

### Content

1. Editorial
2. *In vivo* micro-CT Imaging Technology Available at CIF Epalinges
3. Zeiss LSM 700 Confocal Microscope at CIF Dorigny
4. New Powerful Image Processing Setups
5. Time-lapse Setups Available on Confocal Microscopes at CIF Bugnon and Epalinges
6. Training activities
7. Spotlight on: Multiple Regions of Interest Selection with Image J

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### 1. Editorial

It is our great pleasure to present this new edition of the CIF Newsletter. Since the previous edition of the CIF Newsletter, the facility has not been hibernating—quite the opposite! During that period, the third branch of the facility in Epalinges has been brought to full speed, new instrument acquisitions and developments have been achieved as well as several new training activities have been organized. In this issue of the newsletter, we have selected several aspects of the facility's activities to provide you with an update on the platform current structure and possibilities. We also propose a step-by-step tutorial that, we hope, you will find helpful. I wish to warmly thank my colleagues who contributed to the writing of this issue of the newsletter (Y. Krempp, F. Morgenthaler, A. Paradis), and the users of the facility for their continuous interest in imaging and optical microscopy.

We hope you will enjoy reading this CIF Newsletter and welcome your feedback.

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*CIF Coordinator*

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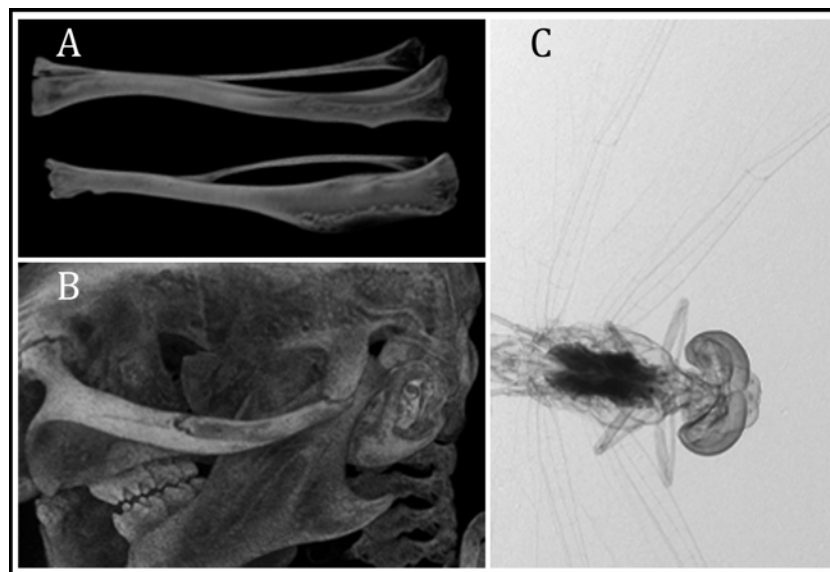
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## 2. *In vivo* Micro-CT Imaging Technology Available at CIF Epalinges

A scanner for Computed Tomography (CT) called SkyScan 1076 is available in Epalinges in the conventional animal facility. Computed tomography provides non-destructive three-dimensional visualization and characterization images that map the variation of X-ray attenuation within objects (which relates closely to density).

The SkyScan 1076 is a high performance *in vivo* micro-CT scanner for preclinical research. The large format (11 megapixel x-ray) camera gives many combinations of resolution, image field size and scan speed. Image field width up to 68 mm allows rat and mouse imaging, at pixel sizes of 9, 18 and 35 $\mu$ m. Variable x-ray applied voltage and filters provide scanning flexibility to allow imaging of a wide range of samples from lung tissue to bone with titanium implants.

Animal beds for both mice and rats are available and made of carbon fiber or polystyrene foam. The physiological monitoring system contains a temperature sensor on the animal bed, which can be placed near the animal. A pulsed heated airflow is applied to maintain animal body temperature. Current temperature and target temperature are displayed in the physiological monitoring software window. The setup is also implemented with an isoflurane anaesthesia system. Finally, for improving thoracic image acquisition, image acquisition can be gated and synchronized with breathing movements.



**Images obtained with the micro-CT scanner.**

- A. 3-D reconstruction of an *ex-vivo* mouse tibia & fibula. *Courtesy Dr. Frédéric Grosjean.*
- B. 3-D reconstruction of a mouse skull acquired *in-vivo*. *Courtesy Dr. Frédéric Grosjean.*
- C. Single plane scan of a dragonfly.

The full range of SkyScan software package is supplied, including fast volumetric reconstruction, software for 2D / 3D quantitative analysis and for realistic 3D visualization. SkyScan's volumetric reconstruction software "NRecon" uses the set of acquired angular projections to create a set of cross section slices through the object.

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Finally, for those who would like to learn more on all the different applications that can be performed with our SkyScan 1076, the CIF is happy to announce the upcoming seminar:

### High resolution micro-CT in biological and biomedical applications

Dr. Jeroen Hostens, SkyScan application scientist, Kontich, Belgium

April 19<sup>th</sup> at 12:30 PM in room B301 in Epalinges

For more information on this system, for training or access, please contact Florence Morgenthaler, CIF Epalinges Technical Manager (florence.morgenthaler@unil.ch; tel. 021-692-5891).

### 3. Zeiss LSM 700 Confocal Microscope at CIF Dorigny

Since March 2011 we have installed a new Zeiss LSM700 confocal microscope in the Biophore building (Room 4416). This system is replacing the Leica SP2 AOBs at the Biophore that is now no longer part of the CIF.

This Zeiss LSM700 confocal is mounted on an inverted Zeiss Axio Observer Z1 using a motorized XY stage controller. We have 4 solid state laser lines (445, 488, 555 and 639nm) available on this system.



Currently four objectives are on the system: a Plan Apo 10x Ph1 objective with a 0.45 NA, a Plan Apo 25x Oil immersion DIC objective with 0.8 NA, a Plan Neofluar 40x Oil immersion DIC objective with a 1.3 NA and an Achromat 63x Water immersion DIC objective with a 1.2 NA.

Two photomultiplier detectors are available, each coupled with 8 emission filters for spectral flexibility on the detection of the fluorescence signal. An additional transmitted light detector may also be selected to allow the users to acquire a transmitted light image of the samples in confocal mode.

The software running the LSM700 is the ZEN ("Zeiss Efficient Navigation") software. That software allows a really easy and straightforward use of the confocal. Using the so-called "Smart Setup" function, the software will drive you in a few clicks through the light path configuration to set up for your own experiments.

Additionally, the focus stabilizer ("Definite Focus") implemented on this confocal microscope enables maintaining the focus stable in the z axis for in long term (typically time-lapse) acquisitions.

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In summary, the new LSM700 is really easy to use and allows you to do: 3D stacks, multi-position acquisitions, XY tiling, multi channel acquisitions, time lapse imaging, bleaching (FRAP) experiments.

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

#### 4. New Powerful Image Processing Setups

We are faced more and more with very large image data sets consisting for instance of huge mosaics of images at high resolution, or large stacks of images to be processed in 3 dimensions. As the CIF was not equipped to efficiently handle them, we have decided to invest on very powerful cutting-edge computer workstations. Those have been configured in particular to optimally handle the tasks of deconvolution and restoration of large image stacks using the Huygens software, and of 3D volume processing and rendering with the Imaris software. Obviously, other imaging software like Adobe Photoshop will benefit from this new raw power. Here is a summary of the main specifications of the workstations:

<b>CPU:</b>	<b>2x Xeon X5550 2.6 GHz</b>
<b>RAM:</b>	<b>48 Gb RAM</b>
<b>GPU:</b>	<b>Asus nVidia GTX580 DCII 1536 Mb</b>
<b>HDD (System):</b>	<b>2x SSD 120 Gb Raid 0</b>
<b>HDD (Storage):</b>	<b>1x HDD 2 Tb</b>
<b>Display:</b>	<b>24" Eizo Foris</b>
<b>OS:</b>	<b>Windows 7 Pro x64</b>

One such machine will be available in each branch of the CIF to complement the existing workstations before the end of February.

#### 5. Time-lapse Setups Available on Confocal Microscopes at CIF Bugnon and Epalinges

In addition to the three complete time-lapse image acquisition stations set on wide-field microscopes on the three locations of the CIF (Bugnon, Dorigny and Epalinges), the CIF offers now the opportunity to acquire time-lapse movies under confocal microscopes in Bugnon (on the Zeiss LSM 700) and in Epalinges (on the Zeiss LSM 510), as it was already the case in Dorigny (on the inverted Zeiss LSM 510). Moreover, you can bring your living sample in advance and have it settle down in our incubators before starting the actual experiment using our microscope stage-top incubator.

Our multi-dimensional acquisition systems will allow you to follow your living samples over time with a controlled temperature, humidity and CO<sub>2</sub> environment. Each incubator can be fitted with dedicated inserts suitable for Lab-Tek™ chambers (Nunc®), chamber slides (Nunc®/Falcon®), object slides (max. 76x25 mm), glass bottom Petri dishes "60" (d=52-58 mm) and "35" (d=35-38 mm). In addition to the high



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resolution spatial dimensions that can be selected under confocal microscopes, such set-ups also offer the possibility to be configured for multiple wavelengths recordings and for a wide range of different magnifications. Finally, more advanced imaging techniques such as Fluorescence Recovery After Bleaching (FRAP) or spectroscopy can also be combined with time-lapse imaging on these confocal microscopes.

For more information, please feel free of contact a CIF Technical Manager.

## 6. Teaching and Training Activities

In 2011, the CIF has once again proposed several teaching and training opportunities to the research community, including imaging workshops and a new course on Adobe Photoshop, given by Yannick Krempp. This course has received very positive and stimulating feedback from participants.

Teaching activities in 2012 have already started with the annual introductory course on fluorescence imaging by J.-Y. Chatton in January-February with a record attendance.

We are currently defining the topics and dates of this year's workshops. They will likely include the themes of *ImageJ and Image Handling*, *Two Photon Microscopy*, *Advanced Confocal Techniques*, *Whole-animal Bioluminescent and Fluorescent Imaging*.

As mentioned above, the CIF will host a seminar on the CT-Scan approach for biological experimentation, given by Dr. J. Hostens from SkyScan Corp. on April 19<sup>th</sup>, 2012.

We would like to take the opportunity of the Newsletter to remind the readers and users of the facility that we welcome any suggestion of new themes to handle in workshops, seminars, or lectures.

## 7. Spotlight on:

### *Multiple Regions Of Interest (ROIs) selection with Image J*

Probably one of the most common image processing needs covered by the open source software Image J (available in the Image J standard, as well as MacBiophotonics and Fiji versions) consists of measurements of multiple Regions Of Interests (ROIs). Having multiple ROIs is useful if multiple measurements must be made in a single slice. ROIs can be of any shape, can be named and saved, and measurements can be exported.

- **To add one ROI on the ROI manager “manually”:**

To open the ROI manager, click on **Analyze>Tools>ROI Manager...**

Click **Add** in the ROI manager window to add the current selection to the list, it stands for the **Edit>Selection>Add to Manager** command. The ROI manager creates a three part label. The first part (stacks only) is the slice number, the second part is the X coordinate of the selection (it also correspond to the ROI number as long as you do not delete any ROI), and the third part is the Y coordinate. Click on a label to restore the associated selection to the current image. With stacks, the selection is restored to the slice it came from. Alternatively, hold down the alt key to “add and rename”.



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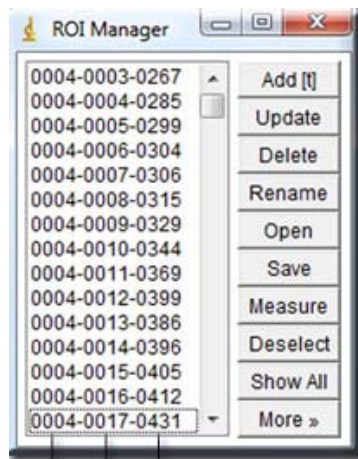

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Y coordinate  
X coordinate  
Slice number (stack only)

**Update** replaces the selected ROI on the list with the current selection.

**Delete** deletes the selected ROIs from the list. *Deletes all if no ROI is selected.*

Use **Rename** to rename the selected ROI.

**Open** opens a ".roi" file and adds it to the list or opens a ZIP archive (.zip" file).

**Save** saves the selected ROI as a ".roi" file. If none are selected, saves all the ROIs in a ZIP archive.

**Measure** measures all the ROIs on all the images in a stack or only the selected ones.

**Deselect** deselects any selected items on the list.

**Show All** causes all selections on the list to be non-destructively displayed on the current image. Click again to stop displaying the selections.

**More»** displays a drop down menu with additional commands (Draw, Fill, Label, Combine, Split, Add Particles, Multi measure, Sort, Specify, Remove Slice Info, Help and options.)

Delete, Save, Measure, Draw, Fill, Label and Combine work with all items on the list when none of them are selected.

Click on a label (selection number) in the image to activate the corresponding selection on the ROI Manager list. Or click on one ROI on the ROI Manager list and you will see it on the image.

**Multi measure:** for simultaneous measurements on several ROIs in a stack. This is very useful for processing dynamic experiments (such as calcium imaging or FM1-43 release over time). Alternatively, "**Measure**" will measure only in the plane where the ROI was created.

- **To add multiple ROIs on the ROI manager at once, for example after having used a threshold for selecting the ROIs:**

Go to the menu: **Image>Adjust>Threshold** to establish a threshold defining your signal as real (higher intensity than the threshold value) or background (lower values). Then, go to the menu: **Analyze>Analyze Particles** and make sure that "Add to Manager" is selected. In the "Analyze Particles" menu, you can also choose to add some more constraints on the ROIs you are interested in (ex.: a size range, exclude particles positioned at the edge of the image...).

These ROIs can now be used in another image (another channel) to measure fluorescence intensity for example, *i.e. select all DAPI positive nuclei and measure the Green intensity in these ROIs.*

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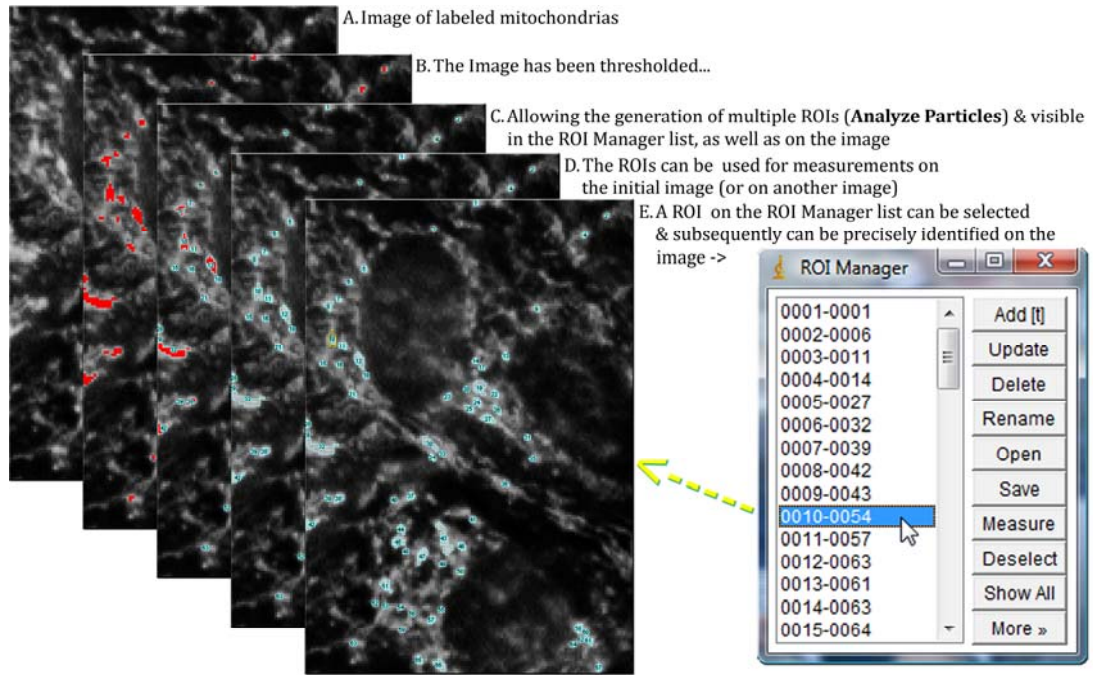


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Anyway, before MEASURING: Go to the menu: **Analyse>Set Measurements** and click on the options (ex. Area, mean Gray value etc...) to be measured. Make sure that "Limit to threshold" and "Display Label" are selected.

Finally, all results can be saved in a Microsoft Excel® compatible format.



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