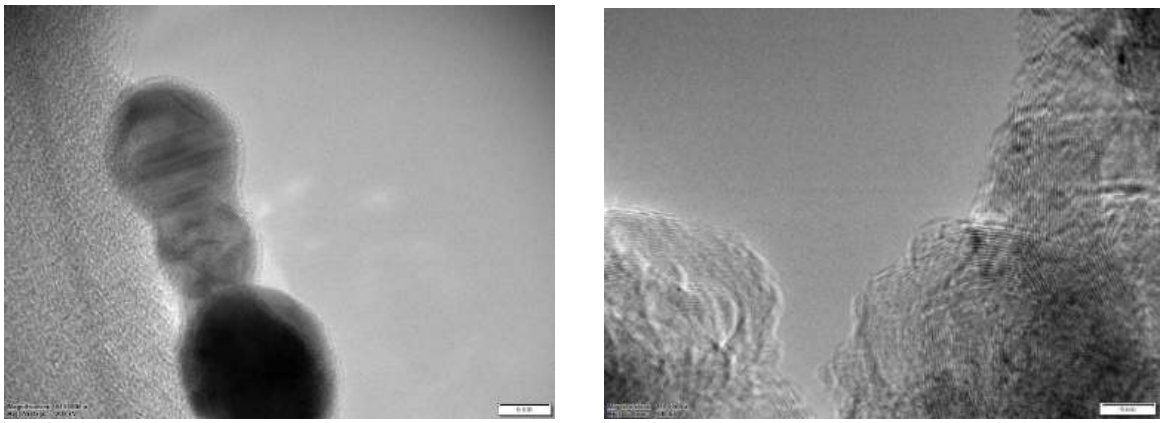

*Figure 18: Gold nanoparticles (left) and graphitized carbon (right) at magnification of 1,1 Mx*

The EM200 microscope is located at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (via Fleming 31/B, Trieste). The instrument is available for 15 percent of machine time for network activities.

Facility Manager: Dr. Susanna Bosi (Master's degree in Chemical and Pharmaceutical Sciences, PhD in Pharmaceutical Sciences with a long experience in carbon nanomaterials and polysaccharides chemistry and characterization).

*JEM 2100f-UHR JEOL TEM Microscope*

The JEM 2100f-UHR Field Emission Transmission Electron Microscope (JEOL) has an accelerating voltage of 80 to 200 kV and can reach resolutions up to a tenth of a nm. It can also be used in STEM mode (0.2 nm resolution), in both dark and bright fields. It is equipped with an energy-dispersive X-ray spectroscopy apparatus (EDS, X-MAX 80T, Oxford instruments).

The microscope can be used in a classical High-Resolution TEM (HR-TEM) mode for imaging the samples with atomic resolution (0.1 nm at 200 KeV) and for diffraction in selected areas (Figure 19). The Dark-field STEM unit can be used for phase identification based on atomic number contrast with a resolution of a few nm. The analysis can be extended to the compositional analysis (EDX spectroscopy or maps). For unstable samples, it is possible to reduce the beam energy to 80 keV. Besides structural, morphological, and compositional analysis, this TEM is also used as one of the standard methods for performing particle size analysis. Other typical applications of the instrument include: composition and defect analysis, investigation of the effects of production processes, and size distribution of (nano)particles in a sample.


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This TEM is available at the Material Research Laboratory of the University of Nova Gorica (room P9, Univerza v Novi Gorici, Vipaska 11c, 5270 Ajdovščina, Slovenia), with an availability of 6 hours per week for ALL-MICRO network activities. Beside standard deposition (from suspension) on TEM grid/supports, the Laboratory can provide preparation of TEM-observable specimens, both for bulk materials and for cross-sections, through all the preparation steps, from cutting to thinning, final polishing and coating.

Responsible for the instrument: Assoc. Prof. Mattia Fanetti. Technical staff in charge: Dr. Blaž Belec.

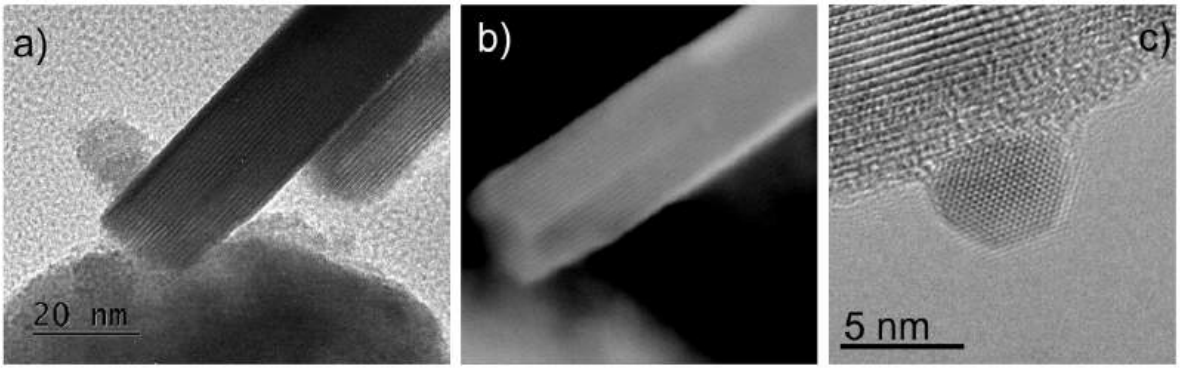


*Figure 19: TEM and corresponding HAADF STEM image of anisotropic $Bi_2Se_3$ nanoplatelets oriented edge-on, with its larger surfaces parallel to the electron beam (a and b, respectively) and HRTEM image of Cu nanoparticle.*


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## SCANNING PROBE MICROSCOPIES

The Scanning Probe Microscopy (SPM) is a family of imaging techniques that allow for high-resolution visualization of material surfaces at the atomic level. Unlike optical microscopy, which uses light to illuminate samples, or electron microscopy, which uses an electron beam, SPM techniques rely on the interactions between a probe that scans the surface and the sample itself. These interactions can involve forces or quantum phenomena, enabling detailed imaging and characterization of materials.

Atomic Force Microscopy (AFM) is one of the most widely used SPM techniques. In AFM, an extremely fine probe is mounted on a flexible cantilever, positioned just a few nanometers above the sample surface. As the probe scans the surface, it measures the forces of interaction—such as van der Waals forces, electrostatic forces, and contact forces—which cause deflection of the cantilever. This deflection is recorded to create a three-dimensional image of the surface.

AFM can operate in various environments: air, solution, or vacuum, making it versatile for different types of materials, from conductors to biological substances. Its applications are extensive, including the characterization of nanotubes, measuring the mechanical properties of materials, and studying biomolecules and cell membranes.

Scanning Tunneling Microscopy (STM) is another key SPM technique that utilizes the principle of quantum tunneling. In STM, a conductive probe is positioned very close to the surface of a conductive or semiconductive material. When the probe approaches within a few angstroms of the surface, a tunneling current is generated between the probe and the sample, proportional to the distance between them. By measuring this current while the probe scans the surface, high-resolution images of the sample's topography can be obtained.

STM is particularly valuable for studying conductive surfaces and gaining detailed insights into the electronic structure of materials at the atomic level. Applications of STM include research in nanotechnology, analysis of semiconductor materials, and study of molecular interactions.

Scanning Probe Microscopy techniques are applied across a wide range of fields. In scientific research, they are used for characterizing new materials, nanofabrication, and analyzing complex biological systems. In industrial settings, SPM can be employed for quality control of surfaces and material research, with applications extending from microelectronics to nanotechnology.


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SPM instruments available through the network:

*ApeResearch Atomic Force Microscope (AFM)*

The AFM (Figure 20) is equipped with modules for standard imaging of topography in contact and non-contact modes, as well as modules that provide imaging in different modes, such as: conductive atomic force microscopy (ConAFM), electrostatic force microscopy (EFM), magnetic force microscopy (MFM). The AFM has a piezo drive that provides a resolution of up to 0.1 nm in the vertical direction and up to tens of nanometers in the lateral direction.

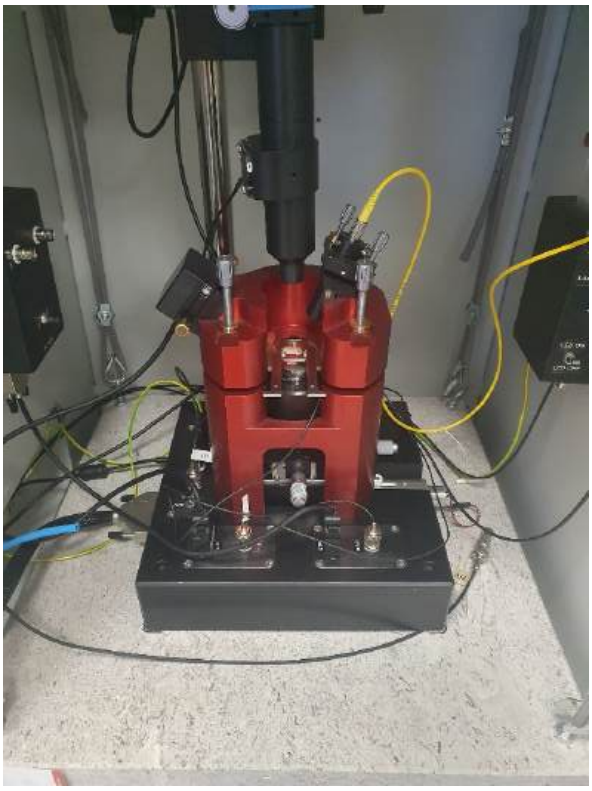

*Figure 20: The AFM apparatus (ApeResearch) available at the Laboratory of Organic Matter Physics, University of Nova Gorica.*

AFM can be used to study surface morphology with sub-nanometric resolution, as well as magnetic, electrostatic, and conductive surface properties of the sample surface. This microscope enables conduction probe and Kelvin probe analysis (Bruker CP-II, APE Research A100) and is designed to investigate the surface topography of solid samples with high resolution (Figure 21).

The AFM apparatus (ApeResearch) is available at the Laboratory of Organic Matter Physics, University of Nova Gorica.


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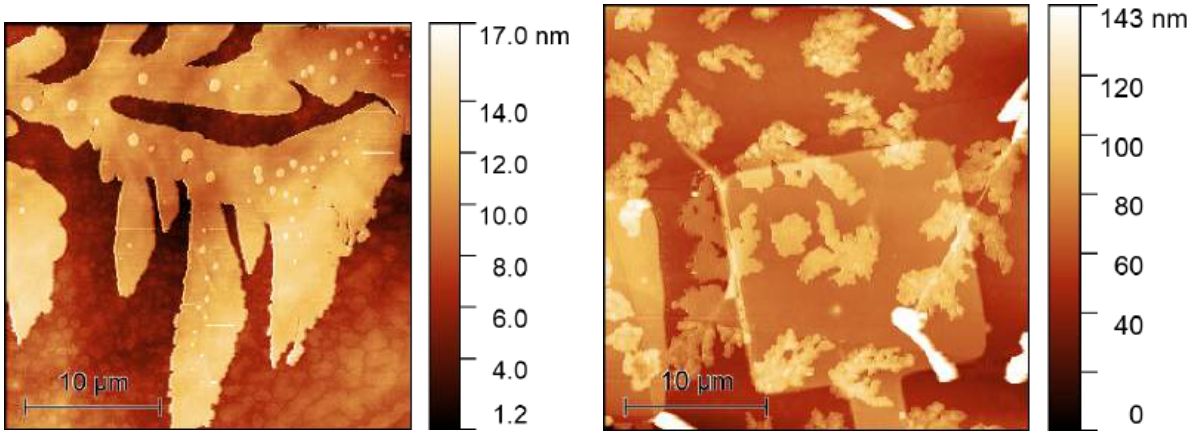


*Figure 21: (a) AFM image showing the morphology of a thin film of organic molecules (C8-BTBT) deposited on hexagonal Boron Nitride (h-BN) substrate. (b) AFM image showing the morphology of organic molecules (C8-BTBT) deposited onto a graphene-based field-effect transistor.*

The instrument is available at the Laboratory of Organic Matter Physics, University of Nova Gorica (Vipaska 11c, 5270 Ajdovščina, Slovenia), 6 hours per week are dedicated to ALL-MICRO network activities.

Responsible for the instrument: Dr. Vadym Tkachuk.

*4-probe STM UHV System microscope*

4-probe STM UHV System is a low-temperature four-probe scanning tunneling microscope (STM) with windows for optical access with laser. It is a unique specialized instrument that merges several advanced features to allow for unique experiments at the nanoscale. The STM is equipped with low-noise amplifiers for measuring tunnelling currents as low as 10-11 A (with bandwidth above 800 Hz) or 10-9 A (above 20 kHz).

Unlike standard STM with a single probe tip, a four-probe STM has multiple probe tips, which allows for local voltage measurements (Figure 22). This feature can be crucial for studying inhomogeneities in samples or extracting resistivity information without the influence of contact resistances. In addition, different biases can be applied to separate probes, allowing for advanced experiments.


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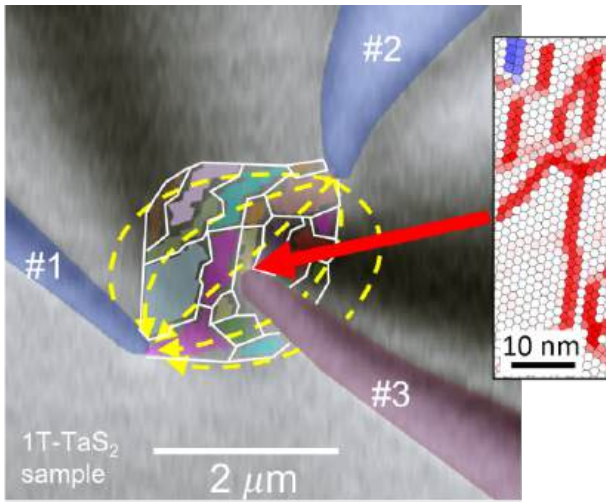


*Figure 22: STM 3-tip experimental setup. Two outer tips (#1, #2) are jammed into the 1T-$TaS_2$ sample and are used to apply electrical current pulses. Tip #3 scans the area in between them to observe the change of polaronic ordering in 1T-$TaS_2$ in relation to the pulses. The inset shows a Wigner-Seitz cell construction on the recorded STM images, where we can observe the dynamics of the domain walls and domain wall crossings.*[6]

A bath cryostat of the STM allows working at the base temperature of 4.2 K for an uninterrupted period of up to 36 hours or at 77 K for up to 120 hours. Precise control of the sample temperature is possible using special sample plates with in-built heater and sensor. –Working at low-temperature has the benefit to reduce thermal noise and enhance the resolution and stability of measurements. Low-temperature phenomena that can be investigated with this instrument include superconductivity, quantum effects, and other temperature-sensitive phenomena. The improved stability of the 4-probe STM reduces thermal drift and enhances the positional stability of the microscope tip.

A scanning electron microscope mounted on top of the STM allows for the precise tip positioning at distances down to 2 um between the tunnelling tips. The optical setup allows for positioning of a laser beam (as small as 50 x 50 um) with a precision down to 20 um for optical excitation of the samples. The samples can be mechanically exfoliated directly in UHV conditions (below 10-10 mB) or thermally treated (annealed) in a separate preparation chamber.

Among the most common applications of this instrument: characterization of surfaces with atomic resolution, measurement of electric characteristic of nanostructures, manipulation of nanostructures and individual atoms, measurement of circuits, study of local density of states (Figures 23 and 24).

The 4-probe STM microscope is housed at the Nanocenter in Ljubljana (Jamova 39, Building C, Jožef Stefan Institute). The instrument is available for 20% of machine time for network activities.

Responsible for the instrument: Dr. Dragan Mihailović.

[6] https://doi.org/10.1038/s41467-023-43800-3


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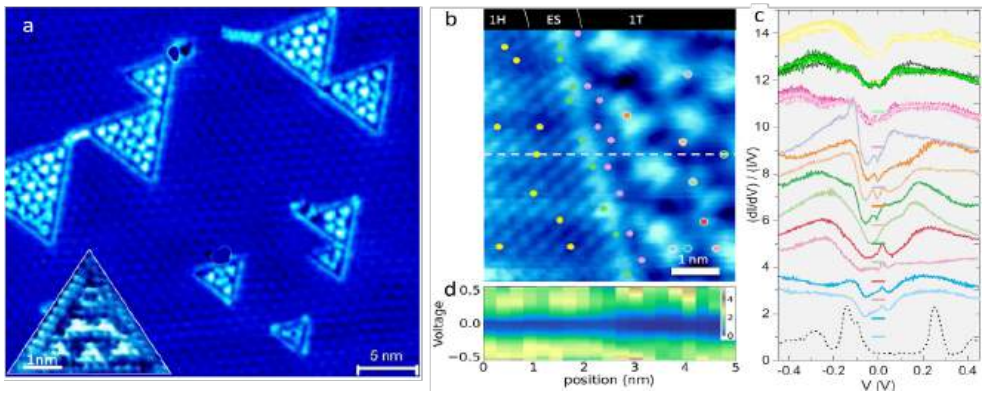


*Figure 23: Laser-pulse-induced nanostructures on the surface of $TaS_2$ crystal, and STM and STS analysis. a) An STM image of different nano-sized 1T-$TaS_2$ triangle structures embedded laterally within a 1H-$TaS_2$ monolayer. b) An STM image of a triangle's edge with indicated positions where STS curves are recorded. c) STS curves (in the corresponding color) for the 1H layer, 1T layer, and the edge states. d) An STS line scan showing the local density of states across the triangle's boundary.*[7]

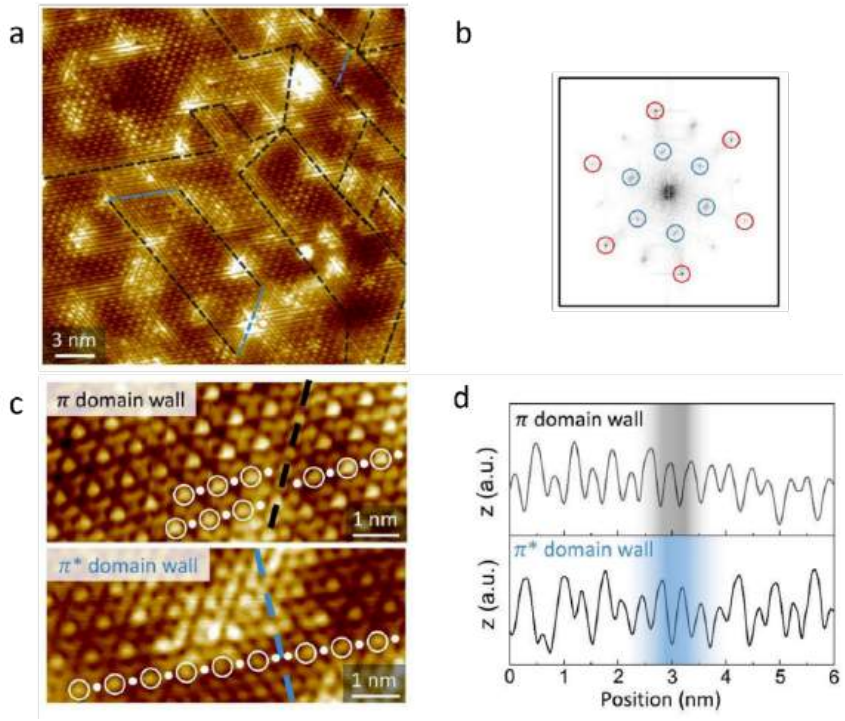


*Figure 24: Studying charge ordering in a dopped 1T-$Ti_{0.98}Ta_{0.02}Se_2$ material. a) STM image with dashed lines marking domain walls. b) Fourier transform of a), where red circles mark lattice peaks and blue circles mark CDW peaks. c) STM image of a phase shift in the domain wall structure, either perpendicular to the CDW direction (i.e., π domain wall) or along the CDW direction (i.e., π* domain wall). d) Line scans for both π and π* domain walls.*[8]

[7] https://doi.org/10.1038/s41467-021-24073-0

[8] https://doi.org/10.48550/arXiv.2407.01261



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## OPTICAL MICROSCOPY

Optical microscopy utilizes visible light to magnify and visualize samples, providing essential insights in fields like biology, materials science, and medicine. Key optical microscopy techniques include linear (e.g., confocal microscopy) and non-linear methods (e.g., multiphoton microscopy).

Confocal microscopy improves resolution and contrast by using a spatial pinhole to exclude out-of-focus light. This technique allows for the collection of high-resolution, three-dimensional images from thick specimens by scanning the sample point by point. Confocal microscopy has applications in cell biology, as the visualization of cellular structures and dynamics, in the detailed imaging of complex tissues and organs. Fluorescence Imaging allows the analysis of interactions between proteins and other biomolecules.

Multiphoton microscopy is a non-linear technique that uses two or more low-energy photons to excite fluorescent molecules, enabling deeper tissue penetration and minimizing phototoxicity. This method provides high-resolution three-dimensional images of live samples and has applications in neuroscience (e.g., imaging neuronal structures and synaptic activities in live brains), developmental biology (e.g., studying embryonic development and cellular interactions in real-time), and cancer research (e.g., monitoring of tumor growth and response to therapies).

In addition to these more common techniques, the ALL-MICRO network comprises a Digital Holographic Microscope. This instrument captures the interference pattern of light reflected from a sample, allowing for quantitative phase imaging. This technique enables a detailed assessment of morphology and refractive index without staining or labeling.

*Nikon C1Si confocal microscope*

The Nikon C1Si instrument is a microscope with a confocal configuration mounted on an inverted Nikon Eclipse stand. The C1Si is equipped with a 4-line laser system, used preferentially for fixed fluorescent samples.

The C1Si microscope is housed at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (room 2, via Fleming 31/A, Trieste). The instrument is available for 15 percent of machine time for network activities.

Responsible for the instrument: Prof. Gabriele Baj (Associate Professor, University of Trieste).


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*Elyra7 Zeiss SIM Optical Microscope*

Elyra 7 is an inverted optical microscope designed to take advantage of the structured illumination (SIM) technique to increase the final resolution of the system, with a linear resolution reaching 100 nm. The microscope allows imaging using laser lines (406, 488, 560, and 640 nm) and a standard epifluorescence lamp using structured or wide-field illumination. A full range of objectives ranging from 5x to 63X is available: 4x air, 10x air, 20x air, 40x oil, 63x oil. SIM microscopy is generally considered a state-of-the-art choice for live imaging and accepts all types of slides and all common media for light microscopy (Figures 25-28). SIM reconstruction allows for an improvement of image resolution of about 2x on standard optical resolution.

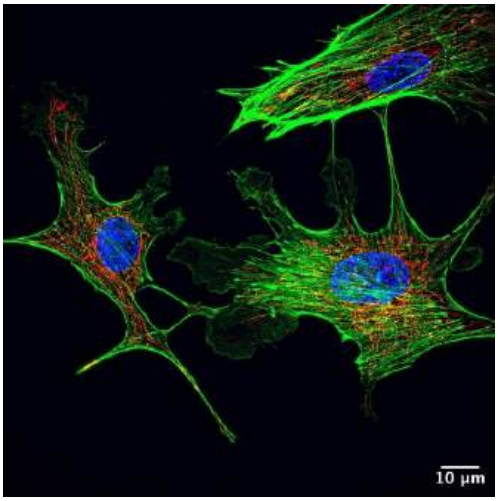


*Figure 25: SIM superresolution image of HeLa cells. Actin in green, mitochondria in red, and nucleus in blue. Courtesy of Agnes Thalhammer.*

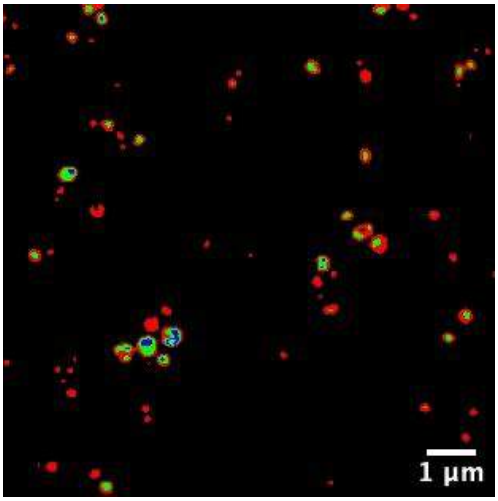


*Figure 26: SIM superresolution image of nanoparticles. Courtesy of Prof Marcor. (Image by Agnes Thalhammer)*

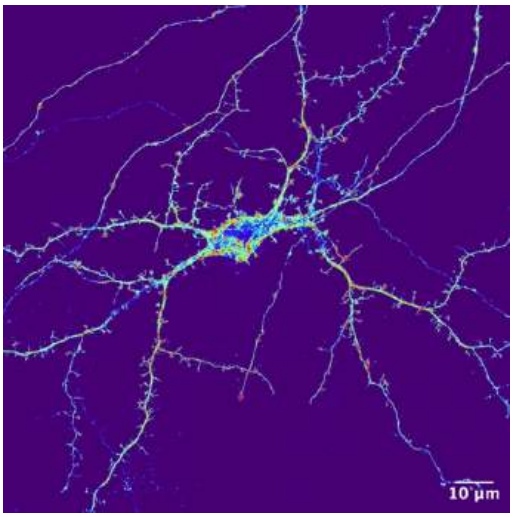


*Figure 27: SIM superresolution image of neuronal cell. Courtesy of Prof Cingolani (image by Agnes Thalhammer).*

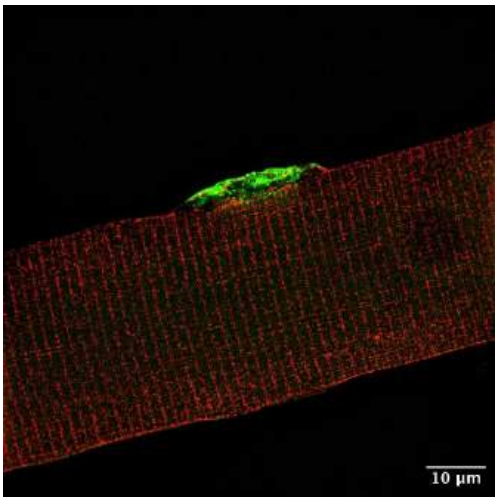


*Figure 28: SIM superresolution image of a muscle cell. Curtesy of Prof Lorenzon (image by Agnes Thalhammer).*


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This microscope is ideal for fluorescently labeled samples or samples expressing fluorescent proteins with a thickness <20 micrometers, such as cell lines, primary cultures, and thin sections. The reconstruction of images obtained in SIM (or Apotome in the case of the 20x) mode works best for cytoskeletal and punctate structures.

The microscope is in addition equipped with a temperature-, humidity- and $CO_2/O_2$ -controlled incubation chamber ideal for live imaging of stained (or fluorescent protein expressing) cells in medium. The microscope further allows usage in Total Internal Reflection (TIRF) mode.

The Elyra7 microscope is housed at the Interdepartmental Center for Advanced Microscopy (CIMA) at the University of Trieste (Room 2, 31/A Fleming Street, Trieste). The instrument is available for 15 percent of machine time for network activities.

Responsible for the instrument: Dr. Agnes Thalhammer (researcher working in the field of molecular and cellular Neurosciences since 1998, with extensive expertise with cell culture, immunocytochemistry, optical microscopy, live imaging and data analysis of optical images).

*Nikon A1R-MP plus Multiphoton Microscope*

The Nikon A1R-MP plus instrument is an upright microscope with a 700-1080 nm pulsed laser (Coherent). The setup is designed to take advantage of illumination at long sample wavelengths to visualize and record thick samples. Specifically, in normal biological samples, the laser can stimulate fluorophores in 800 micron-thick samples. Dedicated detectors can be used to collect all regularly used visible fluorescent tracers. The working distance is up to 2 mm.

This microscope is ideal for fluorescent samples with thicknesses of >250 micrometers up to 2 mm. It works with blue, green, and near-red (TRITC) fluorophores. Examples of samples that are routinely analyzed with the Nikon A1R-MP microscope are sliced organ preparations expressing fluorescent proteins, organoids stained with fluorophores, or tissue sections that are rich in collagen fibers. For the latter sample, collagen can be made visible via second harmonic generation fluorescence without the need for a fluorophore. The microscope can work also on immunofluorescently stained cells of a 3D culture.

Short-term live imaging (<30 min, such as calcium imaging) can be performed on organotypic slices, tissue slices or full organisms (such as C. elegans) that are appropriately labeled (i.e. calcium dyes).


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The A1R-MP plus microscope is housed at the Interdepartmental Center for Advanced Microscopy (CIMA) of the University of Trieste (Building Q, Via Licio Giorgieri 5, Trieste). The instrument is available for 15 percent of machine time for network activities.

Instrument manager: Dr. Agnes Thalhammer (researcher working in the field of molecular and cellular Neurosciences since 1998, with extensive expertise in cell culture, immunocytochemistry, optical microscopy, live imaging, and data analysis of optical images).

*Digital holographic microscope*

The digital holographic microscope (DHM), developed by CNR-IOM (2022), works with transparent samples in static or dynamic mode. In addition to operating as a normal optical microscope, the DHM allows measuring the sample height and/or refractive index. Samples can be in air or immersed in a fluid (e.g., living cells), static, or flowing in a capillary at rates between 10 and 500 uL/min. Holographic movies are recorded at up to 300 frames/sec for dynamic samples.

The DHM instrument allows measurement of the height and/or refractive index of isotropic transparent samples. For example, the height and volume of living cells in the culture medium can be measured and monitored accurately over time. Another example is the measurement of red blood cell membrane fluctuations, which has been conducted using the DHM microscope in the CNR-IOM laboratory and allows the determination of the deformability and mechanical properties of cells. Alternatively, for samples with known height, the local refractive index can be measured. This has been applied to measure the volume of the nucleus relative to the cell, in the case of neutrophils. Another application has been the detection and counting of bacteria, leukocytes, and red blood cells in the stream of urine samples for rapid diagnosis of positives and negatives.

The instrument has a field of view of 250 x 160 mm, with optical magnification of 50X, nominal aperture of 0.6, lateral resolution of 6-700 nm, and vertical sensitivity of 10 nm. The sample is illuminated with a red He-Ne laser with emission at 632.8 nm and a power of 1 mW. The hologram is recorded on a 1920x1024-pixel, 5 mm/pixel CMOS camera. In the current configuration, the DHM instrument is placed on a table with attenuated mechanical vibrations, at room temperature, but the temperature can be controlled if necessary.


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The DHM microscope is located at the CNR-IOM laboratory in Trieste (Area Science Park Basovizza, S.S. 14 Km 163.5, building Q, room T54). The instrument is available for network activities for 25% of the machine time. Sample preparation will be assisted by laboratory managers and will be carried out in the dedicated space for this purpose at CNR-IOM. Data analysis will be supervised by CNR-IOM experts. The amplitude and phase of the object field will be numerically reconstructed after holographic registration using custom-developed software and/or Fiji plug-ins.

Instrument manager: Dr. Danut Adrian Cojoc (Senior Researcher CNR-IOM, specialist in optical and biophysical microscopy, designed and developed the instrument). Technical staff in charge: Catalin Dacian Cubotaru (specialist in optical microscopy and IT).

**MICRO-RAMAN SPECTROSCOPY**

Micro-Raman spectroscopy combines optical microscopy with Raman spectroscopy to provide detailed information about the molecular composition and structure of materials at a microscopic scale. In this technique, a laser is focused on a small area of the sample, and the scattered light is analyzed to identify the vibrational modes of the molecules. This provides chemical information without the need for extensive sample preparation or labeling.

*Microscope with Micro-Raman Apparatus*

This instrument allows analysis in Raman spectroscopy after local excitation with a continuous laser (wavelength 532 nm, power 100 mW), either by point spectroscopy or mapping. The spectrometer is a 320 mm Princeton Teledyne IsoPlane with three gratings (150 lines/mm, 600 lines/mm, and 1800 lines/mm) (Figure 29). The range of the Raman spectrum is between 150 cm-1 and 4000 cm-1. The apparatus is mounted on a reflecting microscope with a motorized XY scanning table (12.5 x 12.5 mm range, 200 nm resolution).

Raman spectroscopy can be used to identify the presence of specific molecules (or crystals), and in some cases also additional properties (i.e., defects, stoichiometry, thickness). The device can be used to obtain spectra of samples in various conditions (solid, liquid, and powder), as well as obtain a spectroscopic line profile and map of the surface.


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*Figure 29: The apparatus for Raman microscopy and spectroscopy available at UNG. On the right, the Princeton Teledyne IsoPlane spectrometer.*

The instrument is available at the Laboratory of Organic Matter Physics, University of Nova Gorica (Vipaska 11c, 5270 Ajdovščina, Slovenia), 6 hours per week are dedicated to ALL-MICRO network activities.

Responsible for the instrument: Dr. Vadym Tkachuk.


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