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Raman spectroscopy in single cell-drug and DNA-drug studies

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Chemotherapeutic anthracycline antibiotics such as doxorubicin (DOX) are antibiotics endowed with cytostatic activity used in anti-tumor therapy of breast cancer, malignant lymphoma, acute leukemia and others. Their mechanism of action involves the formation a stable complex between the anthracycline and DNA helix, which prevents further division and leads to cell death. In the course of chemotherapy, anthracycline antibiotics are administered intravenously over a period of several cycles of several months. It is therefore inevitable that endothelial cells are in direct contact with cytostatics, which is not without an importance for their activity and vascular toxicity. Anthracyclines according to literature data show a strong cardiotoxicity and induce apoptosis of the endothelial cells [1].

In this study Confocal Raman Imaging was used to monitor a cellular composition as a result of DOX exposure on living endothelial cells. Cells were directly grown on calcium fluoride slides (CaF2, 25×2 mm, Pike Technologies, U.S.) and treated with appropriate dose of antibiotic (from 100 nM up to 10 μM) for 24 hours. Raman mapping was performed using Witec alpha 300 Raman spectrometer equipped with a 60× water immersion objective (Nikon Fluor, NA=1).

In the literature have been shown AFM results presenting effect of DOX on double stranded DNA [1]. It was shown that the elevated concentrations of DOX result in DNA chain loops and overlaps are present with higher probability. In this study a combination of high-resolution AFM and Raman spectroscopy was also applied to investigate DOX-DNA intercalation at the level of single double stranded DNA molecule.

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Acknowledgements:

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Raman imaging of vascular endothelial cells within blood vessels in murine models of cancer metastasis and diabetes

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Endothelium, the multifunctional organ building the blood-tissue barrier is involved in inflammation of the vascular wall and its malfunction is related to many western world diseases, including cancer metastasis, type 2 diabetes mellitus (T2DM), atherosclerosis and hypertension [1].

The aim of this work was to investigate the spectroscopic changes in murine endothelium caused by cancer-metastasis and diabetes. Using Raman 3D confocal imaging to investigate individual layers of aorta *en face*, we demonstrated that common feature of these pathologies was chemical imbalance in the protein and lipid levels in the endothelium. Alterations in the endothelial layer in the murine model of cancer metastasis were observed as the statistically significant increase of the protein content accompanied by the slight decrease of the lipid level [2]. On the contrary, a strongly increased lipid level was observed in the endothelium under the development of diabetes type 2 [3]. Due to the fact that biological interpretation of these changes requires further work, the measurements of endothelial cells in their natural environment (including the basal membrane and underlying smooth muscle cells) within functional isolated blood vessels as well as their changes induced by pro-inflammatory stimulation were performed.

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New Types of Nanoresonators for Shell-isolated Nanoparticle-enhanced Raman Spectroscopy

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Surface analysis of various materials (especially *in situ*) is very important from the economic and scientific point of view. Such studies are especially difficult for so-called buried interfaces, which include interfaces of various biological samples in the "natural" environment. The aim of this work was to improve selectivity and sensitivity of a new analytical method of chemical analysis of buried interfaces developed by Tian *et al*. in 2010 [1]. In this approach the analyzed surface is covered with the layer of gold or silver nanoparticles covered by a thin protective layer (for example of silica or alumina), and then the Raman spectrum of the investigated sample is recorded. Metal nanoparticles act as electromagnetic resonators, significantly enhancing the electric field of the incident electromagnetic radiation, and hence leading to very large increase in the Raman signal (in the so-called *surface-enhanced Raman scattering – SERS* effect) from the surface on which nanoparticles have been spread. This allows for Raman analysis of surfaces of various important materials (e.g. catalysts, biological samples). Ultrathin coating (for example from SiO_2 or Al_2O_3) does not damp surface electromagnetic enhancement, however, separates nanoparticles from direct contact with the probed material and keeps them from agglomerating. This new analytical method developed by Tian *et al.* is called *shell-isolated nanoparticle-enhanced Raman spectroscopy − SHINERS*.

To increase the efficiency of SHINERS nanoresonators we synthesized SHINERS nanoresonators with other then semi-spherical plasmonic cores (for example: hollow structures or structures containing many sharp apexes and edges). To develop more efficient nanoresonators for SHINERS measurements in an alkali or an acidic environment we developed the methods of deposition of other oxide layers (e.g., from MnO_2, ZrO_2 or TiO_2) on plasmonic silver cores.

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Resonance Raman Spectroscopy in Studies of Red Blood Cells

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Resonance Raman Spectroscopy (RRS) has huge diagnostic potential also in red blood cells (RBCs) studies[1,2]. RRS gives the possibility of an analysis of the molecular changes even in a single RBC in a small quantity of blood without previous staining or fixation processes what makes this technique a promising tool in a cell alteration studies. Moreover, if a specific molecular change of RBCs has characteristic marker bands on Raman spectrum, the diagnosis could take even up to several seconds[2].

Here we presented the application of RRS supported by complementary techniques for detection of biochemical changes of human and murine RBCs obtained from both, healthy conditions, as well as disease state. RBCs were analyzed mainly from the point of the hemoglobin molecular structure. Studies were focused on the detection, differentiation and visualization of different hemoglobin species/adducts formed in RBCs due to pathologically or chemically induced alterations[2-5].

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Investigation of atherosclerosis using novel fiber-optic Raman probe

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It is known that the state of vascular endothelium determines the condition of the cardiovascular system, and its dysfunction causes development of lifestyle diseases. The influence of the endothelium on blood vessels can be described as "from inside to outside" and is relatively well understood, also from the spectroscopic perspective [1]. Recent studies show that progression of the disease, i.e. atherosclerosis also involves significantly the perivascular adipose tissue (PVAT) that interacts "from outside to inside", due to its paracrine activity. PVAT's adipocytes secrete proinflammatory adipokines which diffuse into the vessel wall and can affect the endothelial layer causing its dysfunction. Moreover, impaired function, damage or excess PVAT directly induce inflammation that intensifies atherosclerosis [2].

The aim of this work was to define the chemical changes arising from atherosclerosis and correlate them with the changes in PVAT (particularly lipid composition of this tissue) what may bring deeper understanding of mechanisms of development of atherosclerosis.

In our experiments, *en face* tissue sections of the mice aorta and PVAT obtained from animals with developed atherosclerosis (the murine model of atherosclerosis ApoE/LDLR$^{-/-}$) and control subjects (C57) were investigated using the fiber optic Raman spectroscopy with the 532 nm excitation. The results show, among others, that the lipid to protein ratio changes along the aorta, increasing in abdominal part which effect deepens upon the development of the disease. Moreover, these changes in the lipid to protein content correlate with changes in the degree of unsaturation of lipids in the PVAT tissue.

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A question of time - estimating the age of bloodstains with Raman spectroscopy

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When was the bloodstain deposited?, Was the questioned blood trace formed before or after the criminal event?, Is the bloodstain, originating from the alleged offender, equally aged as bloodstains deposited by the victim? From the judicature perspective, answers to this kind of questions are often indispensable to strip the criminal act from its mystery, however, they still remain beyond the reach of scientists, as the contemporary suite of forensic analytical tools still remains incomplete [1]. Consequently, a field of bloodstain examination is long overdue for revolution, a revolution to be brought about through developing a method for establishing time elapsed since bloodstain deposition.

The answer to the question of bloodstains age might be delivered by Raman spectroscopy (RS), which has already proven its worth in the field of blood-related studies [2]. Extensive research efforts have evidenced that Raman spectra of hemoglobin (Hb) reflect the oxidation and spin state of iron atoms, as well as the degree of Hb oxygenation [3]. Given that *ex vivo* degradation of blood incorporate changes in above-mentioned Hb features, RS appears to be an appropriate candidate for bloodstains dating studies, as it may provide a molecular insight into the degradation pathways of Hb. Therefore, the main motivation behind this study was to propose an approach based on RS for establishing deposition time of blood traces.

In this study, bloodstains subjected to the analysis were formed by depositing 20 µl of capillary blood - without addition of any preservatives nor anticoagulants - into an Al sample holder. Samples were stored under ambient conditions (temperature: 24.6 ± 1.6 °C, relative humidity: 26 ± 5%) for 30 days. Raman spectra were recorded daily on a Renishaw inVia Raman microscope spectrometer, using a diode laser emitting at 785 nm. The collection optic was set at 20x objective. In order to prevent localized sample heating by the exciting radiation, Raman signal was registered while samples being rotated, utilizing a specially designed rotating device. This solution not only allowed the use of higher exciting energy in comparison to static measurements, but also enabled representative probing of the chemical composition of the heterogeneous surface of bloodstains.

The obtained results demonstrated that chemical composition of bloodstains exhibits a distinct temporal behaviour, since at each time point, over the period of 30 days, different Raman spectra were observed. This let us presume that the current forensic stalemate can be broken, and establishing a method for bloodstains dating is only a question of time. However, Raman spectroscopy cannot be considered as a self-contained panacea for the investigated issue. As chemometric support is vital to extract the desired information from spectroscopic signatures, the subsequent stage of the research would involve incorporation of chemometrics into the interpretation of registered Raman signals.

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TiO_2/MoS_2 heterostructures as a photoelectrodes with long term stability

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MoS_2 powders were obtained under hydrothermal conditions (variable: time, surfactants) and deposited electrochemically (variable: time, voltage) onto TiO_2 nanotubes via new, ecological approach proposed by our group [1].

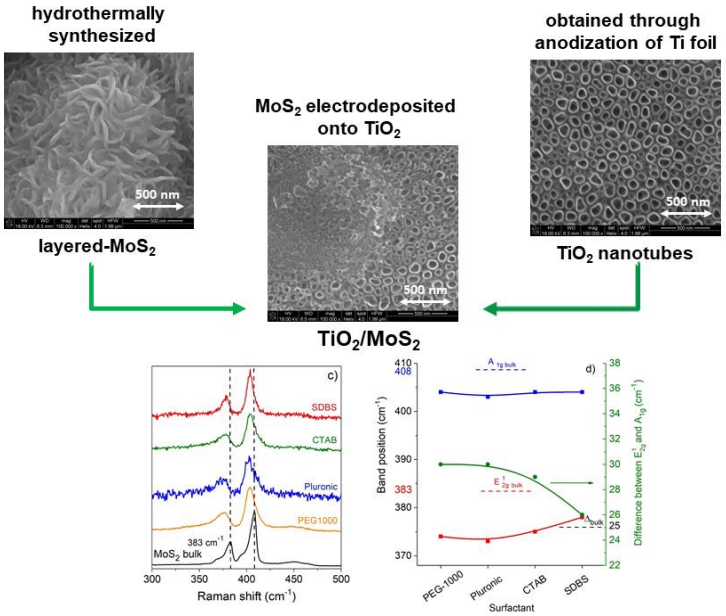

Figure 1. TiO_2/MoS_2 as a type-II semiconductor heterojunction.

The materials were characterized with the use of SEM, XRD, Raman and UV-vis spectroscopy and also characteristics in a photoelectrochemical cell (PEC). Applied techniques allow to confirm a layered structure for MoS_2, which plays as a conductive pathway for electron transport in TiO_2/MoS_2 II-type semiconductor heterojunction.

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Shedding Light on Peptide Aggregation

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Attenuated Total Reflection–Fourier Transform Infra-Red (ATR-FTIR) spectroscopy is one of the most widely used techniques to study secondary structure of proteins in aqueous solution.This method facilitates the investigation of peptide aggregation and amyloid fibril formation. Furthermore, ATR-FTIR can differentiate oligomers from fibrils based simply on their spectral features [1-3].

Here we attempt to identify the potential amyloidogenic amino acids sequences. It's well-known that the peptide conformation is highly dependent on many factors, i.e. the solvent, the pH or temperature [2]. Ideally, the structure study of protein aggregation processesshould be conducted under physiological conditions. The choice of solvent is crucial. Phosphate buffer and phosphate-buffered saline are the ones most commonly used in biological research. Unfortunately, we encountered serious solubility problems.We also observed variable base line distortions in the amide bands region (1720-1380 cm^{-1}), which are crucial for protein structure determination. We eventually decided to conduct experiments with air-dried peptide films.

The main structural features of amyloid aggregates are anti-parallel β-sheets. Amyloid fibrils have a spectral signature clustering between 1611 and 1630 cm^{-1} [2]. The presence of the characteristic relatively intense absorption band in infrared spectra easily distinguishes aggregated proteins. We found that some peptides were incorrectly classified as non-amyloid in literature. These results demonstrate that ATR-FTIR can be used for studying aggregation properties of peptides.

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Applicability of ATR-FTIR spectroscopy to measure protein peroxidation

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Oxidative stress is a state of imbalance between the number of reactive oxygen forms and the body getting rid of it. It results in the oxidation of all cellular elements and, to a large extent, proteins, causing inter alia the formation of carbonyl groups in their structures. We focused on assessment of changes in the oxidative stress level based on changes in the concentration of carbonyl groups in horse plasma.

To measure the amount of oxidative stress, we used a spectrophotometric assay using 2,4-dinitrophenylhydrazine, which we adjusted to measure plasma. Next, we correlated the results with the measurements of the number of carbonyl groups by means of infrared spectroscopy. The test consisted of subjecting the plasma sample to the action of hydrogen peroxide (Sigma-Aldrich, USA). A series of dilutions of 30% (9.8 M) of H_2O_2 were prepared. Then 20 µl of each solution was added to 480 µL of plasma. Samples were incubated for 30 minutes at 37°C in a thermomixer. Then measurements of the concentration of carbonyl groups by the above-mentioned methods were done.

In the peroxidation study of proteins using hydrogen peroxide, both methods showed that with increasing concentration of hydrogen peroxide, the number of carbonyl groups in proteins increased, whereas in the low concentration range spectrophotometric method with DNPH showed a higher coefficient of determination, however in the ATR-FTIR method its value it was also at a satisfactory level, and the correlation coefficient of both methods was 0.99.

We presented the applicability of the ATR-FTIR method to measure the level of protein peroxidation.

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New possibilities to achieve pixel resolution down to 300 nm with FTIR-Imaging

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FTIR spectroscopic imaging provides simultaneous information on the molecular composition of the samples and their spatial distribution with resolution to a few microns. With the development of new optics, objectives and Large Area micro ATR accessories, there has been a drastic improvement in the spatial resolution, sensitivity and overall speed of analysis for a range of new and existing applications. This contribution presents the latest developments in FTIR imaging, providing examples in the analysis of tissues and of very small particles such as microparticles and thin layer structures.

Current state-of-art IR imaging techniques and their capabilities in biomedical sciences

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IR spectroscopic imaging originated nearly 25 years ago, with the successful merging of an IR microscope with an array detector and an interferometer. For almost two decades the instrumentation had been largely similar to this initial setup. Recent advances over the past few years have dramatically changed both instrumentation and availability [1]. Most notably, rigorous understanding of image formation led to emergence of High Definition IR imaging [2], while progress in Quantum Cascade Lasers (QCL) enabled QCL microscopes with high-speed of acquisition [3] and advancements in nanospectroscopy when coupled to an AFM/SNOM microscope. A review of these advances organizing the various developments in terms of spatial, temporal, and information content change will be presented.

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The use of FTIR imaging for ex vivo investigation of physiological and pathological processes of different etiology

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During last decades we have witnessed impressive progress in the applications of FTIR microspectroscopy, particularly in biomedical and life science. The technique is in fact a combination of two methods – light microscopy and infrared spectroscopy. Whilst the first one allows to localizing microscopic details in the analyzed samples, the second one provides information about their chemical composition. The rapid expansion in the biomedical applications of microprobe vibrational spectroscopic analysis results from the fact that large amount of valuable biochemical information can be mined from changes in vibrational spectra recorded for tissues and cells. What is more, FTIR microspectroscopy is a sensitive, non-destructive and label-free method offering diffraction-limited spatial resolution.

In frame of the presentation selected examples of the use of FTIR microscopy and imaging for the investigation of both normal and pathologically changed animal tissues will be shown. The presentation will be focused on the current research carried out in the Department of Medical Physics and Biophysics, Faculty of Physics and Applied Computer Science, AGH UST in the cooperation with the Department of Neuroanatomy, Institute of Zoology and Biomedical Research, JU. Among the discussed topics there will be:

- biomolecular changes of the hippocampal formation connected with postnatal development;
- biochemical anomalies occurring in the brain as a result of the treatment with the high fat diet;
- abnormalities in the distribution and structure of biomolecules appearing in the liver and kidney as a result of SPIO nanoparticles action.

Multi-modal imaging of tumour heterogeneity in a glioma xenograft model

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Imaging modalities are powerful tools for investigating molecular features of tumours both *in-vivo* and *ex-vivo*. In recent years, *ex-vivo* analytical techniques such as Fourier-transform infrared (FTIR) spectroscopy and mass spectrometry imaging have been increasingly employed in cancer research to distinguish healthy from diseased tissue, identify different tumour subtypes and visualise heterogeneity within, and between tumours. Tumours do not behave as a uniform biological entity, and this heterogeneity poses a significant challenge for therapeutic intervention. FTIR and mass spectrometry imaging techniques offer high spatial resolution and an untargeted approach, making them ideal for imaging molecular heterogeneity. Here, a multi-modal imaging approach has been demonstrated for a xenograft glioblastoma, in which FTIR and 3D- desorption electrospray ionisation mass spectrometry (3D-DESI-MS) imaging have been used, alongside positron emission tomography (PET), and immunofluorescence staining to give a comprehensive and complementary insight into tumour heterogeneity. Histological regions including areas of hypoxia are identified within the tumour and molecular information sheds light on the biochemical nature of tumour heterogeneity, which could ultimately inform the development and progression of new treatments in the clinic.

Nano-FTIR nanoscopy for organic and inorganic material analysis

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Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is a scanning probe approach to optical microscopy and spectroscopy bypassing the ubiquitous diffraction limit of light to achieve a spatial resolution below 20 nanometers. s-SNOM employs the strong confinement of light at the apex of a sharp metallic AFM tip to create a nanoscale optical hot-spot. Analyzing the scattered light from the tip enables the extraction of the optical properties (dielectric function) of the sample directly below the tip and yields nanoscale resolved images simultaneous to topography [1]. In addition, the technology has been advanced to enable Fourier-Transform Infrared Spectroscopy on the nanoscale (nano-FTIR) [2] using broadband radiation from the visible spectral range to THz frequencies.

Recently, the combined analysis of complex nanoscale material systems by correlating near-field optical data with information obtained by other SPM-based measurement methodologies has gained significant interest. For example, the material-characteristic nano-FTIR spectra of a phase-separated PS/LDPE polymer blend verifies sharp material interfaces by measuring a lineprofile across a ca. 1µm sized LDPE island (Fig1). Near-field reflection/absorption imaging at 1500cm-1 of the ca. 50nm thin film allows to selectively highlight the distribution of PS in the blend and simultaneously map the mechanical properties like adhesion of the different materials [3,4].

Further, results will be presented that correlate the near-field optical response of semiconducting samples like Graphene (2D) or functional SRAM devices (3D) in different frequency ranges (mid-IR & THz) to Kelvin Probe Force Microscopy (KPFM) measurements. Thus, neaspec s-SNOM systems represent an ideal platform to characterize complex material systems by different near-field and AFM-based methods at the nanoscale.

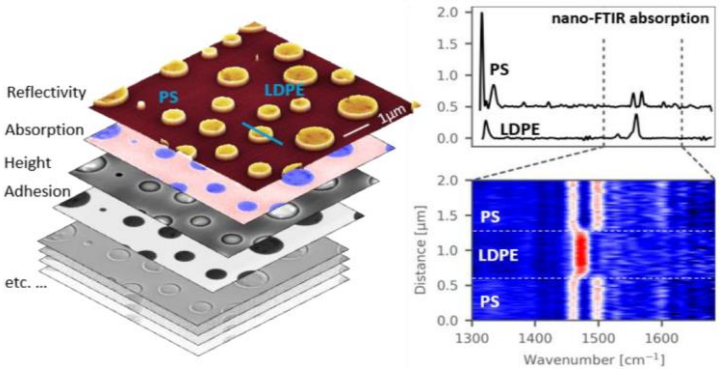

Figure 1. Near-field correlation nanoscopy of a thin PS/LDPE polymer film, highlighting the phase separation of the materials by nano-FTIR measurements as well as the different mechanical properties of the polymers

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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. 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 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.

Vibrational Circular Dichroism as a Tool for Sensing Chiral Molecules in Solution and Solid-state

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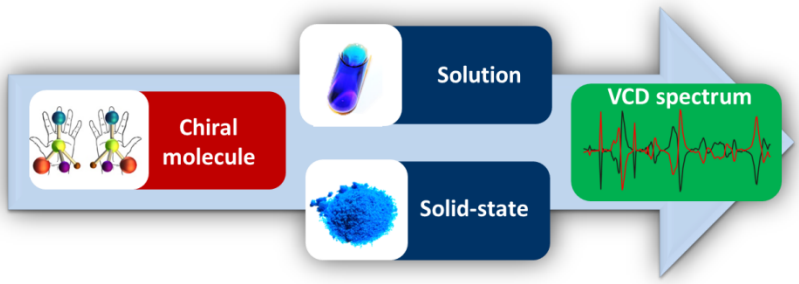
Chirality is omnipresent in nature, both at the molecular and at the supramolecular level and it is generally regarded as a factor of prime importance in the wide range of Life Sciences. The majority of essential compounds of living organisms are chiral. It means that they exist only in a single form among two or more possible options, throughout the whole biochemical world ranging from the simplest viruses to the most complex organisms.[1] Consequently, sensing chiral molecules for determining their absolute structure (configuration/conformation) continues to be an important challenge in many different fields. The most frequently and powerful technique for this purpose is circular dichroism (CD), which is also the first choice method for elucidating chirality, and, in particular, for monitoring and characterizing even smallest changes of molecules in solution and solid-state.[2] CD spectroscopy in the mid-IR range, *i.e.* vibrational circular dichroism (VCD), is considered as a handy tool for molecular probing and for solving diverse stereochemical problems with very different origin.[3]

Within this talk, some recent examples from chiral analysis via use VCD spectroscopy will be demonstrated for solving selected structural problems and also to show challenges posed by this method. Furthermore, based on illustrative examples the comparison of solution VCD data with quantum chemical calculations on DFT level will be shown.



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Biochemical changes occurring in the liver and kidneys of rat after intravenous injection of the D-mannitol coated superparamagnetic iron oxide nanoparticles

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Nanoparticles, defined as the objects with diameter between 1 and 100 nm, exhibit multiple features which make them highly attractive especially for biomedical applications. These qualities include, inter alia, small sizes, comparable with cellular components and biological molecules, high surface to volume ratio, chemical composition of the core and magnetic properties [1]. On the other hand, exactly the same features may be the source of the toxicity of these nanoobjects [2,3]. Among different nanoparticles predominant attention is focused on the biocompatible, biodegradable as well as easy to synthetize and functionalize superparamagnetic iron oxide nanoparticles (SPION) [4].

Despite the opportunities offered by advances in the fields of nanotechnology, the use of nanoobjects in medicine is not possible without prior examination of their impact on the living organism. Thus the first step towards it must be to carry out *in vivo* studies on animal models, that will determine organs in which SPION accumulate as well as structural and functional changes caused by these particular nanomaterials [1].

The Fourier transformed infrared (FTIR) microspectroscopy provides opportunity to evaluate biochemical changes resulting from pathological processes observed in body tissues in various diseases [5,6]. In our study the biochemical changes occurring within liver and kidneys of rats 7 days after administration of the low dose of D-mannitol coated SPION were examined. The study was focused on the liver and kidneys of the animals subjected to the SPION which was dictated by the key role that the organs play in the organism detoxification.

The experiment was done at the SMIS beamline of SOLEIL synchrotron. The FTIR imaging system consisted of an Agilent 620 FTIR microscope and an Agilent 670 FTIR spectrometer with a Globar® light source and a liquid-nitrogen cooled 128 × 128 FPA detector. IR light was transmitted through a 4× objective (N.A. = 0.2). Spectra were collected in the wavenumber range 798 – 3901 cm^{-1}. The chemical mapping was carried out on unprocessed spectra. Univariate spectral maps were performed by displaying either the area of one peak or the area ratio for the two peaks. The Mann–Whitney *U* test was applied for statistical evaluation of the differences between animals treated with SPION solution and control group injected with equal amount of injection medium.

Obtained results revealed that even low dose of intravenously injected SPION may cause significant changes in the relative and absolute content of proteins, lipids and phosphate groups containing molecules within kidneys. Moreover, significant anomalies in protein relative secondary structure were noticed. Interestingly, even accumulation of investigated nanoparticles was observed within liver, almost none statistically relevant biochemical changes were found within the organ.

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Biochemical composition of ovarian tumours: First step into understanding pathogenesis

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Ovarian cancer is the seven most common cancer in women worldwide (18 common cancer overall). Neoplasms tissues derived from ovary are heterogeneous morphology group, including wide spectrum of histopathological scope: more than 300 types. Usually this kind of tumour is detected in late stage, because they do not gives the specific symptoms. The role of molecules and metals is not precisely known in the cancerogenesis process. The aim of this study was to identify the biochemical changes in different type of the ovarian tumours and compare obtained results with control area of the tissue.

Fourier Transform Infrared Spectroscopy (FTIR) was used for biochemical analysis of tumours and X-Ray Fluorescence spectroscopy techniques (XRF) were used for elemental analysis of tissue samples. The samples designed to the elemental and molecular analysis were taken intraoperatively from patients requiring treatment, in co-operation with Jagiellonian University Medical College in Kraków. Specimens were cryosectioned at 20 μm and freeze dried. For each slice an adjacent slice was cut off for histopathological examination. The measurements were carried out at European Synchrotron Radiation Facility Synchrotron – ESRF (FTIR) and at Light Source ANKA Karlsruhe (XRF).

Set of biomolecules (amid massive, lipid massive and fingerprint region) in neoplastic and control area of the tissues were examined. Distributions of biomolecules were presented on 2D maps. The semi-quantitative results indicated the general differences between the various tumours examined, within the range of lipid, protein and phosphate groups absorption bands [1]. The XRF technique revealed minor and trace elements were present in all analyzed tissues. The distribution maps of minor and trace elements were drawn. Significant amounts of elements such as P, S, Cl, K, Ca, Fe, Cu, Zn and Rb were present in all neoplastic tissues analyzed. The study showed significant diversifications in elemental distributions depending on the structure of tissue [2].

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FTIR Imaging as a promising tool in cancer biomarkers discovery. Model studies of changes in distribution of biological components during metastasis process Advanced Techniques of Vibrational Spectroscopy

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Biomarkers are referred to every means of tools which are able to distinguish what is abnormal from what is normal under conditions of physiological homeostasis. Over a last decade, intense interest has been focused on biomarkers discovery and their clinical uses. Especially, cancer biomarker discovery is eminent in this field due to its prospective critical role in early diagnosis, therapy guidance, and monitoring prognosis of tumors. Among cancers, lung cancer is the one showing the highest mortality because of failure in early diagnosis [1]. It is estimated that metastasis is responsible for about 90% of cancer deaths [2]. Unfortunately, despite a great advance in the discovery and development of lung cancer biomarkers with the development of genomics and proteomics technologies, none of them is specific enough to be used in clinical diagnosis [1].

A combination of the FTIR spectrometer, a microscope with Cassegrain objectives and Focal Plane Array (FPA) detector enable simultaneous chemical detection and projecting the distribution of components in an examined sample. This optical system allows for observation of small objects and collection of their FTIR spectra [3]. Such a solution enable imaging of cancer affected lung tissue. Studies were carried out on cross sections of paraffin-embedded lung tissues placed on CaF_2 glass slides derived from murine model, in which tumor cells 4T1 of the breast cancer were orthothropically injected to BALB/cAnNCrl mice. Control tissue and cross-section after 1-5 5 weeks after injection were examined. Infrared spectroscopy was employed to record IR images of cross sections in high magnification mode with pixel size of 1.1x1.1 μm^2 and ultra-high magnification mode with pixel size of 0.66x0.66 μm^2. Measurements were realized in transmission mode. Collected spectra were analyzed with the use of chemometric methods to extract spectral characteristics of cancer metastasis. Hematoxyline & eosine staning was used as a reference.

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Structural characterization of shape-controlled TiO_2 nanomaterials

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TiO_2 with different shapes were obtained via different methods [1,2].

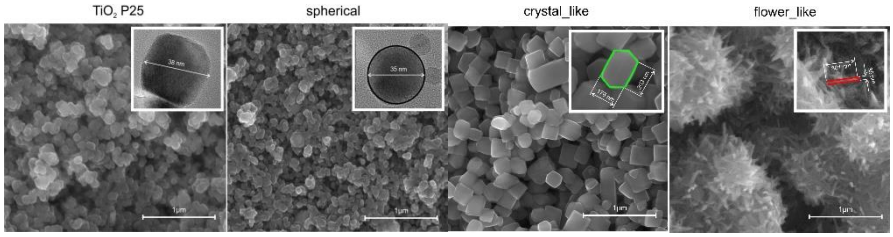

Figure 1. TiO_2 nanomaterials with different shapes.

The materials were characterized with the use of XRD, Raman, FTIR and UV-vis spectroscopy, SEM, TEM, DTA-TG and BET nitrogen adsorption isotherms. The photocatalytic activity was investigated through the decomposition of methylene blue (MB).

Performed measurements allow to infer that shape modifications affect the properties of the obtained nanomaterials, despite the similar phase composition and specific surface area. What is more, different quantities of active centers are present on the nanomaterial surface, due to different synthesis conditions.

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Spectroscopic studies on endoplasmic reticulum stress in endothelial cells induced by tunicamycin

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Endoplasmic reticulum (ER) is a membranous cell compartment, where numerous processes take place, such as proteins folding, proteins and lipids synthesis and detoxification. ER stress is determined as a sequence of enzymatic processes that aim to degrade of accumulated misfolded or damaged proteins. All these processes, which facilitate the restoration of homeostasis in ER, are called unfolded protein response (UPR). A progressive disruption of homeostasis in ER is the factor, which is observed in many diseases (e.g. cancer, chronic inflammation). Tunicamycin (Tu), a drug known from its antiviral and antibiotic properties, evokes pharmacologically ER stress in cells and is widely used in studies focused on ER stress mechanisms.

To track the influence of Tu on human aorta endothelial cells, confocal Raman imaging was applied. For this purpose, cells were exposed to Tu(0,5 and 1 μg/mL) for 24 h. Raman imaging was executed using Witec alpha 300 system with water immersive objective (63x).

Data analysis was conducted k-means cluster analysis (KMCA) and principal component analysis (PCA). Obtained results indicate that Tu enters inside the cells and accumulates in ER area. Additionally changes in general biochemical composition of endothelial cells induced by Tu were also observed. Therefore Raman spectroscopic markers of ER stress were successfully determined.

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FTIR microscopy in early detection of breast cancer development

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Although, the breast cancer can be cured by an early diagnosis, the nature of dissemination and adaptation cancer cells to a tissue microenvironment from distant organs still remains the mystery. Metastasis problem is often impossible to eradicate effectively and makes the cause of 90% deaths from cancer diseases [1]. To prove that metastasis can be detected at the first phase of tumour development in blood plasma, we employed Fourier transform infrared spectroscopy (FTIR) to study its alteration of biochemical composition during cancer progression. Below we represented the scheme of stages of experiment.

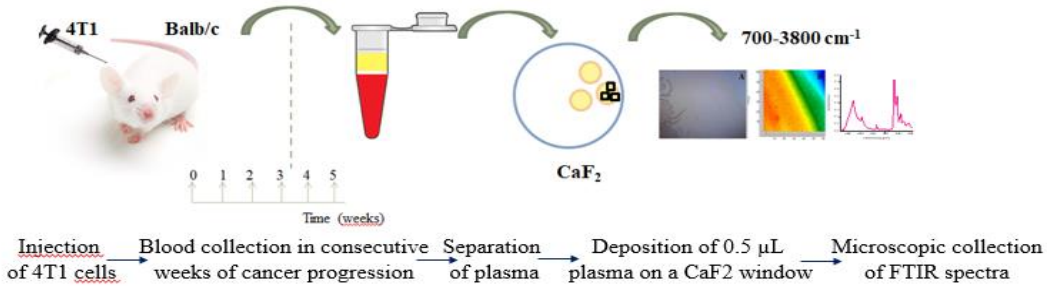

Figure 1. The steps of performed cancer experiment

The breast cancer cells (4T1) were injected to mice body and we collected the blood samples in consecutive weeks of cancer progression. As control we collected samples from healthy mice. FTIR spectra were recorded using an Agilent FTIR microscope. To show specific spectral differences for each metastasis phase we applied PCA and calculation of bands integral intensities and their ratios. Our analysis of FTIR spectra of blood plasma shows that the IR changes occur especially in the wavenumber regions assigned to lipids, proteins and nucleic acids. In our study we observed alterations in total lipid content and phospholipids (where we noticed other tendencies), changes of length of acyl chains and unsaturation of lipids. Moreover, FTIR spectroscopy allowed registering the catabolism of proteins and production of fibrils during cancer progression.

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Raman spectroscopy and Atomic Force Microscopy of human digestive tract

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Almost any type of differentiated cells may lose the ability to control dividing and transform into tumor cells. Tumor transformation is associated with activation of proto-oncogenes and/or inactivation of suppressor genes or abnormal cell differentiation. The imbalance between the production of ROS and the efficiency of antioxidant systems leads to oxidative stress and subsequent damage to important macromolecules such as DNA, proteins and lipids. Tumor transformation can involve also multiple changes in cytoskeleton and cell membranes, as well as in the composition and number of membrane channels, receptors, and enzymes. We will prove that Raman spectroscopy and imaging and Atomic Force Microscopy can be successively used to characterize in detail biochemical and nanomechanical properties of noncancerous and cancerous human digestive tract tissues.

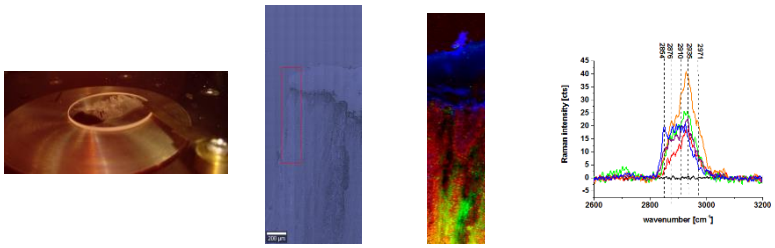

Figure 1. Raman imaging of cancerous human colon tissue.

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Characterization of cancer metastasis in a mice model in means of FTIR imaging spectroscopy. The comparision of sample preparation and spatial resolution

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Sample preparation plays a key role in a potential diagnostic purpose with the use of Fourier transform Infrared spectroscopy imaging spectroscopy. FTIR measurements are carried out mainly on the formalin-fixed (FF) frozen in OCT or paraffin-embedded (PE) tissue cross-sections. Due to the fact that both procedures of sample preparation can affect some spectral regions of IR spectrum, only the combination of those two preparation techniques can provide a complete information about biochemical changes in tissues during cancer progression.

Lung tissues were taken from BALB/cAnNCrl mice after 0, 2-,3- and 5-weeks from orthotropic inoculation with viable 4T1 tumor cells of metastatic breast cancer (N=5). PE and frozen cross-sections of tissues were taken from a half of the height of the left lung and deposited on CaF_2 windows. IR images were recorded in transmission mode with the use of a Agilent 670–IR microscope coupled to a 128×128 focal plane array detector (FPA). An objective of 15x magnification was used for spectral imaging with in standard pixel size of 5.5x5.5 μm^2. After routine preprocessing of spectra, Hierarchical Cluster Analysis was performed in order to study the biochemical profile characteristic for each stage of metastasis for both - PE and frozen samples. The quantitative analysis was also carried out and presented in form of box plots.

Paraffin is known to affect strongly the high-wavenumber region of FTIR spectrum, that is often used for the examination of lipid structure and content. In the case of frozen cross-sections, OCT can obstruct IR tissue features at 1733, 1246 and 1110 cm^{-1} when the removal of OCT from the tissue is not fully successful. Understanding disadvantages and advantages of both procedures gave us a possibility to achieve the global information about biochemical alteration in lung tissue during the process of metastasis of breast cancer to lungs.

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Infrared and Raman study of selected mineral waters from Krynica-Zdrój (Poland)

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Krynica-Zdrój is the biggest spa town in Poland (among 44 towns having the spa status). Mineral waters from Krynica-Zdrój are used in balneotherapy in numerous sanatoriums and bottled for drinking cures. Therapeutic waters from that region have varied composition, and mineralization from 0.6 to 27.2 g/dm^3 [1].

Here, we present the experimental Raman and IR spectra of dry matter of selected natural mineral waters exploited in Krynica-Zdrój *i.e.* Słotwinka, Jan, Kryniczanka and Zuber. Analysis of the spectra in the range 4000 - 400 cm^{-1} show that some component of waters such as SO_4^{2-} and HCO_3^- ions as well as HBO_2 and H_2SiO_3 can be well recognized in the spectrum.

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Raman Optical Activity of supramolecular assemblies exposing induced chirality

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Chirality is an important feature of living matter and nature. At a supramolecular level, supramolecular chirality is the result of biological molecular self-assembly like folding of proteins into efficient systems or storage genetic information in nucleic acids [1]. Aggregation of xanthophyll is also related with the proper functioning of essential biological systems like photosynthetic apparatus and lipid bilayers. It is widely known, that xanthophylls protect cells and tissues from destructive effect of free radicals. Due to their lipophilicity, molecules can form supramolecular structures under the influence of hydrophobic effects and intermolecular forces. Some of them through their aggregation can enhance Raman Optical Activity (ROA) signal. In our recently published work, we reported a new mechanism, where the chirality enhancement is achieved through resonance due to supramolecular aggregation and coupling of the incident laser light with an electronic transition of a supramolecular assembly. This effect brings tremendous enhancement of chiroptical signal and was named Aggregation-Induced Resonance Raman Optical Activity (AIRROA). Up to now, we submitted the AIRROA phenomenon for several xantophylls (astaxanthin,[2] zeaxanthin [3] and lutein [4]).

We have made several trials to optimization the resonance conditions for other supramolecular chiral systems. Our main objective is formation of the superstructures built from structurally similar chiral and achiral components, however the content of achiral ones is much higher. Therefore, the chirality can be induced in achiral molecules due to strong interactions with the chiral ones through noncovalent bonds. An interesting example of chirality amplification is "sergeant and soldiers" effect. When a small amount of chiral compound (the ''sergeant'') is added to achiral ones (the ''soldiers''), a strong ECD signal is obtained similar to that observed for the ''sergeant'' alone [5]. If the ECD absorption band coincides with the ROA excitation wavelength, registration of the RROA spectrum is possible. Using mixture of two naturally occurring xantophylls: astaxanthin (chiral) and β-carotene (achiral), we have obtained supramolecular assembly that exhibits strong chirality and resonance ROA effect. Moreover, achiral β-carotene molecules are forced by chiral addition (astaxanthin) to form a chiral arrangement. Implications of aggregation-induced signal enhancement for chiroptical spectroscopy are still discussed.

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Raman Optical Activity of xantophyll's aggregates

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Xantophylls are responsible for light harvesting in photosynthetic systems, have strong antioxidant activity and may prevent many disease or metabolism dysfunction. Our work is focused on four xanthophylls: astaxanthin, zeaxanthin and lutein. Astaxanthin is a red pigment used in diet of salmonids and crustaceans. It is used also as a dietary supplement, that prevents cardiovascular, immune, inflammatory, and neurodegenerative diseases [1]. Zeaxanthin is a predominant carotenoid pigment of human retina, next to lutein, together influence on proper sight and eye protection due to the blue light absorption [2].

It is widely known that xantophylls have tendency to aggregate. The phenomena of aggregation occurs in biologically important systems such as photosynthetic apparatus and lipids bilayers. Depending on the monomer structure and local environment, there are two types of xantophyll aggregates. The J-aggregate is characterized by the red-shifted absorption band in comparison to the monomer spectrum and loose "head-to-tail" formation. In turn, the H-aggregate is in tight "card-pack" association and their polyene chains are more or less parallel to each other. A compact structure of H-aggregates results in loss of vibrational structure of the electronic absorption spectrum and manifests itself by the blue-shifted UV-Vis band [3].

The type of xantophyll aggregates affects their stability, redox and photochemical properties. We show here that the helical aggregates can exhibit Resonance Raman Optical Activity (RROA), whereas a long distance between chiral centers and unsaturated chain in carotenoid molecules makes the registration of ROA spectrum not possible for a monomer. The RROA spectrum can be recorded only when 1/the polyene chains are arranged in supramolecular chiral structures e.g. helical aggregates that absorb at the wavelength of ROA excitation. So, we report here a mechanism of ROA enhancement, achieved through resonance due to supramolecular aggregation, which we named Aggregation-Induced Resonance Raman Optical Activity (AIRROA) [4-6].

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Spectroscopic evaluation of the effect of alkylating drugs on endothelial cells

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Chemotherapy is often complicated by cardiotoxicity, which can in some cases lead to symptomatic heart failure, and even cardiac death. Past researches have shown that cardiotoxic side effects can occur during: the active anti-cancer therapy (i.e. early cardiotoxicity), within one year after the end of treatment, and many years after the end of chemotherapy. In the case of intravenous chemotherapy, the endothelium (cells that line the inner walls of blood vessels) are the most exposed on the drug. It is therefore inevitable that endothelial cells (single layer of cells lining the inside of blood vessels) are in direct contact with the cytostatics, what in turn is not without significance for its function and homeostasis. Cardiotoxicity can be caused by the damage of these cells during chemotherapy [2].

Studies focused on spectroscopic markers (biomarkers) indicating early changes in biochemical profile of endothelial cells (as results of chemotherapeutic impact) were done. The main purpose of this *in vitro* studies was to analyze the effect of cisplatin and cyclophosphamide on endothelial cells. These drugs are very often used in intravenous chemotherapy, therefore their cytotoxicity can have a direct impact on the inner layer of blood vessels what in turn can led to dysfunction of cardiovascular system. HAoEC cells were cultured on a calcium fluoride slides (CaF_2, 25x2mm) and stimulated with selected doses of anticancer drugs for 24 hours. Raman imaging was performed with WITec Alpha 300 system equipped with 63 x water immersion objective (NA=1) and excitation line at 532nm. The KMC and PCA methods were used for analysis differences between control cells and drug-stimulated cells.

Cisplatin is an alkylation drugs. The mechanism of its action consists in the formation of cross-links between two adjacent guanine molecules. This contributes to the inhibition of replication and transcription of DNA [3] what was confirmed by the presented results. Changes in the intensity of Raman bands for the O-P-O stretching vibrations for DNA /ring deformation modes of nucleic bases (790 cm^{-1}) were observed depending on the drug doses. Other changes were found at the protein level. Cyclophosphamide through alkylation reactions results in the formation of bonds between the two purine bases in the DNA helix. However, according to many publications, this compound is inactive *in vitro* [3]. Results of our studies indicate that it causes changes in the protein composition.

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Raman microscopy studies of endothelium and perivascular adipose tissue in atherosclerosis

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Atherosclerosis, and the subsequent cardiovascular complications are major causes of death in developed countries. In fact, chronic inflammation of the vascular wall present in atherosclerosis is triggered and promoted by endothelial dysfunction[1]. An additional factor responsible for vascular damage is perivascular adipose tissue (PVAT), which secretes proinflammatory factors[2]. So far, the relationship between PVAT accumulation and the development of atherosclerosis is not fully understood and is the subject of research. Due to the key role in the pathogenesis of atherosclerosis, the endothelium and PVAT have (still largely unexploited) significant diagnostic and therapeutic potential.

Raman imaging technique provides specific biochemical information about sample composition. Furthermore, the combination of Raman spectrometer with a confocal microscope allows for reconstruction of the 3D image by measuring individual layers of a tissue.

In this experiment the entire aorta in *en face* section along with PVAT taken from the mice model of atherosclerosis ApoE/LDLR $^{-/-}$ and control mice C57 were measured. Due to the characteristics of chemical changes highlighted by the increased lipid to protein ratio occurring inside the aorta as a result of atherosclerosis, it was possible to correlate obtained results from the vascular endothelium with the changes taking place in PVAT.

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Preliminary biomolecular characterization of skeletal muscle fibres affected by different types of myopathy by means of FTIR

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The aim of presented research was the biomolecular characterization of the myopathic and dystrophic skeletal muscles and compare it with the results obtained for the control group.

Myopathies are diverse group of muscle diseases. This is a sizable group of sickness with diverse grounds, various course and prognosis, but their common trait is a weakness of muscles. Myopathies are divided for inherited (classified separately as dystrophy) or acquired.

Diagnosis of the muscle pathologies begins with the patient's history, clinical picture, which is based on the patient report, neurological examinations and EMG observations. To ultimately identify diseases specialized tests like genetic test (usually blood samples) or muscle biopsy are performed.

The preliminary measurements were done at the Faculty of Physics and Applied Computer Science at AGH University of Science and Technology using the IR microscope (Nicolet Continuum - Thermo Scientific) coupled with FTIR spectrometer (Nicolet 8700 – Thermo Scientific). The measurements' parameters were as follows: the aperture size of 20 μm ×20 μm, there were 100 scans recorded per spectrum (400 scans for background spectrum) within the range of 800-4000 cm^{-1} in transmission mode. There were 14 samples analyzed altogether (4 – myopatic, 5- dystrophic and 5 normal/control samples).

The preliminary results show that there are the differences in lipid content – it seems that it is higher level for dystrophic muscle comparing with myopathic and control group. The differences were also observed within the wavelength range 1180-950 cm^{-1} which is attributed mainly to the carbohydrates.

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Spectrophotometric structure analysis of the natural and styrene-butadiene rubber after exposition to atmospheric conditions

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Rubbers, more precisely natural rubbers, are known to the civilized world since 1493 (K. Columbus observed in Haiti natives playing balls from a secretion of a tree called 'cau-uchu' or a crying tree). However, the production of synthetic elastomers is becoming more popular, and these are mainly butadiene, isoprene, ethylene-propylene and styrene-butadiene rubbers. Despite many similarities between rubbers, their different structures give them characteristics properties [1].

The research was conducted on three natural rubbers with technical specification TSR 10 (Technical Specified Rubber), as well as two styrene-butadiene rubbers classified into the SBR 1500 (Styrene Butadiene Rubber) group.

The rubbers were stored at room temperature, and they were exposed to atmospheric conditions for two and four months. The structural changes in the materials were determined by FTIR and UV-VIS analysis [2,3].

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Spectroscopic and structural characteristics of graphene oxide nanocomposite with embedded nanoparticles of molybdenum disulfide

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Graphene is widely regarded as the material of the future because of its unique physical and chemical properties. Potential application possibilities of graphene and its derivatives, i.e. graphene oxide (GO), are extremely broad and cover various branches of industry, including, among others: energy, electronic, material engineering or the biomedical industry [1-3]. Due to the presence of oxygen functional groups, including hydroxyl, epoxy and carboxyl groups, and the highly developed surface, GO can be modified both by chemical (chemical bonding) and physical (the utilization of van der Waals interactions, π-π interactions and formation of hydrogen bonds) [5,6]. Valuable physicochemical properties of molybdenum disulfide make it an interesting material for the formation of composites with carbon nanomaterials, such as carbon nanotubes or reduced graphene oxide (rGO). The surfaces with a negative charge of molybdenum disulfide react with the edges and surfaces of reduced graphene oxide, resulting in the formation of a bond between MoS_2 and rGO. Thanks to such connections, it is expected that an attractive sorbent with a high adsorption capacity will be provided, which can be successfully used to concentrate heavy metals [7].

A graphene oxide nanocomposite with embedded molybdenum disulfide nanoparticles (MoS_2-rGO) was obtained as a result of the hydrothermal reaction of sodium molybdate with *L*-cysteine in the presence of graphene oxide (GO) [8]. The structure of the obtained nanomaterial was confirmed by infrared (FT-IR) and Raman spectroscopy, transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and energy dispersive X-ray fluorescence spectrometry (EDXRF).

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FT-IR studies of photopolymerization process

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Photoinitiated polymerization is an important process used for a variety of applications in industry. For example, as photocurable coatings, inks and adhesives. A photoinitiating system comprises one or several compounds and is able to initiate a polymerization reaction. Photoinitiating system play a major role in the starting step of photopolymerization [1].

Most of photoinitiators currently used in industry require short-wavelength UV light (below 350nm). UV light sources emitting energy above 350nm adapted in industry are not suitable for these processes because of mismatching between the absorption characteristics of the commercial photoinitiators and the emission characteristics of the light sources. In these cases photoinduced polymerization is inefficient [2].

For this reason photoinitiating systems directly adapted to light-emitting diodes (LEDs) as irradiation devices (especially visible LEDs) are designed and developed. Terphenyl skeleton based compounds were synthesized and used as co-initiators in photoinitiating systems with diphenyliodonium hexafluorophosphate.

The kinetics of the photosensitized cationic and free radical polymerization of monomers were monitored using Fourier Transform *Real – Time* Infrared Spectroscopy. During the photopolymerization, various infrared absorption bands were observed depending on the specific functional group in the monomer.

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Imaging of ovarian cancer tissues using FTIR microspectroscopy. Advanced Techniques of Vibrational Spectroscopy

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Ovarian cancer causes 6% of cancerous death among women [1]. High mortality rate and short survival outcomes are due to lack of specific symptoms [2]. The recognition of pathogenesis of these cancers and processes that occur in tissue – biochemical changes, may contribute to an early diagnosis. Fourier Transform Infrared spectroscopy (FTIR) is widely applied technique that provide insight into disease processes on molecular level. In view of numerous advantages (including simple sample preparation, the ability of gaining complex information) it is powerful tool used to examine biological materials and has potential to support histopathological diagnosis [3,4]. The aim of this study was qualitative analysis of biochemical composition of cancerous and control ovarian tissues. The samples designed for molecular analysis were taken intraoperatively from the patients with the ovarian tumours requiring surgical intervention. The samples were prepared and diagnosed at the Department of Pathology, Jagiellonian University Medical College, Kraków. Three adjacent sections were cut for each sample: the first slice of tissue was placed on a microscope slide for hematoxylin-eosin staining (H&E), while the two adjacent were placed on a MirrIR slide and BaF_2 window. The measurements were carried out at WFiIS, AGH. The Thermo Nicolet Nexus spectrometer combined with the Thermo Nicolet Continuum infrared microscope was used. The microscope was equipped with a MCT detector cooled with liquid nitrogen. Mapping of samples was performed in the XY system with a measurement step of 10 μm x 10 μm and a beam aperture of 16 μm x 16 μm. Spectra from the mid-infrared spectrum 800-4000 cm^{-1} were collected. Spectral analysis demonstrated the differences between analyzed samples: in terms of lipid and amide bands and compounds containing phosphorus groups. The results will be presented and discused on a poster.

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Spectrophotometric and electrochemical study of polymer paint coatings

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Two coating systems on steel substrate were investigated by spectrophotometric technique (FTIR ATR) and electrochemical (EIS). Plates were coated with alkyd resin (base layer - BL) and oil-alkyd enamel (outer layer - OL). Both samples were exposed to outdoor condition for the time of 2 summer months - it allows to investigate the influence of the sunlight radiation as well as real temperature and humidity impact.

Changes taking place in the structure of polymer paint coatings were determined by FTIR analysis, based on the intensity and shape of the peaks obtained. The 2 month exposure on weather condition revealed some changes in the bands' position. The results of spectrophotometric analysis were compared to the electrochemical tests. The EIS data were interpreted with the appropriate equivalent circuit, while the elements of the circuit simulate the electrical behavior of the component of the coating layer. Capacitance measurement allows for determination of the value of ε'' which is useful for evaluating the water uptake in organic coatings, while the pore resistance R_p is related to the barrier properties of the coating to aggressive ions and therefore it is proportional to the number of defects or pores in the coating.

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Comparison of the pancreatic inflammation and malignant tumor classification model based on IR imaging

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One of the major challenges for contemporary medicine is a development of cancer early diagnosis. Up to this day pancreatic cancer is the fourth leading cause of cancer-related death. Due to the difficulty of the methods to detect its early stages, the disease is usually diagnosed at a late stage, which in connection with aggressiveness and somewhat ineffective treatment methods turns into bad long-term therapy results [1].

Infrared imaging is an emerging imaging modality, offering a chemical contrast-based diagnosis of very high accuracy rates (above 05%). The main goal of this work is to use chemical imaging in the form of High Definition IR imaging [2] to perform a comparison of the differences between pancreatic inflammation tissue and the first stage of pancreatic malignant tumors in human biopsies including distinction from healthy tissue. Classification model based on machine learning methods (Random Forest) involves FT-IR data acquisition. The measured tissue microarrays were collected in transmission mode with 1.1 μm projected pixel size. Principles of the approach will be presented along with the plans and preliminary results.

The used method allowed to determine the maximum potential of infrared imaging in distinguishing to inflammation from the early stages of cancer. The use of HD IR imaging in the transmission mode allowed to obtain the best quality spectral and spatial data. Spectroscopic imaging allowed to obtain clinically relevant information in a non-destructive and label-free manner.

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Cationically modified polysaccharides as inhibitors of HSV-1

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Human herpesvirus type 1 (HSV-1), one of the most frequent human pathogens, is known to cause orolabial, genital and corneal lesions. It establishes a latent infection in sensory neurons and may be reactivated by various stress stimuli. The only commercially available therapy is based on viral DNA polymerase inhibitors, acyclovir and its derivatives, which carry some limitations, like low bioavailability and emergence of resistant strains.

Polycationic agents are expected to inhibit HSV-1 entry, by binding to its cellular receptors or adhesion factors. By employing a different mode of action than acyclovir, these compounds may complement conventional anti-herpes therapy.

In this paper we will present the results of our studies on novel HSV-1 inhibitors based on cationic dextrans. The synthesis, properties and mode of action of these systems will be discussed.

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Bipiramidal Au@SiO_2 - Synthesis and characterization of the new type of SHINERS electromagnetic nanoresonators

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SHINERS measurements were introduced by Tian et al. in 2010 as the new approach to analyze various surfaces [1]. In this method Raman spectrum was measured from an investigated substrate covered with gold nanoparticles protected by a thin layer of silica or alumina. Gold nanoparticles act as electromagnetic nanoresonators, significantly enhancing electric field of the incident electromagnetic radiation and hence leading to large increase of the Raman signal from studied surface. The inert shell separates metal cores from direct contact with probed molecules and keeps them from agglomeration. Therefore, such systems might find more analytical applications, also for living cells. In this work, we present the synthesis of bipiramidal Au@SiO_2 core shell nanoparticles as the new type of SHINERS nanoresonators.

The synthesis of bipiramidal Au@SiO_2 nanoparticles, we firstly obtained bipiramidal gold nanoparticles according to a modified method developed by Weizmann [2]. Deposition of thin silica layer on obtained bipiramidal nanoparticles was carried out by the hydrolysis of tetraethoxysilane catalyzed by ammonium solution [3]. Synthesized bipiramidal Au@SiO_2 nanoparticles were presented in *Figure 1*.

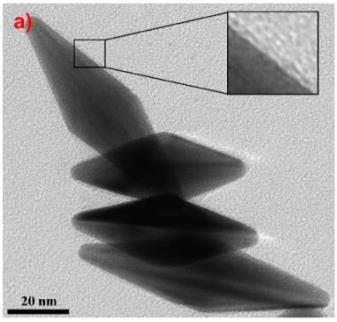

Figure. 1*. TEM micrograph of synthesized* bipiramidal *Au@SiO_2 nanoparticles*

Obtained Au@SiO_2 nanoparticles were afterwards successfully used as nanoresonators in Raman measurements of 4-mercaptobenzoic acid and 2-mercaptoethanesulfonate. Synthesized anisotropic nanoparticles were approximately 10 times more efficient raman nanoresonators than typical spherical ones. Obtained bipyramidal Au@SiO_2 were also used for detection of Thiram pesticide deposited on tomato fruit. Limit of detection of Thiram were estimated to be 1.2 ng/cm^2.

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Study of structure in Gd-Zr-Fe-Co-Mo-W-B alloy with positron lifetime spectroscopy Advanced Techniques of Vibrational Spectroscopy

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In the present work structural studies of the $Gd_xZr_{10}Fe_{58-x}Co_{10}Mo_5W_2B_{15}$ (x= 0, 1, 2, 3, 4, 5) alloy were investigated. The ribbons sample were obtained by melt-spinning technique. Differential scanning calorimetry DSC under noisothermal conditions have been proposed to investigate thermal characteristic of these compositions. Furthermore, positron annihilation lifetime spectroscopy PALS was used to characterize defects and topologically disordered materials without translational oriental symmetry such as metallic glasses. Three components of the positron lifetime τ and their intensities I were obtained. In amorphous alloy the shortest lived component τ_1 is attributed to p-Ps annihilation. The intermediate lifetime τ_2 relates to the free positrons annihilation with electron trapping models. The longest lifetime τ_3 is associated with the ortho- positronium annihilation occurring at the surface between the source and samples.

SERS markers utilizing hybrid magnetic-plasmonic nanostructures with Fe_3O_4 core

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Hybrid nanostructures contribute to development of new multifunctional materials, including those applicable in surface-enhanced Raman scattering (SERS) spectroscopy. Design of the system based on Fe_3O_4 and Ag nanoparticles (NPs) offers combining magnetic and plasmonic properties, which opens a route for new fascinating applications, like simultaneous sensitive SERS detection and recovery of the analyte or production of effective SERS-based chemical "bar-codes".

The aim of this work was to synthesize and characterize hybrid nanostructures, composed of plasmonic Ag NPs attached to magnetic Fe_3O_4 core. Molecules of 2-mercaptoethanesulfonate (MES) were used as a Raman probe, whose main advantage, apart from being a strong Raman scatterer, is photostability. Coating of the hybrid structure with SiO_2 shell was performed, to improve stability of the system.

Fe_3O_4 NPs were prepared by coprecipitation technique [1] and solvothermal method [2]. Next, Ag NPs obtained by reduction with butylamine were attached in a single step. Finally, adsorbed MES layer was protected with polyelectrolytes prior to SiO_2 coating, using Stöber reaction.

Superconducting quantum interference device (SQUID) magnetometry confirmed superparamagnetic character of Fe_3O_4 and Fe_3O_4/Ag NPs and showed preservation of the magnetic properties for the composite. Morphology studies with transmission electron microscopy (TEM) imaging revealed the essential role of degree of dispersion in suspension for solvothermally produced Fe_3O_4 NPs on their decoration with Ag NPs.

SERS signal of good quality was observed for MES probe on non-coated Fe_3O_4/Ag composite, although monodispersity of attached Ag NPs for Fe_3O_4 NPs fabricated by solvothermal synthesis is still being improved. Results of SiO_2 coating aimed at sufficient conservation of SERS activity are encouraging, particularly for the coprecipitated Fe_3O_4 NPs.

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Towards the identification and biochemical characteristics of eosinophilic granulocyte - spectroscopic studies

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Eosinophils, like neutrophils and basophils, belong to the granulocytes produced in the bone marrow in account approx. 2-6% of white blood cells. Their characteristic feature are regular, round shape, bi-segmented nucleus and size below 20 μm.[1] In their cytoplasm are found granules which contains highly cytotoxic proteins deposited in the crystalloid core consisting of Major Basic Protein (MBP) and a matrix composed of eosinophil peroxidase (EPO) etc. Eosinophils possess a wide range of specific functions such as regulating processes of inflamation, antiparasitic activity, bacterial and viral removal, antigen presentation, tumor immunity, the cross-talk between mast cells, dendritic cells and granulocytes. The natural component of eosinophils are lipid bodies (LBs). They usually play a role in cell signaling but in eosinophils they mobilize these cells to generate eicosanoids during the inflammation.[2]

Here we present a unique approach to analyze single eosinophils based on a Raman spectroscopy imaging. We used Raman microscopy for identification and biochemical cell characterization followed by data analysis based on chemometric methods, e.g. KMC-a, PCA etc. This technique allows the identification of eosinophils through the observation of marker bands for eosinophil peroxidase. We observed significant effect of eosinophil peroxidase on spectra from individual classes.

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Spectroscopic and theoretical analysis of supramolecular aggregates of astaxanthin

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Astaxanthin is the major carotenoid in marine animals and it is produced by a few organisms, such as green algae, bacterium and yeasts. Like other chiral xanthophylls, astaxanthin molecules have an unusual tendency to self-assembling in natural and artificial systems (hydrated organic solvents) and formation a wide range of supramolecular aggregates [1,2]. Overall, the pigments form two different types of assemblies: H-aggregates, characterized by a blue-shifted electronic absorption band, and J-aggregates, which exhibit a red-shift in the UV-Vis spectrum in comparison to that observed for the parent monomer [3].

In our new study to improve our knowledge of the astaxanthin aggregation process, we carried out the molecular dynamics simulations (MD) together with the quantum chemical method (ZINDO/S as well as TDDFT) and then we compared received data with experimental one (ECD and UV-Vis spectra). Based on the gained results, we clearly demonstrate that very realistic absorption and ECD intensities were obtained and the simulations reproduced many trends observed experimentally, such as the prevalent sign pattern. What is more, several theoretical models of aggregates of (3S,3'S)-astaxanthin were generated manually from 2 (dimer) 10 (H-aggregate) and 8 (J-aggregate) molecules in the most stable conformation (-GG). Our data showed that once again ZINDO method provided results that are in good agreement with experience.

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An insight into atherosclerotic alterations in mice aorta *ex vivo* studied by unusual fiber optic Raman probe

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The aorta is the most important and also the largest artery in the circulatory system. It begins in the heart and extends down to the abdomen where splits into iliac arteries, thus, the thoracic and abdominal parts can be distinguished. Upon development of vasculatory disease i.e. atherosclerosis, which is promoted by endothelial dysfunction, changes in both parts of the aorta are different. Atherosclerosis predominates in the abdominal part and it is directly related to arterial pressure and wall stress.[1]

The aim of this work was to study the chemical changes in atherosclerotic aorta in comparison to control. Two approaches were investigated: alteration in the thoracic and abdominal region within the aorta and differences arising from the disease.

In our experiments, *en face* tissue sections of the aorta with advanced atherosclerosis (the ApoE/LDLR$^{-/-}$ mice model) and control subjects (C57BL6/J) were investigated using Raman spectroscopy with the newly designed fiber optic probe (equipped with the objective) with the 532 nm excitation.

The results show, *inter alia*, that the aortic segments are heterogeneous with the abdominal part containing more lipids within the vessel wall. Moreover, in this part of aorta, the lipid to protein ratio significantly increase for the atherosclerotic group.

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Study of model biological membranes using AFM and AFM-IR spectroscopy

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AFM-IR spectroscopy [1,2,3] is a new powerful vibrational spectroscopy technique that allows mid IR spectroscopy and imaging with spatial resolution better than the diffraction limit. It was also shown that thanks to its great sensitivity one can obtain an IR spectrum of a layer even a few nanometers thick [3]. That, together with the excellent specificity of IR spectroscopy, makes AFM-IR a useful technique to study thin lipid layers, that can model biological membranes. We have carried out preliminary AFM and AFM-IR measurements on thin films consisting of phospholipid and cholesterol mixtures deposited on silicon and gold surfaces. Using AFM microscopy we are able to see possible phase separation in those lipid films. By means of AFM-IR microspectroscopy we could obtain spectra of both thick and thin lipid layers. We compare AFM-IR spectra with FT-IR spectra of the same lipid mixtures and discuss similarities and differences.

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Halloysite/phosphatase alkaline composite as a component of hydrogel scaffold for bone tissue regeneration

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Alkaline phosphatase (ALP) is an enzyme that actively participates in the biomineralization process, leading to the renewal of bone tissue. ALP catalyzes dephosphorylation processes leading to the formation of free phosphate groups, which in combination with calcium form hydroxyapatite, the main component of bone. Unfortunately ALP, being a protein and enzyme, is susceptible to deactivation and degradation. In order to improve its stability as a bioactive component of a hydrogel scaffold intended for bone tissue repair, the enzyme was first encapsulated in a halloysite nanocarrier. Halloysite, a nanoclay which occurs mainly in the form of nanotubes, was chosen as a carrier due to its high durability and thermal resistance, biocompatibility and unique tubular structure with negative external surface and positively charged interior. Halloysite nanotubes have the lumen, which is large enough to encapsulate ALP, and it is assumed that they should provide very good protection and stabilization for it. The presence of halloysite should also improve the mechanical properties of the subsequently formed hydrogels. The obtained composite material was subjected to physicochemical characterization using SEM, FTIR and XPS techniques. The activity of the entrapped protein was determined using the Bessey and Lowry colorimetric method. The protein was effectively introduced into the halloysite nanotubes and remains active. The enzymatic activity of the halloysite-entrapped ALP in a biomineralization process was also tested using calcium glycerophosphate as a substrate. SEM measurements confirmed the formation of crystallite on the surface of the nanotubes, while EDS analysis confirmed that hydroxyapatite was indeed produced. In the further studies, the methodology for preparing chitosan hydrogels cross-linked with a non-toxic genipin, and containing different amounts of halloysite nanotubes with entrapped ALP, was developed and optimized. Research was also conducted on the influence of the inorganic component on the swelling process of the hydrogel material. The new hybrid system has a potential as a scaffold for the treatment of bone defects.

Spectroscopic analysis of the formation of intermolecular hydrogen bonds in monofluoro derivatives of aniline

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Intermolecular interactions determining the biological activity of drugs by stabilizing the ligand-protein complexes can be finely tuned using substituents containing fluorine. Fluorine, due to its chemical properties is a poor acceptor of hydrogen bonds, but as a substituent leads to an increase in the acidity of hydrogen atoms in a molecule.[1,2] Hydrogen bonds are one of the most important interactions that influence the binding of drugs to a biomacromolecule. A thorough understanding of its unique properties allows a more effective use of fluorine in the rational design of drugs and better modelling of ligand-protein interactions.[2]

Herein, we report systematic studies of the role of fluorine in the formation of hydrogen bonds by aniline in methanol and DMSO solutions using FTIR and NMR spectroscopy.

The results confirm that in both environments, fluorine decreases the strength of hydrogen bonds, especially while in the *meta* position. However, in the case of DMSO *ortho* and *para* fluoroanilines formed stronger hydrogen bonds, which can be attributed to the resonance-inductive compensation.

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MONITORING OF PHOTOPOLYMERIZATION PROCESSES USING REAL-TIME FT-IR TECHNIQUE

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As part of the research work, the possibility of using 1,3-diphenyl-quinoxalin-2-one derivatives as co-initiators in two-component photoinitiating systems has been tested. The main part of the research work were kinetic measurements for model compositions containing tri(ethylene glycol) divinyl ether (TEGDVE) and two-component photoinitiating systems composed of a diphenyliodonium hexafluorophosphate (HIP) and various 1,3-diphenyl-quinoxalin-2-one derivatives. These measurements were carried out using the real-time FT-IR technique. Analyzing the obtained results, the degree of monomer conversion during the polymerization process was determined for each of the analyzed compositions. Obtained values of this parameter in all analyzed cases were very high, on the order of 80-90%. These results prove that the applied systems effectively initiate cationic photopolymerization processes, and the tested 1,3-diphenylquinoxalin-2-one derivatives can be successfully used for the role of co-initiators in this type of systems.

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Studies on ssDNA monolayers on metal substrates by SERS

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Development of an effective technique enabling early diagnosis of genetic disorders, including different types of cancer is still challenging. Unfortunately, many malignancies are detected at a stage of progression beyond when surgical resection or other interventions can be effectively implemented [1].

Surface enhanced Raman spectroscopy (SERS) is a very sensitive and powerful tool for molecular detection upon adsorption on gold and silver nano-structured surfaces (the most common are colloids and electrodes because of relatively easy way of preparation). It relies on the plasmonically amplified electric fields that are excited upon irradiation of with optically resonant light [2]. This amplification enhances the electric field within a few nanometers of the particle surface, magnifying a normally weak Raman signal by a factor of 10^6 and enabling the trace level detection of a wide range of compounds. Due to its high sensitivity, SERS can compete with polymerase chain reaction or different types of blotting which are using routinely for DNA mutation detection as a part of cancer treatment. With appropriately selected SERS measurement conditions, it is possible to detect single-base mismatch in DNA at femtomolar level.

The idea of SERS-based sensors for DNA detection is quite simple: capture DNA (cDNA), complimentary to target DNA (tDNA) with mutation, is immobilized on metal surface, then target DNA is added and this hybrid is labeled with another single stranded reporter DNA (rDNA) modified with an organic dye (Figure 1) [3].

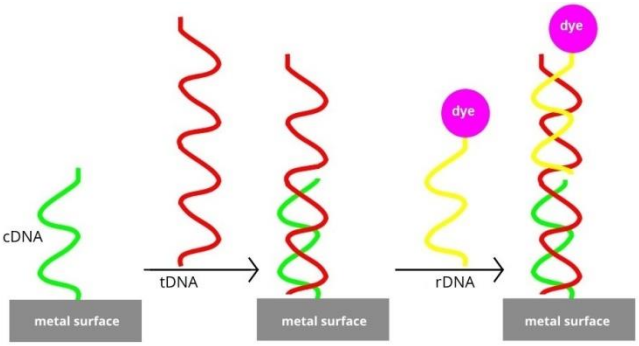

Figure 1. Schematic illustration of fabrication of SERS DNA sensor.

A crucial moment influencing effectiveness of the sensor is immobilization of capture DNA on the metal surface. Here we present the optimization of the formation of ssDNA monolayer on SERS substrate taking into consideration: type of metal surface (gold and silver nanoparticles or electrodes), different concentrations of small spacer molecules between ssDNA (6-mercapto-1-hexanol) and pH. We have also noted factors having damaging impact on nanoparticles and that the hybridization process can be controlled.

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Mid-infrared spectroscopy and imaging of phospholipid bilayers with nanoscale spatial resolution

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We show that nanoscale IR spectroscopy can be used to study the organization of single and multiple supported phospholipid. It was demonstrated previously that it is possible to record nano-FTIR spectra of the protein complement of supported membranes using the sSNOM (scattering-mode Near Field Optical Microscopy) optical configuration. [1] In the current work we use the same technique to measure spectra of phospholipids in individual bilayers in the mid-infrared spectral range. We observe the main absorption bands of the dipalmitoylphosphatidyl choline headgroup above noise level, including ester and phosphate stretching modes. These bands are sensitive to the interaction between phospholipids and their surrounding environment, including the aqueous medium, other lipids and biomolecules external to the bilayer. [2] In addition, we measure absorption bands characteristic for the alkyl chains that are sensitive to the order within the bilayer. We also perform mapping of the phosphate absorption band at 1070 cm^{-1} simultaneously with the AFM topography. We show that it is possible to achieve sufficient image contrast to discriminate between single and multiple phospholipid bilayers. This work opens the way to future studies that use nano-IR spectroscopy to determine the biochemistry of cell membranes and model systems.

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Cellular lipid droplets in primary hepatocytes studied by using Raman imaging

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Non-alcoholic fatty liver disease (NAFLD) is the fastest-growing liver disease in the western world.[1] The increased accumulation of intracellular lipid droplets (LDs) within hepatocytes is a hallmark of liver injury of various etiologies[2], but the mechanisms that lead to the different pathological outcomes are not well defined.[1] Previously, it was assumed that LDs are static energy reservoirs not associated with cellular functions except for those related to lipid homeostasis. Recent research has, however, revealed a new, dynamic face of this organelle. For example, it has been shown that LDs are associated with cell signalling, protein storage, and degradation. Investigation of this organelle will ultimately lead to a better understanding of the cellular and subcellular pathogenesis of associated diseases. *In vitro* formation of cellular lipid droplets is typically induced by the addition of free fatty acids (FFAs) to the cell culture medium. Palmitic acid (PA) and oleic acid (OA) represent the most common saturated and unsaturated dietary fatty acids.[3] Oleic acid supplementation leads to triglyceride accumulation and is well tolerated, whereas excess palmitic acid is poorly incorporated into triglyceride and causes apoptosis. Previous studies have shown that unsaturated fatty acids rescue palmitate-induced apoptosis by promoting palmitate incorporation into triglyceride.[4]

Raman imagining spectroscopy (RS) is a novel and comprehensive approach enabling study and further analysis of various types of liver cells. RS is well-known for its numerous advantages including minimal sample preparation, non-destructivity together with the possibility to obtain information of biochemical composition of the sample and spatial distribution of chosen components at the subcellular level. Subsequent chemometric analysis (*k-means cluster analysis, principal component analysis*) allows investigation of different cellular compartments.[5] This work was focused on the spectroscopic and chemometric analysis of primary hepatocytes' lipid droplets induced by free fatty acids exposure, including determination of lipid droplets' content, size and comparison of fatty acids ratio.

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Cervical cells infected with HPV: identification using Raman spectroscopy

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Raman spectroscopy is a label-free tool to investigate chemical composition of components in samples, based on measurement of inelastic scattering of light. Raman spectra give molecule-unique fingerprints, so provide the possibility of chemical analysis of both inorganic and organic samples. Measurement by Raman spectroscopy enable detection of even subtle differences in the chemical structure of compounds, such as epigenetic changes in DNA methylation[1]. Combining Raman spectroscopy with microscopy gives opportunity to study biological samples with the subcellular resolution. For this reason, this method is very often used for cell or tissue analysis. In this work, Raman microscopy was used to study differences between human cervical epithelial cells acquired from women with cervical cancer, women with cervical dysplasia, as well as women without cervical dysplasia (a control group). Before spectroscopic analysis, samples were subjected to a cytological examination and also tested for HPV infection. Based on these studies, authors divided samples into 4 groups: without dysplasia and HPV, with dysplasia and without HPV, without dysplasia and with HPV and cancer cells with HPV. The aim of the work was finding spectroscopic markers of HPV and dysplasia related to DNA/protein methylation. The preliminary results have showed differences in chemical composition of the control, HPV positive and cervical cells. These results, although require deeper examination, give hope for the possibility of using Raman spectroscopy to diagnose HPV infection and cervical pathology.

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Substituent Effect on Vibrational Spectra of Tricyanoethylene. A DFT Study.

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Tetracyanoethylene (TNCE) charge-transfer complexes have been studied since decades, whereas tricyanoethylene (TCE) ones have been very little looked into. In contrast to TCNE, TCE system can be further functionalyzed. In the centrosymmetric TNCE molecule the vibrational picture of the ν(CN) bands is simple: two ν(CN) bands are present in the IR and the other two in the Raman spectra. The TCE spectral picture is more complicated: three ν(CN) bands are present in either of the spectra. To understand the rules governing the TCE spectra, we calculated series of ca. 25 TCE substituted by electron donor-acceptor groups spanning the substituent effect on both σ- and π-electron system.

However, out of many correlations of the calculated IR and Raman spectra with substituent parameters, we obtained only weak linear trend between mean of the three ν(CN) frequencies and a parameter revealing the substituent effect on σ-electrons. We see at least three reasons why there is no satisfactory correlations for the substituted TCE. (1) The ν(CN) vibrators are coupled and substituents change the degree of coupling differently. (2) Three CN groups in TCE are both σ- and π-electron withdrawing. Therefore in the C=C moiety there is very little of charge to be modified by the substituent. (3) Additionally, the bulky substituents perturb the neighbouring CN vibrators.

To explain the effect in more details we calculated three series of mono cyanoethylenes substituted in the *cis*-, *trans*- and *gem*- positions to the substituent. For them we obtained fairly satisfactory correlations with the ν(CN) frequencies and IR intensities while for the Raman activities the trends were weak if any.

The influence of environment on contact lenses material – spectrophotometric study

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Important aspect of wearing contact lenses (CL) is the proper storage of the lenses with long-time period of usage. The influence of environment conditions on the CL material can be of great importance leading to the irreversible changes in the structure of the polymer [1].

The study presents the changes of properties of three types of CL stored in various solutions (saline solution, commercially available solution for CL storage (CAS), artificial tears (AT)) or in the air. The experimental technique was FTIR ATR, which enables tracking of the changes in the CL material [2,3]. The properties of water in soft contact lenses and the extent to which soft lenses dehydrate during wear, are crucial parameters for oxygen transmissibility characteristics [4].

FTIR ATR spectroscopy scan of the contact lenses was performed on all prepackaged commercial contact lenses with predefined time of usage namely one month (1M), two weeks (2W), one day (1D) CL.

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Vibrational Spectroscopy as a Tool to Investigate Modified TiO_2 with Tailored Shape

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Recently the attention of researchers is focused on shape-controlled TiO_2 NCs due to its unique properties resulting from different exposed facets.

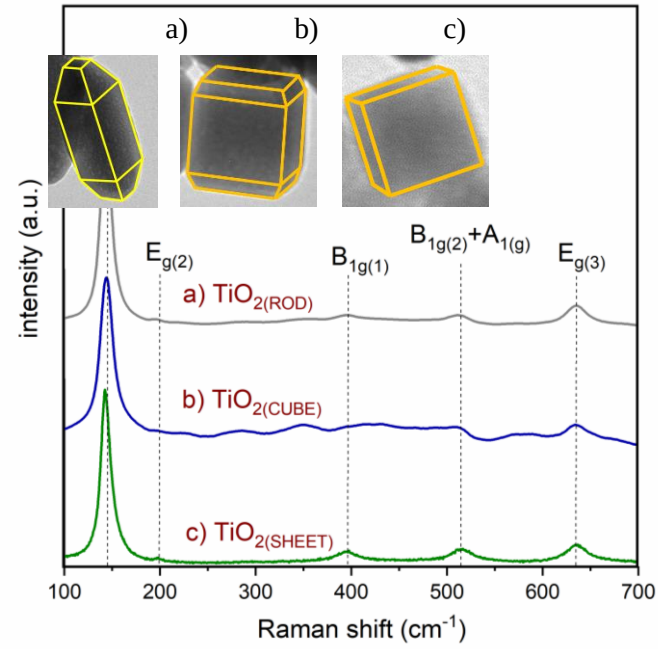

Figure 1. Raman spectra recorded for different types of anatase TiO_2 nanostructures

In this work anatase nanocrystals of various shapes were obtained using hydrothermal method and their surface were modified with narrow-band gap semiconductor and heteropolyacid. The materials were investigated by Raman and IR spectroscopy, X-ray Diffraction XRD, SEM and TEM Microscopy, UV-vis spectroscopy, and Thermogravimetric Analysis TGA.

Acknowledgments:
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Free Fatty Acids uptake by Liver Sinusoidal Endothelial Cells: Insights into Non-Alcoholic Fatty Liver Disease

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LSECs (Liver Sinusoidal Endothelial Cells) play variety of functions including eliminations of macromolecules and small particles, viruses and small immune complexes from the bloodstream, regulation of the sinusoidal blood flow and interactions with tumor metastases [1]. LSECs are also drivers of liver regeneration and determinants of hepatic fibrosis. Noteworthy, the pivotal role of this cells in NAFLD (Non-Alcoholic Fatty Liver Disease) progression was studied more recently. The excessive triglyceride accumulation in the hepatocytes as a results of inappropriate diet rich in saturated fats, is a hallmark of NAFLD. Progression of this state can lead to fibrosis, development of NASH (Non-Alcoholic Steatohepatitis), cirrhosis or hepatocellular carcinoma [2]. It was shown that LSEC injury appeared during simple steatosis phase and may serve as a 'gatekeeper' in the progression to NASH [3]. However, the changes associated with LSEC injury and the exact mechanism of NAFLD are not fully elucidated. Elevated plasma levels of the saturated palmitic acid (PA) and the mono-unsaturated oleic acid (OA) are found in NAFLD patients, and presents the majority of the free fatty acids (FFA) in triglycerides accumulated in liver [4]. The aim of current study was to evaluate the effects of FFA (OA and PA) on LSEC phenotype using Raman imaging technique. Tests performed on LSEC cell line enable to observe the uptake of FFA and accumulation in lipid droplets (LDs). The possibility to link the chemical information with its spatial distribution allow to study the composition of single LD. Additionally, we monitored the chemical changes occurring in cells after 24h from the FFA uptake.

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Acknowledgments:

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Investigation of chemical changes in human valve interstitial cells under the development aortic valve stenosis

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Aortic stenosis (AS) is the most common heart valves disease affecting the 2% - 4% of the population aged over 65 years. Pathophysiology of this disorder is similar to the early stages of atherosclerosis, where chronic inflammation occurs causing fibrosis and leaflets thickening. Further development of AS leads to tissue calcification and valve leaflets stiffness. Interstitial cells present in the valve leaflets, as a result of AS progression, undergo phenotypic transdifferentiation into osteoblasts taking part in formation of calcification mainly consist of hydroxyapatite crystals. Although the mechanism of AS is widely known, the impact of diabetes on the development of the disease is still the subject of research. [1, 2].

In our experiments, valve interstitial cells isolated from human aortic valve (HAVICs) were cultured 7 days in four different media: standard DMEM, DMEM supplemented with $CaCl_2$, β-glycerophosphate and ascorbic acid (osteogenic medium, OGM) and OGM additionally supplemented with glucose in concentrations 1.0 and 4.5 g/l. For those cell cultures measurements of single cells using a confocal Raman microscopy were performed. Based on Raman and chemometric analysis images, we determined changes in chemical composition and distribution of subcellular components. As a result of analysis it was found, *inter alia*, the lack of typical calcification, but stimulating impact of OGM was manifested by an increased level and aggregation of phosphatidylocholine-rich lipid droplets.

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Applicability of new co-initiators for acceleration of cationic and free-radical photopolymerization processes

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Currently cationic photoinitiators can be used only in a limited part of the ultraviolet light usually range from UV-C to UV-B, bypassing the UV-A range and the visible range (Figure 1). Therefore, it is usually necessary to use co-initiators that guarantee the photochemical sensitivity of the initiating system and shift the emission spectrum into longer wavelengths [1].

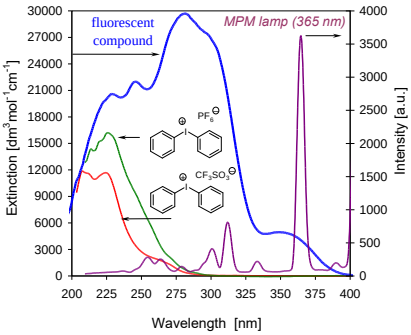

Figure 1. Comparison of the absorption characteristics of commercial iodonium photoinitiators and fluorescent compound with the emission characteristics of industrial light sources (MPM lamp).

In this work we present new fluorescent compounds which can be used as co-initiators for the cationic and radical photopolymerization processes. The research included the basic spectroscopic measurements, including, the measurement of emission and excitation spectra, fluorescence quenching after adding the photoinitiator in the form of the iodine salt, photolysis measurements, Real-Time-FT-IR and Differential Scanning Calorimeter tests.

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Raman imaging studies on primary cardiac microvascular endothelial cells (CMECs)

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Endothelium, the tissue forming the inner layer of all blood vessels in living organisms plays a variety of critical roles in the circulatory system. One of them is the regulatory activity of vascular endothelial cells. Myocardium - cardiac microvascular endothelial cells (CMECs) cross-talk is crucial for maintaining cardiac homeostasis and autoregulation. Factors like oxidative and inflammatory stress, infections, or diabetes which can affect this communication and finally lead to development of different cardiovascular disorders. The relation between endothelial dysfunction and development of cardiovascular diseases is still under investigation [2].

We present results of Raman imaging of CMECs, performed on primary isolated CMECs from murine hearts and transformed endothelial cell line from heart (H5V). The aim of our work was to compare primary cells, primary non-endothelial cells from heart and endothelial cell line in the context of their chemical composition. Primary CMECs are characterized by none or a few lipid droplets, in contrast to the cell line, which showed an ability to develop low-unsaturated lipid droplets. In turn, non-endothelial cells were characterized by high-unsaturated lipid droplets. Due to this feature it was possible to differentiate CMECs in the mixture of all cells isolated from the heart. Using Raman imaging we observed the markers of inflammation (i.e. a formation of lipid droplets) and apoptosis (the changes in amounts of DNA/RNA) in CMECs stimulated with TNF-α.

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Localization of smooth muscle cells in the murine atherosclerotic plaque by iSERS microscopy

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Atherosclerosis still remains under intense studies as it is the leading cause of death and morbidity worldwide. A crucial role in progression of atherosclerosis plays smooth muscle cells (SMCs). During development of the disease they proliferate increasingly and migrate into the intima having a great influence on the formation and stability of plaque[1]. Therefore their identification and quantitative analysis are an important issue in diagnosis. Nowadays, the clinical detection of atherosclerotic compartments relies largely on immunohistochemical staining combined with fluorescence microscopy (IF). However IF are limited by several factors such as autofluorescence, photobleaching of fluorescent labels or overlapping of absorption/emission bands in the multi-target analysis. Immuno-surface-enhanced Raman scattering (iSERS) microscopy has been proposed as a novel and alternative technique overcoming limitations of fluorescent labels due to the fact that the generation of SERS signals of Raman reporters is highly photostable and offers simultaneous quantification of few biomarkers due to a small width of Raman bands [2].

In our work we used iSERS for the detection of one of the marker of atherosclerotic plaques: SMCs in mice brachiocephalic artery (BCA) from a ApoE/LDLR$^{-/-}$ model. For the detection of SMCs we employed SERS labels consisting of gold nanostars covered by 4-nitrobenzoic acid (4-NTB) as a Raman reporter and SH-PEG-COOH as a linker. This moiety was conjugated with a primary antibody directed against α-actin of smooth muscle cells (direct iSERS) and with a proper secondary antibody (indirect iSERS). The presence of the SMCs antigen was identified by collection of a SERS signal of the Raman reporter (ca. 1340 cm-1). SERS image was then compared with an IF image of the adjacent slice of the tissue (Figure 1). This comparison clearly showed a very similar distribution of the studied marker in the aorta wall and atherosclerotic plaques regardless the method of iSERS staining, i.e. direct and indirect methods. Also a quantitative analysis of iSERS and IF images gave a similar number of pixels related to SMCs in artherosclerotic plaque.

We demonstrated that iSERS could be a powerful alternative method for the detection of markers in the atherosclerotic plaque. The identification of smooth muscle cells in BCA tissue by the iSERS biosensor is possible, and we can use both direct and indirect method of staining.

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Synchrotron based microanalysis of live cell metabolism at Diamond; A dynamic flow cell for biomedicine at MIRIAM beamline B22

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One of the remarkable exploitations of the extremely bright synchrotron infrared light is probing single, isolated cells and tissues at sub-cellular resolution, collecting molecular information with excellent spectral quality via a diffraction limited microbeam. Studying individual cells reveals the cell-by-cell biochemical differences (e.g. due to cell cycle or biological variability), otherwise averaged together in conventional IR imaging or spectroscopy. Nowadays, the research frontier in the field is the *in vivo*/*ex vivo* analysis of living cell metabolism.

The combined requirements of high spatial resolution, rapid data acquisition/high spectral quality and high photon brightness (necessary to overcome the strong absorption by water) make synchrotron radiation an invaluable microanalysis tool for IR fingerprinting.

The production of time-lapse FTIR images at subcellular spatial resolution is a powerful tool for studying localized time-dependent intracellular changes in protein structure *in vivo*. A significant limitation of this technique has been the relative difficulty with which it can be applied to living systems in dynamic mode.

The emerging and rapid development of microfluidic technology has presented an ideal solution to this problem. It is a technique that is promising for non-invasive studies of mammalian cells, capable of providing insight to disease progression, drug discovery, and even cell regulation and development.

B22: Multimode InfraRed Imaging And Microspectroscopy (MIRIAM) is the IR user facility located at the Diamond Light Source synchrotron in Oxfordshire, UK. MIRIAM is accessible to academic and industrial users, and serves a wide range of science areas.

We present recent developments in a dynamic flow cell device that allows long-term, continuous IR measurement of adherent cells, up to 24 hours.

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SERS blinking on anisotropic nanoparticles

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The SERS blinking effect of thiacyanine cation on anisotropic Ag nanoparticles (NPs), synthesized in a seed mediated method with cyclodextrins used as a capping agent were investigated by a power law and truncated power law analysis. The dark state probability distribution dependencies on time duration differ from its analogues for the conventional aggregated spherical nanoparticles covered by citric anions in most cases. The truncation of the power law has multiple nature in the significant percent of probability distribution graphs and/or the truncation exponent is often higher than 1 as represented by $P_{off}(t) = At^{\alpha^{off}}\exp(-(t/\tau)^{\beta}), (\beta \geq 1)$.The reason of it can be found in the heterogeneity of NP dimensions and its tops and edges existence, which all are additional, independent factors affecting probability distribution of the dark state. When a dye molecule moves on a spherical NP, the distance to the possibly met junction with other NP is limited by the sphere shape in every dimension. On anisotropic NP the molecule random walk can be carried out on pre-shortened or pre-extended distance to the hot spot (shorter and longer axis of nanorod for example) and the hot spots can optically trap the molecules with a different energy (edges and tops with different dimensions, junction in aggregated NP). In the consequence in the sums of the amount of dark events held on in the particular times, so in the probability distribution some perturbation occurred and/or some of truncations became a very strong effect. Remains of cyclodextrins on NP surfaces, which can trap the dye molecule inside of its "basket" additionally could increase the truncation time.

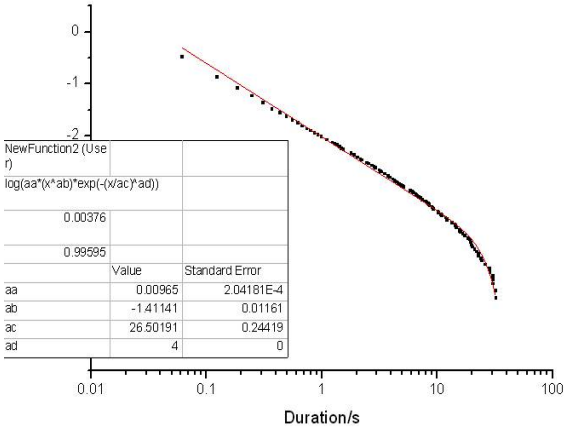

Figure 1. The probability distribution for dark SERS events plotted against their duration