Scientific Report

First name / Family name	Dmitry Sorokin
Nationality	Russian
Name of the *Host Organisation*	Masaryk University Faculty of Informatics
First Name / family name of the *Scientific Coordinator*	Prof. Michal Kozubek
Period of the fellowship	01/01/2012 to 31/12/2012

I – SCIENTIFIC ACTIVITY DURING YOUR FELLOWSHIP

My research interests lay within the field of mathematical methods of image processing and biomedical image processing and analysis, namely object tracking and cell images registration. During my master and PhD studies I worked on developing mathematically well-founded methods of image analysis with application to general real-life and synthetic images. During my ERCIM fellowship I had been working at Centre for Biomedical Image Analysis, Faculty of Informatics, Masaryk University under the supervision of Prof. Michal Kozubek.

The first 1.5 months of my stay at the host organization was devoted to acquaintance with the new field of research – microscopy image processing and analysis. I was given a very broad and expert overview of the current projects being under development by the members of the group. After getting the overview I was able to choose the suitable problem to solve. Since then I had been working on elastic cell images registration and single particle tracking in live cell images. The work was done in close collaboration with Dr. Pavel Matula (CBIA, Faculty of Informatics, Masaryk University), who provided me the mathematical and informatical support and Dr. Eva Bartova (Institute of Biophysics, Academy of Sciences of the Czech Republic), who provided us with real microscopy data and biological expertise.

The main goal of the project was observation of local motion of intracellular structures which allows building of quantative characteristics (e.g. MSD curve) that biologists are interested in. In other words, having the sequence of time-lapse images of one live cell as input, one needs to obtain a set of trajectories of intercellular particles and make some automated analysis of these trajectories. Normally the processing steps are:

- Global motion compensation (registration of cell images)
- Segmentation of intercellular objects
- Tracking of intercellular objects
- Analyzing the trajectories

The most complex and important step is usually the global motion compensation. Different approaches used for this step can affect results drastically.

Although the topic is quite hot and is being researched by several groups there is still no universal solution. The ability of using particular method highly depends on the data even if the method is very well founded and validated.

The goal of the project was to improve existing approach which was developed in the host organization for specific data received from collaborators. As the cornerstone of particle tracking problem is cell images registration method, I was concentrated on development of non-rigid registration techniques. Namely, I worked on the development of non-rigid registration method that was suitable for the data with invisible cell body as well as on implementation and improvement of non-rigid registration method that was suitable for the data with visible cell body.

The method for cells with visible bodies has already shown its efficiency in biological application, namely in tracking and colocalization study of 53BP1 and PML proteins in blood cells [1] and the study of nucleoli dynamics [2]. The work on the method for cells with invisible bodies developed during the fellowship continues. The method is being finalized and tested at the moment in frame of my current research project which I applied for after the end of the fellowship.

II – PUBLICATION(S) DURING YOUR FELLOWSHIP

[1] Veronika Foltánková, Pavel Matula, Dmitry Sorokin, Stanislav Kozubek and Eva Bártová. Hybrid detectors improved time-lapse confocal microscopy of PML and 53BP1 nuclear body co-localization in DNA lesions. *Microscopy and Microanalysis, Saarbrücken: Cambridge University Press, 2013. ISSN 0958-1952*
[ACCEPTED, IF=3.007]
Abstract:
We used hybrid detectors (HyDs) to monitor the trajectories and interactions of promyelocytic leukemia (GFP-PML) nuclear bodies (NBs) and mCherry-53BP1-positive DNA lesions. 53BP1 protein accumulates in NBs that occur spontaneously in the genome or in gamma-irradiationinduced foci. When we induced local DNA damage by ultraviolet irradiation, we also observed accumulation of 53BP1 proteins into discrete bodies, instead of the expected dispersed pattern. In comparison with photomultiplier tubes (PMTs), which are used for standard analysis by confocal laser scanning microscopy, HyDs significantly eliminated photobleaching of GFP and mCherry fluorochromes during image acquisition. The low laser intensities used for HyD-based confocal analysis enabled us to observe NBs for the longer time periods, necessary for studies of the trajectories and interactions of PML and 53BP1 NBs. To further characterize protein interactions, we used resonance scanning and a novel bioinformatics approach to register and analyze the movements of individual PML and 53BP1 NBs. The combination of improved HyD-based confocal microscopy with a tailored bioinformatics approach enabled us to reveal damage-specific properties of PML and 53BP1 NBs.

[2] Lenka Stixova, Dmitry Sorokin, Petra Sehnalova, Sona Legartova, Jana Suchankova, Stanislav Kozubek, Pavel Matula, Ivan Raska, Eva Bartova. Irradiation induces UBF recruitment to DNA lesions and changes UBF localized movement. *Journal of Structural Biology, Elsevier, 2013. ISSN: 1047-8477*
[SUBMITTED, IF = 3.805]

III – ATTENDED SEMINARS, WORKHOPS, CONFERENCES

[1] Summer school:
"Summer School on Image Processing 2012" organized by MU Vienna, TU Vienna, IST Austria, Vienna, Austria, June 4-13, 2012
School participant. Working on project "Analysis of 3D tubular tree structures of human lungs"

[2] Workshop:
"Advanced Confocal Microscopy and Living Cell Studies" organized by Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic, October 15-19, 2012
Oral presentation / practical tutorial: Pavel Matula, Dmitry Sorokin "Single Particle Tracking Analysis"

Abstract:
Single particle tracking (SPT) allows us to track the motion of proteins in cells. Individual molecules or small clusters are fluorescently labelled and observed in microscopes. The main goal of SPT is to determine the trajectories of particles. Knowing the trajectories one can resolve modes of motions of individual particles on the basis of mean square displacement (MSD) curve. We discuss the important steps in SPT analysis, i.e., particle detection, image registration (compensation for cell movements if intracellular mobility is of the interest), particle association and trajectory analysis.

IV – RESEARCH EXCHANGE PROGRAMME (REP)

REP 1:

REP Organisation:	Vienna University of Technology
Country:	Austria
Department:	Information Management and Preservation Lab
Local scientific coordinator:	Dr. Allan Hanbury
Dates:	03.07.2012 - 16.07.2012

Experience:

I became acquainted with the structure and development of the biggest European biomedical image analysis and retrieval project (Khresmoi, http://www.khresmoi.eu/). I was also acquainted with the process of organization of scientific summer school for Ph.D. students and early stage post-doc researchers. Along with that, I was able to take part in the "Summer School on Image Processing 2012" as a participant where I got new expertise and very broad overview in the field of medical imaging as well as developed my research contacts network. In frame of the summer school I took part in the development and management of the project "Analysis of 3D tubular tree structures of human lungs".

REP 2:

REP Organisation:	École Polytechnique Fédérale de Lausanne
Country:	Switzerland
Department:	Biomedical Imaging Group
Local scientific coordinator:	Prof. Michael Unser
Dates:	24.09.2012 - 29.09.2012

Experience:

I gave a talk "Tracking of Intercellular Objects in Live Cells with Visible and Invisible Bodies" on BIG weekly seminar. I also acquainted with the workflow and the research topics of the one of the world's leading groups in the field of biomedical imaging and mathematical methods of image processing. The trip was a very good opportunity to develop my research contacts network.