BIO-OPT-XUV (BOX) Research Team Advancement at the Faculty of Biomedical Engineering, Czech Technical University in Prague

WORKSHOP #2

May 27-28, 2014

Book of Abstracts

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Dear Colleagues,

Welcome to Kladno and to the second Workshop organized at the very end of the MEYS ESF Project CZ.1.07/2.3.00/20.0092: BIO-OPT-XUV (BOX) Research Team Advancement. The project has been carried out during the past three years and most of you took part in.

We are very pleased that we had a chance to collaborate with several highly respected academic institutions in the Czech Republic and abroad.

The program of Workshop #2 consists of two parts. The first part includes an overview of major results obtained in the course of this project and communicated by project participants and by successful PhD students.

The second part of Workshop #2 is a round table discussion on the theme “Future Research Collaboration”, which starts 10 a. m. on Wednesday 29, May 2014. Everybody is cordially invited to participate and to help us to extend and optimize our future research activities. We would especially appreciate new suggestions for future research cooperation beyond this project.

We look forward to seeing you at the Workshop!

With best regards,

Miroslava Vrbová
Project coordinator

May 22, 2014

Abstracts

May 27, 2014

Education of the team in the domain of XUV-UV-VIS-BIO interaction (KA1)

Miroslava Vrbová (Faculty of Biomedical Engineering, Czech Technical University in Prague)

The aim of the project was to strengthen education and research at the Faculty of Biomedical Engineering CTU with a focus on the interaction of the optical (OPT) and extreme ultraviolet (XUV) radiation with biological (BIO) objects. At the beginning, the focus of research and quality was guaranteed by experienced academics who have gradually come to the faculty. In the frame of the project the research team was strengthened by reintegration of a Czech professor coming from abroad, by participation of several foreign scientists who gave the consultancy to our researchers and PhD student. Our cooperation both with foreign laboratories, and laboratories of the Czech Academy of Sciences helped us to improve the quality of PhD and Mgr. student education. The team have integrated new young academics, past doctoral students and graduates of master's program Instruments and methods for biomedicine.

The solution of the project was realized in 8 following parts (“Key Activities (KA)”) under the guidance of colleagues, the name of which are presented in brackets.

KA1: Education of the team in the domain of XUV-UV-VIS-BIO interactions (M. Vrbová)

KA2: Training of specialists in the field of XUV laboratory sources (Jančárek)

KA3: Laboratory infrastructure development (P. Kučera, V. Fidler)

KA4: New methods in biomedical imaging (V. Fidler)

KA5: New combined TR/OPT/UV/XUV spectroscopy (V. Fidler)

KA6: Photobiology and photo-medicine of (non-)neoplastic mucosal affections (P. Kučera)

KA7: Fluorescence spectroscopy and microscopy of bio-membranes (R. Macháň)

KA8: Fiber optics for fluorescence biosensors (M.Pospíšilová)

The overview of achievements is reported separately for every Key activity.

In the frame of KA 1 we have organized opening Workshop #1 (October 3, 2011) to specify detail program and closing (current) Workshop #2 (May 27, 2014) to summarize our results. In the meantime we have prepared 5 condensed courses or seminars: X-Ray and XUV Plasma Sources and Applications (November 2012), Fundamentals of Plasma Physics (October, November 2012), Advanced Methods for Diagnostic Imaging (January 2013), Fluorescent Nanoparticles and Molecules for Nanomedicine (September 2013), Advanced Methods for Diagnostic Imaging (January 2014). Generally, these courses were delivered by our cooperating professor from abroad and followed by seminars of our students in the relating subject.

In frame of cooperation with foreign institutes and institutes of the Czech Academy of Science we have specified actual themes of PhD theses and dissertations. We have organized seminars with our master students where they reported procedure in their research activity. Four of our PhD students successfully defend their theses and joint our research group, next four PhD students are currently schooled.

New methods in biomedical imaging (KA4)

V. Fidler (Faculty of Biomedical Engineering, Czech Technical University in Prague)

Activities in this field were made possible thanks to the already available equipment in the BOX laboratory in Kladno as well as the already existing collaboration between the department of Chemistry at Brown University, USA, and the Faculty of Biomedical Engineering at the CTU in Prague. These favorable conditions allowed us to achieve important results in all the areas of KA4, including substantial publications.

In the XUV laboratory in Kladno, new hardware parts were implemented into the instrumentation for XUV imaging. New software was also developed for advanced data collection and analysis during the period of this project. Simultaneously close collaboration with Professor Rose-Petruck's laboratory at Brown University allowed 2 PhD students and 2 diploma FBME students to perform parts of their experimental work there. Their theses combine the results from both BOX laboratory in Kladno and the Rose-Petruck laboratory. This work, with balanced contribution from both sides, resulted in high level publications.

More successful results in teaching and pedagogy were brought about by two joint advanced courses organized at the FBME. Lecturers from partner universities in USA, Switzerland, and United Kingdom, from Institutes of the Czech Academy of Sciences, and local speakers ensured a top-notch and up-to-date materials for those advanced courses.

Scientific and medical importance of biomedical imaging on the one hand, and the well-established international collaboration on the other clearly demonstrate that both joint advanced courses and joint scientific research deserve further financial support to continue.

X-ray scatter-based imaging: from cell studies to clinical imaging

C. Rose-Petruck (Department of Chemistry, Brown University)

Spatial Frequency Heterodyne Imaging (SFHI) uses the x-radiation scattered off tissue and nanoparticle immuno-labels for image formation. This modality does not require any x-ray optics between the object and the x-ray source. As a consequence, it is suitable for clinical imaging of low-absorption contrast, soft tissue.

We used SFHI for the detection of liver cancers labeled with nano-particles. We demonstrated that SFHI detects NP-labeled cells with sensitivity nearly equal to that of Magnetic Resonance Imaging (MRI). Considering that MRI is under development and use for nearly 50 years while we develop SFHI for about 4 years, we believe that SFHI has great promise to develop into a superbly sensitive imaging modality for nanomaterials. Recently, we carried out SFHI studies at the Massachusetts General Hospital using clinical x-ray imagers.

XUV spatial harmonic imaging

Petr Bruza (Czech Technical University in Prague)

The development and experimental results of the Soft X-ray Heterodyne Imaging (SFHI) in the soft x-ray water window will be reviewed. SFHI permits the computational decomposition of the images acquired with a single-shot exposure into (i) an x-ray absorption image, (ii) two dark-field images, and (iii) two differential phase contrast images. During the BIO-OPT-XUV project the theoretical simulations and experimental results were assessed and found to be in a good agreement. SFHI is demonstrated to deliver more detailed sample information than conventional x-ray absorption imaging. The presented work was performed at the Faculty of Biomedical Engineering, CTU in Prague, and the department of Chemistry at Brown University, USA.

Photobiology and photomedecine of (non)tumor affections

P. Kučera (Faculty of Biomedical Engineering, Czech Technical University in Prague)

The long term projection of KA6 activity has been to create at the FBMI conditions allowing development of new technologies in a rapidly expanding domain of photodiagnosis and phototherapy concerning diseases such as tumours, inflammations and allergies.

During the period of the project BOX, the activity was based on exchange of experience between Czech and foreign teams. This resulted in organisation of top level courses for young FBMI students and researchers, realisation of master diploma works, proposal of PhD theses (in collaboration with KA8) and training in laboratory techniques. Last but not least, in collaboration with KA3, the activity also led to implantation at the FBMI of a new and indispensable laboratory that makes now possible research on living samples. This laboratory, open to all interested FBMI research groups, is fully functioning and produces normal and tumour cell cultures that are already used in several new research projects.

With respect to the future, these basic achievements should stimulate a conception of long-term multicentric and pluridisciplinary collaborations with national and international specialists in the domain of interaction between radiation and living systems. The final aim could even be to create at the FBMI a reference centre for photobiology.

Is there a future for optical imaging in medicine?

Norbert Lange (University of Geneva)

Already today optical imaging toolboxes offer clinicians and researchers in medical sciences a large panel of low-cost methodologies enabling improved diagnostics and patient management.

Nowadays, one couldn't imagine conducting preclinical trials ranging from subcellular levels to whole animals without optical assessment. Market surveys estimate that the optical imaging market will be worth 18 billion dollars in 2018. The future impact of this technology will largely depend on improvements with respect to light sources, detection devices, and contrast agents. In this presentation some major principles of optical imaging that have to be respected in the future will be discussed.

Furthermore, based on currently available technologies it will be detailed why these methods gained their way to clinical practice. Finally, some perspectives for potential improvements will be described shortly.

Proteolytically triggered drug delivery systems for selective photodynamic therapy

Jordan Bouilloux, Andrej Babic, Imene Ateb, Viktorija Herceg and Norbert Lange (University of Geneva)

New carriers have been designed to bring photosensitizers (PS) towards cancer cells in view of selective photodynamic therapy. These PS, are inactive in their native configuration, once meeting cancer-associated proteases restore their activity and can lead upon irradiation with specific wavelength to production of reactive oxygen species.

To this end, cyclopeptides have been chosen as the starting vehicles, because their distinct design and molecular weight permit to know the exact positions and number of loaded PS on the delivery vehicle. In order to observe the best self-quenching effect of the native molecule, different loadings of PS have been tried onto these templates. Also, different sizes of poly(ethylene glycol) have been tested to vary water solubility, bioavailability and pharmacokinetics. All couplings were successfully achieved with high yields using copper-free "click"-chemistry as an efficient and biocompatible chemical reaction. Quenching values were obtained measuring the native fluorescence of the different products as compared to a mono-substituted non-quenched compound. Furthermore, fluorescence increase after trypsin digestion of peptidic linkers between the PS and the template was measured. In vitro assays were performed using PC-3 prostate cancer cells. Finally, a preliminary screening of pharmacokinetics of promising compounds was done in the chick chorio-allantoic membrane model.

The main results of KA8 and a focus on further research.

M. Pospisilova (Czech Technical University in Prague), G. Kuncova (Institute of Chemical Process Fundamentals, AV CR, v.v.i), V. Matejec (Institute of Photonics and Electronics of the ASCR, v. v. i.)

The main aim of KA8 was to build up an optic fiber sensor (OFS) laboratory as a support for education and investigation of OFS. The developed OFS laboratory is now equipped with a basic unit of laser light sources and detection systems for the visible part of light spectra, opto-mechanical parts and optical fibers for the investigation of OFS and with new biomedical methods using special optical fibers. First laboratory tasks for students of CTU FBME were realized in academy years 2013/2014. In the period of the KA8 solution 6 student's theses were successfully developed: 3 Bc. Theses in cooperation with ICPF AS CR, 3 Master ones in cooperation with IPE AS CR and ICPF AS CR.; 2 students continue their studies of whole cell biosensors as PhD students. Special optical elements have been developed and produced in IPE AS CR for OFS - whole cell bioluminescence using optical fiber elements and GMO (Genetic Modified Organelles), pH measurements based on absorption transmitted light (U-shape OF) and fluorescence light (V-taper) and for enzymatic sensors (V-taper). Preliminary results of these studies, which have been presented on international and domestic conferences, opened new research issues in the field of whole cell bioluminescence OF sensors, pH and enzymatic sensors such as performance of active layers on sensing elements (GMO, fluorofores etc), light intensity levels which define the OFS sensitivity, etc. It was shown that active layers with Ru complex immobilized on the tip of a V-taper of 40μm in diameter are able to give a detectable response to oxygen concentration (in cooperation with ICPF ASCR). Developed pH OFS based on special optical fiber elements will be further employed as a diagnostic method for measurements of pH of human saliva in cooperation with 1MF CU and General University Hospital Prague. PCS OF were used in a laboratory laser backscatter method for the investigation of cilia activity (in the cooperation with KA7 prof. MUDr. Kucera, PhD., Master Theses 2013). Preliminary results show the need to investigate and realize a new control electronics and detection system to achieve a better signal/noise ratio. The system will allow us to analyze the shape of backscatter signal which brings more information's than value of frequency only. The viability of human cells is starting to be studied by interactions of light with a wavelength of 340nm with samples of cells. Research activities mentioned above will be further solved in the cooperation of FBME and ICPF AS CR (development of enzymatic sensor using special optical fiber element, pH OFS) and with IPE AS CR (optical fibers for such measurements).

Enzymatic optical sensors

Gabriela Kuncova (Laboratory of Immobilized biocatalysts and optical sensors (IBO), Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic)

In the laboratory of immobilized biocatalyst and optical sensors of the Institute of Chemical Process Fundamentals the students of the Faculty of biomedical Engineering run basic training comprising measurement with UV/VIS and fluorescent spectrophotometers, luminometers, preparation of buffers, culture media and organic-inorganic materials for sensitive elements of optical sensors. Special training was divided into two parts; whole cell biosensors and enzymatic sensors. IBO laboratory is equipped with instrumentation for determination of yeast vitality with the instrument VitalFluor based on monitoring of intensity fluorescence of cofactors nicotine amide dinucleotide (phosphate) NAD(P)H during aerobic-anaerobic transition and optical fiber sensor MATINOES for determination of concentration of oxygen, glucose and biogenic amines.

The determination of cell vitality with the instrument VitalFlour is based on the measurement of a time-scan of the intensity of fluorescence of NAD(P)H during aerobic-anaerobic switch. The ratio NAD+/NADH is a measure that reflects both the metabolic activities and the health of cells. Only reduced form of NAD is fluorescent with maximum excitation wavelength 340 nm and emission wavelength 460 nm. This allows us to non-invasively follow the intracellular changes of the NAD+ to NADH ratio. Microbial vitality is a quality measure, which strongly influences the course of processes of the food industry as beer and wine fermentation, dough leavening or cheese making. The instrument VitaFlour was originally tuned for determination of vitality of yeast, which is the most important industrial microorganism but the VitalFlour tenet might be applied for other types of cells, in case of determination of cell vitality without cell staining and cell destruction is needed.

The MATINOES sensor is based on the measurement of oxygen consumption due to oxidation of glucose catalyzed by enzyme glucose oxidase. The sensor is composed from a coated lens (Fig.1), a lightguide bar and a bundle of optical fibers connected to a light source and detector. The sensor is generic. On the same sensor principle we can measure the concentrations of sucrose, fructose and other sugars as well as oxygen. Ruthenium complex serves as an optical transducer. Its fluorescence is quenched proportionally with oxygen concentration. Covalent attachment to a carrier protects the enzyme against harsh conditions after mixing with polymer and during the UV curing of the sensitive layer on the acrylate lens. Both sensitive parts - the enzyme and the complex are coated in chemically and mechanically stable organic- inorganic polymer ORMOCER® that has a siloxane network and is UV curable.

MATINOES and VitalFluor optical sensors were developed for industrial application but in combination with tiny optical fibers they have potential for measurement of oxygen, glucose and biogenic amines in small spaces and tissue. Monitoring of these compounds in body fluids is assumed as a tool for early detection of health problems.

Two students FMBI have been taught the measurement and preparation of sensitive layers of enzymatic sensor MATINOES during team project and bachelor thesis. Nowadays they continue with diploma thesis. Their experimental work includes preparation of sensitive layers on lenses and V tapered optical fibers and determination of analytical characteristics of prepared biosensors.

Whole-cell Biosensor for Detection of Environmental Pollution

H. Kalabova, M. Pospisilova (Faculty of Biomedical Engineering, Czech Technical University in Prague), M. Jirina (Faculty of Information Technology Czech Technical University in Prague), G. Kuncova (Institute of Chemical Process Fundamentals, AV CR, v.v.i.)

On-line in-situ monitoring of the environmental pollution in remote localities is enabled by whole-cell optical fiber sensors. An optical fiber element (OFE) comprising tapered up quartz fiber with an active layer of immobilized bioluminescent bioreporters on broader end of the OFE ensures effective coupling of low intense light produced by cells to an optical fiber. OFEs were granted by Institute of Photonics and Electronics of the AS CR, v.v.i. Bioluminescent bioreporters are genetically engineered microorganisms that produced bioluminescence selectively in response of contaminants in the cell environment. With aim to increase detected bioluminescence by modification of OFE shape a mathematical model simulating light transmission applying methods of geometric optics was developed. The simulation shows a part of input rays reaching the detector as a function of broader end and OFE length. Theoretical results were compared with measured transmission by OFE and PCS fiber with core 600 µm. In the demonstration experiments bacteria Pseudomonas fluoresces HK44 selective to presence of salicylic acid and naphthalene were immobilized in the active layers. The measured intensity of bioluminescence using the OFE was six times higher as compared to PCS fiber, which was in agreement with the result of mathematical modeling. The applicability of biosensing was demonstrated by detection of BTEX with Pseudomonas putida TVA8 in twelve samples of the wastewater in laboratory conditions. For future research will be studied properties of active layer, viability of the cells and different techniques of immobilization.

Absorption optical fiber sensors

Jan Zajíc (Czech Technical University in Prague), V. Matejec (Institute of Photonics and Electronics of the ASCR, v. v. i.)

Measurements of pH are very important in medicine, biology, chemistry, environmental protection, etc. For such purposes both bulk measurement methods such as potentiometry and spectrophotometry, as well as different sensors including optical ones are used. The research of optical pH sensors is focused on novel absorption and luminescence pH transducers, pH-sensitive detection membranes, novel optical sensing structures, and on the development of optical sensors making possible the continuous pH detection in patient bodies or in plant and animal cells, etc.

The main focus of this work is on spectrophotometric bulk pH measurements and on characterizations of novel fiber-optic detection elements. Characterization experiments were carried out on U-shaped fiber-optic detection probes, provided with porous detection layers immobilizing an absorption pH indicator (methyl red, methyl orange and bromothymol blue). The probes were characterized by using prepared reference solutions and measured with a glass pH electrode and VIS spectrophotometry. Spectral-response curves of the probes in a range of 300-800 nm were measured by using an assembled experimental setup. Angular distributions of output power, when excited probes by an inclined collimated beam and in some cases time-response curves of probes, were also measured. Calibration curves of the probes were determined from which a pH working range of 4.5-6.5 with a maximum sensitivity of 0.03 1/pH unit were determined.

Training of specialists in the field of XUV laboratory sources (KA2)

A. Jancarek, M. Vrbova (Czech Technical University in Prague), P. Vrba (Institute of Plasma Physics, of the ASCR, v. v. i.)

A brief overview of pre-graduated and post-graduate students trained by KA2 team together with their work in the area of XUV sources is presented. Students were members of FBME and FNSPE community working in favor of BOX grant.

Bright EUV light source for metrology based on pulsed-power technology

S. Zakharov (NAEXTSTREAM, France), V. Zakharov (Keldysh Institute of Applied Mathematics RAS, Moscow, NRC "Kurchatov Institute", Moscow)

The actinic metrology tools in EUVL deployment require very stable high brightness EUV sources with moderate in-band power output. We report on a new compact EUV light source development through extensive computational modelling. The source is based on a fast pulsed plasma discharge created in a gas-filled capillary wall confined structure, triggered through a transient hollow cathode discharge mechanism. Nanosecond scale of the resistive discharge is provided by high voltage pulsed power technology with energy storage line imbedded in the source structure. Modelling of discharge dynamics and non-equilibrium plasma emission is carried out by means of the radiation-magnetohydrodynamic (RMHD)code ZENITH, which is a next generation of the RMHD codes Z* and ZETA widely used for simulations of laser- and discharge-produced plasmas. Computational optimization of the plasma source parameters in complete experimental geometry with the energy storage line is done.

The low-inductance energy storage line is charged to Joule range of electric energy at 20-30kV voltage that provides up to 20kA current through the discharge lasting for 15-20ns. The fast discharge and the dielectric capillary wall confinement provide a small size stably positioned emitting plasma source. Pulsed xenon plasma heated up to 30-50eV temperature has 150nm diameter. It emits up to 7W/kHz of EUV radiation power, averaged at high frequency operation mode, in 2% spectral band around 13.5nm wavelength into 2πsr. The in-band brightness of the considered plasma source can meet the requirements of EUVL metrology tools.

XUV Sources based on Capillary Discharges and Target Laser Plasmas (Brief description and comparison)

Pavel Vrba[1], Miroslava Vrbová[2], S. V. Zakharov[3], V. S. Zakharov[3], A. Jancarek[1], M. Nevrkla[1] and P. Kolar[1]

[1]Institute of Plasma Physics, of the ASCR, v. v. i., [2]Czech Technical University in Prague, [3]NAEXTSTREAM, France

Monochromatic XUV radiation sources emitting the radiation in spectral range $2.2 < \lambda < 4.4$ nm (called “water window” region), may be useful for interaction experiments with watered biological samples. If nitrogen plasma is heated to temperatures 40 – 80 eV, prevailing abundance of helium-like nitrogen ions N^{6+} and strong emission at wavelength $\lambda = 2.88$ nm, corresponding to $1s^2$-1s2p transition are expected. Plasma heated up to the required temperature may be created either by pinching capillary discharge or by laser beam interaction with gas puff target.

Emission properties of non-equilibrium plasma in water-window range

Vassily Zakharov (Keldysh Institute of Applied Mathematics RAS, Moscow, NRC "Kurchatov Institute", Moscow)

Discharge and laser produced plasmas used in soft X-ray sources are non-equilibrium. Understanding of behavior of such plasma and the results on modelling of sources depend on kinetic and spectral parameters of plasma. Mismatch between actual plasma conditions and its theoretical estimations leads to incorrect evaluation of parameters and to impossibility of optimization of sources. Modelling of emission properties of plasmas of various elements is carried out in the approach for kinetic parameters of non-equilibrium plasma, incl. interaction processes with arbitrary electron distribution, based on Hartree-Fock-Slater model. Non-equilibrium kinetic modelling demonstrates high sensitivity of ionization and emission properties of plasma to a presence of non-thermal electrons and to the processes of interaction of radiation with matter. Efficiencies of light sources in required bands are discussed. Optimal plasma conditions for substantial increase of emission intensity are proposed.

Applications of a table-top laser-induced plasma source emitting in the soft x-ray range

Mathias Müller (Laser-Laboratorium Göttingen e.V.)

The spectral range of the 'water window' (λ = 2.3 to 4.4 nm) represents a highly interesting regime for studying carbon-based specimen, due to a 10 times higher absorption of carbon compared to oxygen and water. This opens up a variety of applications, e.g. high resolution microscopy and nearedge x-ray absorption fine structure (NEXAFS) spectroscopy. These studies are typically conducted at synchrotons; however, as the interest in imaging techniques and surface sensitive chemical analytics is growing, there is also a considerable demand for compact lab-based soft x-ray sources.

In this talk, an overview on the soft x-ray activities of the Laser-Laboratorium Göttingen based on laser-driven plasma emission from pulsed gas jets is given. Results of NEXAFS measurements at the carbon K-edge (λ = 4.4 nm) and calcium L-edge (λ = 3.58 nm) are presented, along with a brief description of a table-top soft x-ray microscope using monochromatic radiation at 2.88 nm wavelength.

Capillary Discharge Driver for Investigation of ASE at 13.4 nm in Recombining Nitrogen Plasma.

Michal Nevrkla, Alexandr Jančárek, Fahad Nawaz (Czech Technical University in Prague)

Progress in development of driver capable to deliver >70 kA with rise-time ~30 ns into 22 cm long capillary is presented. Driver was assembled and currently is tested and debugged in order to achieve required capillary current.

Ablative capillary discharge for optical guiding of ultra short laser pulses

M. Tamáš (Czech Technical University in Prague)

Dynamics of capillary discharge in evacuated capillary aspre-formed plasma column for optical guiding of femtosecond laser pulses is studied. The dynamics of the plasma in the discharge is simulated by means of 1-D MHD code NPINCH. The results are discussed and Â compared with experimental results.

New combined TR/OPT/UV/XUV spectroscopy (KA5)

V. Fidler (Czech Technical University in Prague)

Advanced spectroscopy in XUV-UV-VIS-NIR regions is the most important source of new information for many natural sciences, including biomedical research and engineering, and combined techniques in spectroscopy and microscopy are now of the highest priority. Consequently, activities within KA5 were multi-directional in the area of instrumentation, research, laboratory development, creation and teaching of new courses, and scientific research on the home ground and beyond.

More information on steps taken and results achieved by the KA5 team will be part of D. Panek's presentation at the Workshop 2. The accomplishments resulting from KA5 include:

- significant extension of the BOX laboratory research instrumentation in Kladno that allows e.g. combined XUV excitation – UV-VIS-NIR fluorescence or luminescence spectroscopy next to introduction fluorescence confocal microscopy/spectroscopy techniques;
- new teaching laboratories (together with KA3), new courses on basic and advanced spectroscopy, MA projects, diploma works, and PhD theses completed in this field;
- advanced courses led by a collaborating international team from the UK, USA and Switzerland, which were organized in Kladno;
- diploma and PhD collaborative education, e.g., one FBME student diploma thesis jointly completed with Prof. Zimmt laboratory at Brown U.,USA and another diploma thesis completed with Prof. Birch's laboratory at Strathclyde U., UK. (the latter student was subsequently awarded a prestigious stipend for her PhD study at London IC, U.K.),
- a new research area for BOX laboratory in Kladno was establish that is focused on structural study of biological membranes and their interactions with bio-molecules of medical importance

Results of the KA5 in cooperation with KA4, KA3 and KA7 represent a future scientific area where the FBME "in-house" research, together with already established international collaborations, can become a strong and internationally competitive research center to represent the CTU in Prague in its function of a "Research University", supposing that corresponding resources will be provided

Gold nanorods: new opportunities in biological imaging and sensing

Y. Chen (Department of Physics, Strathclyde University)

Intrinsic fluorescence from gold nanoparticles has attracted intensive interest in recent years. It combines with high photostability, low toxicity, tunable absorption band and ability to conjugate to bio-molecules, making gold nanoparticles a versatile probe in biological imaging and sensing. We have studied the Two-Photon Luminescence (TPL) from gold nanorods (GNRs) and found their characteristic short lifetime (less than 100ps) can be used to distinguish gold nanorods from other fluorescent labels and endogenous fluorophores in lifetime imaging. TPL of similar characteristics was also observed from gold nanorods generated using a size-selected cluster source, indicating a surface plasmon enhanced two-photon excitation process is still active at small sizes. In addition, we have found surface plasmon enhanced energy transfer between biological labelling dyes and gold nanorods under two-photon excitation in both solution and intracellular phases, consistent with theoretical simulation using density matrix method. These studies demonstrated that gold nanoparticle-dye energy transfer combinations are appealing, not only in Fluorescence Resonance Energy Transfer (FRET) imaging, but also energy transfer-based fluorescence lifetime sensing of bio-analytes. Internalization of GNRs has been studied via FRET based fluorescence lifetime imaging using GFP labelled early endosome. Observed energy transfer between GNRs and GFP indicates the involvement of endocytosis in GNR uptake. Finally, a novel nanoprobe based on gold nanorod for RNA sensing at the single cell level through energy transfer imaging microscopy will be discussed.

XUV-UV-VIS spectroscopy for biomedical research

Dalibor Pánek (Czech Technical University in Prague)

In this talk, a unique time-resolved spectroscopic tool for characterization of novel scintillators will be reviewed. The method is based on the pulsed SXR excitation from laser-produced plasma and fast luminescence detection in the UV-VIS region. The use of nanosecond low-repetition SXR excitation makes it possible to quantitatively evaluate the contribution of slow decay components (≥0.1 μs) to the integral luminescence. During the

BIO-OPT-XUV project we improved the setup for luminescence decay measurement and subsequent data analysis. The method was applied on the study of several types of novel scintillating materials and thus it is becoming an established technique to study delayed recombination phenomena caused by material defects. Such a rigorous assessment of the effect of material defects is of great importance for the development of modern fast detectors for biomedicine and other fields.

In the talk, current projects on further developments will be briefed together with a new laboratory equipment and capabilities acquired during the project that can be used in a combined experimental setup or complementary measurements.

Fluorescence spectroscopy and microscopy of bio-membranes (KA 7)

Radek Macháň (Department of Biological Sciences and Centre for BioImaging Sciences, National University of Singapore, Singapore)

This contribution summarizes the main outcomes of Key Activity 7 (KA 7) solved in collaboration between FBME and J. Heyrovský Institute of Physical Chemistry of ASCR (JHIPC). 2 bachelor projects, 4 master projects and an ongoing doctoral project have been the main outcome of KA 7. The core of the collaboration and 5 of those projects have been centred around the laboratory of Prof. Martin Hof focusing on bio-membrane dynamics studied by fluorescence methods. However, collaboration has been established also with other laboratories at JHIPC, resulting in 2 master projects (supervised by Dr. Zdeněk Bastl and Prof. Patrik Španěl, respectively) solving topics beyond the initial scope of KA 7. One of those topics is presented in the contribution of Dr. Bastl. Another outcome of KA 7 has been the transfer of Zeiss ConfoCor 2 microscope from the laboratory of Prof. Hof to FBMI. This has allowed the transfer of some of the topics solved initially at JHIPC to FBMI. In this context, it is worth to mention the 2 bachelor projects supervised by Dr. Dalibor Pánek, which established a methodology for vesicle leakage assays by fluorescence lifetime correlation spectroscopy and fluorescence burst analysis. Those assays are being developed further and will be applied to the study of peptide-membrane interactions. Another topic which can be addressed using the transferred instrument is the process of DNA condensation by polycationic compounds. A master project supervised by Dr. Teresa Kral focused on that topic and resulted in valuable information on the interplay between simple inorganic salts and organic polycations in the process of DNA condensation. Another topic with a big potential for further collaboration between FBMI and JHIPC is the interaction between bio-membranes and amyloid peptides. It has been addressed in the master project of Markéta Kubánková; the subsequent contribution by Prof. Hof provides more information on the topic and the recent results obtained at JHIPC.

Diffusion of membrane bound Beta-Amyloid peptides and the influence of GM1 clusters

Mariana Amaro, Radek Sachl, Jana Humpolickova, Gokcan Aydogan, Martin Hof (J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences C.R.)

Aberrant misfolding and aggregation of amyloidogenic proteins is implicated in the onset and progression of devastating neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and others. While the exact molecular factors responsible for these incurable diseases are still largely unknown, it has been found that peptides associated with these disorders bind and disrupt cellular membranes.

However, despite this growing awareness, characterization of the interactions of amyloid peptides with the cell membrane and understanding the biological significance of such interactions are still incomplete. In particular, still open and critical questions are whether there are fundamental membrane targets for amyloid peptides, and what are the relationships between membrane interactions and neurodegenerative diseases' pathologies.

It has been suggested that Beta-Amyloid peptides bind GM1 gangliosides present in lipid rafts and that this complex could be responsible for seeding fibrillation of the peptide. However, such studies were performed in system with high concentrations of GM1, Sphingomyelin, cholesterol and amyloid peptides.

In this work we use single-molecule fluorescence techniques to access if the GM1-Beta-Amyloid interaction remains when using nanomolar concentrations of amyloid peptide and the model systems in study (Lipid bilayers of Giant Unilamellar Vesicles) are less extreme and have a more physiological relevant character.

Functionalized gold surfaces for biomedical applications

Zdeněk Bastl, (J. Heyrovský Institute of Physical Chemistry)

Cellular adhesion and growth on solid surfaces is the principal theme in the development of biosensors and implantable medical materials. The first step in this development is the preparation of suitable surface coatings allowing coupling of biologically active molecules on the solid surface. Such coatings on surfaces can be obtained through self-assembly of thiol group containing molecules (SAMs). These SAMs are known to form well-ordered molecular layers

on gold surfaces and they are easy to prepare. The presence of SAM on gold surface results in properties of an interface entirely different compared to unmodified gold surface. Molecular recognition is another important application for SAMs.

A wide variety of ligands (amine, carboxylate, phosphine,…) form SAMs on gold surface, however thiol surface attachment groups are most frequently used because of strong chemical bond that forms between sulfur and gold. The disadvantage of widely used alkanethiol SAMs is the presence of 6-10% of defects and their limited stability.

Dicarba-closo-dodecaborane-thiols- functionalized inorganic cluster species with structure of nearly regular icosahedron were recently introduced by our teams [1-3] as a new type of stable molecules for self-assembled monolayers on gold and gold nanoparticles. The chemical reactivity of dicarba-closo-dodecaboranes has been extensively investigated over the last few decades and this provides a good source of information about the synthesis of their derivatives with different functional groups (e.g. COOH, OH, SH…) attached to either carbon or boron atoms of the skeleton. Through proper selection of terminal functional group specific surface interactionscan be exploited to immobilize molecules at an interface. The SAMs of this new family of molecules proved to possess several unique features including low concentration of defects and stability offering thus excellent prospects for designing interfaces with improved or novel functions.

Workshop Schedule, Tuesday 27, May, 2014

Time	Speaker	Title
9:30	M. Vrbová	Education of the team in the domain of XUV-UV-VIS-BIO interaction (KA1)
9:50	V. Fidler	New methods in biomedical imaging (KA4)
10:00	C. Rose-Petruck	XUV and X-ray Imaging: New prospects for biomedicine
10:20	P. Brůža	XUV spatial harmonic imaging
10:30		Break
11:00	P. Kučera	Photobiology and photo-medicine (KA6)
11:10	N. Lange	Is there a future for optical imaging in medicine?
11:30	Jordan Bouilloux	Proteolytically triggered drug delivery systems for selective photodynamic therapy
11:40	M. Pospíšilová	Fibre optics for fluorescence biosensors (KA 8)
11:50	G. Kuncová	Enzymatic optical sensors
12:05	H. Kalábová	Whole cell biosensors
12:15	J. Zajíc	Absorption optical fiber sensors
12:30		Lunch
13:30	A. Jančárek	Training of specialists in the field of XUV laboratory sources (KA2)
13:40	S. V. Zakharov	Bright EUV light source for metrology based on pulsed-power technology
14:00	P. Vrba	XUV sources based on capillary discharges and target laser plasmas
14:15	V.S. Zakharov	Emission properties of non-equilibrium plasma in water-window range
14:30	M. Muller	Applications of table-top laser-induced plasma source emitting in soft x-ray range
14:40	M. Nevrkla	Capillary discharge driver for investigation of ASE at 13.4 nm in recombining nitrogen plasma
14:50	M. Tamáš	Ablative capillary discharge for optical guiding of ultra- short laser pulses
15:00		Break
15:20	V. Fidler	New combined TR/OPT/UV/XUV spectroscopy (KA5)
15:30	Yu Chen	Collaborations between CTU and SU
15:45	D. Pánek	XUV-UV-VIS spectroscopy for biomedical research
16:00	R. Macháň	Fluorescence spectroscopy and microscopy of bio-membranes (KA 7)
16:15	M. Hof	Diffusion of membrane-bound b-amyloid proteins
16:30	Z. Bastl	Functionalized gold surfaces for biomedical applications
16:45		Conclusions

Wednesday 28, May, 2014

Time	
10:00	Round table discussion "Future Research Collaboration"
12:30	Lunch