

**Report**

**Microscopy of living cells**

**Mikroskopie živých buněk**

Bilateral Project 63p15

Irene Lichtscheidl

University of Vienna/A

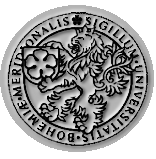
Dalibor Stys

University of South Bohemia in Nove Hrady/CZ

November 2012

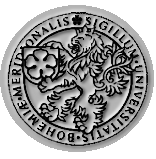
**Institute of   
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**Cell Imaging and Ultrastructure Research**



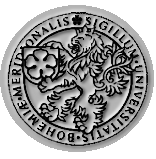
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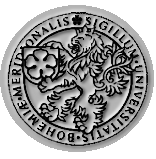
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**A. Ergebnisse**

The Viennese partner is a Core Facility for Light and Electron Microscopy and serves as a multidisciplinary environment for researchers with all kinds of different interests (univie). The Cezch partner is expert in computational sciences and in the development of new softwares (nh). Cooperation of the two partners was especially successful since two software programs could be developed which will allow for quantification of image stacks from the microscope as well as of digital images from plants on culture plates. In addition, we started to introduce a newly developed software from the Czech partners to the laboratory of the Viennese partner which serves for organizing data and protocols, an extremely important tool for a multi-user facility.

Besides these scientific results, we had a strong focus on teaching and on communication with partners from outside our bi-lateral activities.

**A.1. Developing a new program for analysis of images from the fluorescence microscope**

Pictures from the fluorescence microscope have a high potential for gaining insight into structural and functional traits of cells and their organelles. Calculation of the volume of the displayed organelles is, however, difficult; in conventional wide field microscopes it is practically impossible, and there is only rare reliable software for advanced clsms (confocal fluorescence laser scanning microscopes) which gives, however, values which are not realistic (eg DAIME).

Accordingly, Tomas Nahlik (nh) developed a program which is able to calculate the volume from fluorescent images from both conventional wide field microscopes and clsms: NAHRA. For this goal, he spent 6 weeks in univie and worked together with Rabia Ghaffar (univie) about her object, large chloroplasts from deep shade plants. She is an expert in fluorescence microscopy and she provided images of all different kinds which were the base for the computational analysis.

First comparison with the DAIME program gives a big difference; chloroplasts appear smaller in the new NAHRA software. Rabia Ghaffar therefore started to make pictures of the chloroplasts in the electron microscope, when Tomas Nahlik was in Vienna for a second stay of 6 weeks; analysis seems to prove that the real size of objects is mirrored by the newly developed NAHRA program rather than by DAIME.

For further proof of the suitability of the new software, we need now to try several test objects; we have to document them by different microscope techniques and evaluate their size in the different programs. We want to do this in the coming year, so that we then can publish this program.

**A.2. Developing a new program for estimating the size of plant cultures on agar plates**

Plant and tissue cultures often serve as a basis for the estimation of stress reactions of plants. Often some stress reagents, eg heavy metals, are added to the agar, and changes of plant growth indicate the severeness of a possible damage. So far, pictures were taken from the agar plates and the size of the plants was estimated by ruler, which is of course not extremely precise.

During his two stays in Vienna, Tomas Nahlik therefore developed a computer program which can recognize automatically the size of the plants; accordingly, it gives the area that is covered by the plants and allows for estimation of plant growth in a given time. At the moment being, we have several cultures growing in our growth cabinets in Vienna, so that we can test the new software and its suitability within the following months. Thereafter, the program can be published.

**A.3. Preparing and testing software for data management and data storage**

The Czech partner Petr Cisar has developed a new software for the storage and management of data in laboratories (BioWes project); this shall now be tested in the multi-user facility in Vienna to find out about applicability and probable need for improvement. Petr Cisar has introduced the program to the group in a seminar in Vienna and in the Minisymposium in Nove Hrady. He will now install the software during December at computers and servers of the Core Facility of univie. The research staff of the facility will be trained in their use for image enhancement, image analysis, data organisation.

It will be applied in the course of the coming year by themselves and their guests.

**A.4. Lecturing and practical training in the subject of image analysis and microscopy techniques including some theoretical aspects of the problem.**

Concerning bi-lateral exchange of ideas and development of new techniques, we have made visits to the partner laboratories, Tomas Nahlik has spent two periods of 6 weeks each in Vienna, we made a Mini-Symposium in Nove Hrady and an International summer school in Vienna, and we had several lectures in seminars. In addition, we had a strong focus on lecturing and teaching our young researchers; accordingly, students from both countries participated in all research activities described below, and an international microscopy course was given.

**Nove Hrady** - According to plan, we prepared a ***Minisymposium*** – “Microscopy of living cells” - which took place in Nove Hrady 24.9.-26.9.2012. During lectures we presented methods of microscopy of mammalian cells, construction of our new microscope and different techniques of image processing. Lectures of people from University of Vienna were about cryotechniques in optical and electron microscopy, dynamics of living plant cells and the potential of new fluorescent tracer dyes.

Names of participants from Vienna: Marieluise Weidinger, Ingeborg Lang, Rabia Ghaffar, Stefan Sassmann, Siegfried Reipert, Wolfram Adlassnig, Irene Lichtscheidl

Names of participants from Nove Hrady: Dalibor Štys, Petr Císař, Tomáš, Náhlík, Karina Romanova, Anna Zhyrova

During December is planed ***training in AFM*** (atomic force microscopy).

Names of participants from Vienna, who plan to join: Marieluise Weidinger, Ingeborg Lang, Rabia Ghaffar, Stefan Sassmann, Siegfried Reipert, Wolfram Adlassnig, Irene Lichtscheidl

Names of participants from Nove Hrady: Dalibor Štys, Petr Císař, Tomáš, Náhlík, Karina Romanova, Anna Zhyrova, Jan Urban, Vladimír Kotal, Ali H. Reshak

**University of Vienna (Univie) –**Univie prepared an ***international course about different microscopy techniques*** for 4 ECTS points (copy of certificate is in appendix)

Names of participants: Karina Romanova, Anna Zhyrova, Tomas Nahlik (participating as student during may, as lecturer during September), Dalibor Stys (lecturer) (all Nove Hrady/Cz), Jacquie Montanaro-Punzenöhler (Meduni Wien), Elisabeth Stein (Meduni Wien),, Kasia Wezowicz (University of Krakow/Po), Barbara Bock, Yasmin Weiss, Anselm Pavlik (all Univie).

There was also ***3 months stay of PhD student Tomas Nahlik (nh) in univie***. During this stay was established cooperation between students. Tomas Nahlik developed new software for Rabia Ghaffar for analysis of chloroplast images (see above). They plan further experiments and adaptation of the software for 3D reconstruction and volume measurement of chloroplasts images in a further period of cooperation.

Tomas Nahlik in addition developed a new software for the analysis of photographs of mosses in culture plates – measuring the area and grows rates.

Another most important activity of Tomas Nahlik was his involvement in the International Microscopy Trainingscourse, where he lectured about image analysis.

***Visits and seminars in Vienna***: Dalibor Stys, Petr Cisar, Jan Urban and Tomas Nahlik had lectures about their work in Univie:   
Dalibor Stys presented lecture about Self-organizing systems.   
Petr Cisar presented DoE (Design of Experiment) and his project for data management.   
Jan Urban had lecture about Distinguishability and discriminability in experiment.   
Tomas Nahlik presented lecture about Image analysis and setup experiments according to high quality image.

**B. Nutzung und Verbreitung der Ergebnisse**

An important task of our project is the communication of our results to our colleagues, especially also to guests of the Microscopy Facility in univie, as well as the education of young researchers in both partner universities.

**B.1. Publication of scientific results**

Our research and newly developed programs were presented in the Minisymposium about Microscopy of living cells in Nove Hrady, and in seminars and hands-on training in univie (see above, point A.4).

The newly developed programs and results achieved by their application will be possible after a test phase in the second half of the coming year.

**B.2. Educational activities**

Students from both countries participated in all research activities described above. In addition to the Minisymposium and to the seminars and lectures, an International trainings-course in light microscopy was organized in univie (see above, A.4). The practical experience and the multidisciplinary approach provided a synoptic environment that will be helpful for the future scientific work of students, but also of staff members of our groups.

**B.3. Communication with partners from outside our bi-lateral activities**

We extended our cooperation to our partners working in other Institutions and invited a guest student from University of Olomouc/Cz, David Zalabak, to join in the hands-on training gin microscopy. He is presently in Vienna and analyses the roots of his plants in the fluorescence microscope and in the electron microscope.

Another guest was Juraj Piovar from the University in Kosice/Sk; he involved in the program of Tomas Nahlik in univie and got training for his object, lichens, in the light and fluorescence microscope in order to estimate stress caused by heavy metals.

**C. Durchführung**

Welche konkreten Änderungen gegenüber der Planung ergaben sich hinsichtlich Inhalte und Mitarbeit/Anzahl der Teilnehmer während des Projektverlaufs?

No substantial changes

**D. Bewertung**

Bitte fűhren Sie besonders positive, aber auch durchaus negative Beobachtungen und Erfahrungen an. Wir akzeptieren gerne, dass nicht alles immer positiv sein kann. Beziehen Sie in diese Beobachtungen auch mögliche langfristige Auswirkungen Ihres Projekts, die Übertragbarkeit auf andere Projekte und die Zusammenarbeit mit dem Projektpartner mit ein.

We appreciate the opportunity for this kind of project to promote mobility for research and education. We experienced that cooperation by physical closeness and personal meetings is far more efficient than communication only by email and internet, and we appreciated also the possibility to have close cooperation with our young researchers by hands-on training, lectures and courses. We hope that we can get a prolongation of this mobility.

Thanks for the good attitude of SAIA and ÖAD staff in course of our project!

**E. Perspektiven**

Hat sich eine Fortführung der Kooperation ergeben?

1. Welche geplante Fortführung gibt es?

We need to continue our cooperation in order to evaluate our new programs and to publish the in good international scientific journals. We therefore apply for a second year of cooperation in the frame of the AKTION/ÖAD, and we hope that this will give us the time need for the tasks described below.

1. Welche konkrete Fortführung gibt es?

1. NAHRA software has to be evaluated for different objects and various microscope techniques. Therefore, Rabia Ghaffar will generate new images from the light- and fluorescence microscope as well as from the electron microscope. Also model objects of some of the guests of the Microscope Facility in Vienna will be evaluated.

2.Software for measuring the area of plants on culture plates must be evaluated with different kinds of plant and tissue cultures

3.BIOWES software must be applied by univie for its applicability in a multi-user facility

4.Publications about NAHRA and area measurement must be written

5. Together with Prof. Dalibor Stys from the group Nove Hrady, a WWTF/Austria project between University of South Bohemia and University of Vienna shall be worked out together with Wolfram Weckwerth/univie.

According to part 3 we are preparing elongation of this project and other new joint projects for exchange of scientific workers.

6.We are planning three joint projects for Summers Schools with Irene Lichtscheidl, Wolfram Adlassnig and Stefan Sassmann as co-leaders and some students from Univie as participants.

7.Since the International Microscopy Course was such a big success, we would like to do it again. In addition, we would like to offer a new course about correlative microscopy.