# Progress Report for 2010

# ( 4.1.1. POPIS ŘEŠENÍ PROJEKTU )

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| IDENTIFICATION NUMBER | LC06063 (2006-2010) |
| TITLE OF THE PROJECT | Fluorescence microscopy in biological and medical research |
| COORDINATOR | Hof Martin Prof. Dr. rer.nat., DSc. |
| PARTICIPANTS | Kubínová Lucie RNDr. CSc.<br>Hozák Pavel Prof. RNDr. DrSc.<br>Palková Zdena Prof.RNDr. CSc. |
| ASSOCIATED PARTICIPANTS | Mareček Vladimír Prof. Ing. DrSc.<br>Svoboda Petr Doc. RNDr. DrSc.<br>Blahoš Jaroslav MUDr. Ph.D. Doc. |
| NUMBER OF APPEARED AND ACCEPTED FULL PUBLICATIONS WITHIN FIRST 58 MONTHS | 87 (average impact factor 3.4) |
| OBTAINED ACADEMIC DEGREES WITHIN FIRST 58 MONTHS | 30 (3 BC, 8 MGR, 13 PHD, 1 DOC, 2 DSC, 3 PROF.) |

## A. Overview on Scientific Activities in 2010

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 improvements. 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), fluorescence lifetime correlation spectroscopy (FLCS), multi-focus fluorescence correlation spectroscopy and a variety of specialized single molecule fluorescence analysis methods. Methods for enhancement of resolution in microscopy above the diffraction limit have been developed recently and one of the methods (DSOM) has been implemented in the laboratory of prof. Hof.

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 search for their first biologically relevant applications. These issues are summarized in the aims V001, V002, and V004 in the original grant proposal (see contract pages 8-18). They are actually assigned to two work places located at the Academy of Sciences of the Czech Republic, the J.Heyrovský Institute of Physical Chemistry (M. Hof) and the Institute of Physiology (L. Kubínová). The first partner specializes in development and novel applications of advanced “single molecule” approaches like single- and multi-channel FCS, FLCS, and super-resolution fluorescence microscopy. Moreover, the J. Heyrovský 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 a 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. The individual activities related to those 4 work packages in 2010 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2010” in the report:

a) Using FLCS as an unique and new tool for investigation of phase separation in lipid membranes – stage 4
b) Characterization of protein (peptide)-membrane interactions-stage 4: Combination of Ellipsometry, laser scanning microscopy, Z-scan fluorescence correlation spectroscopy, FLCS, and fluorescence anti-bunching experiments
c) Establishing energy transfer as a tool for determination of co-localisation in membranes of living cells and model membranes-stage 3
d) Development and application of a confocal fluorescence microscope with spectral resolution and enhanced spatial resolution - stage 2
e) Testing of new condensing agents and DNA carriers by FCS, FLCS and solvent relaxation technique- stage 4
f) Application of advanced fluorescence techniques in the elucidation of the structure function relationship of dehalogenase proteins and Cu II-azurins- stage 2
g) Experimental and computational investigation of lipid dynamics in biological membranes (all Hof)
h) Preparation of mercury film electrodes on solid amalgam surfaces (Mareček)
i) Development of methods for pre-processing of image data acquired by confocal and two-photon microscopy – stage 5
j) Methods combining image analysis and stereological approach for evaluation of 3D microscopic image data – stage 5
k) Analysis of data acquired by confocal and two-photon microscopy using different fluorescence microscopy techniques – stage 4 (all Kubínová)

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 investigation of 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 the current 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,), Z. Palková (Faculty of Nature Sciences, Charles University), P. Hozák and J. Blahoš (both Institute of Molecular Genetics, Academy

of Sciences of the Czech Republic). All four teams are applying new strategies for controlled labeling of systems of interests with appropriate markers, and are performing fluorescence measurements on living cells in the laboratories equipped with those advanced fluorescence microscopy techniques. Moreover the know-how in cell handling was transferred to the laboratories headed by M. Hof and L. Kubínová. These activities are directly connected to the aims V005 - V008 formulated in the original proposal (see contract pages 8-18). The individual activities aiming for the application of advanced fluorescence microscopy in biosciences in 2010 were (for details please see "AKTIVITY USKUTEČNĚNÉ v roce 2010" in the report:

1. Structural and dynamic organization of TRH-R-eGFP molecule in plasma (cell) membrane
2. Dependence of delta-opioid receptor action on the cell membrane integrity (all Svoboda)
3. 
4. CFP based evaluation of metabotropic glutamate receptor-G protein relations using CFP
5. FRET changes in CFP-YFP pair in single subunit of metabotropic glutamate receptor during activation of the receptor complex (all Blahoš)
6. 
7. Analysis of multimerization of individual Ato proteins using FRET.
8. Identification of domain(s) important for interaction of Ato proteins. Analysis of their role for Ato protein function.
9. Development and application of the technique for monitoring of volatile ammonia and other amines on pH of different cellular organelles.
10. Two-photon confocal microscopy analysis of colonies of wild yeast strains: Development of new staining approaches. (all Palková)
11. 
12. Development of a tool for characterization of spatial properties of filamentous structures in the cell
13. Testing the PIP2 - NM1 interactions in the nucleus by fluorescence tools.
14. Definition of nuclear structures involved in NM1-PIP2 interactions.
15. Describing dynamic properties of two nuclear isoforms of the myosin 1C.
16. Assessing the relationship between gene positioning and its activity within chromosomal territories. (all Hozák)

When looking back at the 5 years of existence of the centre, we can formulate following conclusions. In the case of laboratories focusing on advanced fluorescence microscopy and mathematical image processing located at J.Heyrovský Institute of Physical Chemistry and at the Institute of Physiology, the existence of the centre provided an important support for their relevant to biological applications of fluorescence (objectives V001-V004). The resulting, relatively large, number of publications consists to a large extent of methodological work, although own research containing biologically relevant applications of the methods is also included (typically in objective V002). In the case of biological laboratories at the Institute of Molecular Genetics, Charles University and the Institute of Physiology, the centre stimulated advance in development of genetically modified organisms with selective fluorescent labelling (objectives V005-V008), which are crucial for investigation of processes in living cells by fluorescence microscopy. The most successful collaborations within the centre have been then established between the laboratories of prof. Palková and Dr. Kubínová, those of prof. Hof and doc. Svoboda and also Prof. Hozák and Dr. Kubínová. It is probable that the established collaborations will result in further published result and will continue beyond the existence of the centre.

## B. Other Activities

### i) Students involved in 2010

PhD students: 6 (Hof) + 2 (Palková) + 2 (Kubinova) + 7 (Svoboda) + 5 (Blahoš) + 7 (Hozák)

Mgr., Ing. or Bc. students: 2 (Hof) + 4 (Palková) + 3 (Svoboda) + 1 (Hozák)

### ii) Teaching Regular Courses at Universities in 2010

M. Hof: Fluorescence Spectroscopy (Charles University in Prague, Faculty of Science)

M. Hof: Spectroscopy (University Olomouc, Faculty of Science)

J. Sýkora, M. Hof: Fluorescence Spectroscopy in Biology and Medicine (South-Bohemian University České Budějovice)

J. Humpolíčková: Physical Chemistry Medicine (South-Bohemian University České Budějovice)

R. Macháň: Fluorescence Spectroscopy in Biology and Medicine (Czech Technical University in Prague)

P. Svoboda: Molecular Pharmacology (přednášky a praktická cvičení), Molecular Pharmacology (přednáška), Faculty of Science, Charles University in Prague

L. Kubínová and J. Janáček: Image analysis and stereological methods for biologists („Metody analýzy obrazu a stereologie pro biology“, Faculty of Science, Charles University in Prague)

J. Blahoš: Medical pharmacology, 2nd Medical School Charles University in Prague and Medical Faculty Charles University in Hradec Kralove

P. Hozák: Microscopy methods in three PhD courses (Faculty of Science, Charles University in Prague)

Z. Palková: Molecular Biology (“Molekulární biologie”, Faculty of Science, Charles University in Prague)

Z. Palková: Cell Cycle and Signalling (“Buněčné cykly a signály”, Faculty of Science, Charles University in Prague)

## iii) Organized Conferences in 2010

M. Hof: 5th Prague-Wroclaw Seminar on Biophysics of Lipids, November 16-19, 2010

P. Hozák: Prague, 1 - 4 September, 2010, Advanced imaging techniques in biomedicine: from molecules to organisms.

## iv) Invited Lectures at International Conferences, Seminars, Workshops, or Courses in 2010

M. Hof:

- Invited lecture: Hydration and mobility in membranes: Membrane Symposium to the honour of the 60th birthday of Prof. Paavo Kinnunen, Helsinki, Finland, 21.5.2010
- Invited lecture: Hydration and mobility in lipid bilayers detected by the fluorescence solvent relaxation technique: Hofmeister series and oxidised phospholipids, 7e congrès du GERLI , Biarritz, France, 4.10.2010
- Invited lecture: Fluorescence Lifetime Correlation Spectroscopy, 7th Advanced Imaging Methods Workshop, Berkely, USA, 21.1.2010

- Invited lecture: Advances in Fluorescence Correlation Spectroscopy, Advanced imaging techniques in biomedicine: from molecules to organisms, Prague, 2.9.2010

A. Benda:

- Invited lecture: New possibilities offered by EM-CCD detection in classical confocal microscope, 16th International Workshop on "Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences" PicoQuant Berlin, September 17, 2010

J. Humpolíčková:

- Invited lecture: Dynamic Saturation Optical Microscopy, 16th International Workshop on "Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences" PicoQuant Berlin, September 16, 2010
- Invited lecture: Dynamic Saturation Optical Microscopy, 7th Prague Workshop on Photoinduced Molecular Processes (PIMoP), Prague , March 24 2010

R. Macháň:

- Invited lecture: Investigation of Moleular Diffusion in Biological Membranes, Seminar series on super-resolution microscopy, Institute of Medicinal Biology, Singapore, July 15, 2010.

L. Kubínová:

- Invited lecture: "The error in measurement of capillary supply of skeletal muscle fibres from thin cross sections and its reduction by 3D method using confocal microscopy" at the "52$^{nd}$ Symposium of the Society for Histochemistry", 1 - 4 September 2010, Prague.
- Invited lecture: "3D image analysis of microscopic structures by stereological and digital methods" at the Lorentz Workshop "Modeling with Images in the Life Sciences", 29 November - 3 December 2010, Leyden, Netherlands.

J. Janáček:

- Invited lecture: "Variance of length or surface area estimates using periodic grids" at the 28$^{th}$ European Meeting of Statisticians, 17 - 22 August 2010, Piraeus, Greece.

P. Hozák:

- Invited lecture: "Nuclear myosin 1 – transcriptional involvement?", National Institute of Genetics, Kyoto University, Japan

## v) Obtained Academic Degrees

- PhD: Anna Kulakowska,
- Mgr: Z. Kubínová
- Bc: P. Marášek

## vi) Web-site referring to this project

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

## vii) Awards

- Dr. Jana Humpolíčková: Stipendium L'Oréal pro ženy ve vědě.
- Mgr. Burdíková: Fellowship for Junior Scientists to attend 17th International Microscopy Congress (Rio de Janeiro, Brasil, September 2010)

## viii) Miscellaneous

P. Hozák is a member of the executive committee of European Microscopy Society, and the president of the European "Society for Histochemistry," and Czechoslovak Microscopy Society.

Martin Hof is the main editor for Europe of Journal of Fluorescence and was appointed as the Series Editor of the "Springer Series on Fluorescence"

J. Blahoš is in committee of Sigma-Aldrich Conference for Young Chemists and Biochemists.

L. Kubínová serves as a vice-chairman of EMB (Engineering in Medicine and Biomedicine) Chapter of Czechoslovakia Section of IEEE. In 2010 L. Kubínová was appointed director of the Institute of Physiology ASCR.

P. Svoboda is the head of Laboratory of Neurobiology, Department of Physiology, Faculty of Science, Charles University.

Moreover, 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.

C. **Full publications acknowledging** LC06063 appeared (17), in press (13) or submitted (7) in 2010

1) Kulakowska-Zań A, Jurkiewicz P, Sýkora J, Benda A, Mely Y, Hof M. Fluorescence Lifetime Tuning-A Novel Approach to Study Flip-Flop Kinetics in Supported Phospholipid Bilayers. Journal of Fluorescence (2010) 20, 563-569. (IF=2.0)
2) Macháň R, Miszta A, Hermens W, Hof M. Real-time monitoring of melittin induced pore and tubule formation from supported lipid bilayers and its physiological relevance. Chemistry and Physics of Lipids (2010) 163, 200-206. (IF=2.1)
3) Miszta A, Macháň R, Hermens W Th, Hof M. Peptide-Membrane Interactions Studied by Ellipsometry, Laser Scanning Microscopy, and Z-Scan Fluorescence Correlation Spectroscopy. Membrane Active Peptides. Methods and Results on Structure and Function. (Castanho M, Ed.) La Jolla: International University Line (2010) 219-245. ISBN 978-0-9720774-5-3. - (IUL Biotechnology Series)
4) Humpolíčková J, Benda A, Macháň R, Enderlein J, Hof M. Dynamic saturation optical microscopy: employing dark-state formation kinetics for resolution enhancement. Physical Chemistry Chemical Physics (2010) 12, 12457-12465. (IF=4.1)
5) Kral T, Leblond J, Hof M, Scherman D, Hersovici J, Mignet N. Lipopolythiourea/DNA interaction: A biophysical study. Biophysical Chemistry (2010) 148, 68-73. (IF=2.3)
6) Hovorka O, Šubr V, Větvička D, Kovář L, Strohalm J, Strohalm M, Benda A, Hof M, Ulbrich K, Říhová B. Spectral analysis of doxorubicin accumulation and the indirect quantification of its DNA intercalation. European Journal of Pharmaceutics and Biopharmaceutics (2010) 76, 514–524. (IF=3.2)
7) Barucha-Kraszewska J, Kraszewski S, Jurkiewicz P, Ramseyer Ch, Hof M. Numerical studies of the membrane fluorescent dyes dynamics in ground and excited states. Biochimica et Biophysica Acta-Biomembranes (2010) 1798, 1724-1734. (IF=4.0)
8) Beranová L, Cwiklik L, Jurkiewicz P, Hof M, Jungwirth P. Oxidation Changes Physical Properties of Phospholipid Bilayers: Fluorescence Spectroscopy and Molecular Simulations. Langmuir (2010) 26, 6140-6144. (IF=3.9)

9) Huranová M, Ivani I, Benda A, Poser I, Brody Y, Hof M, Shav-Tal Y, Neugebauer K M, Staněk D. The differential interaction of snRNPs with pre-mRNA reveals splicing kinetics in living cells. Journal of Cell Biology (2010) 191, 75-86. (IF=9.6)

10) Yosypchuk B., Fojta M., Barek J.: Preparation and Properties of Mercury Film Electrodes on Solid Amalgam Surface, Electroanalysis, 2010, 22(17-18), 1967–1973. (IF=2.6)

11) Vyskočil V, Daňhel A, Fischer J, Novotný V, Deylová D, Horáková E, Barek J, Yosypchuk B, and Wang J: Silver Amalgam Electrodes – A Look Back at the Last Five Years of Their Development and Applications. Sensing in Electroanalysis. Vol. 5 (2010) University Press Centre, Pardubice (K. Vytřas, K. Kalcher, I. Švancara, Eds.), pp. 13-31. ISBN 978-80-7395-348-5

12) Pavek P, Pospechova K, Svecova L, Syrova Z, Stejskalova L, Blazkova J, Dvorak Z, Blahos J. Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene Biochem Pharmacol. 2010 Jan 1579(2):277-87 (IF=4,3)

13) Vopalenska I, Stovicek V, Janderova B, Vachova L, Palkova Z (2010), Role of distinct dimorphic transitions in territory colonizing and formation of yeast colony architecture. Environ Microbiol,12, 264–277. (IF = 4.9)

14) Philimonenko A., Janacek J., Snyers L., Almeder M., Berger W., Schmidt W., Schöfer C., Hozák P., Weipoltshammer K., Chromosomal dynamics of cell cycle regulator gene p21 during transcriptional activation., J Struct Biol. 2011 Feb173:382-90 (IF = 3.7)

15) Čebašek, V., Eržen, I., Vyhnal, A., Janáček, J., Ribarič, S., Kubínová, L.: The estimation error of skeletal muscle capillary supply is significantly reduced by 3D method. Microvascular Research 79(1): 40-46, 2010. (IF = 3.1)

16) Burdíková, Z., Čapek, M., Ostašov, P., Machač, J., Pelc, R., Mitchell, E.A.D., Kubínová, L.: Testate Amoebae Examined by Confocal and Two-Photon Microscopy: Implications for Taxonomy and Ecophysiology. Microscopy and Microanalysis 16: 735-746, 2010. (IF = 3.0)

17) Filová, E., Burdíková, Z., Rampichová, M., Bianchini, P. , Čapek, M., Košťáková, E., Amler, E., Kubínová, L.: Analysis and three-dimensional visualization of collagen in artificial scaffolds using non-linear microscopy techniques. Journal of Biomedical Optics 15(6): 066011, 2010. doi:10.1117/1.3509112. (IF = 2.5)

18) Michálek, J., Čapek, M., Kubínová, L.: Compensation of inhomogeneous fluorescence signal distribution in 2D images acquired by confocal microscopy. Microscopy Research

and Technique. Article first published online: 3 DEC 2010 – in press. DOI: 10.1002/jemt.20965 (IF = 1.9)

19) Janáček, J., Cvetko, E., Kubínová, L., Travnik, L., Eržen, I.: A novel method for evaluation of capillarity in human skeletal muscles from confocal 3D images. Microvascular Research, 2011 – in press. DOI: 10.1016/j.mvr.2010.11.012 (IF = 3.1)

20) Eržen, I., Janáček, J., Kubínová, L.: Characterisation of the capillary network in skeletal muscles from 3D data - a review. Physiological Research 60, 2011 – in press. (IF = 1.4)

21) Vohník, M., Burdíková, Z., Vyhnal, A., Koukol, O. Interactions Between Testate Amoebae and Saprotrophic Microfungi in a Scots Pine Litter Microcosm. Microbial Ecology – in press. (IF = 3.3)

22) Hájková, Z., Bugajev, V., Dráberová, E., Vinopal, S., Dráberová, L., Janáček, J., Dráber, P., Dráber, P. STIM1-directed Reorganization of Microtubules in Activated Mast Cells1, Journal of Immunology – in press. Prepublished online 15 December 2010; Doi:10.4049/jimmunol.1002074 (IF = 5.6)

23) Pelc, R., Hostounský, Z., Otaki, T., Katoh, K.: Conventional, apodized and relief phase-contrast microscopy. In: Neurohistology & Imaging: Basic Techniques (chapter, "Neuromethods", Doucette, J.R. & Walz, W., Editors), Humana Press (Springer), Totowa, NJ, USA – in press.

24) Kozina, V., Geist, D., Kubínová, L., Bilić, E., Karnthaler, H. P., Waitz, T., Janáček, J., Chernyavskiy, O., Krhen, I., Ježek, D. Visualization of Reinke´s crystals in normal and cryptorchid testis. Histochemistry and Cell Biology – in press. (IF = 3.0)

25) Michálek, J., Kubínová, L., Čapek, M. Non-rigid Registration of CLSM Images of Physical Sections with Discontinuous Deformations. Microscopy and Microanalysis – in press. (IF = 3.0)

26) Štefl M, James N G, Ross J A, and Jameson D M. Applications of phasors to in vitro time-resolved fluorescence measurements, Anal. Biochem. (2010 - accepted) (IF=3.3)

27) James N G, Ross J A, Štefl M, and Jameson D M. Applications of phasor plots to in vitro protein studies, Anal. Biochem. (2010 - accepted) (IF=3.3)

28) Macháň R, Hof M, Chernovets T, Zhmak M N, Ovchinnikova T V, and Sýkora J, Formation of arenicin-1 microdomains in bilayers and their specific lipid interaction revealed by z-scan FCS, Analytical and Bioanalytical Chemistry (2010 -accepted) DOI: 10.1007/s00216-011-4694-z. (IF=3.5)

29) Pembouong G, Morellet N, Kral T, Hof M, Scherman D, Bureau M-F, Mignet N. Triblock copolymer L64 destabilises lipidic membranes and does not interact with DNA, Journal of Controlled Release (2010 - accepted) (IF=5.9)

30) Daňhel A, Yosypchuk B, Vyskočil V, Zima J, Barek J: A Novel Paste Electrode Based on a Silver Solid Amalgam and an Organic Pasting Liquid. Journal of Electroanalytical Chemistry (2010 - accepted). (IF=2.3)

31) Sokolová L, Williamson H, Sýkora J, Hof M, Gray H B, Brutschy B, Vlček A Jr. Pseudomonas aeruginosa Azurin Oligomers, J. Phys. Chem. (2010 - submitted)

32) Yosypchuk B, Mareček V: Properties of thiolate monolayers formed on different amalgam electrodes. Journal of Electroanalytical Chemistry (2010 - submitted).

33) Yosypchuk B, Barek J, Yosypchuk O: Preparation and Properties of Reference Electrodes based on Silver Paste Amalgam. Electroanalysis (2010 - submitted).

34) Brejchova, J., Sykora, J., Roubalova, L., Ostasov, P., Vosahlikova, M., Hof, M. and Svoboda, P. Fluorescence spectroscopy studies of HEK293 cells expressing DOR-Gi1α fusion protein the effect of cholesterol depletion. BBA-Biomembranes, submitted 2010

35) Pineda Rodo A. Váchová L. Palková Z. Universal Confocal Approach to the Experimental Determination of Protein Topology and Organellar pH Estimation, J. Biol. Chem. (submitted)

36) Váchová L., Šťovíček V., Hlaváček O., Chernyavskiy O., Štěpánek L., Kubínová L., Palková Z. Flo11p, MDR transporters and extracellular matrix cooperate in creating and protecting of biofilm yeast colony. Curr. Biol. (submitted)

37) Dzijak R., Yildirim S., Kahle M., Novák P., Hnilicová J., Venit T., Hozák P., Specific NLS within the light chain binding domain directs both Nuclear myosin I and Myosin Ic to the cell nucleus. (submitted)

### D. **Full publications acknowledging** LC06063 appeared in 2009

1) Humpolickova J, Benda A, Beranova L, Hof M. Compaction mechanism of intermediate-sized DNA elucidated by fluorescence lifetime correlation spectroscopy. Chemicke Listy 2009; 103(11):911-914. (IF=0.6)

2) Humpolickova J, Benda A, Enderlein J. Optical Saturation as a Versatile Tool to Enhance Resolution in Confocal Microscopy. Biophysical Journal 2009; 97(9):2623-2629. (IF=4.7)

3) Sykora J, Bourova L, Hof M, Svoboda P. The effect of detergents on trimeric G-protein activity in isolated plasma membranes from rat brain cortex: Correlation with

studies of DPH and Laurdan fluorescence. Biochimica et Biophysica Acta-Biomembranes 2009; 1788(2):324-332. (IF=4.2)

4) Jesenska A, Sykora J, Olzynska A, Brezovsky J, Zdrahal Z, Damborsky J, et al. Nanosecond Time-Dependent Stokes Shift at the Tunnel Mouth of Haloalkane Dehalogenases. Journal of the American Chemical Society 2009; 131(2):494-501. (IF=8.1)

5) Stefl M, Kulakowska A, Hof M. Simultaneous Characterization of Lateral Lipid and Prothrombin Diffusion Coefficients by Z-Scan Fluorescence Correlation Spectroscopy. Biophysical Journal 2009; 97(3):LO1-LO3. (IF=4.7)

6) Sachl R, Mikhalyov I, Hof M, Johansson LBA. A comparative study on ganglioside micelles using electronic energy transfer, fluorescence correlation spectroscopy and light scattering techniques. Physical Chemistry Chemical Physics 2009; 11(21):4335-4343. (IF=4.0)

7) Blanco-Rodriguez AM, Busby M, Ronayne K, Towrie M, Gradinaru C, Sudhamsu J, et al. Relaxation Dynamics of Pseudomonas aeruginosa Re-I(CO)(3)(alpha-diimine) (HisX)(+) (X=83, 107, 109, 124, 126)Cu-II Azurins. Journal of the American Chemical Society 2009; 131(33):11788-11800. (IF=8.1)

8) Huranova M, Jablonski JA, Benda A, Hof M, Stanek D, Caputi M. In vivo detection of RNA-binding protein interactions with cognate RNA sequences by fluorescence resonance energy transfer. RNA-A Publication of the RNA Society 2009; 15(11):2063-2071. (IF=5.0)

9) Olzynska A, Jurkiewicz P, Hof M. Fluorescence solvent relaxation in cationic membranes. In: Geddes CD, editor. Reviews in Fluorescence 2007. New York: Springer; 2009. p. 119-138.

10) Holub K, Janchenova H, Stulik K, Marecek V. Proton transfer across a liquid/liquid interface facilitated by phospholipid interfacial films. Journal of Electroanalytical Chemistry 2009; 632(1-2):8-13. (IF=2.5)

11) Vachova L, Chernyavskiy O, Strachotová D, Bianchini P, Burdiková Z, Ferciková I, Kubinova L, Palkova Z (2009) Architecture of developing multicellular yeast colony: spatio-temporal expression of Ato1p ammonium exporter. Environ Microbiol. 11: 1866-1877. (IF = 4.7)

12) Bourova, L., Stöhr, J., Lisy, V., Rudajev, V., Novotny, J. and Svoboda, P. (2009) G-protein activity in percoll-purified plasma membranes, bulk plasma membranes and low-

density plasma membranes isolated from rat cerebral cortex. Medical Science Monitor 15(4), BR111-122 (IF=1.607)

13) Karen, P., Ševanec, M., Smerdu, V., Cvetko, E., Kubínová, L., Eržen, I.: Software for muscle type classification and analysis. European Journal of Histochemistry 53(2): 87-95, 2009. (IF = 1.6)

14) Čebašek, V., Eržen, I., Vyhnal, A., Janáček, J., Ribarič, S., Kubínová, L.: The estimation error of skeletal muscle capillary supply is significantly reduced by 3D method. Microvascular Research 79(1): 40-46, 2010. (IF = 3.0)

15) Griffiths, P.J., Isackson, H., Pelc, R., Redwood, C.S., Funari, S.S., Watkins, H., Ashley C.C.: Synchronous In Situ ATPase Activity, Mechanics, and Ca2+ Sensitivity of Human and Porcine Myocardium. Biophysical Journal 97(9): 2503-2512, 2009. (IF = 4.683)

16) Pelc, R., Ishii, N., Ashley, C. C.: Laser flash photolysis of diazo-2, a caged calcium chelator: The relationship between the extent and rate of smooth muscle relaxation. Journal of Laser Applications 21(1): 32-38, 2009. (IF = 0.549)

17) Vohník, M., Burdíková, Z., Albrechtová, J., Vosátka, M.: Testate amoebae (Arcellinida and Euglyphida) vs. ericoid mycorrhizal and DSE fungi: A possible novel interaction in the mycorrhizosphere of ericaceous plants? - Microbial Ecology 57: 203-214, 2009. (IF=2.9)

18) Čapek, M., Brůža, P., Janáček, J., Karen, P., Kubínová, L., Vágnerová, R.: Volume reconstruction of large tissue specimens from serial physical sections using confocal microscopy and correction of cutting deformations by elastic registration. Microscopy Research and Technique.72: 110-119, 2009. (IF=1.8)

19) Chernyavskiy, O., Vannucci, L., Bianchini, P., Difato, F., Saieh, M., Kubínová, L.: Imaging of mouse experimental melanoma in vivo and ex vivo by combination of confocal and nonlinear microscopy. Microscopy Research and Technique 72: 411-423, 2009. (IF=1.8)

20) Janáček, J., Čebašek, V., Kubínová, L., Ribarič, S., Eržen, I.: 3D visualization and measurement of capillaries supplying metabolically different fiber types in the rat extensor digitorum longus muscle during denervation and reinnervation. Journal of Histochemistry & Cytochemistry 57(5): 437-447, 2009. (IF=2.8)

D. **Full publications acknowledging** LC06063 appeared in 2008

21) Adam Miszta, Bas van Deursen, Roy Schoufs, Martin Hof, and Wim Th. Hermens "Absence of Ethanol-Induced Interdigitation in Supported Phospholipid Bilayers on Silica Surfaces" (2008) Langmuir, 24, 19-21. (IF=3.9)

22) Adam Miszta, Radek Machan, Aleš Benda, Andre J Ouellette, Wim Th. Hermens, Martin Hof "Combination of ellipsometry, laser scanning microscopy and Z-scan fluorescence correlation spectroscopy elucidating interaction of cryptdin-4 with supported phospholipid bilayers" (2008) Journal of Peptide Science, 14 (4): 503-509.(IF=1.8)

23) Jana Humpolíčková, Aleš Benda, Jan Sýkora, Radek Macháň, Teresa Kral, Barbara Gasinska, Joerg Enderlein and Martin Hof "Equilibrium Dynamics of Spermine-induced Plasmid DNA Condensation Revealed by Fluorescence Lifetime Correlation Spectroscopy" (2008) Biophys J, 94(3), L17-9. (IF=4.8)

24) Jana Humpolíčková, Miroslav Štěpánek, Teresa Kral, Aleš Benda, Karel Procházka, Martin Hof "On Mechanism of Intermediate-sized Circular DNA Compaction Mediated by Spermine: Contribution of Fluorescence Lifetime Correlation Spectroscopy" Journal of Fluorescence, 18(3-4, 679-684 (IF=2.6)

25) Humpolickova, J., L. Beranova, M. Stepanek, A. Benda, K. Prochazka, and M. Hof, Fluorescence Lifetime Correlation Spectroscopy Reveals Compaction Mechanism of 10 kbp and 49 kbp DNA and Differences between Polycation and Cationic Surfactant. Journal of Physical Chemistry B, 2008, 112, 16823-16829. (IF=4.1)

26) Noppadon Adjimatera, Aleš Benda, Ian S. Blagbrough, Marek Langner, Martin Hof, Teresa Kral "Fluorescence Correlation Spectroscopic Studies of a Single Lipopolyamine-DNA Nanoparticle" (2008) kapitola z knihy "Fluorescence of Supermolecules, Polymers, and Nanosystems", Springer Ser Fluoresc, 4, 381-413.

27) Piotr Jurkiewicz, Čestmír Koňák, Vladimír Šubr, Martin Hof, Petr Štěpánek, Karel Ulbrich. Investigation of nanoparticle coating by fluorescence correlation spectroscopy. Macromolecular Chemistry and Physics, 2008, Roč. 209, č. 14, s. 1447-1453. (IF=2.0)

28) Agnieszka Olżyńska, Piotr Jurkiewicz, Martin Hof "Properties of Mixed Cationic Membranes studied by Fluorescence Solvent Relaxation" Journal of Fluorescence, 18(5), 925-928 (IF=2.6)

29) Blanco-Rodríguez Ana Maria, Ronayne Kate L., Záliš Stanislav, Sýkora Jan, Hof Martin, Vlček Antonín Jr. "Solvation-Driven Excited-State Dynamics of [Re(4-Et-pyridine)(CO)3(2,2´-bipyridine)]+ in Imidazolium Ionic Liquids. A Time-Resolved

Infrared and Phosphorescence Study" Journal of Physical Chemistry A, Journal of Physical Chemistry A, 2008, 112(16), 3506-3514 (IF=3.0)

30) Valenta, J., Fucikova, A., Vacha, F., Adamec, F., Humpolickova, J., Hof, M., Pelant, I., Kusova, K., Dohnalova, K., Linnros, J. Advanced Functional Materials, 2008, 18(18): 2666-2672 (IF=7.5)

31) Kratochvílová, Irena; Nešpůrek, Stanislav; Šebera, Jakub; Záliš, Stanislav; Pavelka, Matěj; Wang, G.; Sworakowski, J. New organic FET-like photoactive device, experiments and DFT modeling. European Physical Journal E, 2008, 25, 299-307.(IF=2.0)

32) Kubát, Pavel; Lang, Kamil; Lhoták, P.; Janda, Pavel; Sýkora, Jan; Matějíček, P.; Hof, Martin; Procházka, K.; Zelinger, Zdeněk. Porphyrin/calixarene self-assemblies in aqueous solution. Journal of Photochemistry and Photobiology. A - Chemistry Section, 2008, 198, 18-25.(IF=1.9)

33) Durchánková, D., Novotny, J. and Svoboda, P. (2008) Time-course of agonist-induced solubilization of trimeric Gq/G11 alpha proteins resolved by two-dimensional electrophoresis. Phys. Res. 57, 195-203 (IF=1.5)

34) H. Jänchenová, K. Štulík, V. Mareček, Adsorption and ion-pairing interactions of phospholipids in the system of two immiscible electrolyte solutions - Part III: The behaviour of a lecithin layer at the water/1,2-dichloroethane interface at interfacial potential differences lower than the zero-charge potential difference, J. Electroanal. Chem. 612 186 (2008).(IF=2.6)

35) K. Maeda, T. Maekawa, Y. Yoshida, T. Okugaki, S. Kihara, V. Mareček, Synergistic barrier effect of phosphatidylcholine and phosphatidic acid on the ion transfer across a polarized liquid–liquid interface and its electrochemical stability, J. Electroanal. Chem. 619-620 53 (2008).(IF=2.6)

36) Pelc, R., Hostounský, Z., Otaki, T.: Correlation between off-axis illumination and apodized phase-contrast: two complementary microscopic phase-imaging modes. Journal of Biomedical Optics 13 (5): 054067, 2008. ISSN 1083-3668.(IF=3.0)

37) Jirkovská, M., Janáček, J., Kaláb, J., Kubínová, L.: Three-dimensional analysis of capillary bed and its angiogenic activity in terminal villi of normal term placenta. Placenta 29(10): 892-897, 2008. ISSN 0143-400. (IF=3.2)

38) Kumpost J, Syrova Z, Kulihova L, Frankova D, Bologna JC, Hlavackova V, Prezeau L, Kralikova M, Hruskova B, Pin JP, Blahos J. Surface expression of metabotropic glutamate receptor variants mGluR1a and mGluR1b in transfected HEK293 cells. *Neuropharmacology*, **55**:409-418, 2008 (IF=3.2)

39) D. Durchánková and P. Svoboda (2007). Time-course of agonist-induced solubilization of trimeric Gqα/G11α proteins resolved by two-dimensional electrophoresis. Physiol Res. 2008 57:195-203 (IF=2,1).

## E. **Full publications acknowledging** LC06063 appeared in 2007

40) Jan Sýkora, Karin Kaiser, Ingo Gregor, Wolfgang Bönigk, Günther Schmalzing, and Jörg Enderlein "Exploring Fluorescence Antibunching in Solution To Determine the Stoichiometry of Molecular Complexes" (2007) Anal. Chem 79, 4040-4049. (IF=5.6)

41) J. Hohlbein, M. Steinhart, C. Schiene-Fischer, Aleš Benda, Martin Hof, and C.G. Huebner „Confined Diffsuion in ordered nanoporous Aluminia membranes“ (2007) SMALL, 3, 380-385. (IF=6,4)

42) Rieber K, Sýkora J, Olżyńska A, Jelinek R, Cevc G, Hof M"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, 1768, 1050-1058. (IF=3.6)

43) A. Olżyńska, A. Zan, P. Jurkiewicz, J. Sýkora, G. Gröbner, M. Langner, M. Hof"Molecular interpretation of fluorescence solvent relaxation of Patman and 2H NMR experiments in phosphatidylcholine bilayers" (2007) Chem. Phys. Lipids, 147, 69-77. (IF=2.4)

44) Sýkora Jan, Slavíček Petr, Jungwirth Pavel, Barucha Justyna, Hof Martin "Time-Dependent Stokes Shifts of Fluorescent Dyes in the Hydrophobic Backbone Region of a Phospholipid Bilayer: Combination of Fluorescence Spectroscopy and Ab InitioCalculations" (2007) Journal of Physical Chemistry B, 111, 5869-5877. (IF=4.1)

45) Albrechtová, J., Janáček, J., Lhotáková, Z., Radochová, R., 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 58 (6): 1451-1461, 2007 (IF=3,6).

46) Čebašek, V., Kubínová, L., Janáček, J., Ribarič, S., Eržen, I.: Adaptation of muscle fibre types and capillary network to acute denervation and shortlasting reinnervation. Cell and Tissue Research 330 (2): 279-289, 2007 (IF=2,6).

47) Kubínová, L., Kutík, J.: Surface density and volume density measurements of chloroplast thylakoids in maize (Zea mays L.) under chilling conditions. Photosynthetica 45(4): 481-488, 2007. (IF=0,8)

48) 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., 601 101 (2007). (IF=2,3).

49) H. Jänchenová, K. Štulík, V. Mareček, Adsorption and Ion-pairing Interactions of Phospholipids in the System of Two Immiscible Electrolyte Solutions. Part II. Formation and Behaviour of a Lecithin Layer in the Presence of Multivalent Anions in the Aqueous Phase, J. Electroanal. Chem., 604 109 (2007). (IF=2,3).

50) Greg Moakes, Leslie T. Gelbaum Johannes Leisen, Jiri Janata, Vladimír Mareček, Luc L. Daemon, Self-organization of water in lithium/nitrobenzene system, J. Phys. Chem. B, 111 7312 (2007). (IF=4.1)

51) Ostašov, P., Bourova, L., Hejnova, L., Novotny, J. and Petr Svoboda (2007), Disruption of the plasma membrane integrity by cholesterol depletion impairs effectiveness of TRH receptor-mediated signal transduction via Gqα/G11α proteins. J Recept Signal Transduct Res. 27, 335-352 (IF=2.0)

52) Janderová-Rossmeislová L, Nováková Z, Vlasáková J, Philimonenko V, Hozák P, Hodný Z.: PML protein association with specific nucleolar structures differs in normal, tumor and senescent human cells. J Struct Biol., 2007, 159, 56-70. (IF=3.5).

## F. **Full publications acknowledging** LC06063 appeared in 2006

53) 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. (IF=3.9)

54) 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. (IF=4.8)

55) 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 (IF=3.9)

56) 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 (IF=3.9)

57) 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. (IF=1.7)