ABSTRACTS

ASC Summer School

20 – 24 June 2022

Lecture 1

Lecturer: dr Joanna Gryboś

Topic: Introduction to Transmission Electron Microscopy

Within this contribution, the focus is to provide the participants with basic concepts of transmission electron microscopy. The first part of the lecture is focused on the description of the physical basics of electron beam–matter interactions. Further, the lecture is focused on typical methods of sample preparation. Strong attention will be devoted to emphasizing the sample requirements as well as the limitations of the microscopic technique. To better understand the physics behind electron microscopy and spectroscopy, a brief description of the electron microscope construction is presented, as well as the typical contrast types obtained on the microscopic images. The further part of the lecture is devoted to the most important microscopic techniques such as the **High-Resolution Transmission Electron Microscopy** (HR TEM) to present its ability to reveal the ultimate details of the structure of nanocrystalline materials. The chief advantage of the HR TEM 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. Since the HR TEM allows for emphasizing the most powerful imaging abilities of the microscope, the technique is used for presenting the limitations of the imaging system.

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 wave function. 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 microscopic imaging parameters. What the electron microscopists are looking for is the projected potential of the structure. Unfortunately, due to the strong scattering of the electron by the crystal potential, only the wavefunctions transmitted by very thin crystals are the close to 1:1 image of the projected potential. Moreover, the imaging system of the electron microscope suffers from aberrations (spherical, chromatic, limited bandpass, etc.) so that, depending on the amount of misfocus, images quite different from the projected potential are obtained. Thus, image simulation, commonly based on a multislice algorithm, should be used for retrieving the non-distorted wave function and the projected potential of the examined crystal structure. Proper analysis of HR TEM images provides reach information on the nanocrystal structure with a picometric precision, allowing for analyzing, e.g., defects, surface structure relaxation, and reconstruction.

Another crucial area of use of the TEM is **Electron Diffraction**. Within this part, the lecture is focused on presenting the physical basics of the pattern formation, the difference between X-ray – matter and electron beam – matter interactions, that influence the most electron diffraction, and the presentation of an exemplary use of selected electron diffraction techniques.

The last microscopic technique described during the lecture is **Scanning Transmission Electron Microscopy** (STEM). The presentation is mainly focused on the type of data collected during the STEM experiment and its applications for the analysis of the morphology of the nanocrystals.

Besides techniques based on elastic scattering, a transmission electron microscope can provide spectroscopic information about the specimen. The spectroscopic description of the sample is obtained while the microscope is operating in one of the **analytical modes**: **EDX** (Energy Dispersive X-ray Spectroscopy, **Figure 1**) signal consists of characteristic X-ray photons emitted when sample ions excited by electron beam return to their ground state. Analysis of the photons' energy allows for identifying the elements of the sample.

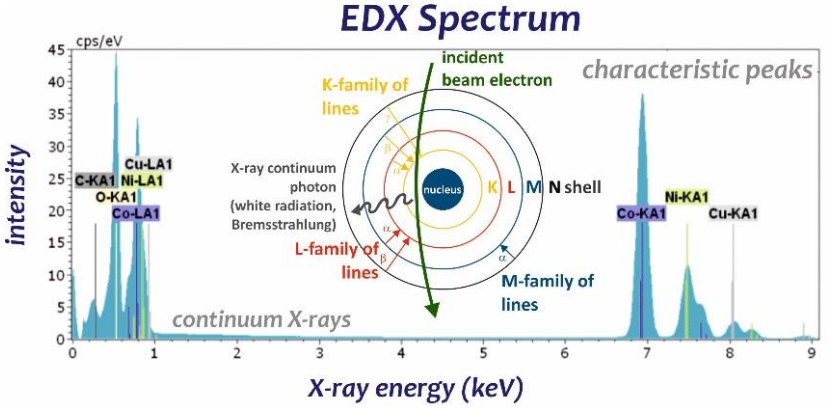


Figure 1. Exemplary EDX spectrum.

EELS (Electron Energy Loss Spectroscopy, **Figure 2**) energy losses due to inelastic scattering of the electron beam transmitted through the specimen are measured and recorded as an energy loss spectrum providing information on atom type, local bonding, coordination, valence state, orbital hybridization, and structural distortions of the atom in a crystal structure, **EF-TEM** (Energy-Filtered Transmission Electron Microscopy): a microscopic image is formed, using a post-column GIF (Gatan Energy Filter), by the inelastically scattered electrons, over selected energy ranges of the EEL spectrum additionally providing chemical information to the TEM images.

These methods, including their physical background, type of provided information, sample requirements, and limitations will be presented in the last part of the lecture.

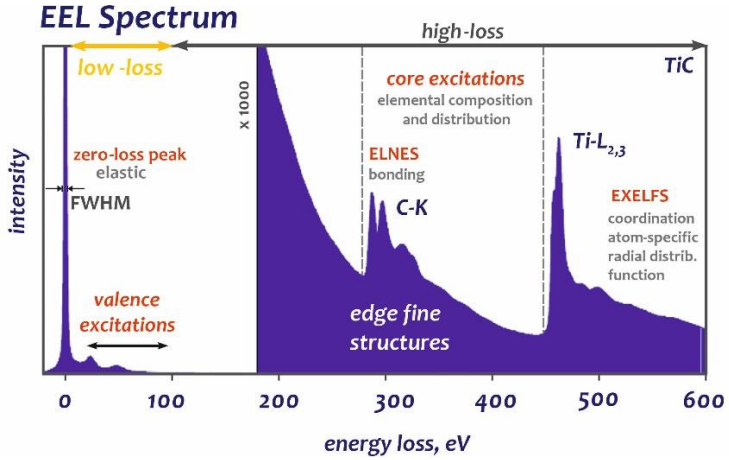


Figure 2. Exemplary EEL spectrum.

Lecture 2

Lecturer: dr Aušra Domanska

Topic: CryoEM basics and application

Cryogenic electron microscopy (cryoEM) is a general term for the observation of frozen specimens under an electron microscope. In transmission electron microscopy (TEM), electrons that pass through the sample are detected, whereas in scanning electron microscopy (SEM) electrons that reflect from the sample are recorded. In TEM, two modes of data collection are possible: imaging and diffraction. TEM image is referred to as a projection and is a sum of a density through the macromolecule. The diffraction-based cryo-EM techniques include 2D electron crystallography and microcrystal electron diffraction (MicroED). SEM image is a distribution map of the intensity of the signal being emitted from the scanned area of the specimen; the intensity of the signal depends on the surface topology.

CryoTEM approaches operating in imaging mode are especially suited for structure determination of biological macromolecules, which are purified or in the context of cells and even tissues. Due to recent advances, cryoEM is a powerful technique in structural biology generating high-resolution density maps of macromolecules, alongside to X-ray crystallography. CryoEM imaging and single particle analysis (SPA) is suitable for studying isolated identical molecules embedded in random orientations in thin layer of vitrified ice. In SPA, thousands of images that represent single molecule of interest, from different orientations, are aligned and combined to produce a high-resolution reconstruction. If molecules have no regular structure, for example pleomorphic viruses or cells, cryo electron tomography (cryoET) can be used. In cryoET, sample is tilted in respect to the electron beam and images at different angles are taken from the same object. These images are then processed to produce a 3-dimensional (3D) volume of the object. CryoET gives very valuable biological insights into how molecules are arranged within the cells or tissues, but the resolution of this method is usually lower than that of SPA, 2D electron crystallography or MicroED. Sub- tomogram averaging is used to gain resolution of repeating objects in the tomograms.

In 2D electron crystallography and MicroED, crystalline material is used. In 2D crystallography, crystals are 2-dimensional, whereas in MicroED crystals are 3D (but very small if compared to those used in X-ray crystallography), thereby causing differences in data collection and processing approaches. Studying 2D crystals, electron diffraction data and electron microscope images are taken from 2D crystals that are used to determine structure of the macromolecule. Henderson and his colleagues were first to use this technique in solving structure of a membrane protein bacteriorhodopsin, which naturally forms 2D crystals. Nowadays, 2D electron crystallography is mainly used for structure determination of membrane proteins as they can form 2D protein arrays in the lipid sheets. MicroED technique combines approaches both used in cryoTEM and X-ray crystallography. The cryo-sample preparation and use of electron microscope are similar to cryoTEM procedures, whereas crystal growth, data processing and refinement resemble those in X-ray crystallography. In MicroED, electron-diffraction data is collected from very small crystals at an ultra-low dose that allows many exposures from a single crystal. Thus, MicroED can complement structural biology techniques when only micron sized 3D crystals are available.

In recognition of advances in cryoEM, the 2017 Nobel Prize in Chemistry was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson for their contributions to method's development.

Lecture 3

Lecturer: prof Elisabetta Venuti

Topic: Vibrational modes in molecular crystals: the synergy between Raman experiments and computed structures and spectra

Molecular crystals are solids in which molecules retain their chemical identity because intermolecular interactions, arising from van der Waals forces as well as hydrogen and halogen bonds, are much weaker than the intramolecular ones (i.e. the covalent bonds of the chemical species). The large difference in the strength of the two kinds of interactions involved is mirrored by the vibrational properties of the system, which typically span two distinct energy regions. The so-called "lattice" or external modes lie in the 10–150 cm^{-1} range, while the "intramolecular" modes are generally found at higher wavenumbers. The lattice modes are cooperative motions in which the molecules in the unit cells can be described as rotating and translating with respect to each other, as a whole, without getting deformed. The intramolecular modes, instead, correspond to the vibrational modes of the isolated molecule, combined and slightly perturbated by crystal interactions. The most interesting feature of the lattice modes is their dependence on the specific pattern of intermolecular interactions which characterize each crystalline structure and makes them the ideal spectroscopic probe of the structure itself. Thus, their investigation can be used for structural identification and polymorph screening as the resulting spectra represent the fingerprint of a specific crystal structure. Also, and importantly, the knowledge of the relationships between crystal packing motifs and intermolecular vibrations has direct implications on all properties that on such vibrations depend, such as charge and heat transport.

The focus of the lecture will be on how the lattice modes at Γ point can be investigated by micro-Raman spectroscopy in the low wavenumber range[1], and how the interpretation of the experiments is made easier by the development of highly accurate density functional theory (DFT) calculations which reproduce the low-frequency Raman patterns of the molecular solid, just starting from a given or assumed crystal structure[2].

Micro-Raman spectroscopy in the low wavenumber range is a conventional Raman technique in which the scattering signal can be collected over an energy range that is very close, ideally by only a few wavenumbers, to the intense line of the elastic Rayleigh scattering. The method has proved to be fast, non-destructive and allows for in situ measurements.

The most common Raman spectrometers on the market use holographic notch filters, characterized by considerable bandwidths, typically >200 cm^{-1}. With such a value, both the Rayleigh scattering and a large portion of the low-wavenumber range of interest are cut out. To experimentally achieve satisfactory low-wavenumber detection limits can be achieved by means of two different strategies, which will be briefly presented: triple stage mono-chromators or, more recently, volume Bragg gratings as ultra-narrow optical filters, also known as Ultra-Low frequency Filters (ULF).

From a computational point of view, the possibility of predicting correctly the vibrational properties in molecular solids depends on the accuracy of the model which describes the intra- e inter-molecular forces. In fact, (semi)local DFT functionals do not account for the long-range attractive part of vdW-interactions and appear unsuitable to describe the vibrations of these systems in the lattice phonon spectral region. With a posteriori vdW-correction schemes, however, an accurate calculation of the vibrational spectra of organic molecular crystals is actually feasible and the whole vibrational range[3], from the highest intramolecular frequencies (4000 cm^{-1}) down to the lowest modes around 10 cm^{-1}, is well described (Figure 1).

Combining the experimental and computational approaches constitutes a valid method for polymorph characterization alternative/complementary to X-ray diffraction techniques and can be applied to the study of functional molecular materials.

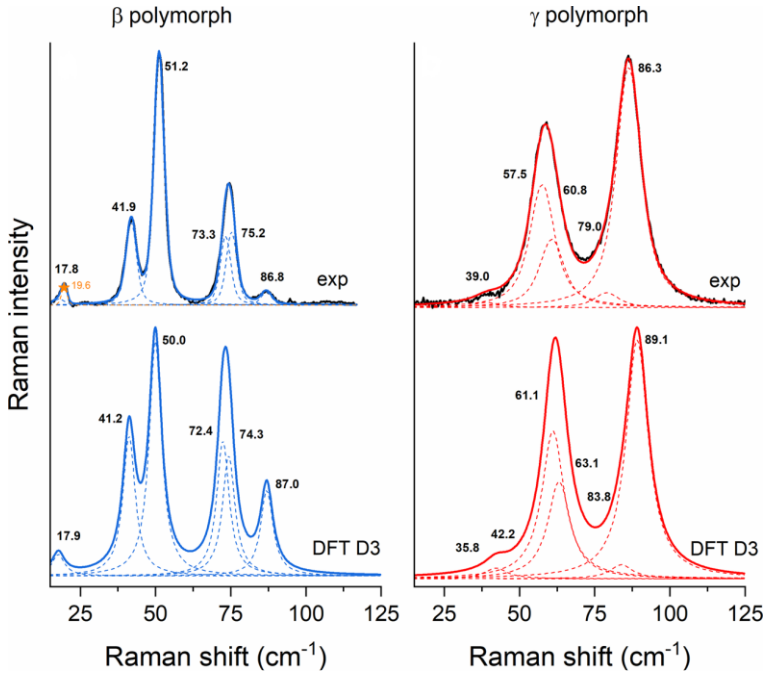


Figure 1 DFT-vdW calculated lattice phonon Raman spectra of γ (right) and β (left) polymorphs of the model system coronene are compared to the experiments at the temperatures where the two crystal forms are thermodynamically stable

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Lecture 4

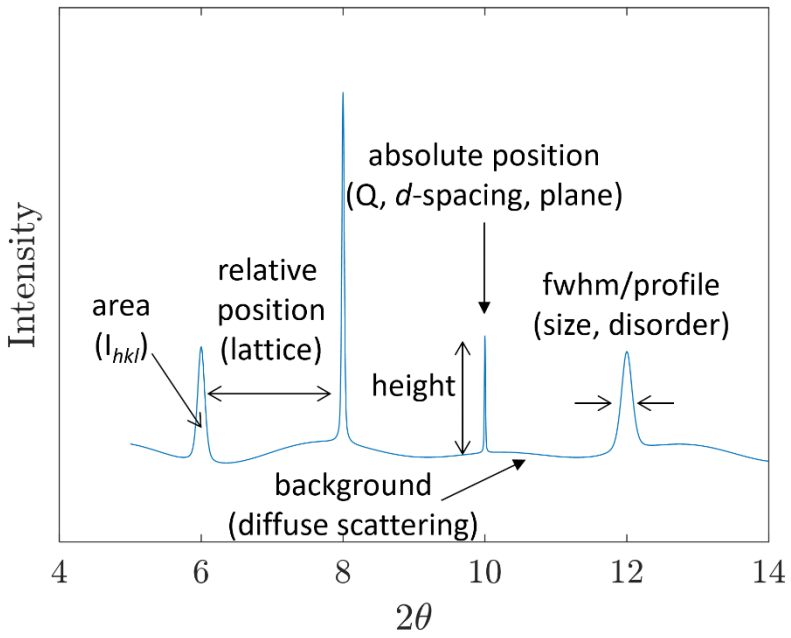
Lecturer: dr Mattia Gaboardi

Topic: X-ray powder diffraction methods at synchrotron light-sources

The study of materials and their mechanistic understanding is often underpinned by a minimum knowledge of their structure. Nowadays, large-scale research infrastructures facilitate the investigation of crystalline materials and nanostructures in all forms and shapes for applications encompassing all fields in materials science, physics, chemistry, biology, and cultural heritage.

Powder diffraction is a powerful tool employed in most cases to obtain basic information at small laboratories. At synchrotron light-sources this quintessential technique is taken to its extreme limit, enabling non-standard experiments where full tunability, large sensitivity, high resolution, and fast data acquisition become natural traits. These advantages can be largely useful when studying materials upon variation of external parameters (*in situ*) such as temperature, pressure, or mechanical stress or during standard working conditions (*operando*), such as humidity, voltage, or interaction with gases.

A basic awareness of crystallography and diffraction theories is a useful element to run simple experiments and for a minimum data analysis. Deeper understanding of powder diffraction techniques available at synchrotrons, an extended perception into common-practice analytical tools, and information hidden in the Bragg peaks will be important part of this seminar and are meant to provide much needed opportunities for young researchers approaching this area.



Lecture 5

Lecturer: prof Kinga Góra-Marek, dr Karolina Tarach

Topic: Time-resolved IR spectroscopy: from adsorption and diffusion processes to reaction intermediates identification

If modifications to the catalyst structure, pore hierarchy, or chemical environment have changed the site speciation, which one offers the best activity/selectivity? Can we modify or create a new tool to recognize the working sites and count active species in real catalysts? These and similar questions must be answered concerning the formulation of effective catalysts, most often with the use of spectroscopic tools.

The time scale of phenomena specific to the catalytic reaction, i.e. diffusion, adsorption, the transformation of substrates into intermediates and final products, is an indispensable factor to figure out the reaction mechanism. Time-resolved spectroscopic studies of unstable intermediates and reactive moieties conducted over a temporal range spanning from seconds to several dozen milliseconds can cross beyond the boundaries of the conventional approach. The possible insight into the elementary steps and the reaction pathway with spectroscopic methods depends on both the lifetimes of the intermediate species and the conditions required for the respective process investigation (*in situ* and *operando* conditions). However, meeting the criteria mentioned above results in gaining the spectra sequences, which are characterized by high complexity and very low, even extremely low intensity of the bands. Conventional analysis of difference spectra does not give, therefore, the proper answer. Implementation of two-dimensional correlation analysis to IR and UV-vis spectroscopic data (2D COS IR and UV-vis) attained for catalytic processes makes it possible to identify intermediates “hidden” in the spectra as well as the sequence of the intermediates' appearance on the catalyst surface. The 2D COS analysis allows for simplifying the complex spectra consisting of many overlapped peaks, enhancing spectral resolution by spreading peaks over the second dimension, and finally identifying various inter- and intramolecular interactions through selective correlation of IR bands. A combination of two-dimensional correlation spectroscopy and rapid scanning techniques is thought to give an insight into mutual relationships between reagent and catalyst in the transformation of highly reactive molecules and those with poor IR characteristics as well.

Acknowledgment

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Lecture 7

Lecturer: prof Szczepan Zapotoczny

Topic: Atomic Force Microscopy for Surface Imaging and Beyond

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 8

Lecturer: dr Dariusz Marek

Topic: Industrial Use of IR and Raman Spectroscopy

Vibrational spectroscopies, i.e. infrared absorption spectroscopy and Raman spectroscopy, are commonly used by chemists and physicists in the academia for the characterization of the molecular structure of studied samples. However, various features of these techniques, in particular a short analysis time, lack of sample preparation, the possibility of measurement *via* packaging, easy automatization, and often one-click operations, make them also attractive for commercial applications. Therefore, vibrational spectroscopies are increasingly used by various branches of the industry for a plethora of different applications. Among others, these techniques are applied for quality control by food and feed, polymer, petrochemical, pharma, cosmetics, and dairy industries. The technique that is particularly suited for quality control is near-infrared absorption spectroscopy (NIR) as the recorded spectra are usually quite simple, which facilitates the chemometric analysis providing not only a qualitative but also a quantitative output. Moreover, the measurement area of the NIR spectroscopy is significantly larger compared to the area measured in the mid-infrared and Raman spectroscopy, giving more representative information about studied samples. Similarly to Raman spectroscopy, NIR can also be used to record the spectra *via* the package and, if properly configured, also for in-line applications.

An example of a common application of NIR is quality control in poultry farming, where this method is used at every step of meat production including controlling feed ingredients, greasers, premixes, feed production, meat quality, etc. Exemplary NIR spectra of blood meal (a high protein animal feed) with the chemometric analysis of the ash concentration, recorded and analyzed in order to build the quantitative model, are presented in **Fig. 1**.

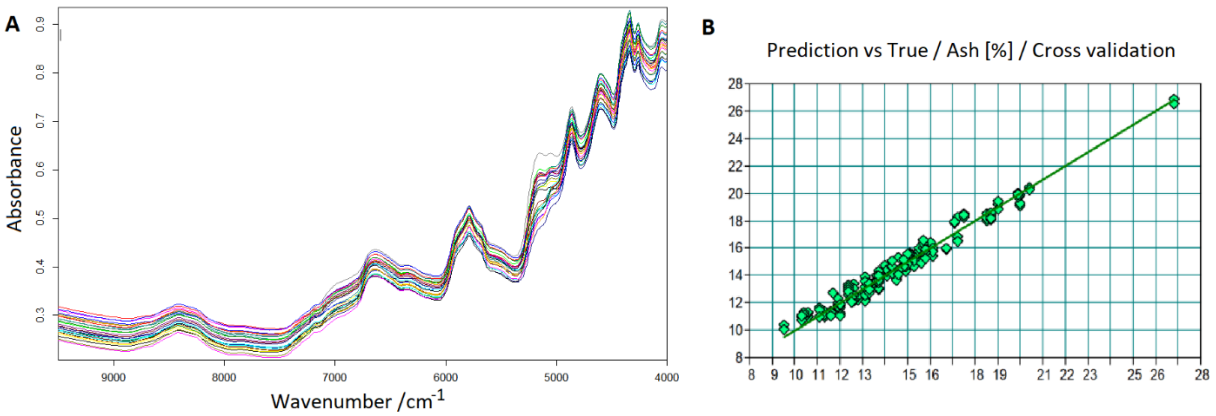


Figure 1. NIR spectra of blood meal (A) and chemometric analysis of the ash content in blood meal samples (B).

Other common applications of NIR are control of batch homogeneity, in-line control of reaction processes, and the control of the active pharmaceutical ingredient (API) concentration in tablets.

However, the significant advantage of mid-IR and Raman spectroscopies over NIR is the high spatial resolution, which gives the opportunity to determine the distribution of selected components in the microscale *via* the microscopic approach. Hence, IR, and particularly Raman spectroscopy, can be applied for analysis of sample contaminations (pills, liquid crystal displays, and others), API distribution in tablets, analysis of paint coatings, layers analysis of polymer laminates, determination of gemstone and signature falsification, etc.

Currently, spectroscopy is replacing various conventional methods, requiring sophisticated preparation steps and longer analysis times. In particular, spectroscopy enables to move the quality control from the laboratory directly to the production process *via* the in-line approach, which gives the opportunity to optimize production processes in the real time.

Lecture 9

Lecturer: prof Krzysztof Kruczała

Topic: Electron Paramagnetic Resonance Imaging - basic principles, hardware, software and applications

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:

$$h\nu = g_e\mu_B(B + xG_x) \qquad (1)$$

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)*:

$$Y(B) = \int_{-\infty}^{+\infty}(S(B - x \cdot grad_X B) \cdot P(x) \cdot C(x))dx \qquad (2)$$

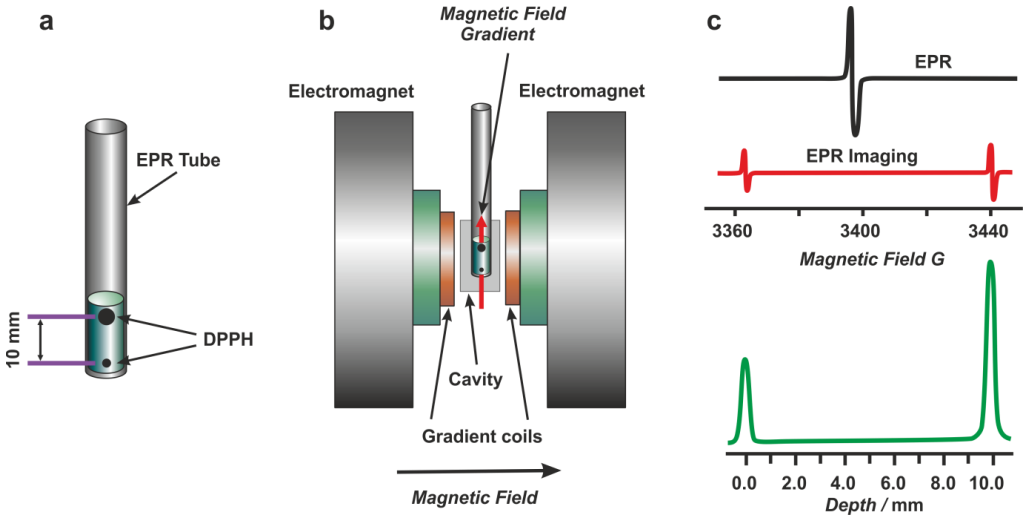


Figure 1. Scheme of the EPRI experiment. The application of a magnetic field gradient allows to determine the concentration profile of paramagnetic species within the sample.

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.

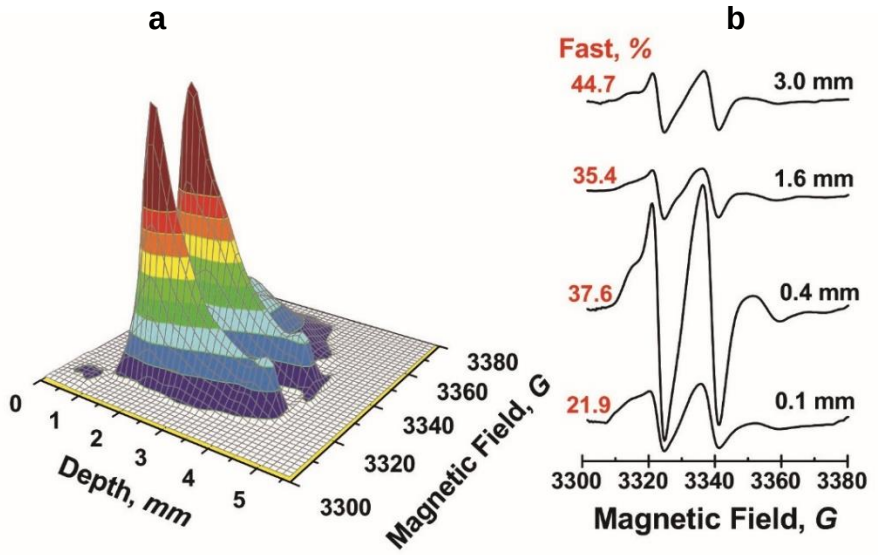


Figure 2. (a) 2 D spectral-spatial perspective plot of nitroxide radicals derived from hindered amine stabilizer doped co-polymer of ethylene and propylene. (b) Spectral slices determined nondestructively from 2D plot. The co-polymer sample was UVB-irradiated for 20 hours.

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 10

Lecturer: dr Bartosz Leszczynski

Topic: X-ray microtomography

Nowadays, tomographic methods such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) are the gold standard in imaging diagnostics of many diseases and injuries. The aim of clinical tomography is to maximize spatial resolution and at the same time shorten the examination time while maintaining the patient's safety, especially to reduce the radiation dose in CT and PET. Current CT devices achieve a spatial resolution of 0.24 [1].

There are preclinical equivalents of clinical scanners designed for small samples or small laboratory animals for ex vivo and in vivo examination. Due to imaging of a significantly smaller volume of several cubic centimeters and much less restriction on the radiation dose or the value of magnetic field induction, it is possible to achieve resolution at the micrometer level. X-ray microtomography (micro-CT) is an excellent example. Micro-CT is a dynamically developing nondestructive imaging technique. The most important difference between micro- and clinical CT is their optimization for specific applications.

From a technical point of view, both clinical and micro-CT require an X-ray source. There are micro-CT laboratories operating on synchrotron lines that take all the advantages of synchrotron radiation, such as monochromaticity and high radiation intensity. However, due to the limited availability of synchrotrons, the majority of clinical CT and micro-CT scanners use X-ray tubes. The second integral part of the CT system is the X-ray camera. Therefore, CCD or CMOS arrays combined with a scintillator transforming X-rays into visible light are used. Micro-CT high-resolution cameras allow you to obtain an image with a resolution of up to 4920 x 3264 pixels.

The detector captures an image of X-ray attenuation within the sample. Computed tomography is an indirect imaging method. Recorded projections are used in the image reconstruction process, which enables the calculation of the three-dimensional distribution of the linear X-ray attenuation coefficient in each part of the sample. This distribution is normalized and presented as cross-sectional images of the test sample. Typically, these are images presented in 256 shades of gray.

The versatility of the technique requires optimization of the experimental procedure for a particular task. In general, the optimization process begins with sample preparation and selection of the most effective scanning parameters. In the next step, knowledge of the structure and composition of the sample is helpful in minimizing imperfections and imaging artifacts considering the image reconstruction process. The final important step is to optimize the procedures related to image processing, analysis, and 2D and 3D visualization methods. Micro-CT has a wide range of applications, from material analysis to soft tissue imaging.