Importance and relevance of the research topic

The project integrates state-of-the-art laser technology, Raman spectroscopy and research in diagnosis of human biological tissue. The scientific field of the proposed project belongs to cross-disciplinary research at the border of physics, chemistry and biology/medicine. Biologists have never before had such methods for studying the molecular structure of biology. Last decade brought to dramatic advances and led to creation light with completely new qualities, which are not found in nature. The ability to control the coherence of light led to exciting results: the shortest pulses (femtoseconds $10^{-15}$ and below), the highest peak powers (petaWatts, $10^{15}$ W), the highest stability in amplitude and frequency ($10^{-15}$), the highest modulation rates (terabit/s) and the average powers of coherent light of some 10kW. Commercially available Kerr lens mode locked Ti:sapphire oscillators provided ultrashort pulses with high energies, broader tenability, excellent pulse-to-pulse stability and ease of operation. Equipment for the generation and analysis of picosecond and femtosecond laser pulses has left the laboratory stage and is now commercially available. Similar progress has taken place in non-linear optics, which stimulated the further development of laser spectroscopy and is an important ingredient in the design and fabrication of new optical materials and devices. In addition, ultrashort optical pulse lasers have become more useful as advances have taken place in amplifiers, parametric devices, detection techniques, fiber technologies and imaging.

Extensive research to apply Raman spectroscopy for medical diagnostics has been developing in many international laboratories. Medical researchers hope that the instrument based on Raman scattering will be the most sensitive of its kind in the world and will help them to identify proteins in human blood serum that foretell the susceptibility, presence and diagnostics of disease such as cancer. 23 October, 2003 Intel Corporation (the world’s largest chip maker) and Fred Hutchinson Cancer Research Center, home of two Nobel Prize laureates, and recognized internationally for its pioneering work launched the effort to build an Intel Raman Bioanalyzer to develop a method of studying, diagnosing and preventing cancer. “This collaboration is unique and exciting interaction” – said Dr. Lee Hartwell, Nobel Laureate and center president and director. “Biologists have never before had such a method for studying the molecular structure of biology. This is true discovery based research, we don’t know what we will see or learn. It may lead to a new era of molecular diagnostics and improved methods of early disease detection”.

The research topic is directly related to studying ultrafast elementary events that occur on the picosecond and femtosecond time scale such as: dynamics of vibrational motions, electronic relaxation, photodissociation, solvent assisted reactions, isomerization, proton and electron transfer, solvation dynamics, solvated electron dynamics and other chemical reactions, many of them of biological importance. The specific aim of the proposed project is to provide an experimental and theoretical basis for understanding the ultrafast electronic dynamics, vibrational dynamics, H- bond dynamics and photochemistry of the early intermediates for a class of proteins known as rhodopsins and the phthalocyanine derivatives by laser spectroscopy methods, mainly femtosecond and picosecond transient absorption, picosecond time-resolved coherent anti-Stokes Raman spectroscopy and low temperature Raman spectroscopy. The class of rhodopsins is responsible for highly specialized biological processes like vision and proton pumping from the cytoplasmic side of the membrane to the extracellular surface. We will concentrate on bacteriorhodopsin (BR) and its retinal modified analogs. The second class of our interest – phthalocyanine derivatives are important potential photosesitizers of the second- and third-generation in the photodynamic therapy of cancer in medicine.
The project combines basic research and useful applications for cancer therapy and diagnostics by laser spectroscopies. The project will provide an approach to the solution of challenges of photochemistry in of III generation photosensitizers including phthalocyanine derivatives and their metal complexes that are used in medical diagnostics including photodynamic therapy and Raman spectroscopy as a diagnostic method.

The long-term goal of the project is to develop methods of spectral diagnosis for in vivo analysis of medical samples such as blood serum or breast tissue. The project will help to identify proteins in human blood serum and to create spectra that reveal the chemical structure of molecules in healthy and diseased cells. The goal is ambitious as the stage of commercialization has not been reached yet. Fred Hutchinson Cancer Center and Intel Corporation has just started building the Intel Raman Bioanalyzer whereas Palo Alto Research Center (Stanford University) in cooperation with Xerox Corporation and Scipps Research Institute have launched the effort to build the fast cytometer for screening and monitoring cancer cells through a blood test. Although financial means engaged in the equivalent projects in USA through the financial support of big corporations such as Intel or Xerox are significantly larger than we can expect to get on our research, we hope we can offer new insights into the basic mechanisms of molecular changes associated with tissue malignancy and new targets for cancer therapy and diagnostics by Raman spectroscopy.

The objectives of this project is threefold:

1. **first**, to understand photochemistry and mechanisms of vibrational relaxation of the second and third generation photosensitizers, phthalocyanine derivatives and its metal complexes (X=Cu, Zn, Mo, Mn, Co, Li, Mg) in liquid solutions, glasses, crystals and in human blood by Raman spectroscopy and time resolved laser spectroscopy,

2. **second**, to determine molecular mechanisms of the photochemical path for the early intermediates of BR photocycle: H, I(460), J(625) and reveal the role vibrational relaxation, intramolecular energy transfer, structural changes in the retinal, the protein, and/or their respective interactions that are required to facilitate specific reactive pathways in the BR photocycle by femtosecond pump-probe absorption and CARS spectroscopy,

3. **third**, to establish the empirical relations between the Raman and emission spectra and the biochemical composition of the human blood and breast tissue. So far specific Raman spectra related to breast cancer, bladder cancer, lung cancer, leukemia, colonic carcinoma, melanoma have been obtained in LLSM

We hope that only projects at the border of physics, chemistry and biology/medicine can trigger further development and progress in laser spectroscopy. The progress is possible if we overcome barriers created by specialization- danger of entering into a dead-end-streets: duplicating research efforts, missing important research and activities, lack of inter-disciplinary communication and inability to understand the specialized language used to describe of the recent ideas and achievements by laser specialists. Traditional methods of homogeneous scientific research become less and less efficient. This gap can be overcome by these of laser- laboratories where people of various specializations can meet, communicate, train, teach and participate in networking activities. LLSM meets the criteria for inter-disciplinary research. The team consists of physicists and chemists with strong theoretical and experimental background in laser spectroscopy working for many years in the Department of Chemistry and collaborating with medical centers and hospitals. LLSM gives an excellent opportunity to continue Ph.D careers because of good infrastructure in the field of laser science. The long time effect of the projects is unknown but due to interest in the field of laser physics, Raman spectroscopy and diagnostic optical methods there are many opportunities for synergy with hospitals. The LLSM has a long lasting interest, expertise, and achievements in the field of H-bonded systems, solvation dynamics, vibrational dynamics in crystals, supercooled liquids, glasses, photochemistry and femtosecond dynamics in the second and third generation photosensitizers and in mechanisms of the primary events in the bacteriorhodopsin photocycle. The theoretical model for H-bonded systems proposed by H. Abramczyk at the beginning of 90 is still one of the most complete descriptions of the molecular mechanisms responsible for the extraordinary IR spectral properties and femtosecond vibrational dynamics in H-bonded systems. Recently proposed model for bacteriorhodopsin photocycle introduces a serious revision of currently accepted picture. It is believed that LLSM has expertise in experimental laser spectroscopy including femtosecond spectroscopy and
Raman spectroscopy with strong theoretical background in nonlinear spectroscopy will help to interpret the laser spectroscopy results obtained in LLSM.

Research topic

The specific aim of the proposed project is to provide an experimental and theoretical basis for understanding the ultrafast electronic dynamics, photochemistry and vibrational dynamics of the early intermediates in some phthalocyanine derivatives and biologically important proteins, mainly bacteriorhodopsin (BR) and its retinal modified analogs by laser spectroscopy methods including femtosecond/picosecond transient absorption and Raman spectroscopy. The substances of our interest, phthalocyanine derivatives, are important potential photosensitizers of the second- and third-generation in the photodynamic therapy of cancer. Bacteriorhodopsin (BR) belongs to the broad group of retinal proteins, which are responsible for very important biological functions, such as vision, photosynthesis and proton pumping. Bacteriorhodopsin photocycle induced by light absorption in the retinal chromophore results in the transfer of proton from the cytoplasmic to the extracellular surface of the bacterium cell. The proton transfer generates the electrochemical gradient across the membrane, which triggers ATP (adenosine triphosphate) synthesis used for metabolism in the bacterium.

The objectives of this project are threefold and are presented in the first part of the application where the nature of the research is discussed. Regarding the first objective we have to emphasize that determining the mechanisms of photochemical reactions upon light excitation is the primary step in understanding photobiological mechanisms of interaction between the photosensitizers and human tissue, such as mechanisms of selectivity, mechanisms of tissue necrosis. Understanding the primary photochemical and photophysical events is the key step in understanding photobiology and the mechanisms of selective interaction between the photosensitizers and human tissues. The aim of the first objective of the project - understanding photochemistry of the phthalocyanine macrocycles - will help to establish mechanisms of energy dissipation, particularly the contribution from the I and II type of oxidation. This knowledge is crucial in efficiency of the photodynamic therapy (PDT).

The first objective of the project will be implemented using the following measures and research methods:

- determining mechanisms of photochemical and photophysical processes in phthalocyanine derivatives and their metal X complexes (X=Zn, Li, Mn(II), Cu(II), Fe(II)) in liquid solutions, crystal and glassy states and in the human blood. Studies will be concentrated on mechanisms of energy dissipation, electron transfer between ligand and metal, electron transfer between ligands of the adjacent macrocycle rings, mechanisms of generation of protonated isomers in phthalocyanine radicals and formation of adducts and peroxo compounds. The project is going to establish influence of person’s bloodstream on the polymerization degree in phthalocyanine complexes, which is crucial in estimation of effectiveness for the photodynamic therapy.

- To enhance the output of the first objective the project will be performed in synergy with hospitals to analyse samples of human blood (with and without phthalocyanine photosensitizers) and breast tissue delivered by the needle biopsy. The long term goal of the project is to establish the empirical relations between the Raman and emission spectra and the biochemical composition of the human blood and specific spectra related to different types of cancer. So far we have tested around 100 patients suffering from pathological processes such as breast cancer, bladder cancer, lung cancer, leukemia, colonic carcinoma, melenoma and 100 healthy patients. This goal of the PhD project will be performed in the full correlation with the research performed so far in LLMS in Poland.

The aim of the second objective is to provide an experimental and theoretical basis for understanding mechanisms of primary events in bacteriorhodopsin and its retinal modified analogs upon light excitation. Hence, the aim of the second objective is to provide basis for understanding ultrafast dynamics in proteins that gives rise to the femtosecond spectral changes. Given the femto/picosecond time scale for the processes involved in electronic relaxation as well as electron transfer in phthalocyanine macrocycle a detailed elucidation requires femtosecond/picosecond time resolution. The experimental methods that will be used to achieve the goal of the first objective are Resonance Raman spectroscopy, low temperature Raman spectroscopy, pump-probe transient electronic absorption, pump-probe transient IR absorption.