

SPIN-Lab Centre for Microscopic Research on Matter - basic lectures

A summer school student is required to attend all lectures in his or her primary discipline.

Contents

1. X-ray microtomography – a non-destructive 3D/4D imaging technique.....	1
2. Scanning Electron Microscopy (SEM): Operating Principles and X-ray Microanalysis (EDS & WDS).....	2
3. Sample preparation methods for electron microscopy: principles, SEM-FIB, ultramicrotomy	4
4. Transmission Electron Microscopy: Principles, Instrumentation and Applications	5
5. Other advanced techniques for structural and chemical analysis of materials. Raman/AFM/confocal microscopy.....	6

1. X-ray microtomography – a non-destructive 3D/4D imaging technique

Jolanta Rajca, MSc Eng.

1. Introduction to X-ray microtomography

- X-ray radiation:
 - radiation sources,
 - interaction of X-rays with matter,
 - origin and types of imaging artifacts.
- Components of a microtomograph (based on the UnitomHR scanner): X-ray source, detector, rotation stage, control software.
- Elements of the imaging process:
 - sample preparation and mounting,
 - data acquisition – selection of scanning parameters,
 - reconstruction of microtomographic data,
 - image processing and analysis.

2. Image processing and three-dimensional analysis

- Segmentation methods:
 - thresholding,
 - contouring-based segmentation,
 - watershed segmentation,

- segmentation assisted by deep learning methods.
- Morphological and logical (Boolean) operations in spatial structure analysis.
- Surface and volumetric reconstruction – generation of 3D models.
- Spatial analysis and quantitative evaluation – measurements of volume, porosity, layer thickness, and phase distribution.

3. Practical aspects of microtomography – applications in science and industry

- Applications of microtomography in various fields:
 - biomedicine: imaging of bone structures and implants,
 - biology: analysis of organism morphology and cellular structures,
 - materials engineering: studies of composites, porosity, and defects,
 - food industry: quality control and product structure assessment,
 - other areas: geology, automotive engineering, cultural heritage conservation.
- In-situ experiments – real-time imaging of physical and biological processes (4D imaging).

2. Scanning Electron Microscopy (SEM): Operating Principles and X-ray Microanalysis (EDS & WDS)

Łukasz Feldo-Grudziński BSc, Daniel Wójcik MSc

1. Introduction to Scanning Electron Microscopy (SEM)

Overview of SEM Technology: Historical development, evolution from optical to electron microscopy, and modern applications in materials science, geology, biology, and engineering.

SEM Instrumentation Components: Electron gun types (thermionic, field emission), electromagnetic lenses, apertures, scan coils, detectors, and vacuum systems.

Electron–Matter Interactions: Primary and secondary electron production, backscattered electrons, X-rays, and other signals generated by electron beam–sample interaction.

Imaging Modes and Contrast Mechanisms: Secondary electron imaging for topography and backscattered electron imaging for compositional contrast.

2. Principles and Mechanism of SEM Operation

Electron Beam Generation: Mechanisms of electron emission and control of beam parameters (current, spot size, energy).

Beam–Sample Interaction Volume: Depth and lateral extent of interaction, dependence on accelerating voltage and atomic number.

Signal Detection and Image Formation:

- Secondary electron detector (Everhart-Thornley) for surface morphology.

- Backscattered electron detector for atomic number contrast.
- Other detectors: in beam detectors, transmitted electron detectors and EBSD systems.

Image Resolution and Artifacts: Factors affecting resolution (probe diameter, working distance, astigmatism), and common image artefacts with troubleshooting approaches.

3. X-ray Microanalysis in SEM: Energy-Dispersive Spectroscopy (EDS)

Principles of EDS:

- Characteristic X-ray generation via inner-shell ionization.
- Energy measurement by silicon drift detector (SDD) or Si(Li) detector.
- EDS spectral interpretation and identification of elements.

Quantitative and Qualitative Analysis:

- Peak identification, background subtraction,
- Limits of detection and quantification accuracy.

Mapping and Line Scans: Elemental distribution mapping and compositional profiling.

4. Wavelength-Dispersive Spectroscopy (WDS)

Principle of WDS:

- Diffraction of characteristic X-rays by crystal spectrometers (Johann/ Johansson geometries).
- Role of analyzing crystals (TAP, PET, LiF) in wavelength discrimination.

WDS vs EDS:

- Higher spectral resolution and accuracy; slower acquisition speed.
- Complementary use in identifying overlapping peaks in EDS spectra.

Quantitative Analysis Using WDS:

- Calibration procedures with standards.
- Correction factors and precision in trace element measurement.

3. Sample preparation methods for electron microscopy: principles, SEM-FIB, ultramicrotomy

Jakub Jała MSc Eng., Daniel Wójcik MSc, Joanna Lis-Kłoda MSc Eng.

I. Sample preparation for electron microscopy techniques

1. *Introduction to sample preparation for electron microscopy techniques*

- Electron microscopy capabilities
- Electron beam – sample interactions
- Necessities and limitations in scanning electron microscopy
- Necessities and limitations in transmission electron microscopy
- Considerations for proper choice of sample investigation technique

2. *Sample preparation methods for scanning electron microscopy*

- Important aspects for sample preparation methodology
- Holder types and their application
- Conventional sample preparation (conductive samples)
- Preparation of non-conductive samples
- Uncommon samples preparation

3. *Sample preparation methods for transmission electron microscopy*

- Holders and materials for transmission electron microscopy
- Nanoparticle sample preparation
- Preparation of thin films for TEM investigation
- Biological sample examination

II. Fundamentals of the FIB-SEM Technique

1. *Introduction to FIB-SEM*

- History and types of FIB systems
- Construction and components of a FIB instrument
- Principle of operation and comparison of ion species (Xe, O, N, Ar, Ga) — advantages and limitations of each
- Capabilities and limitations of FIB
- Sample preparation for FIB-SEM analysis
- Gas Injection System (GIS)

2. *Fundamental Sample Analysis Techniques Using FIB-SEM*

- Cross-section milling
- Preparation of ultrathin sections (lamellae)
- 3D tomography
- Side effects of ion beam interaction — redeposition, curtaining, surface topography changes
- Methods for minimizing artifacts during FIB processing

III. Ultramicrotomy:

1. *Theoretical fundamentals of ultramicrotomy*

- The concept of microtomy and ultramicrotomy – scope of applications
- Principle of operation of the ultramicrotome – construction elements (cutting block, sample holder, diamond/glass knife, cooling system, drive mechanism)

2. *Sample preparation for ultramicrotomy*

- Stages of sample preparation: fixation, dehydration, embedding in resins

- Selection of supporting material and embedding media (epoxy and acrylic resins)
- Factors influencing cutting quality (material hardness, temperature, cutting speed)
- 3. *Types of ultramicrotomes and their applications*
 - Classical ultramicrotomes and cryo-ultramicrotomes
 - Applications in transmission electron microscopy (TEM), atomic force microscopy (AFM), and surface analysis
- 4. *Operation and maintenance of the ultramicrotome*
 - Classical ultramicrotomes and cryo-ultramicrotomes
 - Applications in transmission electron microscopy (TEM), atomic force microscopy (AFM), and surface analysis
- 5. *Applications of ultramicrotomy in scientific research*
 - Structural and morphological studies of nanomaterials, polymers, composites, and biological tissues
 - Combining ultramicrotomy with other techniques (TEM, SEM, AFM, EDX)
 - Modern trends in ultramicrotomy – automation, cryo-sectioning, integration with electron microscopy

4. Transmission Electron Microscopy: Principles, Instrumentation and Applications

Muhammad Farooq Saleem, PhD, Ania Hercog, MSc Eng.

PART I: fundamentals of transmission electron microscopy

- Operating principles and physical basics of TEM
- Microscope components and their functions
- TEM operational modes and analytical techniques
- Output data interpretation
- Sample preparation methods

PART II: introduction to cryo-tem microscopy

- Fundamental concepts of Cryo-TEM
- Technical requirements and method advantages
- Applications in various scientific fields
- Cryo-sample preparation process
- Analysis of typical data

5. Other advanced techniques for structural and chemical analysis of materials. Raman/AFM/confocal microscopy

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I. PART 1: Raman/AFM

1. Raman Spectroscopy

- Raman effect vs. Rayleigh scattering; Stokes and anti-Stokes bands (technique basics).
- Molecular vibrations; differences between Raman spectroscopy and IR.
- Parameters affecting band position and intensity.
- Spectrometer setup: light source, spectrograph, detector.
- Raman imaging and mapping.

2. Atomic Force Microscopy (AFM)

- Construction of an atomic force microscope; operating principle.
- Types of interactions in AFM: van der Waals, electrostatic, capillary forces.
- AFM operating modes: contact, non-contact, tapping.
- Imaging physicochemical surface properties.

3. Applications of the discussed techniques and their correlation

- Applications of Raman spectroscopy and AFM.
- Correlation of Raman spectroscopy and AFM (correlation of data/maps): capabilities.
- Limitations of Raman and AFM (outline of issues and pitfalls).

II. PART 2: Confocal microscopy

1. Sample preparation for confocal microscopy

- Material preparation
 - Fixation (chemical and physical methods)
 - Permeabilization (imaging intracellular structures)
 - Blocking (increasing binding specificity)
- Fluorescent staining
 - Types of fluorophores (fluorescent dyes, labelled antibodies, fluorescent proteins)
 - Staining sequence (e.g., cell nucleus, cytoskeleton, membranes)
 - Principles of fluorophore selection
 - Reduction of photobleaching
- Autofluorescence and in vivo observations
 - Natural compounds and structures exhibiting autofluorescence
 - Dyes for intravital observations
 - Ensuring conditions that maintain viability (CO₂ conc., temperature)

2. Principles of confocal microscopy

- Optical microscopy – introduction (optical microscope elements, resolution, magnification, contrast)
- Optical aberrations (chromatic, spherical, distortion, astigmatism, coma)
- Confocal microscopy (lasers, detector types, pinhole, inverted microscope)

- Basic parameters and settings (numerical aperture, excitation and emission wavelength)
- Fluorescence – phenomenon (ground and excited singlet states, Jablonski diagram, Airy disk, point spread function)
- Numerical aperture, immersion
- Photobleaching and photoquenching
- Fluorescence lifetime
- Data analysis (measurements in ImageJ, creation of 3D projections)