cellular imaging facility
newsletter

N° 8 — November 2009

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Content

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1. Editorial

The CIF platform has gone through major developments over the last couple of years, mobilizing the efforts of our staff. This perhaps explains why the last CIF Newsletter was published one year and a half ago! We will give in this issue an update on the platform current structure, new acquisitions, and possibilities.

Undoubtedly, the most notable development to highlight is the recent opening of a new --third (!) -- imaging platform on the Epalinges campus. You will read below that this new structure offers a very complete panel of top-level imaging equipment and support, and substantially increases the capacity of the CIF as a whole.

Another highlight of this year is the acquisition of the first two-photon microscope accessible to CIF users. This instrument has been installed at the Dorigny facility. This high-end technology, which enables imaging deep into thick specimens, opens new perspectives for plant, animal, and insect biology experimentation.

All these exciting developments of the facility, that you and your research directly benefit, have been made possible by the decisive and continuing support of the Faculty of biology and medicine, for which we are very grateful.

Finally, this CIF Newsletter is published for the first time in English, as an increasing number of researchers interested in our CIF activities do not speak French. We hope that you will enjoy reading it and welcome your feedback!

Jean-Yves Chatton
CIF Coordinator

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2. A third CIF platform on Epalinges campus

Last May 2009, a new branch of the CIF platform opened on the Epalinges campus. The necessity of establishing a strong and well equipped imaging facility had become urgent as the Epalinges campus had started a profound mutation a couple of years ago. It will also soon include the arrival of several new research groups.

To take care on a daily basis of this CIF facility on the Epalinges campus, a new technical manager, Florence D. Morgenthaler, has been hired. Her main roles consists in teaching and training users, maintaining the setup, advising users regarding their questions on biological imaging, taking part to the workshops, handling the booking calendar in Epalinges.

Florence has studied the heterogeneous properties of synapses in single neurons using a wide range of different microscopes (widefield, confocal, and electron microscopes) and obtained her Ph.D. degree in 2003. She worked as a postdoc in Switzerland as well as in the USA and, more recently, completed her knowledge of bio-imaging techniques by using nuclear magnetic resonance to study brain glycogen metabolism. You can contact Florence Morgenthaler either by phone (021 692 5891) or by E-mail (Florence.Morgenthaler@unil.ch).

The CIF Epalinges facility is located in the biochemistry building (ch. des Boveresses 155). As it is the case for the two other CIF facilities located in Bugnon and in Dorigny, this new facility is open to all researchers of the university and CHUV. In practice, it means for instance that, if you already have a CIF account and are a user of the CIF Bugnon or Dorigny, you can use the resources in Epalinges and save your data in your folder on the Atlas server. However, we kindly ask you to contact the technical manager of the platform prior first use to make sure that you have the appropriate training and know the particularities of the new setups.

Here is a list of the different resources you can use in Epalinges:

- **Two confocal microscopes**: one mounted on an upright microscope that can scan in a faster mode for live imaging (Leica SP5 Tandem), and one attached to an inverted microscope (Zeiss LSM 510 Meta). That one will soon receive an incubation chamber for imaging living cells over longer period of time.

- **Two wide field microscopes**: an upright microscope essentially dedicated to imaging slides of fixed samples (Zeiss AxioImager) and an inverted microscope with incubation chamber and XY motorized stage allowing time-lapse imaging (Zeiss AxioObserver). Both microscopes allow fluorescence and bright field image acquisition on CCD cameras.

- **One stereo-microscope** allowing acquisition of **fluorescent** and/or color images with a final magnification between 7.6 to 160x (Leica M205 FA).

- **One laser capture microdissection** setup (Arcturus XT) for dissecting and retrieving cells from a desiccated tissue slice (either using transmitted or fluorescent light for tissue visualization).

- **One i*n vivo* whole animal** imaging setup for rodents kept under isoflurane anesthesia (Xenogen IVIS Lumina II). Both luminescent (after luciferin injection) and fluorescent imaging can be performed.

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- **Two workstations** giving access to 3D visualization and measurement software (Imaris), to image processing and analysis in 2D (Metamorph), and to other general purpose imaging software.

You will find detailed information on these new setups on our website (http://cifweb.unil.ch, select Epalinges resources).

3. A new LSM 710 confocal coming soon to CIF Bugnon

We have the great pleasure to announce that a new confocal of the latest generation, a Zeiss LSM710, will be delivered at the end of this year. This new instrument, which holds the promise of highly increased sensitivity and spectral flexibility, will replace the first confocal microscope that the CIF platform acquired the Zeiss LSM510 Meta for the Bugnon facility. The new confocal microscope will give access to a wide range of laser lines (405, 458, 488, 514, 561, and 633nm) covering the entire visible spectrum. It will be equipped with the new Quasar spectral detector, a motorized XY stage, and a system for long-term focus stabilization. The installation and testing are scheduled for early January 2010.

4. Two photon technology accessible at CIF Dorigny

A new two-photon microscopy setup has recently been installed at CIF Dorigny. It is the successful result of a partnership between two FBM professors, Profs. Geldner and Benton, and the CIF that enabled obtaining a grant from the Swiss National Science Foundation to finance a high-end two-photon system.

Multiphoton microscopy enhances standard confocal microscopy by using a pulsed infrared laser to excite the fluorescent dyes. The two-photon excitation of the fluorophores by infrared light only occurs at the focal plane where the photon density is maximal. As a result, this mode of excitation is intrinsically confocal and offers the advantage that pinholes used in confocal microscopes are no longer needed resulting in considerable increase in fluorescence detection efficiency. As the infrared excitation is hardly absorbed by biological tissues, multiphoton microscopy offers the possibility to penetrate significantly deeper into the tissues. This new two-photon microscope will benefit users who need to perform imaging deep into thick specimens, where they will be able to produce optical sectioning with high quality.

Our setup ...

The Zeiss LSM 710 NLO consists in a conventional confocal scan head equipped with the laser lines 458, 488, 514, 561 and 633 nm. The infrared laser Ti:Sapphire

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Chameleon Ultra II that can be tuned from 690 nm to 1040 nm with an maximum output power of 4W at 800 nm. The microscope is an fixed stage upright Zeiss AxioExaminer Z1. Currently three objectives are available on the system: Achroplan 10x W objective with a 0.3 NA, a Plan-Apochromat 20x W objective with a 1.0 NA and an Apochromat 63x Water-Oil immersion objective with a 1.2 NA. The emitted fluorescent light may be detected in the confocal scanhead (internal or 'descanned' detection), on external 'non-descanned' detectors (NDD), or on a GaAsP non-descanned detector (GaAsP NDD), that is positioned very close to the objective in the light path. The GaAsP NDD is the ultimate level of sensitivity for detection on multiphoton systems, with highly efficient photon detection and negligible dark noise.

For more information about this system, for training or access, please contact Arnaud Paradis, CIF Dorigny Technical Manager (arnaud.paradis@unil.ch; tel. 021-692-4090).

5. Update on current charges for resource usage

We have recently modified the hourly charge of several CIF resources:

- The Stereomicroscopes, previously CHF 15.-/h, are now charged **CHF 10.-/h** on all three facility branches.
- The Xenogen IVIS 3D (CIF Dorigny) has been reduced from CHF 50.- to **CHF 30.-/h**. The reason is that we are now able to purchase consumables at significantly lower price, which we can transfer on the final hourly cost.
- The two-photon microscope Zeiss LSM710 NLO (CIF Dorigny) can now be booked and is charged **CHF 30.-/h**.
- Image processing workstations are **now free of charge** on all three facility branches. Please note that the **booking of the workstations is still mandatory**.
- **Epalinges**: After a period during which the instruments have been installed, the online booking system and the general infrastructure have been implemented, the CIF will start billing for the use of the Epalinges resources starting on **January 1st , 2010**. The hourly rates for the CIF Epalinges resources will correspond to those used for the other branches of the facility; they are listed in detail on the CIF website under "Usage and Charges".

6. Courses and workshops

- Five workshops have been programmed in 2009 by the CIF. The topics of this year were/are:

  (***1***) **Image J** (16 June 2009): basics, macros, description of plugins developed at CIF.

  (***2***) **Photoshop** (22 & 29 Sept 2009): basic operations, comparison with scientific (quantitative) image processing, optimizing content for presentation, special functions, artistic features.

  (***3***) **Colocalization** (20 Oct 2009): image acquisition parameters, cross-talk, 2D vs. 3D, non-standard techniques, quantitative estimates.

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(*4*) **Bright field and contrasting methods** (10 Nov 2009): Köhler adjustment, phase contrast, dark field, oblique illumination, DIC, infrared imaging.

(*5*) **Two photon microscopy** (1 Dec 2009): Principle, applications, CIF setup description.

The success that was encountered in terms of participants and of feedback is very stimulating for the organizers. The CIF workshop series will therefore certainly be renewed next year. It is likely that the most popular themes will be repeated, and new ones will be introduced. We always welcome suggestions of new topics to cover!

- The annual introductory course on microscopy, by **Jean-Yves Chatton** (that is also a Privat Docent course), will take place in January and February 2010. The course outline is given below.

INTRODUCTION TO FLUORESCENCE IMAGING FOR THE ANALYSIS OF LIVING CELLS

Place: Petit Auditoire de l'Ecole de Médecine, rue du Bugnon 9 (Upper level)

Schedule: Winter Semester – Tuesdays 12:15 – 14:00

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12 January 2010 : Basics of transmitted light and fluorescence microscopy

19 January 2010 : Confocal microscopy

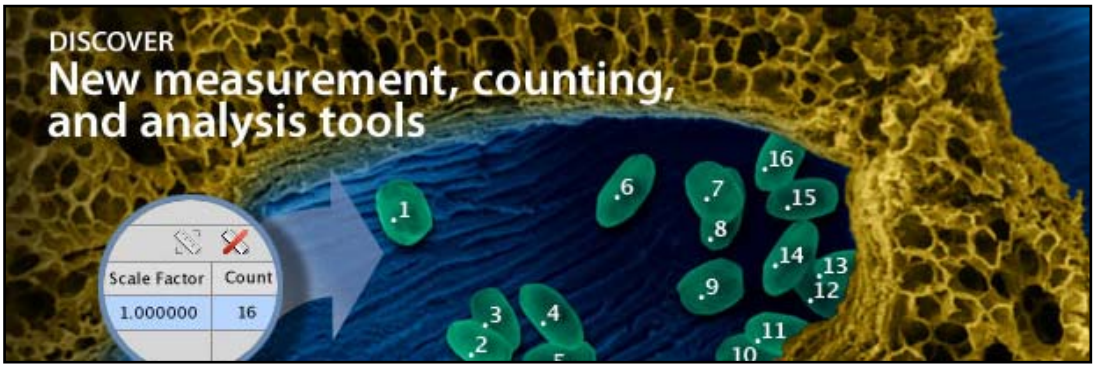
26 January 2010 : Modes of image formation, acquisition, signal sampling

2 February 2010 : Dynamic recording of cellular functions by fluorescence imaging. Intracellular calcium, pH, and sodium and their application to cellular signaling.
Problems related to imaging of living cells

9 February 2010 : Other optical applications (proposed topics):
Fluorescence recovery after photobleaching (FRAP), flash photolysis, multiphoton microscopy, evanescent wave microscopy, laser tweezers, fluorescence resonance energy transfer (FRET), digital holography microscopy, optical contrasting methods (phase contrast, DIC).

As for previous editions, lectures will be given in English. In parallel with theoretical lectures, demonstrations will be proposed on the confocal microscopes of the CIF. The course will give right to ONE credit for students of UNIL doctoral schools (FBM and neuroscience). Admission to the course is free and open to anyone interested; nevertheless, we kindly ask those who wish to attend to **register to jean-yves.chatton@unil.ch**.

7. Spotlight on Adobe Photoshop CS4



Adobe Photoshop is well known for its huge collection of features dedicated to picture enhancement and editing. What is less known is that it also has some interesting features for the scientist. Some of these features include **image calibration, count tool, scale bars, ROI analysis.**

While most of you are using Photoshop only for basic tasks such as resizing images or adding text, there's a lot more you can do to improve your pictures.

The **last CIF workshop on Photoshop covered almost all of these features** plus more, and **you should soon be able to watch some video tutorials on this topic on the CIF web site**.

In the meantime, you can try by yourself (**you need Photoshop CS4 Extended** or above) on our image processing workstations, and/or contact yannick.krempp@unil.ch for support on a specific question about Photoshop.

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