Group of Microscopy for Biomedicine

Department of Electron Microscopy

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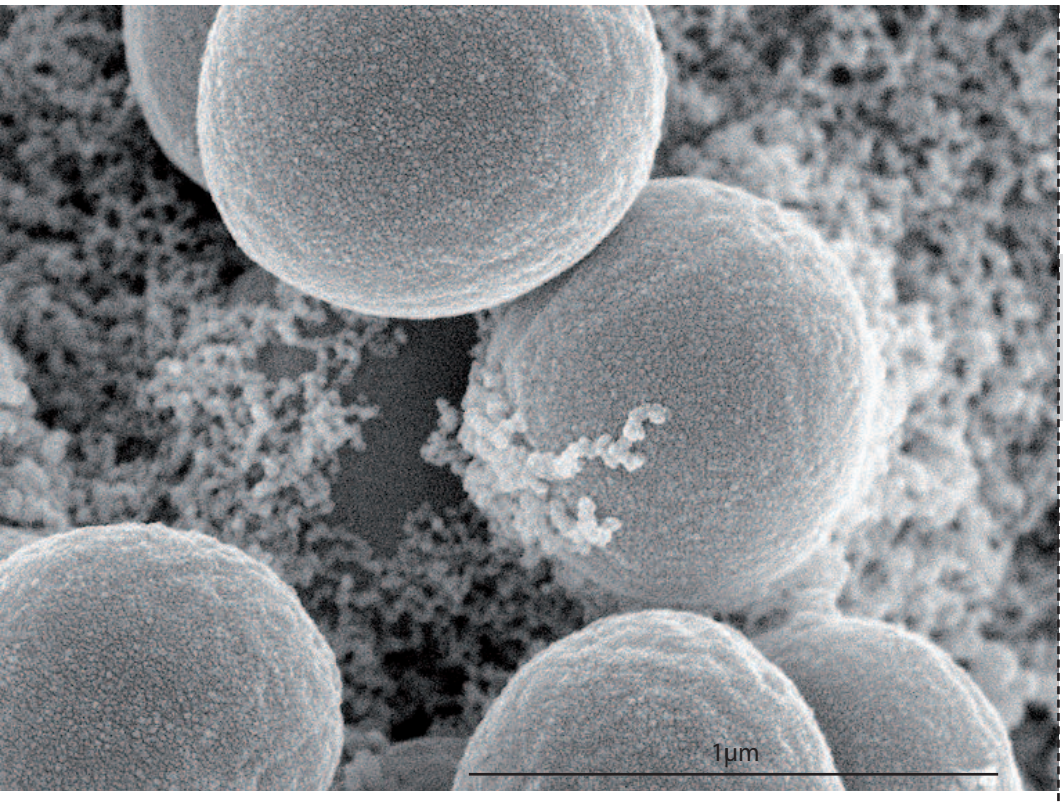


SEM micrograph of Staphylococcus epidermidis and its biofilm (combined chemical and physical sample preparation)

THEMATIC RESEARCH FOCUS

Research area

– Scanning electron microscopy (SEM)
– Scanning transmission electron microscopy (STEM)
– Quantitative imaging
– Cryo-electron microscopy
– Cathodoluminiscence

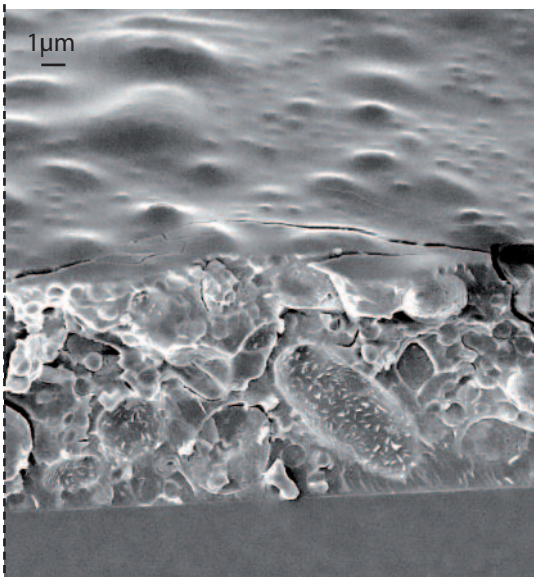
Excellence

– Quantitative imaging using annular dark-field mode in SEM, detection of very low signals in S(T)EM for quantitative imaging, mass measurement of macromolecular complexes
– Cathodoluminescence and photon collection in scintillators and screens for image formation in electron microscopy

Mission

– Development of quantitative imaging using SEM/STEM including hardware and software developments and specific sample preparation of biomedical and soft matter samples
– Improving the performance of imaging systems in electron microscopy and in other electron beam instruments

Freeze-fracture of microbial biofilm (Staphylococcus epidermidis, Candida parapsilosis)



Content of research

– Theoretical and experimental activities related to quantitative imaging using SEM/STEM of very thin samples, nanoparticles and macromolecular complexes
– Cryo-techniques in SEM/STEM
– Novel techniques in correlative microscopy
– Imaging of biomedical and soft-matter samples using SEM/STEM
– Cathodoluminescence kinetics of fast single crystal scintillators
– Design of scintillation detection systems with high efficiency of signal photon collection

Main capabilities

Basic research

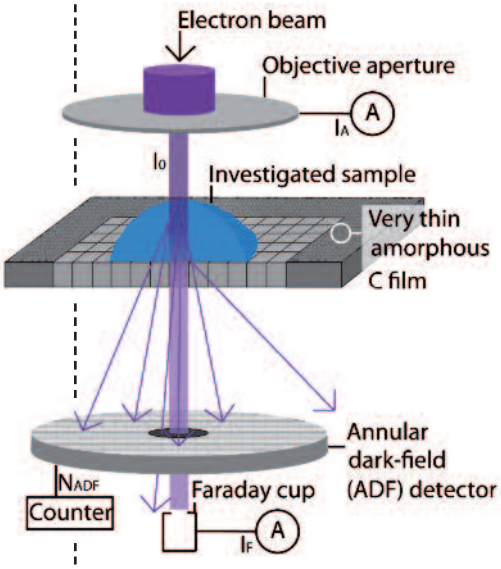
– Theoretical simulations of electron scattering for quantitative imaging
– Study of sample preparation in relation to quantitative imaging
– Efficiency and kinetics of cathodoluminescence using time-resolved spectroscopy in a large temperature range
– Study of very weak cathodoluminescence using Time Correlated Single Photon Counting (TCSPC)

Applied research

– New detectors for SEM/STEM of very low signals for quantitative imaging
– Cryo-preparation techniques
– Applications in quantitative imaging like mass-thickness mapping and molecular mass measurements
– Applications in cryo-SEM

Fields of research results application

– Electron microscopy
– Cryo-electron microscopy
– Measuring instruments
– Materials sciences (polymers, soft matter, etc.)
– Life sciences (molecular biology, tumor biology, biochemistry, etc.)
– Nanotechnology
– Microbiology
– Nuclear radiation detection



Principle of quantitative imaging in SEM using an annular dark-field detector

KEY RESEARCH EQUIPMENT

– Electron beam excitation unit with an electrostatic deflection system and a blanking diaphragm
– UV light collection and transmission system with a Horiba Jobin Yvon iHR320 spectrometer
– Utilization of equipment of the Group of Microscopy and Microanalysis
– Cryo-equipment extending the SEM Magellan 400 (FEI) to cryo-SEM

ACHIEVEMENTS

We started employing new methods of S(T)EM microscopy at ISI focused especially on the observation of biological objects. Related results from the Institute of Medical Physics and Biophysics at the University of Münster where the group leader worked are also included in the following list:

Quantitative electron microscopy

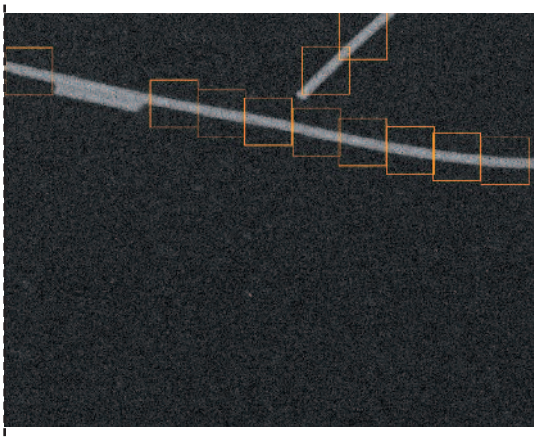
- Kouyianou, K.; De Bock, P.–J.; Müller, S. A.; Nikolaki, A.; Rizos, A.; Krzyžánek, V.; Aktoudianaki, A.; Vandekerckhove, J.; Engel, A.; Gevaert, K.; Tsiotis, G.; *The Chlorosome of Chlorobaculum tepidum: size, mass and protein composition revealed by electron microscopy, dynamic light scattering and mass spectrometry-driven proteomics.* Proteomics 11, (2011), p. 2867–2880
- Pfaff, M.; Miller, E.; Klein, M. F. G.; Colsmann, A.; Lemmer, U.; Krzyžánek, V.; Reichelt, R.; Gerthsen, D.; *Low-energy electron scattering in carbon-based materials analyzed by scanning transmission electron microscopy and its application to sample thickness determination.* Journal of Microscopy 243, (2011), p. 31–39
- Krzyžánek, V.; Müller, S. A.; Engel, A.; Reichelt, R.; *MASDET–A fast and user-friendly multiplatform software for mass determination by dark-field electron microscopy.* Journal of Structural Biology 165, (2009), p. 78–87
- Krzyžánek, V.; Reichelt, R.; *High-Resolution Scanning Electron Microscope for Mass Determination: Progress in the Development of a New Nanoanalytical Tool.* Microscopy & Microanalysis 13 (Suppl. 3), (2007), p. 80–81

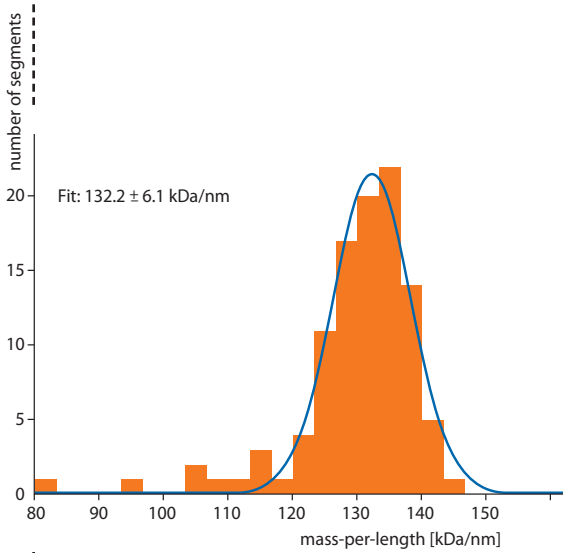
Characterisation of biological, biomedical and soft-matter samples using SEM and TEM techniques

- Groscurth, S.; Müller, B.; Schwan, S.; Diekstall, F.; Senft, M.; Kendall, A.; Kommor, B. A.; Neumann, U.; Kalischuk, M.; Kawchuk, L. M.; Krzyžánek, V.; Heilmann, A.; Stubbs, G.; Twyman, R. M.; Prüfer, D.; Noll, G. A.; *Artificial Forisomes Are Ideal Models of Forisome Assembly and Activity That Allow the Development of Technical Devices.* BioMacromolecules 13, (2012), p. 3076–3086
- Hillebrand, A.; Post, J. J.; Wurbs, D.; Wahler, D.; Lenders, M.; Krzyžánek, V.; Prüfer, D.; Schulze Gronover, C.; *Down-Regulation of Small Rubber Particle Protein Expression Affects Integrity of Rubber Particles and Rubber Content in Taraxacum brevicorniculatum.* PLoS ONE 7, (2012), e41874
- Ernst, A. M.; Jekat, S. B.; Zielonka, S.; Müller, B.; Neumann, U.; Rüping, B.; Twyman, R. M.; Krzyžánek, V.; Prüfer, D.; Noll, G. A.; *Sieve element occlusion (SEO) genes encode structural phloem proteins involved in wound sealing of the phloem.* PNAS 109, (2012), E1980–E1989
- Eissenberg, J. C.; Ilvarsonn, A. M.; Sly, W. S.; Waheed, A.; Krzyžánek, V.; Pohlmann, R.; Waschkau, D.; Kretzschmar, D.; Dennes, A. C.; *Drosophila GGA model: an ultimate gateway to GGA analysis.* Traffic 12, (2011), p. 1821–1838
- Krzyžánek, V.; Sporenberg, N.; Keller, U.; Guddorf, J.; Reichelt, R.; Schönhoff, M.; *Polyelectrolyte multilayer capsules: Nanostructure and visualisation of nanopores in the wall.* Soft Matter 7, (2011), p. 7034–7041.
- Edlmayr, J.; Niespodziana, K.; Popow-Kraupp, T.; Krzyžánek, V.; Focke-Tejkl, M.; Blaze, D.; Grote, M.; Valenta, R.; *Antibodies induced with recombinant VP1 from Human Rhinovirus exhibit cross-neutralization.* European Respiratory Journal 37, (2011), p. 44–52
- Grote, M.; Krzyžánek, V.; Reichelt, R.; *Bundles of hexagonally arranged tubules in timothy grass pollen: Detection of a novel pollen component using anhydrous fixation and image analysis techniques in transmission electron microscopy.* Journal of Microscopy 228, (2007), p. 34–39

We are engaged in the preparation and observation of hydrated samples by SEM, cryo-SEM, FIB-SEM and correlative microscopy.

- Dobranská, K.; Růžička, F.; Nebesářová, J.; Wepf, R. A.; Samek, O.; Krzyžánek, V.; *Characterisation of bacterial/yeast biofilms by scanning electron microscopy.* In: Proc. 15th European Microscopy Congress EMC2012, 16–21. September 2012, Vol. 1 Physical Sciences Applications, p. 671–672





Annular dark-field micrograph of Tobacco mosaic virus (TMV) and the mass distribution within 104 segments

We are the pioneers of algorithms of Computer Optimized Design of scintillation detectors for electron microscopy.

- Schauer, P.; *Extended Algorithm for Simulation of Light Transport in Single Crystal Scintillation Detectors for S(T)EM.* Scanning, 29, (2007), p. 249–253

We have developed and introduced into practice unique methods for the characterization of solids using cathodoluminescence.

- Schauer, P.; *Optimization of decay kinetics of YAG:Ce single crystal scintillators for S(T)EM electron detectors.* Nucl. Instr. Meth. B 269, (2011), p. 2572–2577
- Bok, J.; Schauer, P.; *LabVIEW-based control and data acquisition system for cathodoluminescence experiments.* Rev. Sci. Instrum. 82, (2011), p. 113109.
- Schauer, F.; Schauer, P.; Kuřitka, I.; Bao, H.; *Conjugated Silicon–Based Polymer Resists for Nanotechnologies: EB and UV Meditated Degradation Processes in Polysilanes.* Materials Transactions, 51, (2010), p. 197–201
- Horák, P.; Schauer, P.; *Cathodoluminescence as a method for the study of degradation of polysilanes.* Nucl. Instrum. Methods Phys. Res. Sect. B – Beam Interact. Mater. Atoms 252, (2006), p. 303–307

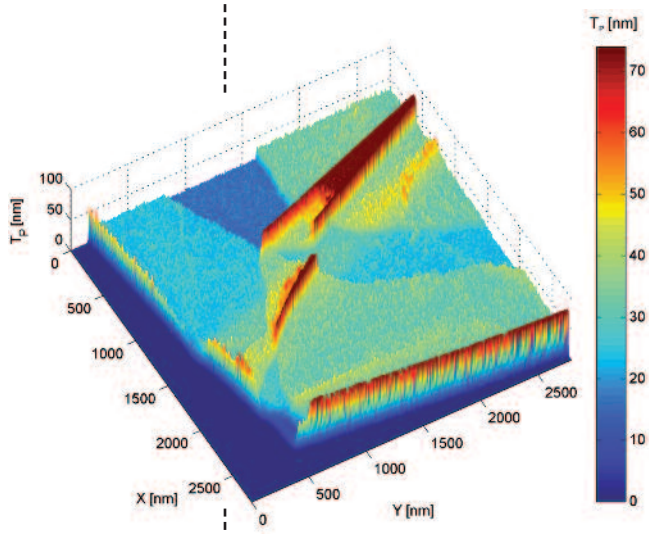
MAIN COLLABORATING PARTNERS

Collaboration with academic partners

University of Münster (Münster, DE)
University of Basel (Basel, CH)
ETH (Zurich, CH)
National Institute of Health (Bethesda, USA)
Physikalisch-Technische Bundesanstalt (Braunschweig, DE)
University of Manchester (Manchester, GB)
Brno University of Technology (Brno, CZ)
Masaryk University (Brno, CZ)
St. Anne's University Hospital (Brno, CZ)
Biology Centre of the ASCR (České Budějovice, CZ)
Institute of Physiology of the ASCR (Praha, CZ)
Institute of Macromolecular Chemistry of the ASCR (Praha, CZ)
Institute of Physics of the ASCR (Praha, CZ)
Tomáš Baťa University in Zlín (Zlín, CZ)

Collaboration with companies

Hitachi High-Tech (Tokyo, JPN)
TESCAN (Brno, CZ)
Crytur (Turnov, CZ)

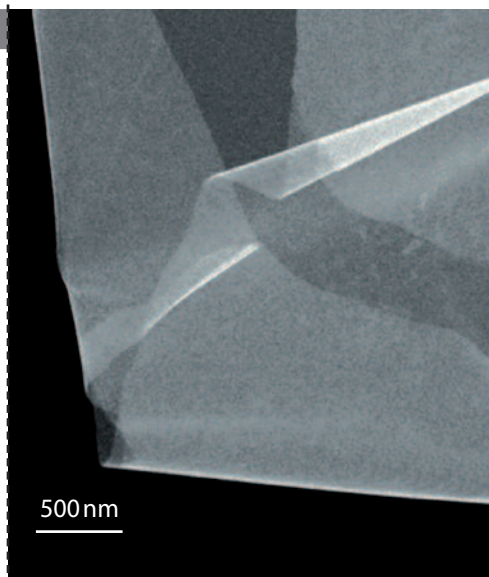


Randomly folded very thin carbon film: annular dark-field image (bottom) and projected thickness map (upper)

EXPECTATIONS

Offers

We offer our know-how in the areas of our expertise.

Requirements

We look for cooperation with academic partners as well as companies in the fields of electron microscopy, nanotechnologies, applications of SEM/STEM techniques including cryo-techniques to biological, medical and soft matter research, material characteristics, applications of scintillators and imaging screens.