Group of Microscopy for Biomedicine

Department of Electron Microscopy

Institute of Scientific Instruments
The Czech Academy of Sciences

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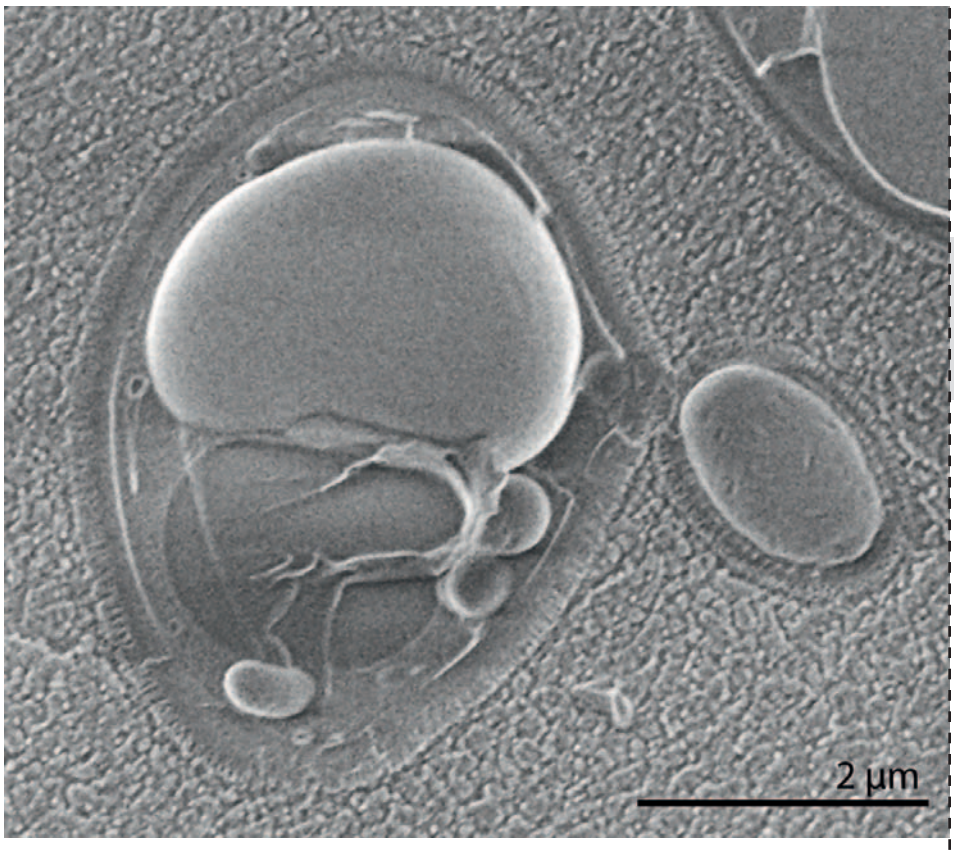


Cryo-SEM micrograph of Sporobolomyces shibatanus

THEMATIC RESEARCH FOCUS

Research area

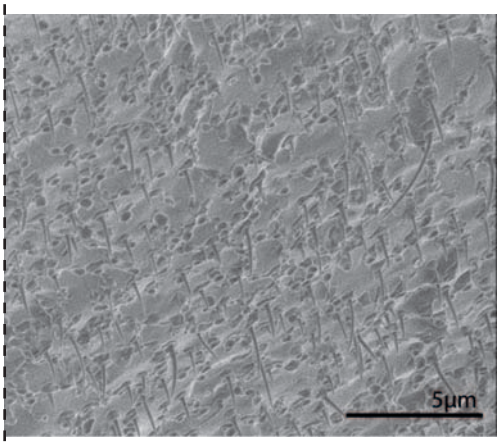
- Scanning electron microscopy (SEM)
- Scanning transmission electron microscopy (STEM)
- Cryo scanning electron microscopy (cryo-SEM)
- Quantitative imaging
- Cathodoluminiscence (CL)

Excellence

- Methodology and instrumentation of cryo-SEM of hydrated samples
- Quantitative imaging using annular dark-field mode in SEM, detection of very low signals, mass measurement with STEM
- Cathodoluminescence and photon collection in scintillators and screens for image formation in electron microscopy

Mission

To improve the performance of imaging and analytical systems in electron microscopy including developments of hardware, software and specific cryo sample preparation methods for soft and full-hydrated materials



Freeze-fracture of bacteria Cupriavidus necator containing PHB granules, elastic under LN2 temperatures

UP-TO-DATE ACTIVITIES

Research orientation

- Theoretical and experimental activities related to quantitative imaging using SEM/STEM of very thin samples and nanoparticles
- Development of cryo-techniques in SEM encompassing sample processing and imaging using combined signal detection
- Conventional SEM of biological and beam sensitive samples including EDX and CL analysis
- Cathodoluminescence kinetics of fast scintillators including thin and bulk single crystals
- Scintillation detection systems design with high signal photon collection efficiency

Main capabilities

Basic research

- Theoretical simulations of electron scattering for quantitative imaging
- Experimental activities related to sample preparation for quantitative imaging and low temperature SEM (physical fixation of hydrated samples, sublimation measurements etc.)
- Study of very weak cathodoluminescence using Time Correlated Single Photon Counting (TCSPC)
- Examination of cathodoluminescence efficiency and kinetics using time-resolved spectroscopy in a large temperature range

Applied research

- Design and production of cryo-devices for sample processing and detection systems for quantitative imaging
- Applications in cryo-SEM of biological and chemical samples, and in quantitative imaging such as mass-thickness mapping and mass loss measurements of soft materials
- Cathodoluminescence characterization of new scintillator materials

Sub-fields of group activities

- Electron microscopy
- Cryo-electron microscopy
- Materials sciences (polymers, soft matter, etc.)
- Life sciences (molecular biology, tumor biology, clinical and environmental microbiology)
- Nanotechnology
- Solid state physics
- Nuclear radiation detection

KEY RESEARCH EQUIPMENT

List of devices

- Cryo-equipment extending the SEM Magellan 400 (FEI) to cryo-SEM
- Cryo-high-vacuum preparation chamber ACE600 with freeze-fracture system, and cryo-vacuum transfer system VCT100 (Leica microsystems)
- Cryo-fixation systems (plunger, propane jet)
- Detection systems for quantitative STEM imaging
- Equipment for chemical processing of biological samples (chemical fixation, critical point dryer, etc.)
- 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

Equipment for cryo-SEM sample preparation

ACHIEVEMENTS

We have been focused on instrumental and methodological research in the fields of signal detection with its theoretical description and calibration, sample preparation and sublimation experiments for the low temperature SEM. In the last five years we published about 30 papers in impacted journals with a good citation response, and about 40 longer contributions in conference proceedings or local journals.

Cryogenic scanning electron microscopy (cryo-SEM)

Instrumental and methodological developments in cryo-SEM, e.g. combination of high-pressure-freezing on sapphire discs with cryo-SEM imaging.

- V. Krzyžánek, K. Hrubanová, J. Nebesářová, F. Růžička: "*Cryo-SEM of Perpendicular Cross Freeze-Fractures Through a High-Pressure-frozen Biofilm*", Microscopy and Microanalysis **20**, S3, 1232–1233, 2014
- FEI/CSMS scholarship awarded to Kamila Hrubanová (2015–2017) Structure investigations of bacterial and yeast biofilms using cryo-SEM
- O. Samek, S. Bernatová, J. Ježek, M. Šiler, M. Šerý, V. Krzyžánek, K. Hrubanová, P. Zemánek, V. Holá, F. Růžička: "*Identification of individual biofilm-forming bacterial cells using Raman tweezers*", Journal of Biomedical Optics **20**, 051038:1–6, 2015

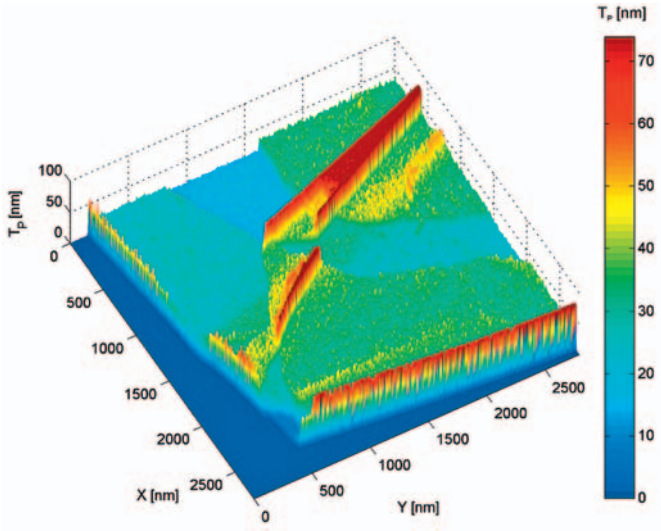
  Investigations of PHB granules produced by bacteria under specific conditions
- S. Obruca, P. Sedlacek, V. Krzyzanek, F. Mravec, K. Hrubanova, O. Samek, D. Kucera, P. Benesova, I. Marova: "*How accumulation of poly(3-hydroxybutyrate) helps bacterial cells to survive freezing*", PLoS ONE **11**, e0157778, 2016

Quantitative imaging using the transmission mode in SEM

Instrumental and methodological developments in quantitative imaging including samples preparation, electron scattering simulation, data recording with required calibrations.

- S. Tacke, V. Krzyzanek, H. Nüsse, R. Wepf, J. Klingauf, R. Reichelt: "*A versatile high-vacuum cryo transfer system for cryo microscopy and analytics*", Biophysical Journal **110**, 758–765, 2016
- V. Krzyžánek, S. Tacke, K. Hrubanová, R. Reichelt: "*Beyond Imaging: Scanning Electron Microscope for the Quantitative Mass Measurement*", Microscopy and Microanalysis **19**, S2, 130–131, 2013

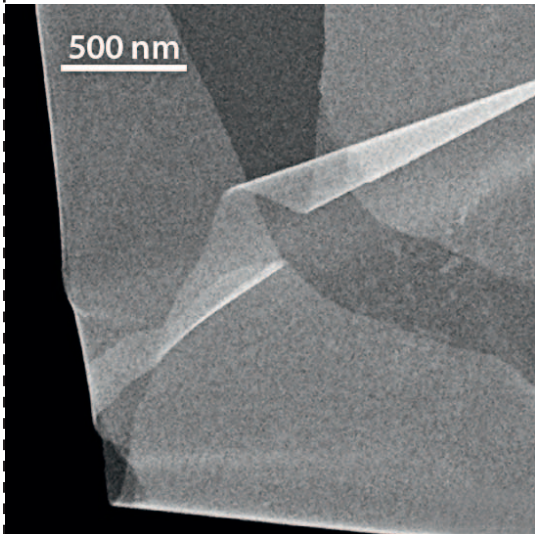
  Applications of STEM quantitative imaging.
- J. Hajduová, K. Procházka, V. Raus, M. Šlouf, V. Krzyžánek, V. M. Garamus, M. Štěpánek: "*Structure of polymeric nanoparticles in surfactant-stabilized aqueous dispersions of high-molar-mass hydrophobic graft copolymers*", Colloids and Surfaces. A - Physicochemical and Engineering Aspects **456**, 10–17, 2014
- V. Novotná, K. Hrubanová, J. Nebesářová, V. Krzyžánek: "*Investigation of Electron Beam Induced Mass Loss of Embedding Media in the Low Voltage STEM*", Microscopy and Microanalysis **20**, S3, 1270–1271, 2014



Randomly folded very thin carbon film: ADF STEM image and projected thickness map

Conventional electron microscopy of biological, medical and chemical samples

- S. Voběrková, S. Hermanová, K. Hrubanová, V. Krzyžánek: "*Biofilm formation and extracellular polymeric substances (EPS) production by Bacillus subtilis depending on nutritional conditions in the presence of polyester film*", Folia Microbiologica **61**, 91–100, 2016
- Y. Resch, K. Blatt, U. Malkus, C. Fercher, I. Swoboda, M. Focke, K.-W. Chen, S. Seiberler, I. Mittermann, C. Lupinek, A. Rodriguez-Dominguez, P. Zieglmayer, R. Zieglmayer, W. Keller, V. Krzyzanek, P. Valent, R. Valenta, S. Vrtala: "*Molecular, Structural and Immunological Characterization of Der p 18, a Chitinase-Like House Dust Mite Allergen*", PLoS ONE **11**, e0160641, 2016



Cathodoluminescence (CL) measurements
New method of performance characterization of SEM detectors.
- J. Bok, P. Schauer: "*Performance of SEM scintillation detector evaluated by modulation transfer function and detective quantum efficiency function*", Scanning **36**, 384–393, 2014
  Promising scintillator improving SEM detectors efficiency.
- J. Bok, O. Lalinský, M. Hanuš, Z. Onderišinová, J. Kelar, M. Kučera: "*GAGG:ce single crystalline films: New perspective scintillators for electron detection in SEM*", Ultramicroscopy **163**, 1–5, 2016
  Strong innovation of the CL apparatus enabling much deeper CL studies thanks to the temperature-controlled specimen holder.
- J. Bok, P. Schauer: "*Apparatus for temperature-dependent cathodoluminescence characterization of materials*", Measurement Science and Technology **25**, 075601, 2014
  Cooperation with CRYTUR company on improving scintillators.
- J. Bok, P. Horodyský, V. Krzyžánek: "*Effect of oxidation annealing on optical properties of YAG: Ce single crystals*", Optical Materials **46**, 591–595, 2015

For more details and publications see www.isibrno.cz/bioem.

MAIN COLLABORATING PARTNERS

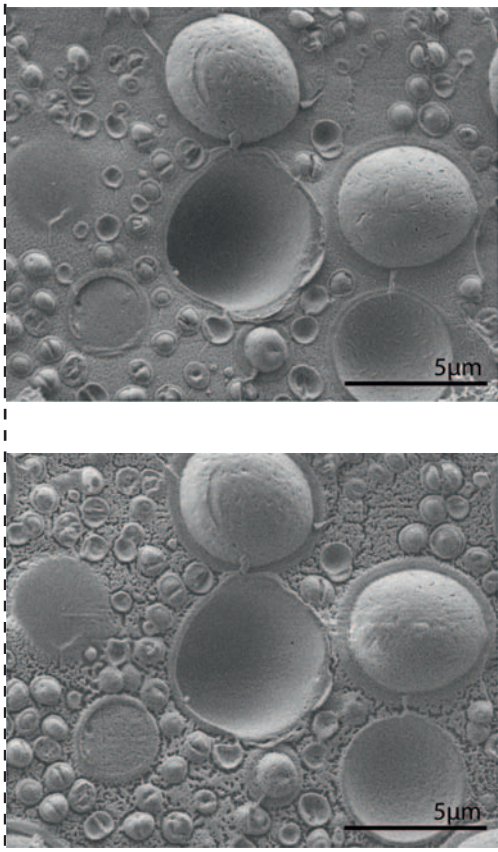
Collaboration with academic partners
- University of Münster (Münster, DE)
- ETH (Zurich, CH)
- National Institute of Health (Bethesda, USA)
- The University of Queensland (Brisbane, Australia)
- Brno University of Technology (Brno, CZ)
- Masaryk University (Brno, CZ)
- St. Anne's University Hospital (Brno, CZ)
- Biology Centre of the CAS (České Budějovice, CZ)
- Institute of Macromolecular Chemistry of the CAS (Praha, CZ)
- Charles University (Praha, CZ)
- Institute of Physics of the CAS (Praha, CZ)
- Tomáš Baťa University in Zlín (Zlín, CZ)

Collaboration with companies
- CRYTUR (Turnov, CZ)
- Microscopy Improvements (Eisenstadt, Austria)
- Leica Microsystems (Vienna, Austria)

EXPECTATIONS

Offers
- We offer collaboration in our areas of expertise
- Partnership in international projects

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 in biological, medical and soft matter research, material characteristics, applications of scintillators and imaging screens.



Progressive sublimation exposing inner structure of microbial biofilm (Staphylococcus epidermidis, Candida parapsilosis)