JAGIELLONIAN UNIVERSITY
IN KRAKOW

ASC Winter School 2018

Imaging and spectroscopy of materials

26.II-2.III.2018 Krakow

Book of abstracts

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Programme

February, 26th (Monday)

16:00 – 19:00 Registration – Faculty of Chemistry

February, 27th (Tuesday) – Faculty of Chemistry, lecture hall A1-01

8:15 Opening

8:30 – 10:00 Lecture 1, *Introduction to electron microscopy* (Z. Sojka, JU Krakow)

10:00 – 11:30 Lecture 2, *TEM imaging and diffraction* (J. Gryboś, JU Krakow)

11:45 – 13:00 Lecture 3, *Analytical transmission electron microscopy - EDX and EELS* (P. Indyka, JU Krakow)

13:00 – 13:30 Meeting with EACEA Officer (J. Eversoniene, EU Brussels)

Lunch break – Aparthotel Vanilla

15:15 – 16:45 Lecture 4, *Three-dimensional characterization of nanomaterials by electron tomography* (J.C. Hernandez Garrido, Univ. Cadiz)

16:45 – 18:15 Lecture 5, *Scanning electron microscopy (SEM) - tool for nanotechnology* (B. R. Jany, Inst. Phys., JU Krakow)

18:30 Dinner – Aparthotel Vanilla

19:30 Chemistry evening, Aparthotel Vanilla – conference hall,
Forensic chemistry lecture (M. Woźniakiewicz, JU Krakow)

February, 28th (Wednesday) – Aparthotel Vanilla – conference hall

8:30 – 9:45 Lecture 6, *Raman imaging – principles and biological applications* (A. Kaczor, JU Krakow)

10:00 – 11:15 Lecture 7, *Surface- and tip-enhanced Raman spectroscopy (SERS and TERS): fundamentals and biomedical applications* (K. Małek, JU Krakow)

11:30 – 12:45 Lecture 8, *Resonance Raman spectroscopy - fundamentals and biomedical applications* (K. Marzec, JCET Krakow)

Lunch break – Aparthotel Vanilla

14:00 – 18:00 Scientific excursion - Wieliczka Salt Mine

18:30 Dinner – Aparthotel Vanilla

March, 1st (Thursday) – Faculty of Chemistry, lecture hall A1-01

8:30 – 10:00 Lecture 9, *Introduction to electron paramagnetic resonance (EPR) spectroscopy* (P. Pietrzyk, JU Krakow)

10:15 – 11:45 Lecture 10, *Electron paramagnetic resonance imaging ‑ basic principles, hardware, software, and applications* (K. Kruczała, JU Krakow)

12:00 – 13:30 Lecture 11, *EPR Tomography* (T. Czechowski, noviLET, Poznan)

Lunch break – Aparthotel Vanilla

15:00 – 18:00 Practical session,
Laboratories of Faculty of Chemistry (TEM, SEM, Raman, EPR)

18:30 Dinner – Aparthotel Vanilla

19:30 Chemistry evening, Aparthotel Vanilla – conference hall,
ASC poster session, alumni meeting and photo contest
Knowledge transfer and dissemination (M. Aksamit-Koperska, Univ. Warsaw)

March, 2nd (Friday) – Faculty of Chemistry, lecture hall A1-01

8:30 – 10:00 Lecture 12, *Atomic Force Microscopy for Surface Imaging and Beyond* (Sz. Zapotoczny, JU Krakow)

10:15 – 11:45 Lecture 13, *X-ray microtomography* (B. Leszczyński, Inst. Phys., JU Krakow)

12:00 – 13:30 Lecture 14, *Future leadership programs in industries and needed communication skills* (K. Chereddy, Novartis, Basel)

13:30 Closing ceremony and lunch – Aparthotel Vanilla

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Lectures

Lecture 1
Introduction to Electron Microscopy

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Since its invention over 80 years ago by Knoll and Ruska the transmission electron microscopy become the most versatile and powerful method for comprehensive characterization of solids. Owing to long progressing scientific and technical developments modern electron microscopy cay provide a wealth of quantitative information about the shape, structure and chemical composition of the investigated materials down to an angstrom scale, which is hardly matched by any other single research technique. Imaging, diffraction and chemical mapping capabilities of the electron microscopy have reached a mature state that allows for wide range of applications in catalysis, nanochemistry and physics, materials science and biology.

This lecture covers basic concepts of ray and wave optics, image formation principles, introductory electron optics, description of electron microscope construction and operation modes, and an overview of diffraction pattern and image contrast formation. Requirements for the samples and interaction of the electron beam with the sample, from the wave and corpuscular point of views, will also be addresses. The aim is to provide a suitable conceptual background for more specialized lectures on imaging and chemical analysis by EM of this school.

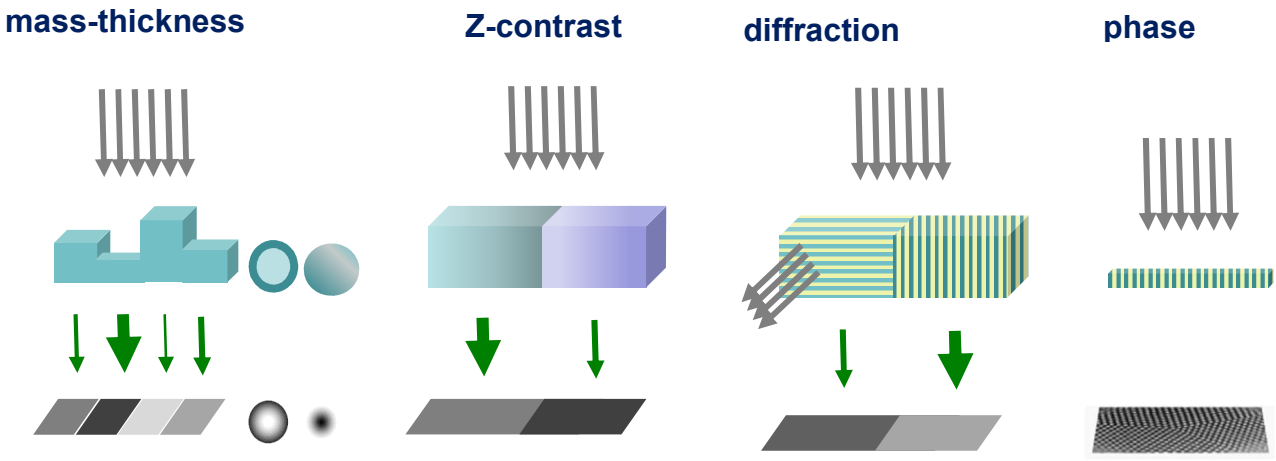

Fig. Basic types of the image contrast in transmission electron microscopy

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Lecture 2
TEM Imaging and Diffraction

Joanna Gryboś
Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Kraków, Poland

The role of a heterogeneous catalyst in chemical reaction consists in creating temporary connections between the catalyst surface and the reactant molecules that facilitate bond breaking or making processes. Depending on the exhibited planes, the exposed atoms exhibit different chemical characteristics. In vast majority, real catalysts are multicomponent systems whose characterization demands an insight at the atomic level into their composition heterogeneities governing the structure-activity relationships. One of the most effective methods to elucidate the morphology, surface structure, and phase stability of oxides nanocrystals is HR-S/TEM technique.

Within this contribution the main focus is devoted to High Resolution Transmission Electron Microscopy Technique to present its ability to reveal ultimate details of the structure of nanocrystalline materials, making possible to interpret how they work as a catalyst. The chief advantage of the Transmission Electron Microscopy originates from its ability to give localized information about the sample structure and composition. Nevertheless, it is important to realize that interpretation of the obtained data is not always straightforward.

Electrons are sensitive to the crystal potential and consequently HREM images are related to the distribution of the electrostatic potential inside the crystal. Due to the strong interaction of high energy electrons with matter (including scattering), the resultant image depends on the specimen thickness. The imaging system of the electron microscope is characterized by a transfer function that modifies the amplitude and phase of the wavefunction. As a result of the strong scattering and the information transfer by the microscope optics, HREM images depend mainly on the thickness of the investigated sample and the transfer function of the microscope. Good HREM images are obtained when the principal diffracted beams are “in phase” with the transmitted beam in the image plane of the objective lens of the microscope. This is a rather strong condition that is satisfied only for specific thicknesses and a proper set of the microscopic imaging parameters. What the electron microscopists are looking for is the projected potential of the structure. Unfortunately, due strong scattering of the electron by the crystal potential, only the wavefunctions transmitted by very thin crystals is the close to 1:1 image of the projected potential. Moreover, the imaging system of the electron microscope suffers from aberrations (spherical, chromatic, limited band pass, etc.) so that, depending of the amount of misfocus, images quite different from the projected potential are obtained. Thus, image simulation, commonly based on multislice algorithm, should be used for retrieving the non-distorted wavefunction and the projected potential of the examined crystal structure. Proper analysis of HR TEM images provides reach information on the nanocrystals structure with a picometric precision, allowing for analysing, e.g., defects, surfaces structure relaxation and reconstruction.

Another crucial area of use of the Transmission Electron Microscope is the analysis of morphology, by using HAADF STEM imaging mode. The shape-retrieving method involves combined use of HAADF STEM imaging and DFT ab-initio calculations together with 3D morphology determination using Wulff construction.

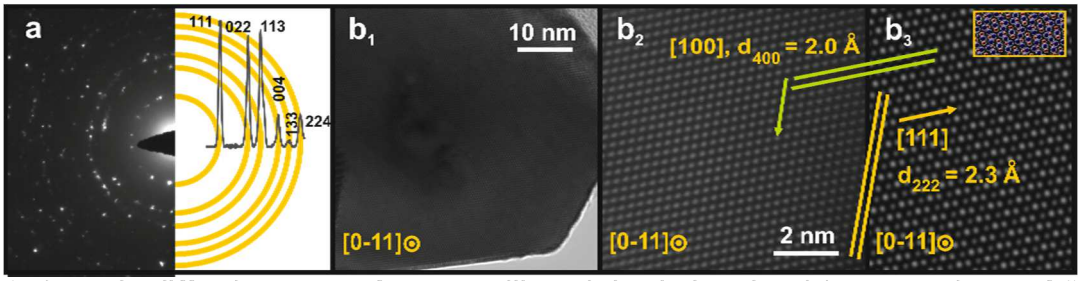

Fig. 1. a) Powder diffraction pattern of nanocrystalline cobalt spinel catalyst. b_1) HR TEM image of Co_3O_4 nanocrystal oriented along [0-11] axis. b_2) Experimental and b_3) simulated image of the structure of spinel nanocrystal viewed along [0-11] axis. Green and yellow lines mark [100] and [111] lattice planes, respectively. For the sake of comparison its atomic structure is imposed on the simulated HR TEM image of the Co_3O_4 nanocrystal ($b_{3\ insert}$).

The experimental part of the shape analysis is based on the acquisition of HAADF STEM images with an intentionally increased electron probe size. The high-angle elastic scattering signal is registered as a fraction of the incident probe current by a calibrated detector. Data analysis consists of digital processing of the image contrast gradient leading to the edges determination of the observed nanocrystals. The obtained edge pattern is used for retrieving the tentative shape by mean of convex hull algorithm. This step allows also for obtaining local orientation of the nanocrystals from 2D image. The image intensity of the HAADF STEM response has to be calibrated against the sample thickness using numerical simulations of several randomly oriented specimens. Using the detector response calibration, the sample thickness profile may be extracted from the measured grey value variations in the STEM image (on the 256 levels of grey scale). By combining retrieved local orientation of the nanocrystals and the thickness profile tentative morphology can be retrieved. The final 3D shape of nanocrystals is deduced from the thickness profiles in comparison to energetically favourable morphologies as suggested by DFT calculations

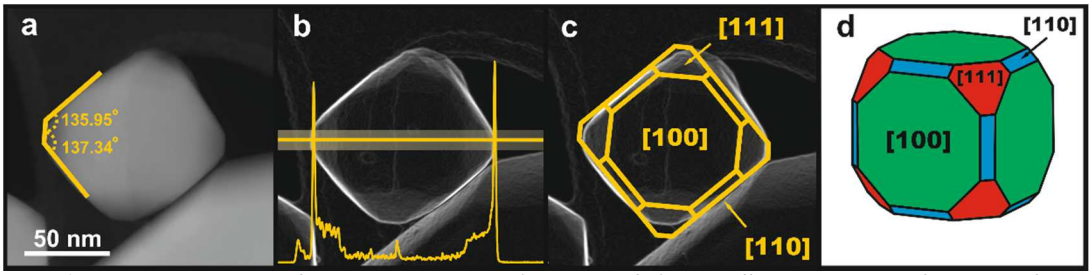

Fig. 2. a) Co_3O_4 nanocrystal HAADF STEM image and its gradient representation complemented by (b) gradient analysis and (c) superimposed Wulff shape projection along [001] axis. d) The retrieved rombicubooctahedral morphology of the observed Co_3O_4 nanograins

Lecture 3
Analytical Transmission Electron Microscopy - EDX and EELS

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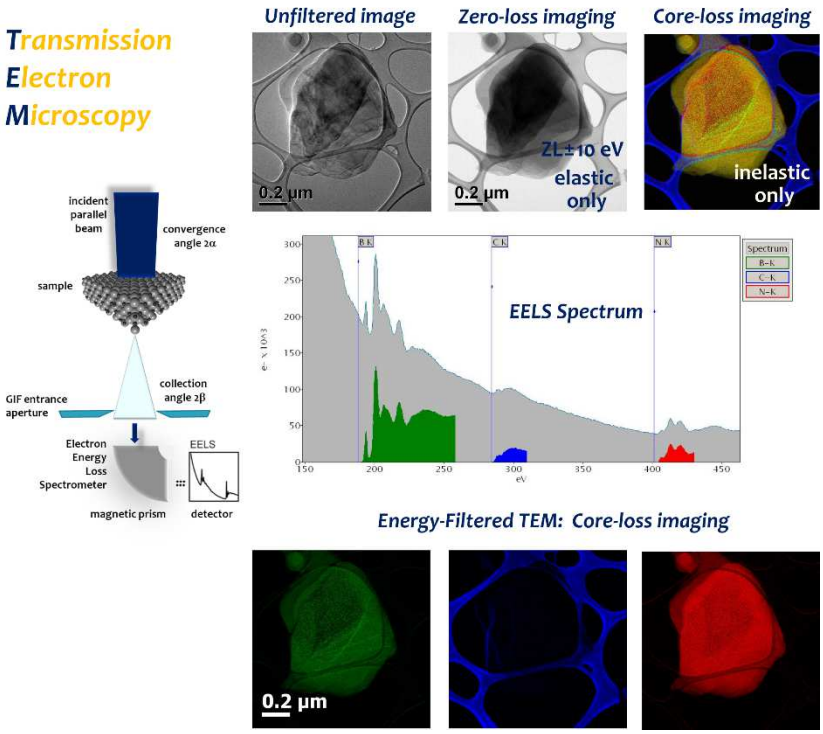
Transmission electron microscopy (TEM) is a powerful technique for imaging and microanalysis of solid state materials. A TEM instrument uses fast electrons (typically of the order of 60 - 300 kV) as a probing radiation source providing structural and spectroscopic information for specimen characterization. The strength of a TEM is that not only can it provide high-resolution images that can contain information down to atomic level but it can also operate in various **analytical modes**: EDX (Energy Dispersive X-ray Spectroscopy), EELS (Electron Energy Loss Spectroscopy) or EF-TEM (Energy-Filtered Transmission Electron Microscopy). These methods are referred to as microanalysis and will be explored in detail within the EELS/EDX panel.

EDX: X-ray photons emitted when ionized atoms of the sample return to their ground state are detected as characteristics peaks. Measuring the X-ray spectrum (plots of X-ray counts versus X-ray energy) allows identification of the sample local composition.

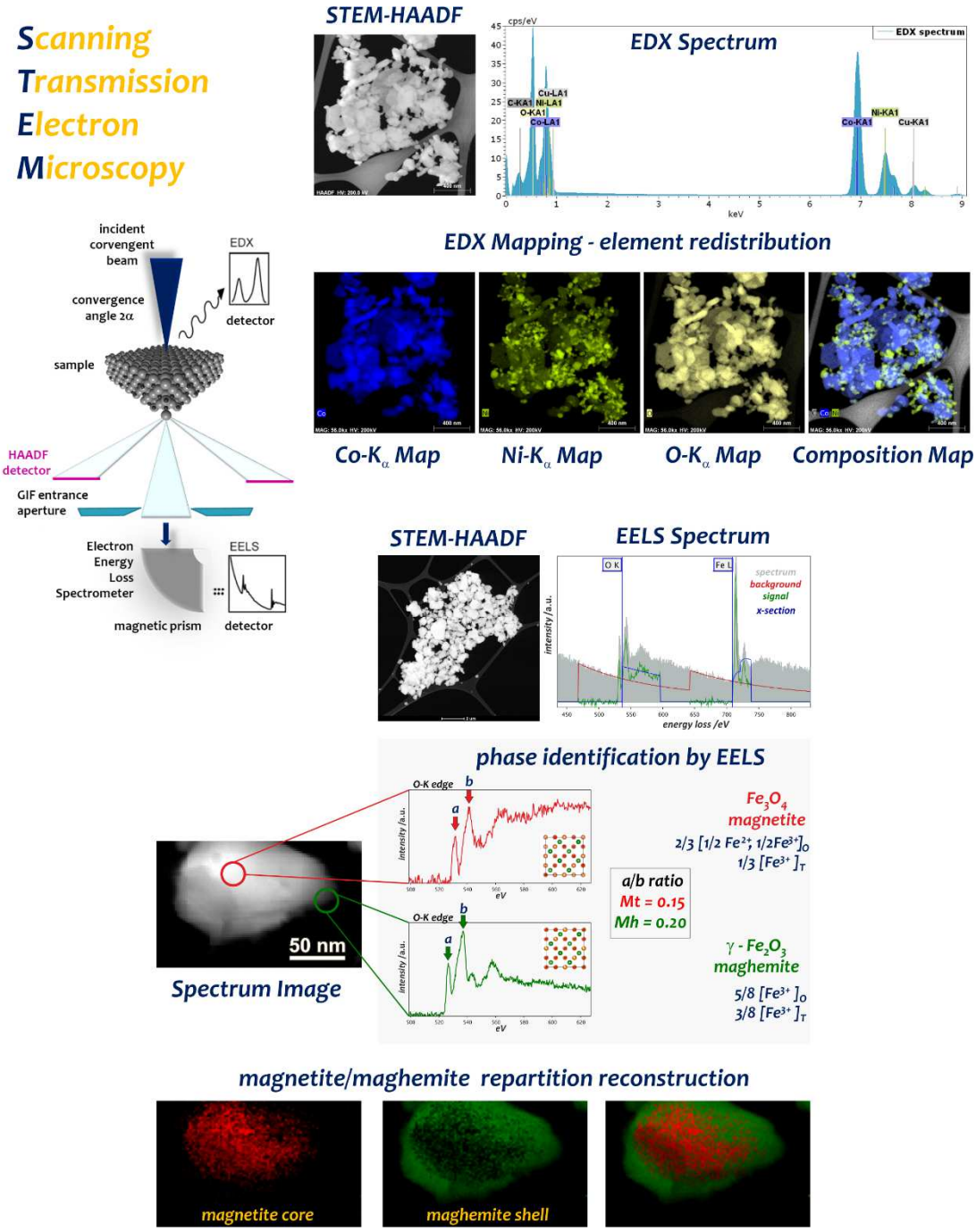
EELS: energy loses due to inelastic scattering of the electron beam transmitted through the specimen are measured in the energy loss spectrum providing information of atom type, local bonding, coordination, valence state, orbital hybridization and structural distortions of the atom in a crystal structure.

EF-TEM: an image from the inelastically scattered electrons over selected energy ranges of the EEL spectrum can be formed using a post-column GIF (Gatan Energy Filter) additionally providing chemical information to the TEM images. EF-TEM exemplary results are shown below, together with the instrumental set-up:



The use of small nano-probe allows performing EDX and EELS analyses with a very high spatial resolution (probe sizes smaller than 0.3 nm are possible using Tecnai FEG

microscopes). When combined with STEM mode (Scanning TEM) compositional line-scan profiles and elemental maps can be recorded. STEM mode configuration set-up and exemplary results are given below:



The measurements were performed on a Transmission Electron Microscope FEI Tecnai Osiris (200kV) equipped with X-FEG Schottky field emitter, Super-X windowless EDX four silicon drift (SDD) detector system and Gatan Quantum image filter (model 963). STEM imaging was performed in high angle annular dark-field (HAADF) mode.

Lecture 4
Three-dimensional characterization of nanomaterials by electron tomography

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Electron tomography is a technique that retrieves three-dimensional (3D) structural information from a tilt series of 2D projections. During the last years, it has been implemented as a key tool for the 3D characterization for both organic and inorganic materials. This is due to the growing interest in emerging fields such as molecular nanotechonology, electronic devices o heterogeneous catalysis, for the fabrication of nanostructures with tailored properties. In particular, the morphological control of nanoparticles has become an important issue because many of their physical and chemical properties are highly shape and morphology dependent.

Historically, the origin of this technique is dated on 1968 since the main works devoted to use Bright Field (BF) Transmission Electron Microscopy (TEM) images for the characterization of the 3D structures of biological systems such as bacteria, virus and cells. [1] The application in the field of materials science is much more recent, just about two decades ago, initially focused on polymers and later on porous catalytic structures. [2] However, due to the crystallographic nature of the majority of the solid-state materials, the choice of bright field imaging (BF) could be inappropriate. In conventional BF-TEM, the complex dynamical interactions of the electron beam with the crystal potential can lead to artifacts on the BF images like Fresnel effects in the sample edges, or diffraction contrasts that produces contour effects and/or stripes/bands due to the thickness. Thus, all of these effects are not complying with the *projection requirement*, [3] widely used in the tomographic reconstruction algorithms, which is based on the premise that the intensity in the projection is a monotonic function of the physical quantity to be reconstructed.

In this sense, the Scanning – Transmission Electron Microscopy (STEM) image mode, using high-angle (~50 mrad) annular dark field (HAADF) detectors, has allow to overcome such imaging limitations providing images – projections of crystalline materials, free from Fresnel effects and minimum diffraction contrasts, which largely satisfy the projection requirement.

In this talk, the principle basics about the HAADF-STEM tomographic reconstruction will be described, illustrating its successful application through a variety of materials and structures. These applications will be focused on a wide range of materials like, for example, mixed oxides materials, with and without supporting noble metal nanoparticles, of special interest in heterogeneous catalysis Indeed, the actual development of nanoparticulated heterogeneous catalysts relies on a better understanding of their structure – properties relationship, at three-dimensional (3D) atomic resolution. More examples will be discussed including metallic and oxide nanoparticles, from the interface between life science and materials science.

Thanks to electron tomography, scientists can use such a powerful tool to address this 3D-characterization facilitating the nanometrological analysis of these systems, in order to quantify physical-chemical parameters closed-related to the activity, selective and performance of these materials like: specific surface area, metallic dispersion, etc. [4,5]

In general, electron tomography requires the acquisition of many projections to generate a reliable 3D reconstruction. However, some limitations reduce the tilt range for the tomographic acquisition; the majority of them related with the instrumentations (microscopes & specimen holders). In this sense, a great effort has been devoted by the scientists to enhance those aspects related with the project acquisition, alignment and processing, previous to the mathematical reconstruction procedure. The development of accurate reconstruction algorithms is also concentrating lots of effort, mainly searching for the minimization of artifacts due to the lack of projections caused by the limited tilt range. During the recent years, many approaches have been proposed with success. [6,7]

In parallel, although the HAADF-STEM mode has been established as the most suitable imaging mode for electron tomography, alternative imaging modes for the projection acquisition have been used more recently, with similar degree of success: a 2D elemental distribution image can be formed using energy-filtered TEM images (EFTEM), or more recently, using the X-Ray Energy Dispersive Spectroscopy (XEDS or EDX or EDS) to obtain 2D chemical composition – maps to be used as projections for every tilt, so the 3D chemical information can be reconstructed. [9]

Finally, this talk will also contain a section devoted to introduce the state-of-art of this technique, as well as to discuss about those aspects that will boost its applications during the next years, illustrating thus its versatility and wealth for the actual research trends in Chemistry.

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Lecture 5
Scanning Electron Microscopy (SEM) - tool for Nanotechnology

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Scanning Electron Microscopy (SEM) has become an important technique for sample characterization at the nanoscale level due to the usage of Field Emitter Guns (FEGs) as a sources of electrons. It is unique since it delivers non disturb high quality information on samples surface topography, by imaging in secondary electrons (SE) mode [1] Fig. 1a, and simultaneously by collection of backscattered electrons (BSE), the information on local chemical composition changes at nanoscale is obtained Fig. 1e. Very ofter the SEM microscope is equipped with Energy Dispersive Spectroscopy (EDS/EDX) detector system, which can collect characteristic x-ray spectra induced by primary electron beam. Analyzing the collected spectra one obtains the quantitative chemical composition of the examined area, by employing Machine Learning one can retrieve also the quantitative information at the nanoscale level [2]. The SEM microscope could be also used for structural studies by using Electron Backscattered Diffraction (EBSD) technique. This is particularly interesting for the applications to the nanocrystalites, for which the crystallographic phase and orientation could be obtained [1].

The SEM when equipped with Focused Ion Beam (FIB) is used for nanomanipulation tasks in particularly for thin samples preparation for the Transmission Electron Microscopy. This together makes SEM a perfect tool for Nanotechnology.
The mentioned techniques related to the SEM will be presented together with their applications to particular nanotechnology examination problems [1,2].

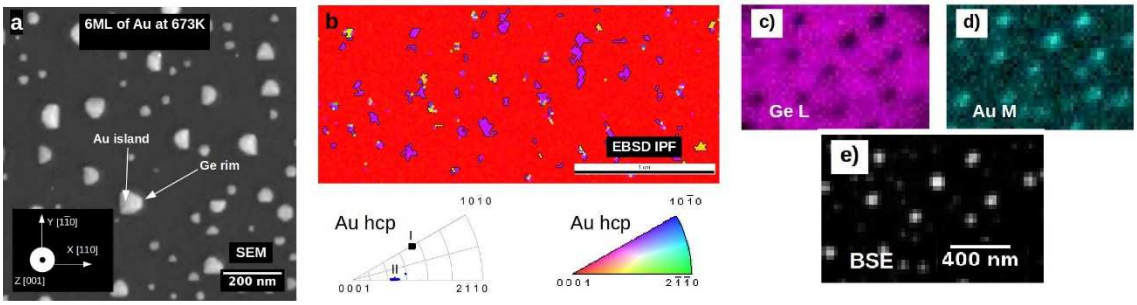

Fig. 1. Nanostructures made of Au hcp phase (hexagonal gold) resulted from thermally induced self-assembly of 6 mono layers (ML) of Au on reconstructed Ge(001) surface [1]. a) high resolution SEM (SE) micrograph, showing morphology of the surface b) SEM EBSD Inverse Pole Figures (IPF) map, showing crystallographic orientation of nanostructures, c)-d) SEM EDX maps together with corresponding SEM (BSE) micrograph e), showing sample composition at nanoscale [2].

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Lecture 6
Raman imaging – principles and biological applications

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Raman imaging is a label-free, high-content tool of analysis of components' distribution in a studied volume of a sample. Registration of Raman spectra in the chosen sample volume is followed by the analysis of marker bands characteristic for compounds of interest in the obtained dataset of Raman spectra. Calculations of the integral intensity of marker bands in these spectra result in obtaining false-color Raman distribution images of compounds related to these marker bands in the studied volume of the sample (Fig. 1).

Raman imaging is a non-destructive technique that does not require sample preparation and gives simultaneously information about multiple components in the sample unless their marker bands overlap. Due to its label-free nature and high spatial resolving power, it is an alternative for confocal fluorescence microscopy (Fig. 1) and a potent tool to study biological samples such as cells [1,2] and tissues [3].

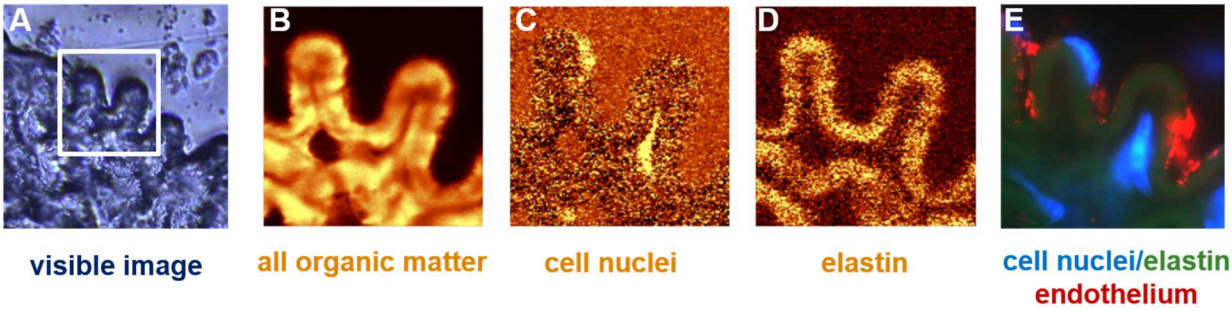

Fig. 1. A microphotograph of a fragment of the vessel wall cross-section (A), Raman distribution images of organic specimens (B), cell nuclei (C), and elastin fibres (D) obtained by integration of bands in the 2800-3100 cm^{-1}, 1560-1630 cm^{-1}, and 520-540 cm^{-1} range, respectively, compared with confocal images obtained after immunohistochemical staining of the tissue (E).

The endothelium is a monolayer of cells, on average of a total surface area of 4000-7000 square meters (*ca.* 20-36 tennis courts) and a weight of about 1 kg that regulates function of the cardiovascular system; haemostasis, permeability, leukocyte trafficking, innate and acquired immunity, and vasomotor tone. [4,5] Endothelial dysfunction is associated with the early stages of cardiovascular diseases, hence the endothelium has enormous therapeutic and diagnostic potential. Multiple examples of applications of Raman imaging to study development of endothelial diseases both in *in vitro* cell cultures and murine models of endothelial diseases are discussed showing tremendous potential of this method in studies of pathophysiology of the endothelium.

References

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Lecture 7
Surface- and tip-enhanced Raman spectroscopy (SERS and TERS): fundamentals and biomedical applications

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Since 1974 when Fleishmann and co-workers observed enhanced Raman scattering of the pyridine molecules adsorbed on electrochemically roughened silver electrodes, surface- and tip-enhanced Raman spectroscopy has developed into a mature and advanced field with a special interest in biomedical applications. To enhance the Raman signal, the interaction of light with nanoscopic silver or gold particles is required. This occurs due to the excitation of surface plasmon resonances (SPR) induced by electromagnetic field in small metal nanoparticles of size between 10-100 nm. The oscillation frequency depends on dielectric function of Ag/Au NPs and their shape and size. There is a plethora of SERS substrates; hydrosols (colloids) easily synthesized by wet chemistry methods, roughened electrodes, metal islands or periodic nanostructures but still many efforts in nanotechnology are currently directed toward the design of highly sensitive and reproducible SERS/TERS substrates. SERS and TERS are exceptionally selective and nondestructive spectroscopic methods that offer sensitivity at a level of single molecules and therefore a variety of bioanalytical applications in life sciences and medical field have been developed. TERS, in addition, opens exciting perspectives for imaging with nanometric spatial resolution.

First of all, this lecture discusses the theoretical background of the surface and tip enhancement of Raman signal by nanoplasmonic structures. To understand fully advantages and obstacles of SERS and TERS, a detailed overview of metallic nanostructures is also required, including the type of substrates, their shapes, size and other factors affecting magnitude of enhancement factor. Finally, the lecture reviews some recent applications of both enhanced spectroscopies in biology, biotechnology and biomedicine to detect an ultra-low content of biomolecules and to monitor biochemical processes *in vitro* and *ex vivo*. To demonstrate a great potential of SERS and TERS, different strategies of biosensing are also described.

Lecture 8
Resonance Raman Spectroscopy - fundamentals and biomedical applications

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This lecture is devoted to resonance Raman spectroscopy (RRS) which has an advantage in many analytical studies over normal RS because of the strong enhancement (by a factor of 10^3-10^6) of specific bands originating from the chromophore group[1,2]. RRS is useful in the study of different pigments and dyes and therefore finds application in art history, archeology and forensics studies. It is successfully applied in nanotechnology and materials science in order to study and characterize structures of such materials as carbon nanotubes, graphite and graphene[1]. This non-invasive and non-destructive method also allows for the detection and assessment of the distribution of various biomolecules inside cells and tissues. This makes RRS extremely useful in biomedical studies, which is the topic of this lecture.

As RRS has long been applied to monitor the molecular dynamics of different metalloproteins[3], the majority of this lecture will be focused on the studies of the proteins with chromophoric heme prosthetic group with the use of RRS combined with complementary techniques. The *in vitro* studies of human red blood cells (RBCs) will be presented with the focus on detection and differentiation of different hemoglobin (Hb) forms including the nitrite-induced, and NO-induced Hb adducts[4], and the impact of the sodium dithionite on the oxygen uptake[5]. RRS provides excellent signal–to–noise ratio spectra with very high reproducibility from single RBC and therefore the studies of various hemopathies are possible which will be presented in the example of malaria infected RBCs[6,7]. Examples of the detection, analysis and visualization of 2D and 3D distributions of heme in both cells and tissues[8-10] will be presented, including also a high–resolution Raman imaging of the single cell[11]. Additionally, some examples of the application of RRS to other groups of chemicals will be discussed. We may include here RRS studies of the retinols content and its changes due to atherosclerosis or cancer progression in liver and lung tissues[12-13].

ACKNOWLADGEMENT

Financial support of the Polish National Science Centre (UMO-2016/23/B/ST4/00795) and the Polish Ministry of Science and Higher Education (grant No IP2015 048474) are greatly acknowledged.

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Lecture 9
Introduction to electron paramagnetic resonance (EPR) spectroscopy

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The term Electron Paramagnetic Resonance (EPR) encompasses a family of techniques based on the absorption of electromagnetic radiation in the microwave frequency region by a paramagnetic sample placed in a magnetic field. EPR has a broad range of applications in catalysis and related fields as it represents a very powerful tool for investigating paramagnetic entities. The first EPR experiment was performed by E. K. Zavoisky in 1945, observing the resonance of $CuCl_2 \times 2H_2O$ with a radiofrequency source operating at 133 MHz and a magnetic field provided by a solenoid. The limitation of EPR is evident from the previous definition in that diamagnetic systems, which represent the majority of the substances, are excluded from EPR investigations. However, this limitation is also an advantage since, for instance, reactive paramagnetic intermediates or paramagnetic centers belonging to a complex chemical system (e.g. a paramagnetic ion in a diamagnetic matrix of a porous material) can be studied without any interference. The relevant information provided by EPR concerns the nature, symmetry, and electronic structure of the paramagnetic center and of its surrounding. A second advantage of EPR, beside its specificity, is its high sensitivity which, in the investigations of solid surfaces, allows the detection of paramagnetic species in concentrations well below monolayer coverage.

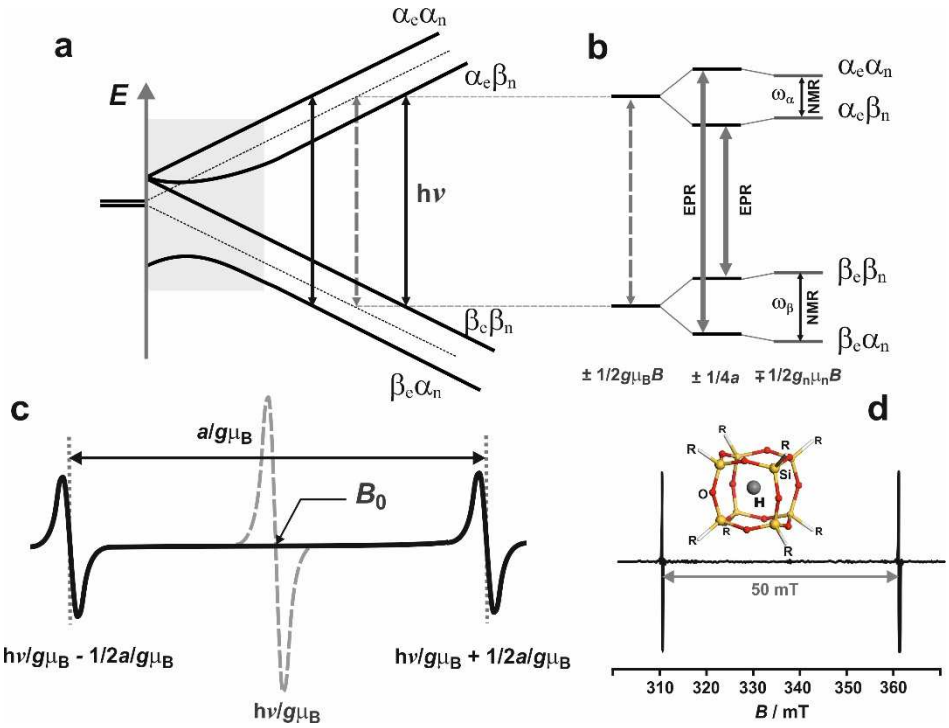

Figure 1. (a) Energy levels splitting for a $S = 1/2$ and $I = 1/2$ system as a function of magnetic field. (b) The energy level diagram at a constant field, and (c) the resulting isotropic EPR spectrum (dotted line shows the virtual Zeeman signal). (d) Experimental EPR spectrum with doublet hyperfine structure due to hydrogen atom ($I = 1/2$) encaged in octasilsesquioxane.

Electron paramagnetic resonance occurs when a magnetic-field component of an electromagnetic wave of suitable frequency (usually in the microwave region) interacts with the sample immersed in a magnetic field and causes the inversion of the spin (and thus of the magnetic moment) of the unpaired electrons, so that certain amount of the electromagnetic energy is absorbed by the system. In quantum mechanical terms the description of the electron

spin resonance can be reduced to the description of the behavior of a single unpaired electron undergoing, under the effect of an external magnetic field, an energy splitting between the two allowed spin states (so called Zeeman interaction). In such conditions an electron in the lower state is promoted to the upper one by absorption of a photon of suitable energy. Yet, another source of information is the interaction between magnetic moments of the unpaired electrons and those associated with the magnetic nuclei present in the system. This interaction, called the hyperfine coupling. gives rise to an EPR spectrum with a series of lines with well-defined separation.

During this lecture basic concepts concerning behavior of electron spin in a magnetic field, spin Hamiltonian and its symmetry, principal types of EPR spectra, and interpretation of spectral parameters will be presented. Selected typical applications of EPR spectroscopy in coordination chemistry, dosimetry, and catalysis will be briefly discussed. The aim is to provide a suitable background for more specialized applications of EPR technique for imaging and tomography discussed during this school.

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Lecture 10
Electron Paramagnetic Resonance Imaging - Basic Principles, Hardware, Software, and Applications

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In a classic experiment EPR the applied static magnetic field is homogeneous and the signal is derived from the entire sample volume. EPRI allows to determine spatial distribution of paramagnetic centers in the sample, and thereby to track the macro heterogeneity without destroying the sample [1,2]. Traditional EPR spectroscopy can be transformed into imaging technique (EPR Imaging or EPRI) by applying a magnetic field gradient at a site of the sample. The magnetic field gradient forms a link between the position in the sample and a resonance field, which results in coding the information on the spatial distribution of paramagnetic centers in the EPR spectrum. Decoding of this information requires the use of advanced numerical procedures [3,4]. In the presence of a magnetic field gradient (generated by an additional coils) only paramagnetic species present in a thin layer of the sample fulfill the resonance condition (Equation 1) at a given value of the homogeneous magnetic field *B*. In the case of the gradient parallel (G_x) to the sample axis the resonance condition is modified in the following manner:

$$h\nu = g_e \mu_B (B + x G_x) \qquad \text{Equation 1}$$

where:

x - the x coordinate (position in the sample)

From a mathematical point of view recorded spectrum, *Y(B)*, is a convolution of spectrum without gradient *S(B)*, concentration profile of paramagnetic species *P(x)* and profile of the cavity sensitivity, *C(x)*:

$$Y(B) = \int_{-\infty}^{+\infty} (S(B - x \cdot \mathbf{grad}_x B) \cdot P(x) \cdot C(x)) dx \qquad \text{Equation 2}$$

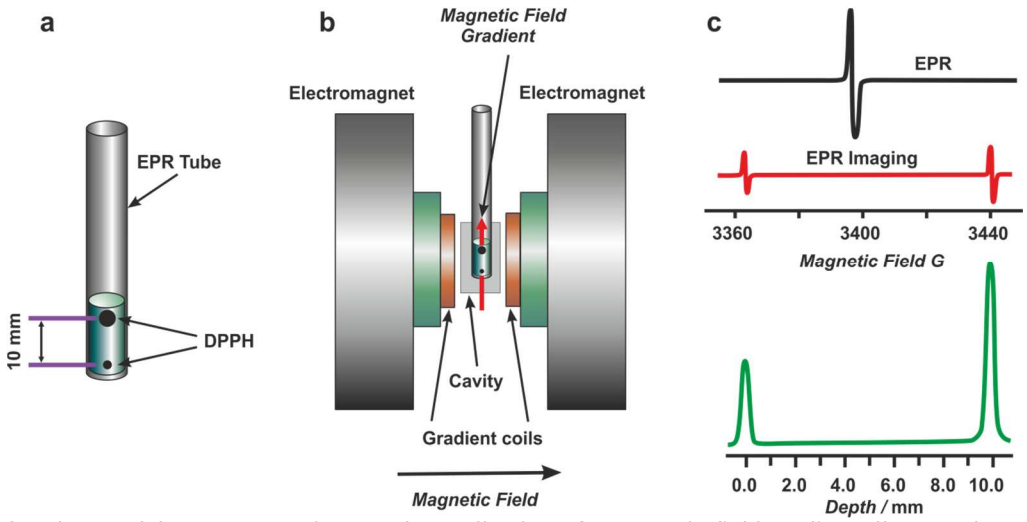

Figure 1. Scheme of the EPRI experiment. The application of a magnetic field gradient allows to determine the concentration profile of paramagnetic species within the sample.

The idea of EPRI experiment is presented in Fig. 1. On the left side (Fig. 1a) a model sample of two DPPH (2,2-diphenyl-1-picrylhydrazyl) crystals of different sizes, placed at a distance of 10 mm from each other is given.

In a classical EPR experiment the spectrum of this sample consists of one line (Fig. 1c, top). When magnetic field gradient is applied, the resonance occurs at two values of homogeneous magnetic field. With an appropriate deconvolution algorithm the concentration

profile of paramagnetic species can be determined (Fig. 1c, bottom). The obtained profile carries information about the spatial distribution of paramagnetic centers, making possible to deduce about the chemical processes take place in the sample [5]. The method could be applied to investigate polymer degradation, diffusion processes as well as in oxygen and spin dosimetry [2,5,6].

To investigate microheterogeneity of a polymer, however, it is necessary to apply two-dimensional spatial-spectral EPRI [6]. This experiment requires registration of a series of EPR spectra as a function of magnetic field gradient, and the use of a reconstruction algorithm.

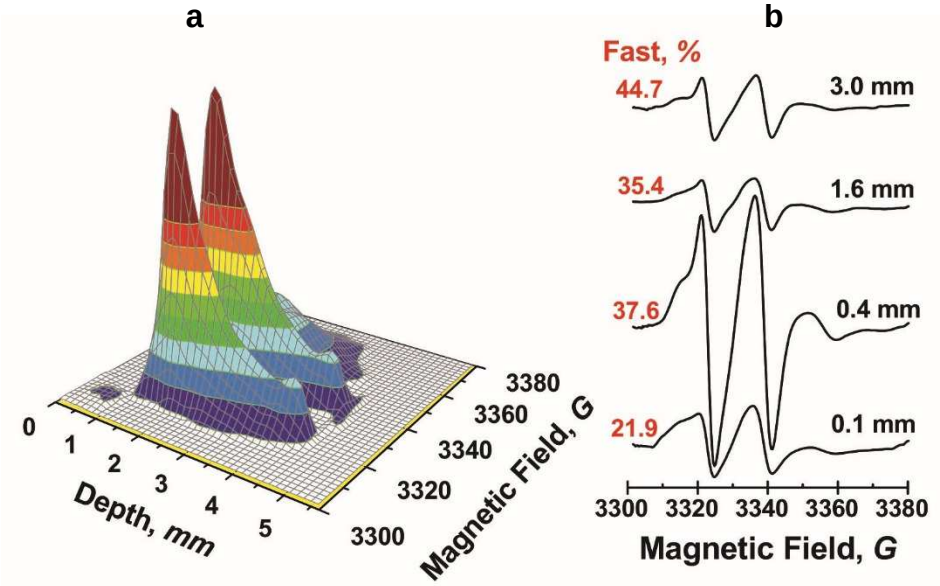

Figure 2. (a) 2 D spectral-spatial perspective plot of nitroxide radicals derived from hindered amine stabilizer doped co-polymer of ethylene and propylene. (b) Spectral slices determined nondestructively from 2D plot. The co-polymer sample was UVB-irradiated for 20 hours.

Two-dimensional (spatial-spectral) imaging (Fig. 2a), not only allows to determine of the location and concentration of paramagnetic species, but also to measure EPR spectra as a function of the position in the sample (Fig. 2b). Variations of 1D profiles and of line shapes deduced from 2D spectral-spatial images allowed the visualization of degradation events on two length scales: in the different phases with a resolution of 1-5 μm, and within sample depth with a resolution of ≈80 μm.

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Lecture 11
EPR Tomography

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One of the rapidly developing methods that provides unique information in the in-vivo setting is electron paramagnetic resonance imaging (EPRI). EPRI is a technique, which through the injection of a nontoxic spin probe with unpaired spin electrons, makes it possible to obtain information about the environment surrounding the highly concentrated spin probe, including the partial concentration of local oxygen, pH changes, the thiol concentration, and the tissue redox state. Since current EPR spectroscopy may be performed in 3D, it is possible to obtain three dimensional maps of tissue micro-environments. For example, oxygen may be mapped by scaling an EPR spectrum line-width of the spin probe, which is proportional to the local oxygen concentration. EPRI oxygen maps of animal tumors or redox state of neuro-degenerative disorders may become a very valuable imaging tool in preclinical studies. This makes EPRI an attractive functional imaging modality for medical applications. However, insufficient anatomical details provided by electron paramagnetic resonance imaging (EPRI), necessitates the use of other anatomic imaging references for the accurate interpretation of EPRI maps, especially in an in-vivo setting, which is similar to PET tomography. The barrier preventing the practical application of EPRI was the long duration of measurement. The development of new detection techniques, in particular based on the use of rapid scan with direct detection, as well as rotating gradients, allows the reduction in duration of the experiment from a couple - to dozens of minutes down to a few seconds. By improving the image reconstruction algorithm, obtained EPR images have higher contrast, resolution, and required lesser number of projections. On the other hand, in classical continuous-wave EPRI, which requires a long detection time, the number of averages could be significantly reduced by using multi-harmonics analysis. All the mentioned techniques combined together allows high-resolution EPR imaging of free radicals and tissue microenvironments.

ACKNOWLEDGMENTS

This work was supported by European Regional Development Found from Smart Growth program under project POIR.01.01.01-00-025/15.

Lecture 12
Atomic Force Microscopy for Surface Imaging and Beyond

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Atomic Force Microscopy (AFM) is a high resolution scanning probe technique that can be applied for imaging surfaces both in liquid and gaseous environments. The method is based on application of an elastic cantilever with a sharp probe (tip) that locally interacts with the studied surface enabling mapping of various surface properties. Precise displacements of the tip with respect to the surface is realized using the piezo crystal scanner while deflection of the cantilevere is commonly followed by reflected laser beam. Various interactions on molecular or atomic scale can be probed using AFM leading to imaging of surface topography but also its mechanical properties, adhesion, electrical conductance, magnetic properties or surface potential with the nanometer scale resolution. Moreover, AFM system may be applied for investigation of the interaction forces on a single molecule scale for e.g. ligand-receptor biological systems (force spectroscopy). The scanning tip may also act as a “dip-pen” in nanolithographic techniques. AFM may be also combined with spectroscopic techniques such as infrared or Raman spectroscopy enabling creation of chemical composition maps that show the spatial distribution of different chemical components with resolution down to ca. 10 nm.

Principles of the AFM technique, general procedures and limitations will be presented together with advanced applications of AFM in mapping of various properties of hard and soft matter samples, force spectroscopy, nanolithography and nano-FTIR applications.

Lecture 13
X-RAY microtomography

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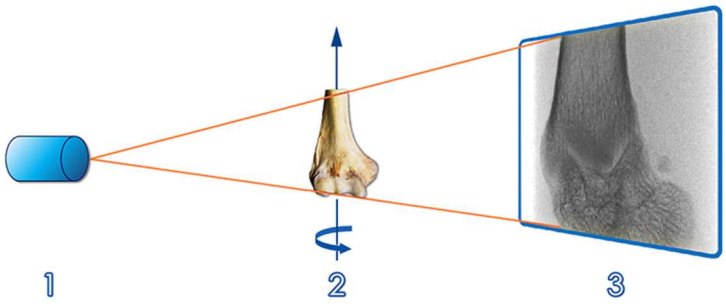
X-ray microtomography (micro-CT) is a non-destructive 3D imaging method that allows to analyze internal structure of small samples with micron resolution. Micro-CT permits to distinguish areas with different values of X-ray attenuation coefficient, which is proportional to the density of the tested material.



X-ray source (1) produces cone beam of X-rays which pass through the sample mounted on a turntable (2). The radiation attenuated within the sample is captured by CCD detector (3) and saved as a projection file. The detector acquires hundreds of angular views with a rotation step lower than 0.5 degree.

In the next step, obtained data set is used for 3D image reconstruction based on Feldkamp algorithm. The reconstruction process results in a stack of cross-section images.
Image processing and analysis provides many morphological parameters describing the sample such as volume, surface, porosity, fractal dimension, structure thickness or structure separation. Micro-CT data can be also used for 3D modeling and finite elements analysis.

Lecture 14
Future Leadership Programs in Industries and needed communication skills

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Future leadership programs are the gateway to enter and develop at multinational companies. Many companies envision consistently developing individuals and improving overall performance to support them in attaining their strategy of business growth. Career development at these companies is therefore considered an essential element and perceived as a shared responsibility of managers, employees and the organization as a whole.

At multinational companies, they believe that you are at the helm of your career and should therefore take primary responsibility for managing it. You are encouraged to be ambitious and carefully plan your career development in order to:

Support your future development
Enhance your strengths and skill sets
Close existing gaps between performance expectations and actual performance
Achieve your current goals
Reinforce your engagement and commitment

This unique work environment supports both your career aspirations and company's business strategy.

Being able to communicate effectively is the most important of all life skill. In order to secure a good position in Industry, a master student should possess strong communication skills.

Chemistry evening
The curious cases from forensic papers

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Forensic chemistry belongs to those disciplines which truly are interested not only to scientists but also to general public enjoyed being thrilled by criminal books or TV shows. Indeed, among scientific papers one may find articles on forensic investigation in curious cases, setting up unusual databases or giving details on experiments which may be further used in a way not predicted by the authors. Within this presentation such three cases will be presented and discussed.

There is no doubt that the spontaneous human combustion belongs to one of the most unusual cases reported in forensic journals. This term refers to the burning of human bodies without any apparent nor visual external source of ignition, and its history of reported cases is at least as long as the beginning of XVIII century, which influenced Charles Dickens to use it in his "Bleak House" novel published in 1853. Till this time numerous cases have been also reported, but treated with scepticism by forensic experts. The mythology of ''spontaneous'' human combustion has been commented by Roger W. Byard in 2016 [1], which enhanced the author to look into this phenomena.

Setting up the databases on various substances, objects, cases, types of evidences is also one of the major tasks of the forensic experts. Various databases are commercially available, such as those for paints, shoes prints, dyes, inks, MS or IR spectra of psychoactive substances etc. Others had to be created by scientists. One of the spectacular and curious database released in 2017 by LokMan Sung is that on morphology of modern arrowhead tips on human skin analog [2]. Apparently, contemporary archery, treated not only as a sport but also as a way of hunting, may be also a subject of forensic investigation. The performed and published experiments enabled to provide a reference and primer for medical examiners and coroners to formulate their opinions and lend support in trial.

The last example confirms that not only scientists read the forensic papers, particularly those related to investigation of designer drugs. In 2007, there was published an article on methcathinone synthesis from pseudoephedrine using some common chemicals [3]. Apparently the author provided so many details as since then his paper has been frequently quoted on drug users forums presenting the recipes "how to produce drugs at home".

Mentioned above examples supports the thesis that reading scientific forensic papers may be fascinating not only for forensic experts. In some cases, like in the last one, it is even inspiring, however it may lead to some illegal activity.

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Students session

Flash oral 1
Characterization of thin polymeric films by means of AFM

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Atomic force microscopy (AFM) is undoubtedly one of the most useful methods for characterization of surfaces at the nanometer scale. AFM working in standard modes (contact or semi-contact) provides information about thickness of a coating, surface morphology, roughness and qualitative mechanical properties. Moreover, there is a large group of modes designed for characterization of more sophisticated sample features, such as conductivity, magnetic properties or surface potential. ResiScope® is an example of innovative AFM mode that enables surface conductivity mapping and capturing of current/voltage plots in selected areas on the sample.
In this work, standard intermittent contact AFM mode was used for thickness measurements of poly(N-isopropylacrylamide) (PNIPAM) and poly(methylmethacrylate) (PMMA) polymer brushes, obtained using surface-initiated atom transfer radical polymerization (SI-ATRP) in the same conditions on four different substrates (Fig.1). SI-ATRP guarantees a precise control over propagating radicals and formation of well-ordered and dense polymeric layers [1]. The influence of surface type on overall ATRP kinetic was investigated towards detection of probable catalytic activity of the substrates components. Besides, ResiScope® mode application is presented for conductivity mapping of conjugated polymers. Commercially available mixtures of PEDOT-PSS (in various ratios) with different conductivity were successfully characterized using this technique (Fig.2).

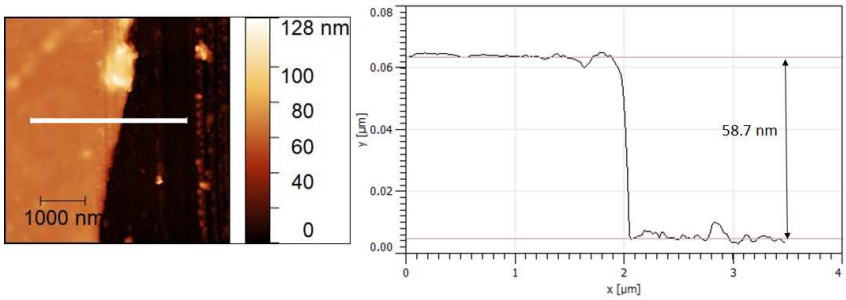

Figure 1. Topographic map of PNIPAM brushes on silica; the white section corresponds to the zone where the nearby thickness profile is extracted.

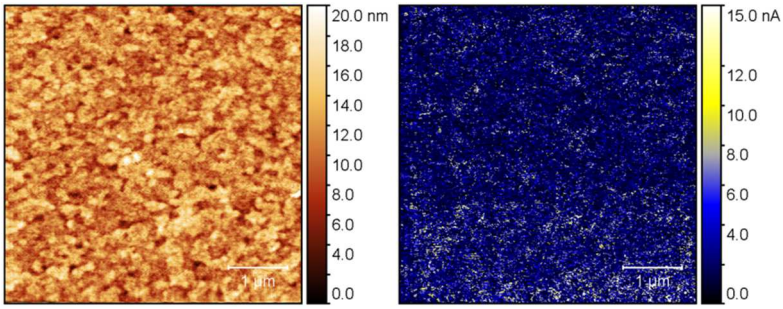

Figure 2. Topography (left) and conductivity (right) maps of PEDOT-PSS sample.

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Flash oral 2
Interaction of small molecules with cobalt spinel nanocrystals

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Cobalt spinel nanocrystals (Co_3O_4) are excellent redox-tunable model catalytic nanomaterials of well-defined shape (Fig. 1a and 1b) and widespread applications. In particular, these materials exhibit an exceptional performance in the low temperature decomposition of nitrous oxide (N_2O). However, catalytic performance of Co_3O_4 can be significantly diminished due to the presence of inhibitory gases, especially nitric oxide (NO). Despite the critical role of NO in the deactivation of Co_3O_4, a comprehensive molecular-level description of its interaction with the catalyst surface is still lacking.

Herein, periodic spin unrestricted, gradient corrected DFT calculations along with first principles thermodynamics were performed to investigate the structure and stability of various NO adspecies on the most abundant cobalt spinel (100) facet, over a wide range of temperatures and pressures. Several conceivable models of NO admolecules were examined to find the most stable adsorption mode. Then, according to the obtained results, the NO molecules were successively added to coordinatively unsaturated cobalt ions in order to obtain full surface coverage corresponding to a monolayer. The results revealed that in the pressure range of typical catalytic reactions (p/p° ~0.01 to 1), at low temperature range (below 50-100°C), two molecules of NO are bound to the surface in a top-on fashion: one to an octahedral center (Co^O) and the other to a tetrahedral center (Co^T), as depicted in Fig. 1c. In higher temperatures (between 50-100°C and 475-600°C), the NO molecule adsorbed on Co^O leaves the surface, and only one NO molecule remains strongly attached to the Co^T (Fig. 1d). The constructed thermodynamic diagram of NO sorption (Fig. 1e) summarizes the surface coverage as a function of pressure and temperature, providing a convenient background for understanding the harmful effect of the presence of NO on the catalytic performance of cobalt spinel.

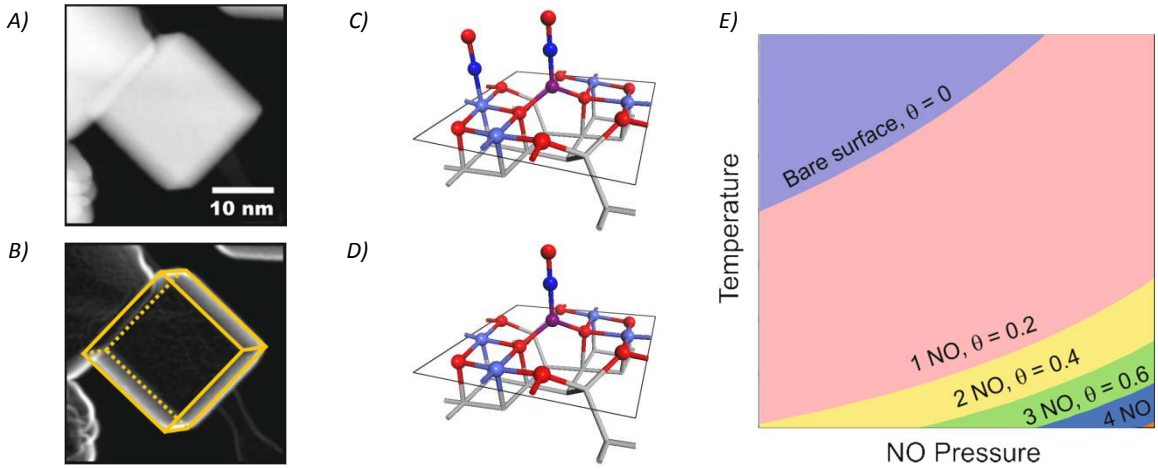

Fig. 1 – A) STEM image of the cobalt spinel nanocrystal exhibiting cubic morphology. B) Gradient of the STEM image, showing the edge pattern used for morphology retrieving. Perspective view of C) two and D) one NO molecule adsorbed on the cobalt spinel (100) surface. Color coding: Co^O – light blue; Co^T – purple; O – red; and N – dark blue. E) Two-dimensional diagram of NO coverage as a function of temperature and partial pressure.

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Poster 1
Luminescent Zr-based containing metal-organic frameworks for application in photocatalysis: synthesis and characterisation

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Metal organic frameworks (MOFs) are currently of great interest because of their well-defined crystalline structures and high porosity, which allow their use in a wide range of applications.[1] Furthermore, low water solubility and the possibility of tailoring their photophysical properties, make them suitable for photocatalysis.[1, 2] Recent studies compare the activity as photocatalyst of Zr-based MOFs with the most commonly used TiO_2, showing higher stability and efficiency.[3] These developments encouraged us to synthesise a new luminescent Zr-based MOF in the perspective of its application as photocatalyst.

Lengkeek et al. (2011)[4]

Figure 1: Synthesis of the target ligand through Heck cross-coupling.

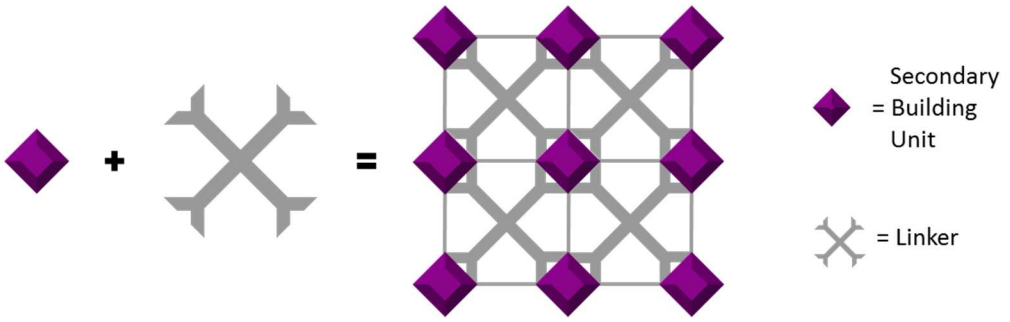

Figure 2: Schematic description of the Zr-based MOF.

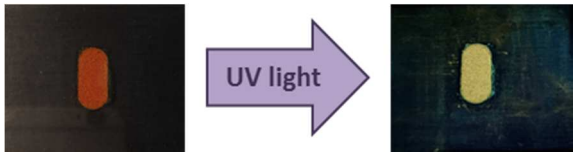

Figure 3: Emission properties of the Zr-based MOF when irradiated with UV-light (254 nm).

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Poster 2
Novel photosensitizing drugs for photodynamic therapy of cancer and biocatalysis

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Porphyrins are a class of naturally occurring tetrapyrrolic macrocycles extensively studied, due to their excellent photophysical and photochemical properties as well as their fundamental role in biological processes. More specifically, their popularity can be accounted for its high quantum yield of triplet state formation[1] and extensive π-electron delocalization that confers them high chemical and thermal stabilities[2]. Several applications have been reported for this class, among them application in photocatalysis and photomedicine[1-3]. According to the required properties, their framework can be modulated (Fig. 1). A common approach is the coordination of metal ions in its cavity and the insertion of different ligands, which enables tuning spectroscopic, electrochemical and photophysical properties, as well as their hydrophobicity, stability and degree of aggregation. Previous studies of the group revealed that, depending of the inserted metal, such as Zn (II), Mn (II), Pd (II) and Co (II), these tetrapyrrolic macrocycles can behave differently upon light irradiation and different media[1,2]. These compounds can react with molecular oxygen in different mechanisms, which determine their biological activity *in vitro* and *in vivo*. Moreover, the functionalization with groups such as sulfonic and sulfonamides modifies their lipophilicity, directly affecting its cellular uptake and accumulation. The present study aimed for the tuning of PDT and biocatalysis relevant sensitizers' properties, through rational synthesis (Fig. 2), followed by spectroscopic and photophysical/photochemical characterization.

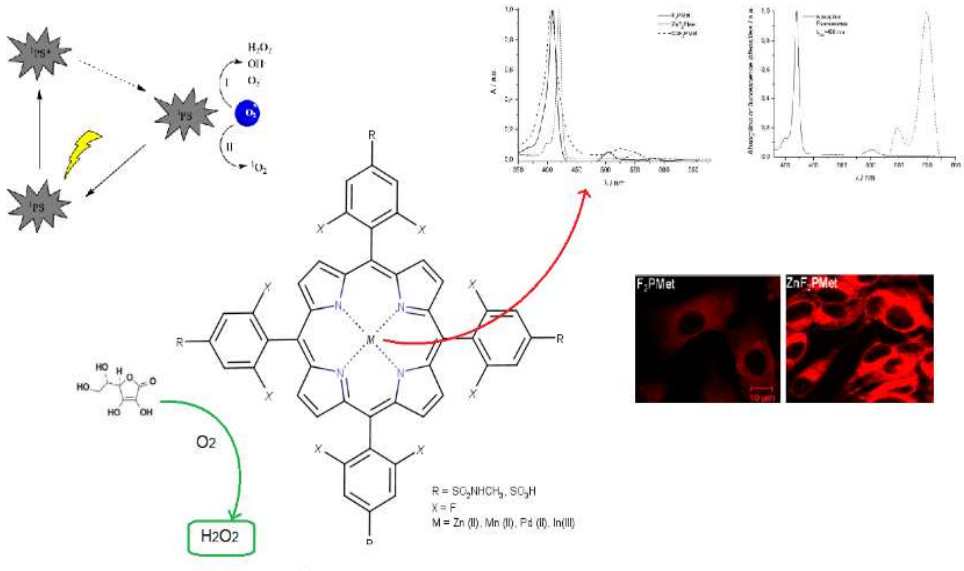

Fig. 1. Framework of a meso-tetraphenylporphyrin (TPP), with different metals and ligands. In the right upper part, spectra of Abs.and Fluor. of free-,Zn- and Co-F_2PMet and in the left, a scheme of the mechanism involved in the formation of Reactive Oxygen Species (ROS) in PDT[1]. In the bottom, example of intracellular distribution of F_2PMet and Zn- F_2PMet[2] and in the left, its application with Ascorbate, in order to produce more H_2O_2 within the cell[3].

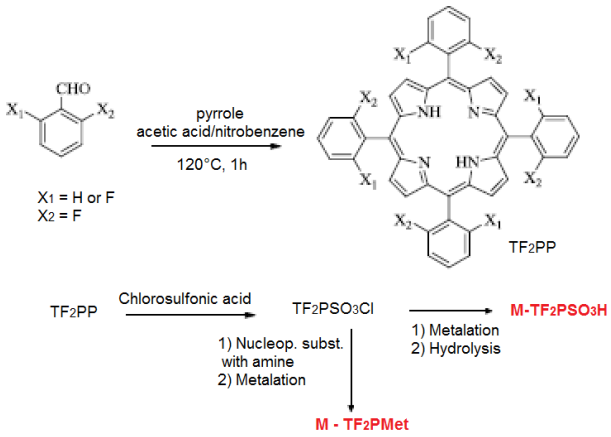

Fig. 2 - Scheme of synthetic route adopted

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Poster 3
Size-modulation of colloidal iron oxide nanoparticles

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Nanosized iron oxide particles have been extensively investigated due to the increasing interest in applications in several areas including nanomedicine. Superparamagnetic iron oxide nanoparticles (SPION), smaller than 20 nm, comprises a superparamagnetic iron oxide core whose surface is functionalized with molecular coatings embodying improved chemical stability, enhanced biological compatibility and high saturation magnetization [1]. This study aimed at to investigate the effect of the molar percentage of Fe^{+2}, in relation to the total iron (Fe^{+2} plus Fe^{+3}) during the coprecipitation of iron salts in alkaline medium of the nanoparticle synthesis process. The employed percentages of Fe^{+2} were equal to 34%, 50% and 75%. The resulting nanoparticulated solid was functionalized with citric acid and dispersed in aqueous medium pH 7, resulting in stable colloidal suspensions named magnetic fluid. The as prepared magnetic fluids were characterized through dynamic light scattering, UV-vis, Infrared and Raman spectroscopies.

Our findings indicated that this synthesis process is efficient to modulate nanoparticle size, obtaining hydrodynamic diameters ranging from 34 nm to 52 nm, increasing as the percentages of Fe^{+2} relative to the total iron used in the syntheses increases. The presence of the citrate at the nanoparticle surface was evidenced by the zeta potential values and analysis of the infrared spectra, indicating that the citric acid molecules are coordinated to the iron at the nanoparticle surface by their carboxylate groups. The Raman spectrum of a synthesized nanoparticle showed vibrational modes at 300, 319, 504, and 694 cm^{-1} characteristic of magnetite phase.

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