# Book of Abstracts

## 5th ASEM Workshop

*Advanced Electron Microscopy*

May 7th and 8th, 2015

Graz

Austrian Society for ASEM Electron Microscopy

# Content

### Scope of ASEM

The aim of ASEM is to provide an open forum for discussion between electron microscopists. This year's workshop on advanced electron microscopy will take place in Graz and will again bring together young and experienced electron microscopists. Two plenary lectures will be held by experts on their cutting research in both biomedicine and physics, and the winners of the 2014 Fritz-Grasenick-Prize will present their awarded work. 32 contributions from life sciences, material sciences, and physics reflect the broad range of applications of advanced electron microscopy.

## The organizing board

**A. Ellinger,** Medical University of Vienna

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# Program

**Thursday, May 7th 2015**

| | |
|---|---|
| 12:00-13:30 | **Registration** |
| 13:30-13:40 | **Opening and Greetings by Gerd Leitinger and Adolf Ellinger** |
| **1st Session** | Chairpersons: **H. P. Karnthaler, M. Pavelka** |
| 13:40-14:10 | **J. Radics** and **L. Königsmaier** (IMP and IMBA, Vienna)<br>*Structure of the Type III Secretion System in Action* |
| 14:10-14:25 | **W. Wallisch** (Vienna University of Technology, Vienna)<br>*SEM and TEM investigation for the characterization of $(Fe,Co)_{2-3}B$ alloys* |
| 14:25-14:40 | **D. Knez** (FELMI-ZFE, Graz)<br>*Electron Beam Induced Oxidation of Nickel Nanoclusters* |
| 14:40-14:55 | **T. Wojcik** (Vienna University of Technology, Vienna)<br>*Investigation of γ' precipitation in Ni-base superalloy PWA1480* |
| 14:55-15:10 | **F. Schmidt** (FELMI-ZFE, Graz)<br>*Plasmon Coupling on Silver Cuboids recealed by Fast Electrons* |
| 15:10-15:25 | **P. Gnauck** (Carl Zeiss Microscopy GmbH, Oberkochen/GER)<br>*A mirror corrected scanning electron microscope* |
| 15:25-16:00 | **Coffee Break** |

# Program

**Thursday, May 7th 2015**

**2nd Session** Chairpersons: **H. Plank, G. Resch**

16:00-16:30 **M. Stöger-Pollach** (Vienna University of Technology, Vienna)
*Determining Optical Properties using Low Voltage EELS and Bessel Beams*

16:30-16:45 **G. A. Zickler** (Vienna University of Technology, Vienna)
*Microstructural TEM-analysis of novel rare earth free permanent magnets*

16:45-17:00 **K. Kornmüller** (Medical University of Graz, Graz)
*Peptide-based architectures: morphology of a supramolecular self-assembled designer-peptide double helix*

17:00-17:15 **L. Konrad** (FELMI-ZFE, Graz)
*EEL and EDX spectroscopic sensitivity factors for the quantitative analysis of hard metals*

17:15-17:30 **A. Mittelberger** (University of Vienna, Vienna)
*Automated Image Aquisition for Low-Dose STEM Imaging*

17:30-17:45 **B. Völker** (University of Leoben, Leoben)
*Microstructure and chemistry evolution due to heat treatments after severe plastic deformation of a CrMnFeCoNi alloy*

17:45-18:00 **G. Raggl** (JEOL)
*Resolution and analysis – the JEOL GRAND ARM and dual EDS*

19:30-20:00 **Appetiser in Krebsenkeller**

20:00 **Conference Dinner**

# Program

**Friday, May 8th 2015**

**1st Session** Chairpersons: **T. Klepal, C. Rentenberger**

8:30-9:00 **W. Kaufmann** (IST Austria, Klosterneuburg)
*Ion channel localization in nanodomains of excitatory synapses in mammalian central neurons*

9:00-9:15 **C. Ebner** (University of Vienna, Vienna)
*2D strain tensor measurement of a metallic glass from elliptic electron diffraction patterns*

9:15-9:30 **H. Fitzek** (FELMI-ZFE, Graz)
*Simulating the pressure limiting system of Environmental Scanning Electron Microscopes using the direct simulation Monte-Carlo method*

9:30-9:45 **U. Hobusch** (Medical University of Graz, Graz)
*3D-reconstructions of a Locust Motion Detecting Pathway with Serial block Face Scanning Electron Microscopy (SBEM)*

9:45-10:00 **J. Kraxner** (FELMI-ZFE, Graz)
*Reliable quantification of X-ray spectra using ζ-factors: from standards to geometry*

10:00-10:15 **S. Noisternig** (University of Vienna, Vienna)
*Structural heterogeneities in Thin Foils of CuZr based Bulk metallic glasses*

10:15-10:30 **E. Korkmaz** (FEI)
*New integrated solution for 3D isotropic volume imaging and reconstruction of biological samples using SEM*

10:30-11:00 **Coffee Break**

**Friday, May 8th 2015**

**2nd Session** Chairpersons: **W. Salvenmoser, A. Ellinger**

11:00-11:15 **T. Ganner** (FELMI-ZFE, Graz)
*Artificial Substrates as Key Element towards Single Enzyme Tracking via High Speed Atomic Force Microscopy in Enzymatic Degradation of Cellulose*

11:15-11:30 **C. Nagelreiter** (University of Vienna, Vienna)
*Electron microscopy in topical drug delivery and cosmetics*

11:30-11:45 **M. Nachtnebel** (FELMI-ZFE, Graz)
*Characterization of microfiltration membranes by in-situ wetting in the ESEM and FT-IR mapping*

11:45-12:00 **T. Heuser** (Vienna Biocenter, Vienna)
*The central pair complex of cilia visualized by cryo-electron tomography*

12:00-12:15 **P. Wan** (Beihang University, Beijing)
*Nitrogen atom shift triggered the structural evolution in chromium nitride ---from hexagonal to cubic phase*

12:15-12:30 **M. Reisinger** (Erich Schmid Institute of Materials Science, Leoben)
*Microbending tests with a PicoIndenter*

12:30-12:45 **R. Winkler** (FELMI-ZFE, Graz)
*High-Fidelity Shapes and Disruption Mechanism during Focused Electron Beam Induced Deposition*

12:45-13:15 **Coffee Break**

# Program

**Friday, May 8th 2015**

**3rd Session** Chairpersons: **U. Lütz-Meindl, F. Hofer**

13:15-13:30 **W. Salvenmoser** (University of Innsbruck, Innsbruck)
*Electron Microscopy and Molecular Biology, two tools for understanding functional biology*

13:30-13:45 **S. Hummel** (University of Vienna, Vienna)
*Amorphization of Graphene on the μ-scale by Electron Irradiation*

13:45-14:00 **A. Orthacker** (FELMI-ZFE, Graz)
*Analytical Electron Tomographic Investigation of an Aluminium Alloy with Nano-Precipitates*

14:00-14:15 **T. Schachinger** (Institute of Solid State Physics, Vienna)
*Generation of Bessel Beams with Extremely High Orbital Angular Momentum*

14:15-14:30 **H. Plank** (FELMI-ZFE, Graz)
*Beyond Current SEM – AFM Solutions: A Highly Flexible in-situ AFM for Correlated Microscopy*

14:30-14:40 **Closing Words**

# Sponsors

ASEM 2015 is supported by:




# Structure of the Type III Secretion System in Action

Julia Radics[1,2] and Lisa Königsmaier[1,2]

(1) *Research Institute of Molecular Pathology, Vienna, Austria*
(2) *Institute of Molecular Biotechnology Austria, Vienna, Austria*

Type III secretion systems (T3SSs) are complex macromolecular machines present in many pathogenic Gram-negative bacteria. T3SSs are able to inject proteins from the bacterium directly into eukaryotic host cells by crossing the inner and outer bacterial membrane and the host cell membrane. The injected effector proteins (toxins) trigger host cellular responses essential for the bacterial pathogenicity, which, depending on the bacterial species, can result in a diverse set of diseases, ranging from mild headaches to fatal outcomes such as the plague or hemorrhagic colitis.

The core structure of the T3SS is the syringe-like needle complex (NC) embedded in both bacterial membranes with a protruding needle filament. Recent structural and biochemical studies have revealed insights into the topology and structure of this ~ 3.5 MDa membrane complex which is assembled of five main structural proteins (InvG, PrgH, PrgK, PrgJ, PrgI) each present in multiple copies. Moreover, a group of membrane proteins (SpaP, SpaQ, SpaR, SpaS, InvA) was identified, which plays a role in the assembly of the substructure within the NC called export apparatus and is essential for substrate secretion. However, little is known about the secretion process itself, presumably because it occurs so rapidly that it is difficult to trace effector proteins during their transport through the T3SS. It is generally assumed that effector proteins are transported through the elongated cavity of the NC, but direct structural evidence for this hypothesis is missing.

In this study, concatemeric substrates of the Salmonella T3SS were designed which were tagged at their C terminus with green fluorescent protein (GFP). The thermodynamically stable GFP domain trapped the substrate proteins inside the NC, enabling to isolate needle complexes with associated substrates by gradient centrifugations and immuno-precipitations.

Single Particle analysis from cryo electron micrographs of NCs and cryo electron tomography of Salmonella expressing GFP-tagged substrates visualized substrates inside the NC, providing direct evidence for the hypothesis that effector proteins are transported through the cavity of the NC. Moreover, immuno-gold labeling of the GFP identified the entry position of substrates into the NC.

Furthermore, data revealed that the secretion process of substrates, based on the substrate SptP occurs in a polar fashion with the N terminus first and that substrates have to be unfolded before they can enter the NC. These data show for the first time a substrate during transport within the T3SS and provide a structural basis for further functional studies.

# SEM and TEM investigation for the characterization of $(Fe,Co)_{2-3}B$ alloys

W. Wallisch[1,2], P. Toson[1], M. Stöger-Pollach[1,2], J. Bernardi[2] and J. Fidler[1]

*(1) Vienna University of Technology, Institute of Solid State Physics, Vienna, Austria.*
*(2) Vienna University of Technology, USTEM, Vienna, Austria.*

An enhanced research of alternatives to permanent magnets containing rare earth elements were provided and the ambition to reduce or to replace the rare earth content is to find new magnetic materials, which are able to compete the current high performing permanent magnet devices [1]. In the recent past, $(Fe,Co)_{2-3}B$ alloys have become the focus of the study due to their high magnetocrystalline anisotropy of $K_1 = 0.41$ MJ/m³ [2].

Cast ingots of different $(Fe_{1-x}Co_x)_{71}B_{29}$ ($0 \leq x \leq 1$) compounds have been synthesized by induction melting in inert atmosphere. Melt-spun ribbons were prepared by a melt spinning facility with various wheel speeds in the range of 18 m/s to 38 m/s under a constant pressure difference of 200 mbar. The microcrystalline microstructure of the cast material as well as of the melt-spun ribbons was analyzed by scanning electron microscopy (SEM, FEI Quanta 200 FEGSEM) and transmission electron microscopy (TEM, FEI TECNAI F20). The SEM investigations of the melt-spun ribbons reveal a homogeneous structure with randomly orientated grains (Fig. 1.a). The bright field image of sample 1700 exhibits precipitates, which decorated the grain boundaries. In the diffraction pattern (Fig. 1.b top-right) different $(Fe,Co)_2B$ grains were identified. In addition, the crystallography of selected ribbons was investigated by X-ray diffraction. Furthermore, the magnetic properties of the cast material and the melt-spun ribbons were measured by vibrating sample magnetometer and compared to density functional theory calculations. In total, $(Fe_{0.7}Co_{0.3})_{71}B_{29}$ alloys are a potential solution for novel hard magnetic materials in the gap between hard ferrites and rare earth magnets [1].

The funding from the European Community's Seventh Framework Programme (FP7-NMP) under grant agreement n° 280670 (REFREEPERMAG) is acknowledged.

[1] Wallisch W., Fidler J., Toson P., Sassik H., Svagera R., Bernardi J., 2015. Submitted.
[2] Iga A., 1970. Japan Journal of Applied Physics 94, 415.

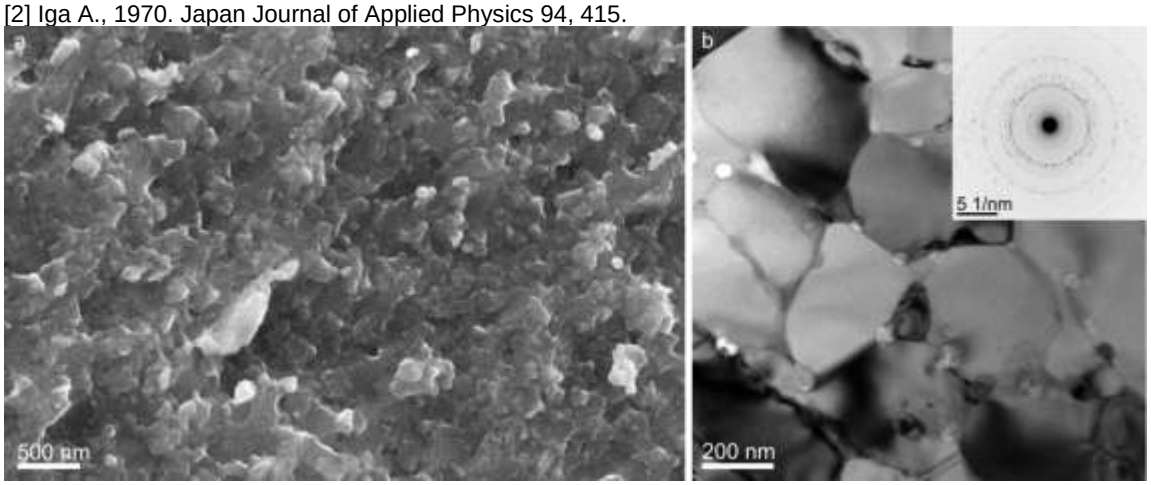


Fig. 1: a) SEM investigation of sample 1700 shows homogeneous structure with randomly orientated grains. The grain size is in the order of 300 nm. b) Bright field image of sample 1700 with a polycrystalline diffraction pattern. The size of the grains is consistent with the SEM investigation.

# ELECTRON BEAM INDUCED OXIDATION OF NICKEL NANOCLUSTERS

Daniel Knez[(1)], Alexander Volk[(3)], Philipp Thaler[(3)], Werner Grogger[(1,2)], Wolfgang Ernst[(3)], Ferdinand Hofer[(1,2)]

(1) *Institute for Electron Microscopy and Nanoanalysis, Graz University of Technology, Graz, Austria*
(2) *Graz Centre for Electron Microscopy, Graz, Austria*
(3) *Institute of Experimental Physics, Graz University of Technology, Graz, Austria*

Electron beam induced oxidation and rearrangement of metallic nanostructures, driven by the nanoscale Kirkendall effect, was reported recently by several authors [1]. However, we report the creation of hollow NiO nanoclusters consisting of less than 2000 nickel atoms with electron beam induced oxidation in a scanning transmission electron microscope (STEM).

Nickel clusters with extremely high purity were grown inside superfluid helium nanodroplets at 0.37 K under ultra-high vacuum (UHV) conditions [2].

We draw conclusions about elemental composition and morphology of these clusters before, while and after oxidation *in-situ* and give new insights into the oxidation mechanisms of nickel clusters on different substrates, which differ in the amount of initially adsorbed oxygen (see Figure 1).

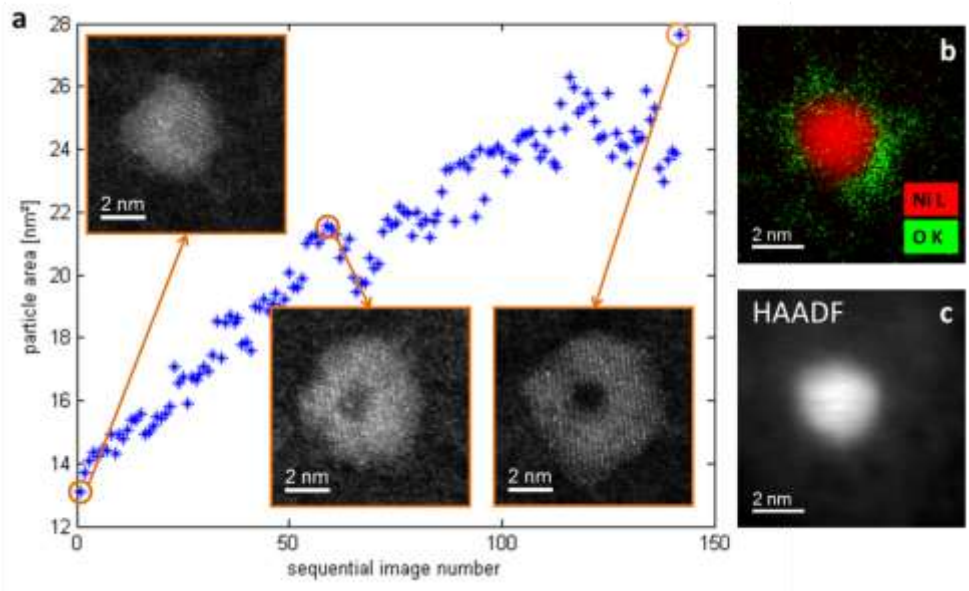


Figure 1: (a) Lateral enlargement of a Ni-Cluster (with ~1500 atoms) over time during electron induced oxidation on 3 nm amorphous carbon, calculated from a HAADF image series (142 images over 447 s, 3 µs dwell time, I~90 pA); Insets show the cluster at 3 different states: initial, after 60 images and final state; (b) EELS elemental map of an unoxidized Ni cluster on Graphene (red: Ni L, green: O K) and (c) simultaneously recorded HAADF signal

[1] E. A. Lewis et al., Nanoscale. 6, 13598–13605 (2014).

[2] P. Thaler et al., Phys. Rev. B. 90 (2014).

# Investigation of γ' precipitation in Ni-base superalloy PWA1480

E. Povoden-Karadeniz[c], M. Kozeschnik[b], T. Wojcik[c], E. Kozeschnik[a,c]

[a] *Christian Doppler Laboratory for Early Stages of Precipitation, Institute of Materials Science and Technology, Vienna University of Technology*

[b] *Department of Physics, University of Technology Graz*

[c] *Institute of Materials Science and Technology, Vienna University of Technology*

Interrupted cooling experiments are capable of discovering the start temperature of the earliest stages of precipitation. With this method, the start temperature of γ' precipitation in single crystal Ni-base superalloy PWA1480 was determined inside 5 K. Solution-treated specimens were cooled stepwise to different temperatures above and below the estimated temperature, where nucleation of γ' occurred first. The microstructures resulting from these cooling experiments were explored by transmission electron microscopy. This $(Ni,Co)_3(Al,Ti)$ hardening phase is coherent with the matrix and was investigated using dark field imaging. Development from initially cuboid, diffuse particles via octo-cubes towards octo-dendrites was shown as a function of interrupting temperature. Differential scanning calorimetry, prior to the interrupted cooling experiments, was used to identify the temperature range of the main event of γ' formation. The experimental heat flow, as well as particle size and distribution showed a good agreement with thermo-kinetic precipitation simulations.

# PLASMON COUPLING ON SILVER CUBOIDS REVEALED BY FAST ELECTRONS

F. P. Schmidt[1, 2], H. Ditlbacher[2], U. Hohenester[2], F. Hofer[1] and J. R. Krenn[2]

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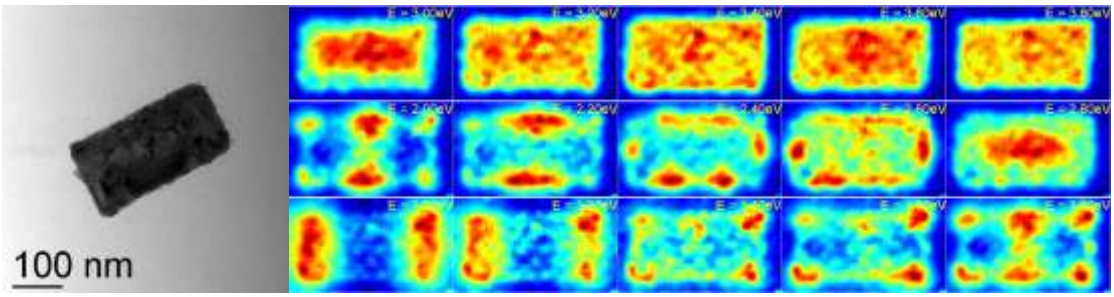
*(2) Institute of Physics, University of Graz, Graz, Austria*

Plasmonic nanostructures allow the concentration of light to nanoscale volumes. They are therefore interesting systems for fundamental research and application oriented studies in optics, photovoltaics and sensor technology. Surface plasmons, i. e., resonant collective electron oscillations play an important role in this quest.

Electron energy loss spectroscopy (EELS) in combination with scanning transmission electron microscopy (STEM) has become a powerful technique to study surface plasmons on metal nanoparticles and is used in this work to resolve the corresponding electromagnetic fields with high spatial resolution. The particle design was realized by electron beam lithography on a 15 nm thin silicon nitride membrane.

While previous work has shown that plasmons on nanometric sized particles can be decomposed into film and edge plasmons [1], here we study a silver nanocuboid to reveal the role of plasmon coupling of those excitations. In addition to experimental results, simulations based on a boundary element approach [2] are presented, which help to understand the rich spectrum of plasmonic eigenmodes in this system and its coupling behaviour. It will be shown that the majority of eigenmodes can be assigned to bonding and antibonding edge modes.

a) b)




a) Lithographed silver cuboids: TEM image of a 30 nm thick silver cuboid on a 15 nm thin $Si_3N_4$ membrane. b) Electron energy loss maps acquired on the particle shown in a) at energies ranging from 1 eV (bottom, left) to 3.8 eV (top, right) with an energy-width of 150 meV.

[1] F.P. Schmidt et al, 2014. Nature Communications 5, 3604.

[2] U. Hohenester and A. Truegler, 2012. Comp. Phys. Commun. 183, 370.

[3] The authors gratefully acknowledge funding from the Austrian Science Fund under grant numbers P21800-N20 and P24511-N26, ESTEEM2 (FP7 project, No.312483), SFB F49 NextLite and the Graz Center for Electron Microscopy.

# A mirror corrected scanning electron microscope

Peter Gnauck, Wolfgang Horn

Carl Zeiss Microscopy, Carl Zeiss Str. 22, 73447 Oberkochen, Germany

In materials science and biological research the Scanning Electron Microscope (SEM) has a long tradition. In recent years the interest in the imaging of sensitive samples and the material contrast at a high lateral resolution has grown. Lowering the primary electron energy helps to reduce the sample damage. On the other hand the interaction volume is decreased, thus increasing the lateral information from the backscattered electron signal.

However, the low primary electron energy is extremely demanding to the electron optics, if not too much of the lateral resolution should be lost due to the increased wavelength of the electrons. In a suitable instrument typically the spherical and the axial chromatic aberration have to be corrected. Additionally, innovative detector schemes can provide enhanced analytic capabilities and can avoid limitations by signal noise and residual instrumental instabilities.

We will discuss a mirror-corrected SEM, offering high-resolution analytics with efficient productivity to visualize even the most sensitive materials by use of electrons with energies far below 1keV. At these energies the resolution of conventional instruments is very poor, but compensating for the primary aberrations of the objective lens can overcome this obstacle. The aberration correction by means of an electron mirror significantly increases the resolution especially for low energies; this has been proven in a unique spectro-microscope.

# Determining Optical Properties using Low Voltage EELS and Bessel Beams

Michael Stöger-Pollach[(1)], Thomas Schachinger[(1)], Walid Hetaba[(1)] and Daniel Abou-Ras[(2)]

*(1) University Service Centre for Electron Microscopy, Vienna University of Technology, Wiedner Hauptstraße 8-10, 1040 Vienna, Austria*
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Very recently, low voltage electron microscopy has transformed into a state-of-the-art technique for materials science [1]. Although its main area of application can be found in the analysis of 2D materials and beam sensitive structures, it becomes more and more relevant in semiconductor science, too. In the present work we discuss applications on valence electron energy loss spectrometry (VEELS) for studying optical properties and band gap variations.

For VEELS of semiconductors low beam energies have a unique advantage over high beam energies: the velocity of the swift probe electron can be kept that small, that the Čerenkov effect can be avoided. Thus only transition radiation has to be taken into account when optical properties have to be determined from the probed material. In the present study we demonstrate the measurement of bandgaps of a Cu(In,Ga)$Se_2$ solar cell, where an In/Ga gradient can be observed within the optically active layer. We use a 20 keV electron beam and a collection angle of 13 mrad for the spatially resolved experiments and find a band gaps in the range of 1.11 eV for $CuIn_{0.82}Ga_{0.18}Se_2$ to 1.55 eV for $CuIn_{0.18}Ga_{0.82}Se_2$.

But for increasing the spatial resolution in electron spectrometry low beam energies are not always an advantage over higher ones. Especially because slower electrons can be focused worse with respect to faster ones and they are more easily deflected by external magnetic fields. Thus small instabilities are the consequence. Additionally the inelastic delocalization is reduced only a little when decreasing the beam energy. We discuss new pathways for circumvent both, the instabilities by using higher beam energies, and the inelastic delocalization by using conical dark-field STEM-VEELS using a Bessel beam. With this new set-up we automatically avoid the collection of electrons having excited Čerenkov photons and improve the spatial resolution of the STEM probe. For the Silicon plasmon loss we gain a delocalization of 5.9 Å (instead of 20 Å being measured under conventional STEM conditions).

[1] U. Kaiser, M. Stöger-Pollach (Eds.) Ultramicroscopy 145 (2014): Special Issue “Low Voltage Electron Microscopy”

# Microstructural TEM-analysis of novel rare earth free permanent magnets

Zickler G.A.[(1)],Toson P.[(1)],Asali A.[(1)],Tozman P.[(2)],Venkatesan M.[(2)],Coey J.M.D.[(2)] and Fidler J.[(1)]

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The search for novel rare earth free permanent magnets is of great scientific and economic interest. The $YCo_5$ material is a promising candidate to meet the requirements of a hard magnet, like a strong magnetic anisotropy and high energy density product.

$YCo_5$ crystallizes in the hexagonal $CaCu_5$-structure with the *P6/mmm* (191) space group and the lattice constants a = 0.4950 nm and c = 0.3973 nm [1]. The magnetic properties of the bulk material with an anisotropy field $H_A$ of 0.13 T can be significantly improved up to 0.83 T by ball milling, flash annealing (FA) in argon, vacuum annealing (VA) and the substitution of Co with Fe, resulting in optimal stoichiometric composition of $YCo_{4.8}Fe_{0.2}$.

TEM/STEM investigations, including selected area electron diffraction (SAED), high resolution high angle annular dark field imaging (HR-HAADF) and EDX analysis, were carried out on an as-milled (AM), a FA (850°C for 2 min.) and a VA (850°C for 2 min.) sample. Due to the small particle size of about 20 µm after the ball milling of the bulk material, the samples hat to be prepared with the focused ion beam lift out technique. The AM sample possesses inhomogeneous mostly amorphous microstructure. During the annealing $YCo_{4.8}Fe_{0.2}$ grains are forming, leading to a homogeneous microstructure and an average grain size of 24 nm in the VA sample (Fig.1a) and 98 nm in the FA sample. The $YCo_5$ structure was identified by SAED imaging with a very good agreement to the simulated X-ray powder diffraction (XRD) spectrum (Fig.1b).

The financial support from the EC-FP7 project ROMEO (no: 309729) is acknowledged. This TEM investigation was carried out using facilities at USTEM, TUW, Austria.

[1] Pareti L., Moze O., Solzi M., Bolzoni F. Journal of Applied Physics **63** (1988) 172-175

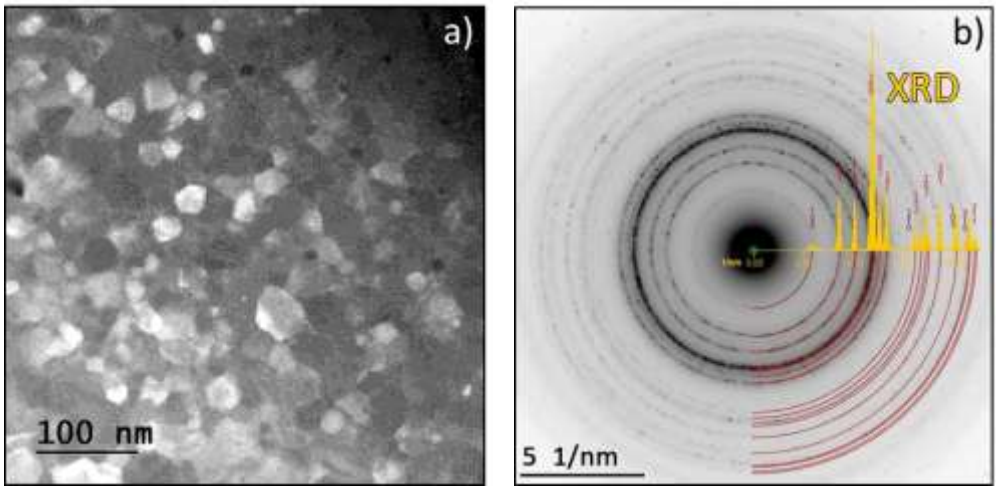


Figure 2: a) STEM image showing the nanocrystalline microstructure of the VA sample;
b) the polycrystalline diffraction pattern in the SAED image is in agreement with the simulated XRD-spectrum.

# Peptide-based architectures: morphology of a supramolecular self-assembled designer-peptide double helix

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*(2) Institute for Electron Microscopy and Nanoanalysis, Graz University of Technology and Graz Centre for Electron Microscopy, Steyrergasse 17, 8010 Graz, Austria*
*(3) Research Unit Electron Microscopic Techniques, Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Harrachgasse 21, 8010 Graz, Austria*
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*(5) Institute of Inorganic Chemistry, Graz University of Technology, Stremayrgasse 9/4, 8010 Graz, Austria*

A very active area of research aims to use peptide-based nanostructured materials as biomimetic artificial matrices in nanomedicine [1]. In our studies, self-assembling amphiphilic designer-peptides serve as building blocks. To fully exploit the potential of these molecules, it requires a deep understanding of the peptides' individual, as well as their collective morphology, the underlying dynamic assembly mechanisms and how these materials act at the interface of synthetic and biological membranes. By combining three different transmission electron microscopy (TEM) techniques (freeze-fracture, negative-staining and cryogenic TEM) and Synchrotron small angle X-ray scattering (SAXS), we studied the concentration-dependent self-assembly of an 8-residue amphiphilic designer-peptide. Above a critical aggregation concentration this peptide forms a variety of structural intermediates and finally develops to unique supramolecular double helices with lengths of several hundreds of nanometers and uniform diameters of about 24 nm. In addition, SAXS allowed a detailed look at the internal organization of the structures: we propose a 3-layered model, where monomers are interdigitated and tightly packed, held together by multiple weak noncovalent bonds. The double helices were finally intertwined to a network, showing hydrogel properties. These double helices remained structurally unaltered for several months [2]. To probe the interaction of peptide-assemblies with artificial membrane mimicks, we performed TEM, SAXS and differential scanning calorimetry (DSC). Even at high concentrations the membrane integrity remained unaffected by the peptides' presence. Based on our results, this peptide has a high potential to meet the needs of a next-generation biomaterial in future medical and technological applications.

[1] Luo, Z., Zhang, S., 2010, *Chem. Soc. Rev.* **41** 4736-4754.

[2] Kornmueller, K., Letofsky-Papst, I., Gradauer, K., Mikl, C., Cacho-Nerin, F., Leypold, M., Keller, W., Leitinger, G., Amenitsch, H., Prassl, R., 2015, *Nano Res.* (in press)

# EEL and EDX spectroscopic sensitivity factors for the quantitative analysis of hard metals

Lukas Konrad[(1)] – Martina Lattemann[(3)] – Johanna Kraxner[(1)] – Daniel Knez[(2)] – Angelina Orthacker[(1)] – Werner Grogger[(1,2)] – Gerald Kothleitner[(1,2)]

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*(2) FELMI Institute for Electron Microscopy and Nanoanalysis, Tu Graz, Steyrerg. 17, 8010 Graz, Austria*

*(3) Sandvik Coromant, R&D Materials and Processes, Lerkrogsvägen 19, 126 79 Hägersten, Stockholm, Sweden*

We are focusing on the analytical characterization of hard metals and ceramics used as tooling materials by electron energy-loss (EEL) and energy dispersive X-ray (EDX) spectroscopy

EELS is often described as an absolute, standardless quantification technique. However, upon quantification, some of the parameters like inner-shell ionization cross-sections, calculated from analytical models (hydrogenic approximations or Hartree Slater oscillator strengths) needed to turn intensities into concentrations turn out to be erroneous. Alternatively, EELS cross-sections can be determined from samples with known composition.

A new approach would be to get these scaling factors out from ab initio multiple scattering calculations, as implemented in the FEFF 9 code [1]. With this tool one can calculate EEL spectra (and energy differential cross-sections) based on Green's functions theory.

For EDX spectroscopy, the ζ–factor approach [2] has proven to be superior for elemental quantifications, particularly when absorption has to be considered. From an experimental point of view, ζ–factors and inner-shell ionization cross-sections (σ) can be converted into each other [3] by: $\zeta*\sigma = [I^E/(I^E_0*I^X)]*[AW/N_{AV}]*D$ (with: $I^E$= core loss intensity; $I^E_0$ = corresponding low-loss integral; $I^X$= X-ray intensity; AW= atomic weight; $N_{AV}$ = Avogadro's number). Hence, by a proper scaling with simultaneously acquired experimental EELS and EDX intensities, EDX quantifications should be possible even for lighter elements, starting from multiple scattering calculations.

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# Automated image acquisition for low-Dose STEM imaging

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The recent development in aberration-corrected electron optics opened the route to imaging beam-sensitive materials with atomic resolution by reducing the acceleration voltage to values below the knock-on damage threshold (e.g. about 80 kV for graphene and related materials) [1]. However, even at beam energies of 60 kV and below, beam driven dynamics prevent the observation of the pristine state of highly beam-sensitive structures like certain defects in graphene or single molecules (functional groups) attached to the graphene surface. Besides using low voltages, a reduction of the the electron dose is a possible way to reduce or circumvent beam damage in such samples.

Recently we have demonstrated a new method to extract information from large, very low dose datasets (using simulated data). Although no atomic structure is visible anymore in the single low-dose images, we have shown that it is still possible to extract information from statistical correlations in the data [2]. In order to make it possible, up to a few µm² have to be scanned with atomic resolution, and at very low dose. Applying this approach to experimental data hence requires an automated way to acquire such a huge amount of images (up to a few thousand). It is of key importance that these regions are not exposed to the electron beam prior to recording the data. Such "low-dose" acquisition methods are commonplace in biological electron microscopy (in particular for single particle analysis), but are difficult to achieve with a precision that allows atomic resolution.

Here we show a low dose acquisition scheme in STEM at atomic resolution, which allows to capture data from a fresh region of interest without exposing it beforehand for focusing or adjustment of astigmatism. We determine the focus height at four points surrounding the region of interest and interpolate the focus at all points in-between. In this way, we can automatically obtain hundreds of images from fresh areas in-between the reference point (Fig. 1), with focus set to the right value with a sufficient accuracy to obtain at least 2 Angstrom and in part of the images 1 Angstrom resolution.

# Microstructure and chemistry evolution due to heat treatments after severe plastic deformation of a CrMnFeCoNi alloy.

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High entropy alloys (HEAs) are a novel class of materials with great application potential. The HEA used in this study is the so called Cantor alloy [1], consisting of equal fractions of Cr, Mn, Fe, Co and Ni. This CrMnFeCoNi alloy is investigated in depth because it shows promising mechanical properties at very low temperatures [2].

In this investigation, it was tried to influence the behavior of the material through severe plastic deformation (SPD), to be precise high pressure torsion (HPT), and following different heat treatments to examine the evolution of the microstructure. To determine the chemistry of the material after the deformation and aforementioned heat treatments atom probe tomography measurements were performed. Evaluating the crystal structure was done via electron diffraction. Finally to determine the microstructure images in the (S)TEM and the SEM were prepared.

It was found that after HPT deformation the material is nano-crystalline and it has a single phase fcc structure like reported in literature [1,3,4]. After the different heat treatments the formation of precipitates in the size regime of up to several hundreds of nm is observed. The formed particles are either enriched in Cr or Ni/Mn or Fe/Co. When the temperature of the heat treatment is high enough the precipitates dissolve again and a single phase fcc microstructure is reached.

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# Resolution and analysis – the JEOL GRAND ARM and dual EDS

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JEOL is one of the largest manufacturer of electron optic equipment and has developed a new 300 kV transmission electron microscope (TEM). The JEM-ARM300F aka GRAND ARM comprises a unique JEOL own ETA spherical aberration corrector and guarantees the world highest HAADF STEM resolution of 63ppm. The GRAND ARM is equipped with a new cold field emission electron source with superior stability and brightness. Together with a newly developed vacuum system, column, stage and detectors an entirely new set of automated software functions is present and allows to easily operate the microscope in an user friendly environment. To improve the capabilities for element analysis with a TEM JEOL launched a dual EDS system. This new set consists of two silicon drift detectors (SDD). The dual EDS system is highly effective in x-ray collection and reduces the dependency on specimen tilt. Due to the systems sensitivity high throughput analyses are guaranteed.

Keywords: JEOL, EDS, TEM

**Figure 1**: JEM-ARM300F GRAND ARM

# Ion channel localization in nanodomains of excitatory synapses in mammalian central neurons

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In central neuron, synaptic transmission occurs when neurotransmitter-filled vesicles fuse with the presynaptic membrane, releasing their content into the synaptic cleft and activating specific receptors at the postsynaptic site. The fusion process is triggered by calcium influx in the presynaptic terminal induced by action potential-mediated depolarization of voltage-gated calcium channels (VGCCs). Efficacy and precision of the process relies heavily on the vesicle fusion machinery with proteins sensing an increase in presynaptic calcium. Hence, unraveling the coupling of synaptic vesicles to VGCCs at sites of vesicle fusion is crucial to understand chemical neurotransmission, in particular neurotransmitter release dynamics.

To investigate this, the ultrastructural topography of VGCCs of the P/Q-type ($Ca_V2.1$) was analyzed in relation to synaptic vesicles in the presynaptic active zone of cerebellar parallel-fibers (PFs) synapsing with spines of Purkinje cells (PC) in the rodent brain. SDS-digested freeze-fracture replica immunogold labeling was applied for the localization of synaptic membrane proteins at high special resolution and electron tomography of pre-embedding immunometal labeled samples was performed for the simultaneous analysis of $Ca_V2.1$ localization and spacing to fusion sites of synaptic vesicles.

Immunogold particles labeling $Ca_V2.1$ were enriched in the active zone of PF boutons, the area where vesicles fuse with the presynaptic membrane and release the neurotransmitters. Immunoparticles were non-homogeneously distributed within the active zone and small aggregations were observed. The number of immunoparticles was highly variable with on average 18±12 particles in a single active zone. Size of synapses was correspondingly variable ranging from 0.04 to 0.36µm$^2$. Quantity of $Ca_V2.1$ virtually depends on synapse size since a positive correlation between $Ca_V2.1$ particle number and active zone area was found. Fusion sites of synaptic vesicles were detected within a 20-30nm distance to $Ca_V2.1$ channels within the active zone compartment.

This indicates localization of $Ca^{2+}$ sensors for vesicular release in close proximity to $Ca_V2.1$ VGCC clusters (<30 nm), and VGCC number per cluster likely determines vesicular release probability. Coupling of $Ca_V2.1$ with synaptic vesicles according to the perimeter release model [1] is anticipated rather than the clustered VGCC-random vesicles placement model [2]. The short coupling distance respectively "nanodomain coupling" seems a general characteristic of cortical synapses involved in high-frequency transmission [3] and indicates synaptic fidelity along with tight coupling.

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# 2D strain tensor measurement of a metallic glass from elliptic electron diffraction patterns

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Elastic deformation as well as residual stresses after plastic deformation lead to strains in the material, which correspond to local displacements of atoms from their equilibrium, stressless state. This implies a local change in scattering condition and therefore a distortion of the diffraction pattern (DP) as compared to a strain free state. Anisotropic strains caused by uniaxial tension lead to an elliptic distortion of the DP.

In the case of a metallic glass whose diffraction pattern consists typically of broad diffuse rings (cf. Fig. 1) the elliptic distortions in the diffraction pattern can be measured by fitting an ellipse to the intensity maxima positions. By subtracting and normalising the strained DPs with respect to an unstrained one, the angular dependence of the strain as well as the 2D strain tensor are obtained [1,2].

The author will present his work on DM Plugins which allow to fit the parameters of the ellipse with a desired angular resolution and additionally obtain the eigenvalues of the 2D strain tensor. The maxima positions of the diffuse ring are obtained from a gaussian fit to achieve sub pixel precision. Nonlinear least square fitting using the cminpack library [3] calculates the ellipse parameters and a plot of the peak positions as a function of the azimuthal angle φ is created. From the data the strain tensor can be extracted and sample parameters as the poisson's ratio can be calculated.

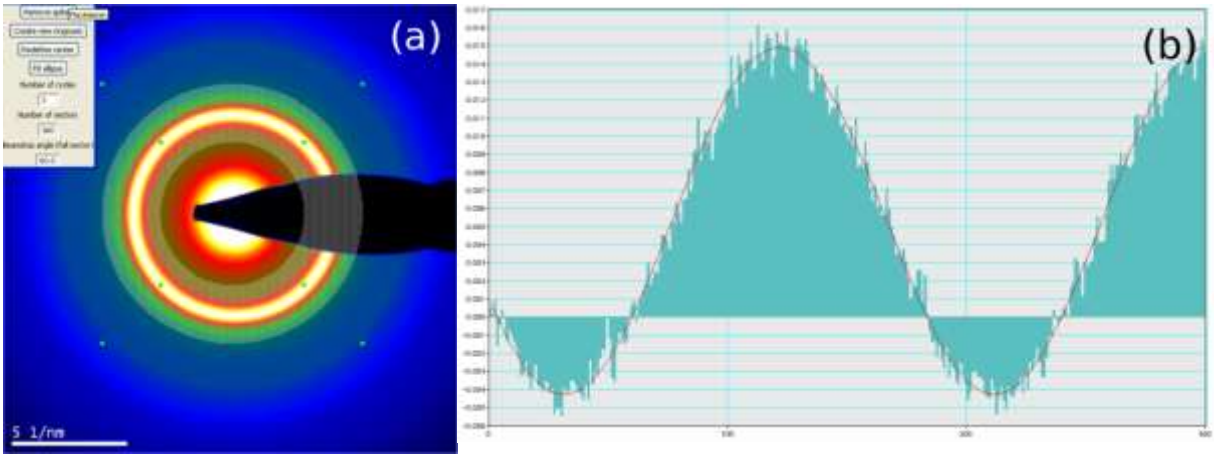


Figure 1: (a) TEM diffraction pattern of an amorphous specimen with a broad continuous ring pattern. The DM Plugin allows to integrate sector-wise and to perform a gaussian fit on the profiles. Using the gaussian maxima positions of all sectors an ellipse is fitted using a non-linear least squares fitting algorithm. (b) Plot and fit of azimuthal strain from which the 2D strain tensor and its eigenvalues can be calculated.

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# Simulating the pressure limiting system of Environmental Scanning Electron Microscopes using the direct simulation Monte-Carlo method

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In environmental scanning electron microscopy (ESEM) high pressure applications are of increasing interest for the investigation of wet and biological samples, because neither sample preparation nor extensive cooling is necessary [1]. Unfortunately the applications are limited by poor image quality, which is mainly caused by the scattering of the primary electron beam in the imaging gas. A significant amount of scattering takes places in the pressure limiting system above the pole piece. Therefore, a detailed understanding of the pressure gradient in the pressure limiting system is essential in order to optimize the performance of ESEM. These pressure gradients, which are virtually impossible to measure directly, can be simulated using the direct simulation Monte-Carlo (DSMC) method [2, 3].

The DSMC algorithm presented in [4] is implemented and used to simulate the pressure limiting system of an FEI Quanta 600 ESEM. Using the simulated pressure gradients, the total amount of scattering in the pressure limiting system is calculated and compared to experimental results. The program presented can simulate arbitrary 2D-geometries and can run reasonably fast on a desktop PC. The program can be used to find deficits in the current design and to evaluate prototypes.

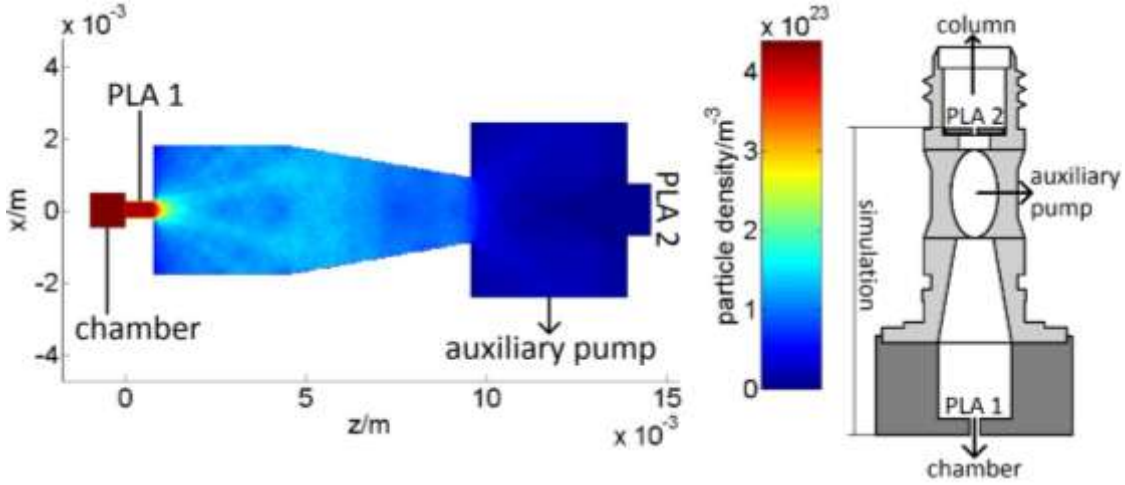


Figure 1: Simulation of the pressure limiting system at a chamber pressure of 2000 Pa (left) and sketch of the pressure limiting system (right)

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# 3D-reconstructions of a neuronal Motion Detecting Sensor with Serial block Face Scanning Electron Microscopy (SBEM)

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Nowadays many scientific fields engage in research into reliable mechanisms found in animal life that are used for bio inspired models and technical applications. Thus, various simple and reliable mechanisms found in animal life have provided inspiration for the growing field of bio inspired design. In our studies we are looking into the neuronal circuit of the locust, its motion detection pathway that warns the animal of imminent collision on the ground and while flying in the air. We aim for understanding the network that will give us the knowledge needed to construct a device that could help blind people in their everyday life.

The optic lobe of *Locusta migratoria* contains two neurons, the Lobula Giant Motion Detector 1 and 2 (LGMD 1 & 2), which can be excited when an object approaches on an imminent collision course. A large number of afferent neurons feed these two cells with input signals from the insect's single eyes (ommatidia). Objects that are not on a direct collision course will not activate enough afferent neurons to excite the LGMD while imminent collision will overcome the LGMDs threshold by stimulating large numbers of afferent neurons [1]. It was previously thought that the afferent neurons receive input from so-called lamina monopolar cells (LMCs), but descriptions of the anatomy of the afferents and their connections with other neurons have so far been lacking. We used Serial Block Face Scanning Electron Microscopy (SBEM), to reconstructed one whole afferent neuron, synaptically connected to an LGMD-branch, and we traced this neuron along its whole length. SBEM (see ref. [2]) was used to scan successive surfaces of a tissue sample embedded in a resin block. For this, an inbuilt ultramicrotome (3ViewTM, Gatan, Inc, Pleasanton, Ca, U.S.A.) repeatedly cuts 40 to 60 nm thick sections off the block while the surface is scanned using an ESEM Quanta 600 FEG from FEI. With this approach hundreds of serial micrographs are obtained within a moderate time frame (see reference [3]). Reconstruction of the whole neuron was performed by software (Amira, FEI). Whereas the afferent neuron overlaps with the LMCs and may be synaptically connected with the LMCs, we observed synapses in an area that the LMCs do not reach, implicating a further neuron involved in the signal cascade.

By tracing the cells that have synapses with the LGMD, information about the wiring and insight how the signals travel in this circuit can be gained. All information collected in this nano-scale environment, connected to physiological and empirical understanding, will lead us to bio inspired design that can be used in various different fields.

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# Reliable quantification of X-ray spectra using ζ-factors: from standards to geometry

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During recent years the ζ-factor method introduced by Watanabe et al. [1] has become a widely used technique for the quantification of energy-dispersive X-ray (EDX) spectra. It offers the ability to perform absolute quantification with a built in absorption correction; so it is also suitable for light element analysis [2]. This method not only provides the concentration of each element but also determines the mass thickness of the sample.

Similar to the Cliff-Lorimer method, well-defined standard materials are the basis for good ζ-factors and hence a reliable quantification. In the case of ζ-factor measurements we use pure element standards with a well-known density if possible. For the mass thickness the measurement of the specimen thickness is still necessary. Therefore we work with a special sample configuration using the focused ion beam instrument to produce a lamella with a rather uniform specimen thickness for the actual measurement and a conical, circular symmetric rod of each standard, which is used for the experimental determination of the inelastic mean free path λ [3]. We work with a FEI Titan³ 60-300 equipped with the ChemiSTEM technology. Hence we have 4 windowless SDD detectors symmetrically placed around the optic axis and we are using the Super-X high visibility holder from FEI. The awareness of the geometrical influence on quantitative results is recently growing (see [4]). Especially using the ζ-factor technique as an absolute quantification method one has to know the given geometrical arrangement of the microscope, the EDX detectors and the sample holder very well. Therefore we made some effort to get a very precise understanding of our EDX system setup used for quantification. We monitored the EDX signal while tilting the sample holder to be able to account for the influence of the holder on the detector signal. Furthermore we work with a precise model of the holder and so we are able to compare the experiment with simulations done in a 3D modelling program (CINEMA 4D by MAXON Computer GmbH).

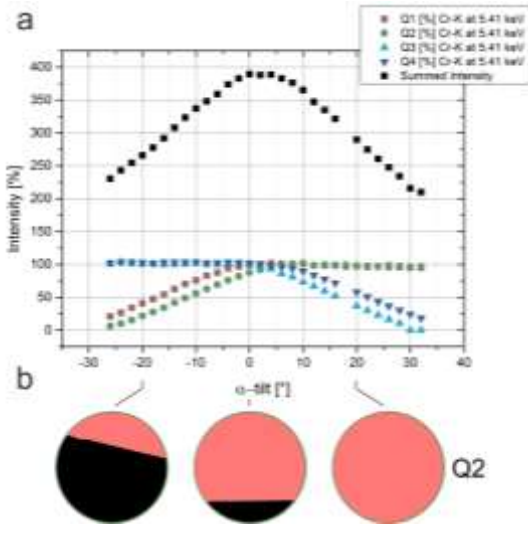


Figure 1: a) α-tilt versus intensity for each detector quadrant and the summed intensity are shown. b) Simulations showing the illumination of Q2 at a holder α-tilt of -20°, 0° and 20°.

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# Structural heterogeneities in Thin Foils of CuZr based Bulk metallic glasses

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Bulk metallic glasses (BMG) are amorphous materials with no long-range order. The unique atomic structures of BMG lead to interesting physical and mechanical properties. To obtain structural information by transmission electron microscopy (TEM) methods thin TEM specimens are of advantage. It is the aim of the present work to show that in very thin areas of a CuZr-based BMG structural heterogeneities compared to the bulk sample can occur.

For our study TEM foils of $Cu_{36}Zr_{48}Al_8Ag_8$ BMG were prepared by electropolishing and ion milling. The TEM foils were studied in a Philips CM200 operating at 200kV. Selected area electron diffraction (SAED) patterns and energy dispersive X-ray (EDX) spectra were acquired from areas of different thickness.

In the thinnest area next to the sample edge the SAED pattern shows two closely spaced diffuse diffraction rings (A, B) at about 3.6 and 4.7 $nm^{-1}$, respectively. With increasing foil thickness a third diffuse ring (C) emerge between A and B at about 4.3 $nm^{-1}$ (cf. Fig. 1) and becomes dominant. This change of the SAED pattern is summarized in Fig. 2 showing the intensity profiles. An integration along rings was carried out using the PASAD software [1]. The position of peak A can be correlated to that of amorphous zirconia. This is consistent with the EDX spectra which reveal an enhanced oxygen content at thinner areas.

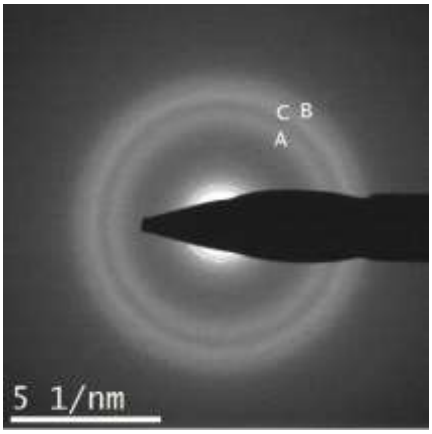


Fig. 1: SAED pattern of the BMG showing the diffuse rings A, B and C.

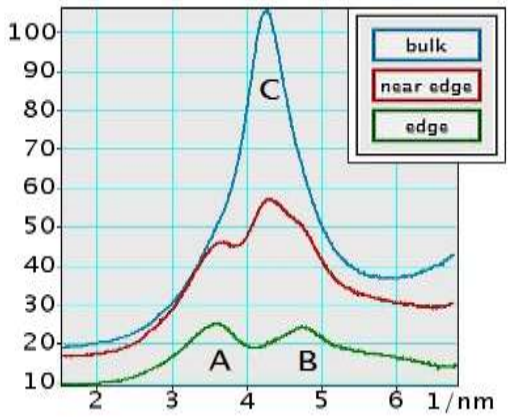


Fig. 2: Integrated intensity profiles of the SAED patterns at different foil thickness.

Our results show that in CuZr-based BMG in foils structural heterogeneities in the form of different structures are present: (i) oxidized amorphous zirconia (peak A) at the surface, (ii) amorphous CuZr structure (peak B) affected by the surface layer (less Zr content) and (iii) amorphous bulk CuZr structure (peak C). The shift of peak position B to C by a change of the Zr content is supported by experimental data of CuZr based BMG [2].

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# New integrated solution for 3D isotropic volume imaging and reconstruction of biological samples using SEM

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The Teneo VS SEM represents a new integrated solution for SEM (scanning electron microscopy) volume data acquisition based on a refined SBFSEM (Serial Block-Face SEM) technique [1]. A combination of hardware and software components allows for automatic data acquisition from selected regions of interest in resin embedded samples stained with heavy metals. The volume information is revealed by physical and/or virtual slicing depending on the required depth resolution and sample conditions. The pure SBFSEM mode is based on alternate slicing and backscatter imaging of the block-face. For virtual slicing a series of images of the exposed block-face is acquired using different accelerating voltages. By their proper selection different backscatter depth emission profiles are created. The collected images may serve as the input for a deconvolution algorithm that computes several subsurface layers. This approach is based on the MED-SEM (multi-energy deconvolution SEM) which is a non-destructive technique capable of high-resolution reconstruction of the top layers of the sample [2]. In the case of physical slicing the minimal slice thickness, and thus the depth resolution, is limited. Virtual slicing is capable of extending it towards the nanometer range and hence high-resolution isotropic datasets can be generated.

The Teneo SEM, in addition to a newly developed in-situ microtome and the reconstruction software form the key components of the workflow used to automatically acquire and process volume data on the different regions of interest. Data throughput depends on sample properties, the desired resolution, as well as on the detection module of the microscope. The Teneo SEM serves as the host tool providing high-resolution backscatter images both in high vacuum and low vacuum. The backscatter electron signal can be detected by in-column detectors or by a newly developed below the lens detector dedicated to low vacuum operation. A compact automated ultramicrotome equipped with a diamond knife is mounted directly on the stage inside the SEM chamber. Easy switching between the dedicated tool and a normal SEM mode is straightforward. The complete workflow is controlled using a software package with a single user interface for setting up and monitoring the 3D acquisition process. Improved ease of use is facilitated through a set of advanced auto-functions for electron column alignment ensuring optimal image quality during data acquisition.

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# Artificial Substrates as Key Element towards Single Enzyme Tracking via High Speed Atomic Force Microscopy in Enzymatic Degradation of Cellulose

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Production of transportation fuels by enzymatic degradation of cellulose, as an alternative to crude oil, proofs to be an interesting concept to face our ever growing energy need. Although known for almost a century, there are still open questions and rate limitations obstructing economic competitiveness. In order to extend current knowledge, direct visualization of the interaction of enzymes (cellulases) and cellulose are absolutely essential. In recent years Atomic Force Microscopy has demonstrated its feasibility to show cellase-cellulose interactions and corresponding effects on a localized, in-situ and real time basis. In this study we present two special tailored cellulose substrates for the investigation of cellulose disintegration, with special focus on the substrate morphology as crystalline and amorphous cellulose have strong impact on degradation kinetics [1]. Both substrates are applied on common problems in cellulase science, like synergism between different cellulases and the investigation of novel cellulose degrading enzymes [2]. Results from in-situ AFM experiments will improve existing models by showing otherwise elusive information of the mechanistic interdependence between cellulases and substrate during degradation.

**Acknowledgements:**

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# Electron microscopy in topical drug delivery and cosmetics

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Drug delivery through the skin is a very promising treatment route for especially local, but also systemic affiliations. However, the investigation of topically applied formulations is of importance for both the pharmaceutical and cosmetics industry. In both fields the structural properties of a formulation need to be investigated as well as its stability. Electron microscopy is an essential tool in these investigations, especially in connection with sunscreens containing nanoparticles as their small size poses a challenge to most methods.

Dermal formulations are available in a wide variety of possible structures as for example: So called nanoemulsions in which the emulsion droplets are in the sub-micron range, double emulsions in which an emulsion is finely dispersed in another outer phase or liposomes composed of phospholipids or formulations with the addition of nanoparticles [1-3]. Due to the sub-micron size of the droplets and particles in these formulations, a visualisation via electron microscopy is very helpful for the investigation of the success of production and the long-term stability of the formulations, thereby providing essential insight into the structure and behaviour of these formulations. The methods employed for these purposes include conventional TEM (transemission electron microscopy) and cryo-TEM together with freeze fracture methods and freeze plunging techniques.

Our group has investigated the aforementioned formulations with regard to their applicability as dermal drug delivery vehicles or cosmetic formulations and has recently developed a method for the screening of sunscreen formulations in accordance with the demand of the European Union to mark the use of nanoparticles in cosmetics. Within this method, laser diffraction is employed to identify samples that are most likely to contain nanoparticles via their enlarged surface area [4, 5]. This screening process singles out the samples that need further investigation of the particle size in order to confirm the occurrence of nanoparticles and thereby minimises the effort due to a prior selection of samples.

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# Characterization of microfiltration membranes by *in-situ* wetting in the ESEM and FT-IR mapping

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Polymeric microfiltration membranes are used in a great variety of applications such as waste water treatment or filtration of colloids and particles in the beverage industry. The increase in the complexity of the structure of these membranes requires also more and more sophisticated characterization methods to gain a deeper insight into the interaction between the pore walls and the applied fluid at the different membrane layers.

Many of the common techniques used for the characterization of the membrane structure and behavior are based on the measurement of parameters which are integrated across the whole membrane cross section. Therefore a new investigation method was developed which delivers spatially resolved results in the µm range [1]. The respective experimental setup enables wetting and drying of the membrane within the specimen chamber of an environmental scanning electron microscope (ESEM). The possibility to record images of the surfaces of wet specimens at high resolution makes the ESEM a perfect tool for the study of physical and chemical processes on the microscale. Additionally, the setup enables the simultaneous recording of the temperatures at both membrane surfaces. These temperature characteristics provide information about both the interior membrane structure and the interaction between the water and the pore walls. Because different membranes differ in the build-up of their layers (thickness and position), each type has its own kind of temperature profile. Correlation of the results gained at the micro- and macroscale gives detailed information of the membrane behavior in real conditions.

Any change in the temperature profiles reveals modifications of the surface properties of the pore walls. Thus, this method enables the investigation of membrane properties changes caused by chemical treatments. In various applications chemicals are used for cleaning and disinfection of the membranes, and the modification of the membrane performance is regularly tested by recording of macroscopic parameters [2]. But it might be interesting to know whether the degradation is homogeneous over the whole cross section or if one of the layers is stronger affected by the treatment than the others. The quoted method enables this possibility, because the temperature profiles comprise also structural information. In fact it could be proven that one of the layers is preferentially degraded. To verify these results, IR mappings at cross sections of pristine and treated membranes were performed by transmission FT-IR. Also these measurements gave an indication on the preferential degradation of the same layer as observed in the ESEM.

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# The central pair complex of cilia visualized by cryo-electron tomography

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Cilia and flagella are universal motility and sensing organelles of eukaryotes. Motility of these highly conserved hair-like appendages is driven by thousands of axonemal dyneins that require precise regulation. One essential regulator of motility is the central pair complex (CPC) found in the center of the typical 9+2 cilia/flagella architecture. The CPC is composed of two singlet microtubules, each with a set of associated projections that extend toward the surrounding nine doublet microtubules. Many CPC defects cause impaired motility or paralysis of cilia/flagella. Several human cilia diseases - collectively called ciliopathies - such as immotile cilia syndrome, show CPC abnormalities, but little is known about the detailed three-dimensional structure and function of the CPC. Using cryo-electron tomography in combination with subtomogram averaging, we visualized the CPC in 3D at molecular resolution in two important cilia model organisms: the green alga *Chlamydomonas* and the sea urchin *Strongylocentrotus*. Their CPCs exhibit both, remarkable structural conservation as well as organism-specific differences including the presence of microtubule inner proteins (MIPs) in *Chlamydomonas*, but not in *Strongylocentrotus*. The overall outlines of the highly connected projection network, which forms a round-shaped cylinder in algae, but is more oval in sea urchin could be an adaptation to the mechanical requirements of the rotating CPC in *Chlamydomonas*, compared to the *Strongylocentrotus* CPC which has a fixed orientation.

Visualizing the 3D structure of the CPC at high resolution in near native conditions is an important first step in understanding how cilia function and why they fail in disease.

# Nitrogen atom shift triggered the structural evolution in chromium nitride ---from hexagonal to cubic phase

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The atomic configurations and structural evolution of disordered $Cr_2N$ have been investigated by $C_S$-corrected high resolution transmission electron microscopy (HRTEM), electron diffraction analysis and energy loss spectroscopy (EELS). Disordered $Cr_2N$ was reported as a hexagonal structure by CBED study [1]. However, our results show some differences. Both diffraction and HRTEM image analysis reveal that the disordered $Cr_2N$ structure possess a hexagonal (*hcp*) arrangement of Cr atoms with N random distribution, and particularly, N atoms shift from the center of octahedral interstices at (001) planes. This shift results in a clear reduction of the relative intensity ratio of (030) to (002) reflection. Considering this shift, the lattice structure is no longer perfect hexagonal.

It was reported that cubic CrN can be formed in a narrow N/Cr ratio between 0.9-1.0 [2]. Here, we found that the disordered $Cr_2N$ can transform into a face-centered cubic (*fcc*) phase at the thin specimen surface with a Cr/N atomic ratio close to be 2:1 as revealed by corresponding EELS analysis. By analyzing the *hcp-fcc* interface, the mechanism of structural evolution is revealed as shearing motion of Cr atoms in (001) planes. Based on the atomic structure of disordered $Cr_2N$ and the observation of *h.c.p. to f.c.c* structural evolution, the effect of nitrogen atoms redistribution on the structure is briefly addressed.

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# Microbending tests with a PicoIndenter

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In this study cyclic in situ bending measurements were performed on ultrafine grained copper using a PicoIndenter 85 from Hysitron. This device has a force resolution of 3nN, which enables cyclic softening to be seen in the stress-strain curve, caused by small microstructural changes. The ultrafine grained copper was processed via High Pressure Torsion. Nanoscaled bending beams with different dimensions were produced via focused ion beam and cyclically loaded. These cyclical fatigue tests were combined with observations of the specimen microstructure. The microstructure of the beams was characterized prior to testing and after a certain numbers of cycles. Various methods of Scanning Electron Microscopy were used for microstructure characterisation include Electron Backscatter Diffraction and backscattered electron imaging. These tests show that the microstructure of ultrafine grained copper can coarsen during cyclic loading, due to the movement of the grain boundaries.

# High-Fidelity Shapes and Disruption Mechanism during Focused Electron Beam Induced Deposition

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During recent years, focused electron beam induced deposition (FEBID) attracts increasing attention due to its mask-less, additive direct-write capabilities with spatial nanometer resolution. This techniques relies on the highly localized nano-synthesis of functional precursor molecules via a nanometer sized, focused electron beam on even non-flat surfaces where classical resist based lithography is complicated or even impossible. While the long lasting purity issues by means of carbon contamination have been recently solved for Pt- and Co-based deposits, highly accurate morphology control is still subject of investigations [1]. However, as emerging applications for nanoscale materials like plasmonics, thin film multilayer devices or high resolution sensor gaps demand precise deposit shape retention from design to deposition, a fundamental understanding for disruption mechanism is indispensable to enable specific control over morphology during FEBID processes.

In this contribution, we discuss limiting factors regarding lateral resolution, proximity deposition and surface shape fidelity. In more detail, we start with fundamental processes influencing the achievable widths of quasi-1D single lines on industrially relevant bulk substrates [2]. We then expand the considerations to 3D deposits and discuss side wall broadening effects together with proximity deposition on the meso-scale [3]. Finally, we focus on the surface properties of 3D deposits by means of flatness and disruption effects [4].

By that this contribution gives a comprehensive insight in the complex interplay between electron trajectories, precursor dynamics as well as process parameters together with its morphological consequences on FEBID deposit from a fundamental point of view. Finally, the presentation demonstrates how ideal shapes can be achieved which is essential for controlled fabrication of highly defined structures from the micro- to the nano-scale [5].

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# Electron Microscopy and Molecular Biology, two tools for understanding functional biology

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Electron microscopy and molecular biology in combination are excellent methods for understanding principals of functional biology. Bio-adhesion is one example of such a fascinating biological function and is widely spread among animal groups. It can be pronounced as permanent or temporary adhesion. Also the biological approach for bio-adhesion is different and can reach from thousands of nanostructures of the scales in geckos to the byssus of blue mussels and finally to a glue produced by gland cells.

In our ongoing project we have started to investigate the fundamental functions of bio-adhesion in the marine flatworm *Macrostomum lignano*. Electron optical investigations revealed that one adhesive organ is composed of only three cell types; an adhesive gland cell, a releasing gland cell and an anchor cell. The adhesive gland cell produces a glue of proteins and carbohydrates which enables the animal to attach to the substrate. Adhesive granules were approx. 270 nm in size and showed typically two components with an inner protein component and an outer glycoprotein ring. The releasing gland in contrast produces a so far unknown substance which dissolves the glue and the animal detaches from the substrate. Releasing granules were much smaller with a size of 70 nm. We have searched the transcriptome database of *M. lignano* for genes involved in either adhesion or releasing and could find some candidate genes. The location of these genes were revealed by in situ hybridization and the function was tested by RNA interference (RNAi). For example *macif1* gene [1] was located in the anchor cells and RNA815_16605 was located in the adhesion gland cell. Both candidate genes were knocked down by RNAi using double stranded RNA and showed a phenotype which could not attach to the substrate anymore. Electron microscopical analysis showed that the knock down of *macif1* led to a loss of intermediate filaments in the anchor cells. This filaments are anchored in hemi-desmosomes at the base of the cell and finally are connected to the actin filaments of the specialized microvilli of the adhesion papilla. TEM of RNA815_16605 knockdown demonstrated a complete loss of the outer carbohydrate containing ring of the granules compared to the wild type. This two examples demonstrate the benefit of using molecular techniques and advanced electron optical techniques to answer complex biological questions.

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# Amorphization of Graphene on the µ-scale by Electron Irradiation

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An experimental study on the amorphization of Monolayer Graphene [1,2] by means of electron irradiation at 200kV is being presented. While electron bombardment of graphene at 200kV leads to drilling of big holes in the membrane at 300K, heating up the sample during irradiation drastically reduces the formation of holes while still allows for creation of a sufficient amount of defects. Employing aberration-corrected scanning transmission electron microscopy at 60kV, we performed ring statistics, i.e. collecting the number of different polygons in the lattice and relate it to the electron dose used for irradiation. Furthermore, enhanced attraction of impurities (Si) by the defects has been observed which leads to possible applications of amorphized graphene, e.g. gas sensors

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# Analytical Electron Tomographic Investigation of an Aluminium Alloy with Nano-Precipitates

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For a thorough understanding of a material, investigations at the nanoscale are often essential. Analytical techniques like electron energy loss spectroscopy (EELS) and energy dispersive X-ray spectroscopy (EDXS) in scanning transmission electron microscopy (STEM) can reveal important chemical information necessary for the development of high-tech materials. The integrative character of the signal acquired through transmission, however, might hide important structural details of the material, relevant for its properties. Those details can be revealed through electron tomography, where the data is acquired at different tilt angles and, after alignment, reconstructed to form a full 3D model of the material under investigation. The combination of both techniques, analytical STEM and tomography, gives full insight into structure and composition of a material.[1] As this is not an established technique many challenges such as sample damage, noisy spectra and the difficult detection of elemental trace concentrations have to be tackled.

In this study an aluminium alloy containing scandium (Sc) and zirconium (Zr) rich nano-precipitates was investigated at different stages of ageing. High resolution STEM and analytical EELS and EDX tomographic investigations were carried out. The resulting 3D elemental reconstructions deliver otherwise inaccessible information on the sample's chemistry and structure. Additionally EDX-spectra were reconstructed channel by channel, resulting in a data cube, where each pixel contains a whole spectrum. By using an HAADF reconstruction to create a mask the pixels of the core and the shell region of a nano-precipitate were extracted and summed to obtain pure spectra of those regions, thus overcoming the intrinsic limitations arising from the integrative character of analytical STEM.

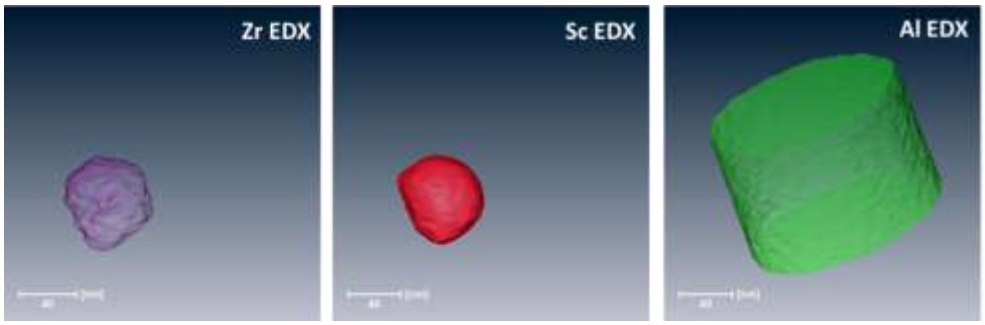


**Figure 3: Segmented volumes from reconstructions of EDX signals of an aluminium alloy with nano-precipitates after ageing for 72h at 500°C. The reconstructions were performed using a total variation minimization algorithm after processing X-ray intensities.**

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[2] The authors thank the Austrian Cooperative Research Facility, the European Union (7th Framework Programme: ESTEEM2) and the Austrian Research Promotion Agency FFG (TAKE OFF project 839002) for funding.

# Generation of Bessel Beams with Extremely High Orbital Angular Momentum

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By placing holographic masks (HM) in the condenser system of a TEM it is possible to shape the electrons' wavefront such that it carries orbital angular momentum (OAM) quantized in units of ħ, as well as magnetic moment [1]. These novel probes are called electron vortex beams (EVBs).

Focused ion beam (FIB) milling is a robust and reliable technique to produce HMs. These masks create a series of EVBs where the different diffraction orders are linearly arranged according to their magnetic quantum number. The EVB radii increase with increasing OAM and thus juxtaposed vortex orders tend to overlap. The separation distance between neighboring orders is inversely proportional to the grating period of the HM and as such poses a technical limit to the generation of beams carrying high OAM. Nevertheless, EVBs up to OAM = 200 ħ have been generated [2]. There, a small central region of the HM, where the feature size could not be resolved anymore by the FIB instrument, was excluded from the FIB milling processes. By extending this Ansatz such that only a small outer ring of the whole HM pattern is milled and by giving up the non-overlapping condition, Bessel beams with even higher OAM values can be generated.

Traditionally the HM are placed in the C2 aperture holder (or in C3 where it applies) of a TEM such that no further manipulation of the EVB is possible. Placing the HM in the C1 (or in C2 where it applies) aperture holder opens up the possibility to separate the overlapping vortex orders by using sub micrometer ring apertures. First tests show that, using optimized milling paths, we can produce HMs allowing OAM = 250 ħ in the first diffraction order. With such a mask, placed in the C1 aperture holder, we have observed EVBs up to the 5$^{th}$ diffraction order, carrying OAM = 1250 ħ, and a huge magnetic moment of 1250 Bohr magnetons.

This is interesting because theoretical investigations suggest that asymmetries in the transition radiation of electrons passing conductive surfaces could be detected if the EVBs' magnetic moment is of the order of 1000 Bohr magnetons [3]. Additionally, the rotational energy of EVBs could become resolvable in EELS for EVBs carrying OAM larger than ~ 625 ħ, corresponding to an electron energy loss of ~ 100 meV [4].

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[5] TS and PS acknowledge financial support by the Austrian Science Fund (FWF) under grant number I543-N20.

# Beyond Current SEM – AFM Solutions: A Highly Flexible in-situ AFM for Correlated Microscopy

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The advent of integrated atomic force microscopes (AFMs) in stand-alone scanning electron microscopes (SEMs) opened new possibilities due to quantitative 3D information even on complex surface morphologies. This does not only reduce turnaround times but also expands the information for both techniques character in a comprehensive manner due to the quasi-simultaneous correlation within one setup. One example in material science is the investigation of slip steps structures and its correlation to the active dislocation sources after nanoindentation of metallic single crystals. The collective density of dislocations and the formation of spatially complex dislocation structures have been studied with a combined AFM and SEM (AFSEM™, GE-Tec, Austria). This system uses a highly flexible, *in-situ* tip scanning system with a wide range of scan-head modules according to customer demands. While the SEM acts as the high resolution navigation tool and can provide structural information via electron backscatter diffraction analyses, the integrated AFM solution provides laterally resolved, quantitative step height information with nm and even sub-nm resolution in XY and Z, respectively. This is an enormous advantage as it is very complicated to distinguish between real surface features on the lower nanoscale and coverage layers via SEM due to the electron beam penetration depth. By that, both techniques complement each other in a straightforward manner providing comprehensive insights practically impossible or extremely complicated via individual technique or two separate instruments, respectively. In the first part of this contribution we demonstrate the technical capabilities of the new solution, its flexibility with respect to sample types and its dimensions together with benchmark measurements using tapping height and phase imaging for correlated microscopy. In the second part we focus on slip steps around nano-indents to give a practical example how the new solution expands the insight in material properties from a fundamental but also technically relevant point of view.