Progress Report for 2006

(4.1.1. POPIS ŘEŠENÍ PROJEKTU)

IDENTIFICATION NUMBER	LC06063
TITLE OF THE PROJECT	Fluorescence microscopy in biological and medical research
COORDINATOR	Hof Martin Doc. Dr. rer.nat., DSc.
PARTICIPANTS	Kubínová Lucie RNDr. CSc. Hozák Pavel Doc. RNDr. DrSc. Palková Zdena Doc.RNDr. CSc. Blahoš Jaroslav MUDr. PhD

A. General Aim of the Project

Establishment of an organizational framework and financial cover for a purposeful collaboration of experts in physical chemistry with researchers in biology and medicine to ensure an effective application of the most advanced and sophisticated methods of physics and physical chemistry, in particular fluorescence microscopy and electrochemistry, to biological and medical research, in particular to problems of cell biology both in vitro and in vivo.

B. Overview on Scientific Activities

Fluorescence illumination and observation is the most rapidly expanding microscopy technique employed today, both in the medical and biological sciences, a fact which has

spurred advances in chromophore and fluorophore technology as well as the development of a series of new fascinating technical developments. These techniques comprise confocal detection, multi-photon and pulsed excitation, laser scanning 3D imaging using piezo- and galvano-scanning units, stereological methods, image analysis as well as deconvolution algorithms, time-resolved imaging, Foerster resonance energy transfer (FRET) analysis, fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP), single- and multi- channel fluorescence correlation spectroscopy (FCS), lifetime fluorescence correlation spectroscopy (LFCS), multi-focus fluorescence correlation spectroscopy and a variety of specialized single molecule fluorescence analysis methods.

Several of these techniques are either not yet fully developed or still are searching for applications in biological and medical sciences. Thus, one aim of this project is to further develop these techniques and to develop first relevant applications of these techniques. These issues are summarized in the aims V001, V002, and V004. They are actually assigned two work places located at the Academy of Sciences of the Czech Republic, the J.Heyrovsky Institute of Physical Chemistry (M. Hof) and the Institute of Physiology (L. Kubinova). The close collaboration between these laboratories has lead to a true centre of advanced fluorescence microscopy. The first partner is specialized on development and novel applications of advanced “single molecule” approaches like single- and multi-channel FCS, LFCS, and multi-focus FCS using pulsed excitation. Moreover, the J.Heyrovsky Institute of Physical Chemistry has outstanding expertise in the application of electrochemical methods for the understanding of the interaction of ions with model membranes (aim V003, V. Mareček). Thus, a further aim of this project is to combine the electrochemical expertise with the know-how in fluorescence, and to apply that combination on model systems and, finally, on living cells. The Institute of Physiology has long lasting experience in laser scanning 3D intensity imaging using confocal detection or 2-photon excitation and has significantly contributed to the development of stereological methods, image analysis as well as deconvolution algorithms. It should be underlined that such complementary expertise together with the complementary fluorescence equipment available is unique for the Czech Republic, and certainly also remarkable when taking international measures. This collaboration is not limited to the availability of complementary techniques, but also has lead to common seminars and mutual visits of scientists aiming to a knowledge transfer between both laboratories. Beside of joining the forces, both laboratories have further developed their techniques and have demonstrated applications which are relevant to this project. The

individual activities in 2006 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2006”):

a) Combining Z-scan FCS with out-of-focus FCS: Quantitative determination of diffusion coefficients in different model membranes [1]
b) Combining Z-scan FCS with out-of-focus FCS: Quantitative determination of diffusion coefficients in OLN-93 cell membranes (oligodendroglioma) [2]
c) The use of the fluorescence lifetime allows for significant improvements of the quality of FCS measurements [3,4]
d) Fluorescence Lifetime Correlation Spectroscopy Combined with Lifetime Tuning: New Perspectives in Biomembrane Research [5]
e) Characterization of liposomes relevant for gene delivery. [6,7]
f) Development of methods for pre-processing of image data acquired by confocal and two-photon microscopy [8,9]
g) Methods combining image analysis and stereological approach for evaluation of 3D microscopic image data [10-14]
h) Analysis of data acquired by confocal and two-photon microscopy using different fluorescence microscopy techniques
i) Adsorption and Ion-pairing Interactions of Phospholipids [15]
j) Dynamics of lithium solvation [16]

It should be pointed out that, in most laboratories, expertise is restricted to some of these specialized techniques, and the full potential of advanced fluorescence microscopy in the investigated biological systems is only partially exploited. Moreover, biologists are often not aware of all possibilities in fluorescence microscopy and, thus, only in some exceptional cases cutting edge technologies are applied in answering questions in cell biology. This project aims to overcome these limitations by promoting the close collaboration of these two complementary fluorescence microscopy laboratories together practically covering the entire needed expertise in advanced microscopy with four teams formulating important questions in cell biology. These teams are headed by following scientists: P. Svoboda (Institute of Physiology, Academy of Sciences of the Czech Republic), Z. Palkova (Faculty of Nature Sciences, Charles University, P. Hozak (Institute of Molecular Genetics, Academy of Sciences of the Czech Republic), J. Blahos (in 2006: Institute of Experimental Medicine, From 2007 on Institute of Molecular Genetics, both Academy of Sciences of the Czech

Republic). All four teams have started i) to implement new strategies for controlled labeling their systems of interests with appropriate markers, ii) to transfer the know-how in cell handling to the laboratories headed by M. Hof and L. Kubinova, and iii) to perform fluorescence measurements on living cells in the laboratories equipped with those advanced fluorescence microscopy techniques. These activities are directly connected to the aims V005 - V008 formulated in the original proposal. The individual activities aiming for the application of advanced fluorescence microscopy in biosciences in 2006 were (for details please see "AKTIVITY USKUTEČNĚNÉ v roce 2006"):

a) Cell cultivation and preparation of samples for confocal fluorescence microscopy
b) Direct effect of detergent on hydrophobic membrane interior of PM and G protein activity [17]
c) The role of plasma membrane integrity in mechanism of hormone action and desensitization [18,19]
d) Preparation of the *Sacccharomyces cerevisiae* strains containing plasma membrane putative ammonium exporters Ato fused with fluorescent proteins [20]
e) Development of two-photon confocal microscopy technique for monitoring of beginning of Ato1p-GFP production in *S.cerevisiae* monocolonies
f) Tagging of target proteins with large fluorophores
g) Introduction of short sequences for small fluorophores covalent labeling into recombinant proteins
h) Preparation of fluorescently tagged myosin molecules
i) Production of fluorescently tagged truncation mutants of myosin
j) Identification of sequences responsible for nuclear translocation of myosin
k) Preparation of vectors for fluorescently labelled PML protein.

Naturally, for the first 10 months of this 5 year project, there is still an imbalance regarding full papers coming from aims V001-V004 on one side and from aims V005-V008 on the other side. Activities connected to V001-V004 can be considered to a major extent to be ongoing projects of the individual laboratories located at the J.Heyrovsky Institute of Physical Chemistry and the Institute of Physiology. Thus a fast and high quality outcome in leading international Journals was guaranteed [1-9,10,15,16]. On the other hand most of the 2006's

activities in the biological orientated laboratories located at the Institute of Physiology, Faculty of Nature Sciences, Institute of Molecular Genetics, and Institute of Experimental Medicine were connected to the introduction of appropriate fluorescent labels to the individual systems and subsequently testing the physiological activities of those labeled systems. These time-consuming experiments are main requirements for the successful application of advanced fluorescence microscopy and are in most of the cases not finished yet. However, first fluorescence microscopy experiments on those systems were performed and some of the results could already be included to full publications [17-19]. It can be expected that the fluorescence microscopy experiments on those systems to be performed in 2007 will lead to an increase of the number of full publications in coming from those laboratories.

C. Other Activities in 2006

i) Students involved

PhD students: 6 (Hof) + 3 (Palkova) + 6 (Kubinova, Svoboda) + 2 (Blahos) + 4 (Hozák)
Mgr. or Ing. students: 3 (Hof) + 2 (Palkova) + 1 (Kubinova) + 2 (Hozák)

ii) Teaching Regular Courses at Universities

M. Hof : Molecular Physics (Czech Technical University in Prague), Spectroscopy (University Olomouc), Fluorescence Spectroscopy in Biosciences (University Ceske Budejovice)

Z. Palková: Molecular Biology (Charles University in Prague, Faculty of Natural Sciences), Cell cycles and signalling (Charles University in Prague, Faculty of Nature Sciences)

L. Kubínová: Quantitative Plant Anatomy ("Kvantitativní anatomie rostlin", Faculty of Nature Science, Charles University in Prague)

P. Svoboda: Molecular Pharmacology (Faculty of Nature Science, Charles University in Prague)

P. Hozák: Structure of the cell nucleus and regulation of gene expression (Faculty of Nature Science, Charles University in Prague)

P. Hozák: organization and teaching of one-week course for PhD students "Acquisition and processing of microscopic images" (a joint ventute of the Institute of Molecular Genetics and the Czechoslovak Microscopy Society).

iii) Organized Conferences

- M. Hof: 3rd Prague Seminar on Biophysics of Lipids (45 participants, 30 from abroad)
- L. Kubínová, J. Janáček: International Conference on Stereology, Spatial Statistics and Stochastic Geometry, Prague (90 participants, 60 from abroad)

iv) Invited Lectures at International Conferences, Seminars, Workshops, or Courses

M. Hof:

- 4th European Short Course on time-resolved fluorescence spectroscopy , Berlin, 11/2006
- 4th Advanced Practical Course on Optical Spectroscopy in Biology , Juelich, 10/2006
- Practical Fluorescence for Life (Scientists), Helsinki, 10/2006
- Workshop "To Raft or not to Raft, that is the question", Leuven, 4/2006

J. Humpolíčková:

- 12th International Workshop on Single Molecule Spectroscopy and, Berlin, 9/2006

A. Benda:

- 12th International Workshop on Single Molecule Spectroscopy and, Berlin, 9/2006

J.Blahos:

- European Synapse Summer School; Bordeaux PENS Training Center, 9/2006

V. Mareček:

- 210th Electrochemical Society Meeting,, Cancun, 11/2006

P. Hozák:

- 16th International Microscopy Congress, Sapporo, 9/2006

v) Obtained Academic Degrees

- Ing.: V. Fagulova
- PhD's: A. Benda, J. Humpolickova, V. Hlaváčková,
- Professorships:
- DSc's: M.Hof , J. Novotny

vi) Web-side referring to this project

http://www.jh-inst.cas.cz/~fluorescence/NRC.htm

vii) Awards

J. Humpolíčková: 2nd best poster (500 €) at the Conference " International symposium on optical analysis for biomolecular machines";
Title of the poster: Employing Z-scan Method in Lateral Diffusion Measurements by Means of Fluorescence Correlation Spectroscopy (FCS): Model Systems versus Cellular Applications

viii) Miscellaneous

There are several other scientific or pedagogic activities of the members of this project. However, it is certainly natural for active scientific groups that their members to present posters at international conferences, review research articles or proposals, organize regular seminars for students, invite international well recognized scientists for lectures or longer stays, or are invited by international well recognized work places for lectures or longer stays. Thus, we believe that it is not necessary to list those activities here in details

D. References

1) Magdalena Przybylo, Jan Sýkora, Jana Humpolíčková, Aleš Benda, Anna Zan, Martin Hof „The lipid diffusion in giant unilamellar vesicles is more than two times faster than in supported phospholipid bilayers under identical conditions" (2006) Langmuir, 22, 9096-9099.
2) Humpolickova J, Gielen E, Benda A, Fagulova V, Vercammen J, Vandeven M, Hof M, Ameloot M, Engelborghs Y„Probing diffusion laws within cellular membranes by Z-scan fluorescence correlation spectroscopy" (2006) Biophysical Journal, 91(3), L23-25.
3) Peter Kapusta, Michael Wahl, Aleš Benda, Martin Hof, Jörg Enderlein „Fluorescence Lifetime Correlation Spectroscopy " (2007) Journal of Fluorescence, 17, 43-48.

4) J. Hohlbein, M. Steinhart, C. Schiene-Fischer, Aleš Benda, Martin Hof, and C.G. Huebner „Confined Diffsuion in ordered nanoporous Aluminia membranes“ (2007) SMALL, in press.
5) Aleš Benda, Veronika Faguľová, Alexander Deyneka, Joerg Enderlein and Martin Hof „Fluorescence Lifetime Correlation Spectroscopy Combined with Lifetime Tuning: New Perspectives in Supported Phospholipid Bilayer Research“ (2006) Langmuir, 22, 9580-9585
6) P. Jurkiewicz, A. Olżyńska, M. Langner, M. Hof „Headgroup Hydration and Mobility of DOTAP/DOPC Bilayers: A Fluorescence Solvent Relaxation Study“ (2006) Langmuir, 22, 8741-8749.
7) K. Rieber , J. Sýkora, A. Olżyńska, R. Jelinek, G. Cevc, M. Hof “The use of solvent relaxation technique to investigate headgroup hydration and protein binding of simple and mixed phosphatidylcholine/surfactant bilayer membranes” (2007) Biochim. Biophys. Acta, in press
8) ČAPEK, M.; JANÁČEK, J.; KUBÍNOVÁ, L. Methods for compensation of the light attenuation with depth of images captured by a confocal microscope. Microscopy Research and Technique, August 2006, vol. 69, no.8, s. 624-635.
9) Freely available (on request from Dr. Čapek, capek@biomed.cas.cz) special SCOM (Series Capture Optimization Macro) macro for compensation of the light attenuation with depth of images captured by a Leica SP2 confocal microscope.
10) ALBRECHTOVÁ, J.; JANÁČEK, J.; LHOTÁKOVÁ, Z.; RADOCHOVÁ, B.; KUBÍNOVÁ, L. Novel efficient methods for measuring mesophyll anatomical characteristics from fresh thick sections using stereology and confocal microscopy: application on acid rain treated Norway spruce needles. Journal of Experimental Botany, 2007 – in press.
11) ERŽEN, I.; KUBÍNOVÁ, L.; JANÁČEK, J.; ČEBAŠEK, V.; RIBARIČ, S. Diversity in muscle fibre type capillary supply. Proceedings of International Conference on Stereology, Spatial Statistics and Stochastic Geometry, Prague, June 26-29, 2006, s. 385-390.
12) ČAPEK, M.; JANÁČEK, J.; KUBÍNOVÁ, L.; SMRČKA, P.; HÁNA, K. Volume visualization of biological tissue specimens using confocal microscopy. Proceedings of YBERC´06, Kladno, July 19-21, 2006, Lékař a technika, 2006, vol. 36, no. 2, s. 240-244.

13) ČAPEK, M.; JANÁČEK, J.; KUBÍNOVÁ, L.; SMRČKA, P.; HÁNA, K. Volume reconstruction of large biological tissue specimens. Proceedings of Advanced Engineering Design, Prague, June 11-14, 2006, CD-ROM.
14) ČAPEK, M.; KUBÍNOVÁ, L.; JANÁČEK, J.; SMRČKA, P.; HÁNA, K. Volume visualization of large biological tissue specimens. Proceedings of 18th Biennial Internatiopnal EURASIP Conference Biosignal 2006, Brno, June 28-30, 2006, s. 230-232.
15) Greg Moakes, Leslie T. Gelbaum Johannes Leisen, Jiri Janata, Vladimír Mareček, Luc L. DaemonSelf-organization of water in lithium/nitrobenzene system J. Phys. Cond. Matter, in press
16) Hana Jänchenová, Alexandr Lhotský, Karel Štulík, Vladimír Mareček Adsorption and Ion-pairing Interactions of Phospholipids in the System of Two Immiscible Electrolyte Solutions. Part I. The Behaviour of Lecithin at the Water/1,2-Dichloroethane Interface, Compared with that of Trimethyloctadecylammonium Cation. J. Electroanal. Chem. 2006, in press
17) Rudajev, V., Stöhr, J., Bouřová, L., Novotný, J. and Svoboda, P. (2007) Increased intrinsic efficacy of GPCR/G protein coupling in detergent-treated, low-density membrane fragments. Manuscript in preparation for FEBS Letters.
18) Ostašov, P., Hejnová, L., Krůšek, J., Svoboda, P. and Novotný, J. (2007) Ca^{2+}-responses to thyrotropine-relasing hormone and angiotensin 2. *Role of plasma membrane integrity and effect of G11alpha protein over-expression on homologous and heterologous desensitization of hormone response.* Manuscript in preparation for Life Sci.
19) Durchánková, D., Ostašov, P., Bouřová, L., Hejnová, L., Svoboda, P. and Novotný, J. (2007) Disruption of plasma membrane integrity by cholesterol depletion impairs effectiveness of TRH-receptor mediated signal transduction via Gq/G11alpha protein. V přípravě pro J. Membrane Biol.
20) Strachotova, D., Palkova, Z. , Vachova, L. (2006) Ato protein production in liquid yeast cultures correlates with ammonia release, SMYTE - 24th Small Meeting on Yeast Transport and Energetics Prague, 31.8.–3.9.2006, poster