JULIEN COLOMBELLI

# Advanced Digital Microscopy Core Facility

The Advanced Digital Microscopy Facility provides researchers at IRB Barcelona and the Barcelona Science Park (PCB) an open-access system to state-of-the-art light microscopy instruments. In 2009, the Facility plans to introduce new applications and to foster scientific collaborations involving modern imaging techniques. Our primary goal is to set up, maintain and make fully accessible a wide range of complementary techniques, mainly based on fluorescence imaging, and to support researchers during the entire imaging process. The 3D imaging of fixed and living samples is supported through spectral confocal microscopy, and soon additional 3D microscopy will be accessible on several platforms, like spinning disk or multiphoton confocal microscopes, to allow optimisation of specific imaging parameters, for example to limit phototoxicity, enhance fast imaging or improve imaging deeper into samples and organisms. In 2008, the Facility, a joint initiative of IRB Barcelona and the PCB, invested significant efforts to set up the laboratory. Designed to offer ideal conditions in which to perform full microscopy experiments and image analysis, the lab space now offers optimised rooms for microscopes. A cell culture room, a wet bench, and computer workstations for image analysis are expected to become operational in 2009. The Facility also focuses on developing instruments and providing a range of custom systems that combine laser-based imaging and manipulation techniques, like FRAP and laser surgery, in order to offer researchers greater flexibility than that achieved with commercially available applications.


***Figure 1.*** *Layout of the ADM Core Facility located in the Barcelona Science Park. 3D render: Arch M Corda.*

## Construction phase and lab

The Advanced Digital Microscopy Core Facility was created in July 2008. From August to December 2008, the new lab space of the Facility was designed and prepared in collaboration with the PCB, as shown in Figure 1. In an area of about 120m$^2$, the lab includes office and desk space for open-access computer workstations dedicated to image analysis. Five wide dark rooms were optimised to host a total of ten systems in optimum working conditions, including stable temperature and electrical supply, mechanical isolation, complete darkness and a dust-limited environment. A shared lab space provides access to a wet bench and to a dedicated space that hosts two fluorescent stereoscopes optimised for sample preparation, manipulation and high magnification fluorescent imaging. A culture room with a sterile environment is also available for critical experiments.

## Services for IRB Barcelona researchers

The Facility offers the following techniques:

**Facility Manager** Julien Colombelli **Research Officer** Lída Bardia

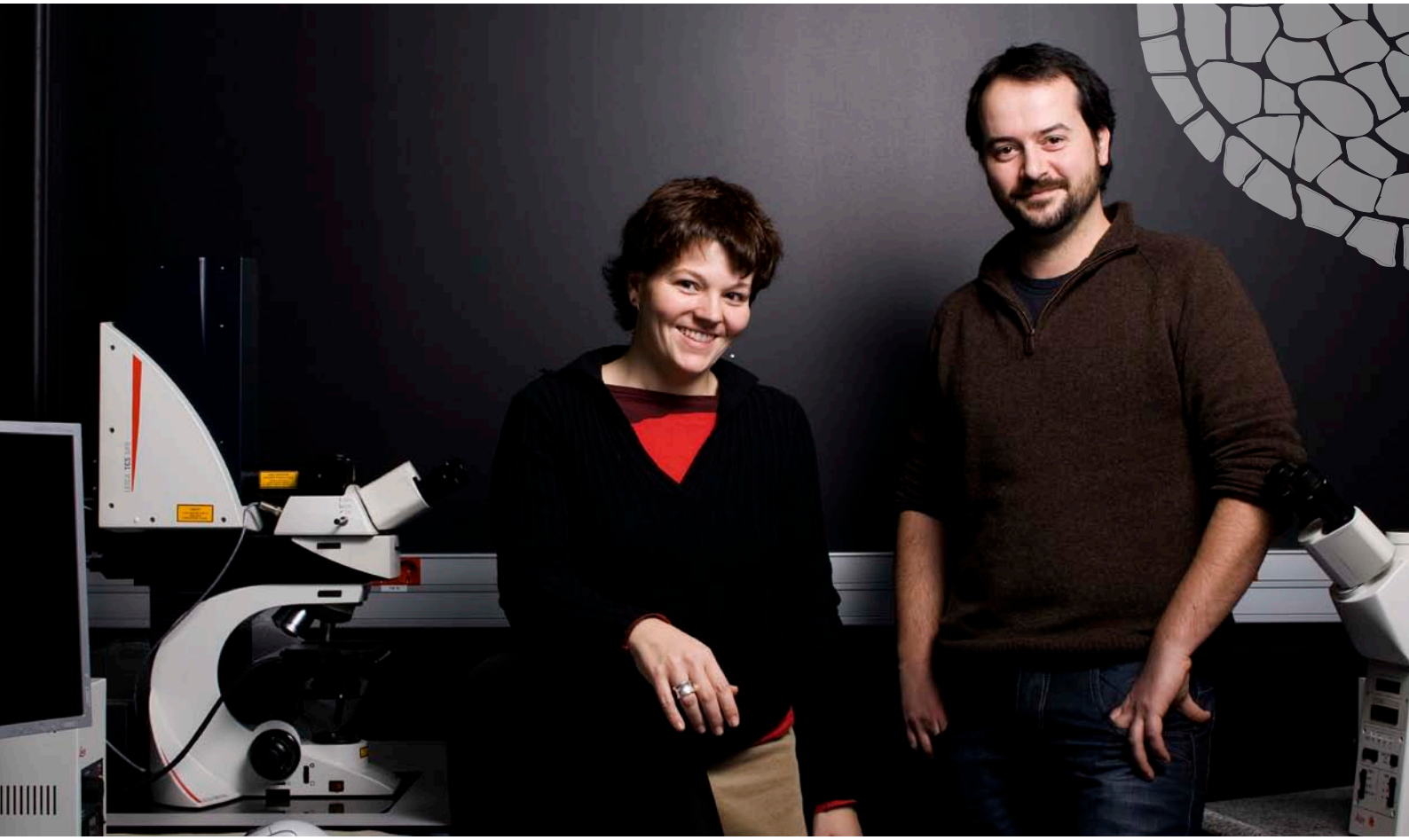

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***Figure 2.*** *Construction work on the Facility in 2008. The room for the spinning disk confocal microscope in August (left) and November (right).*

- **Conventional transmission and fluorescence microscopy.** Bright field, Phase contrast, Differential Interference Contrast (DIC), Dark field and multiple colour fluorescence imaging of fixed samples. Fluorescence stereoscopy for sample manipulation and selection.

- **Spectral confocal microscopy.** 3D, 4D and 5D imaging with optical sectioning, custom spectral detection and resolution, multiple position and incubated environment control for living cells.

- The Facility is currently setting up **image processing workstations** to provide access to high computing power and specialised image analysis software packages to perform 3D image visualisation and quantification, advanced imaging techniques analysis (FRAP, etc.), deconvolution, and general data interpretation and presentation.

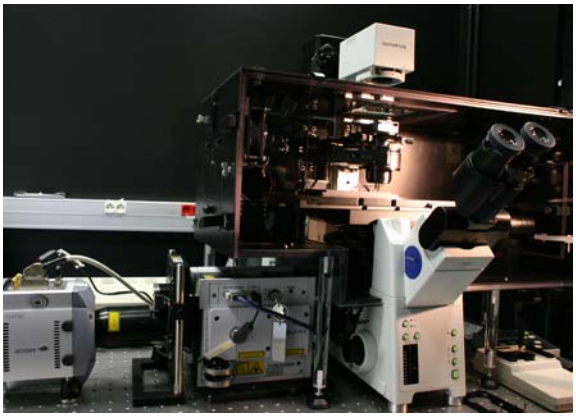

***Figure 3.*** *The new spinning disk confocal microscope from Andor, set up at the Facility. The microscope is aligned on a vibration isolation table and offers an incubation environment for live cell imaging.*

## New instrumentation and perspectives

In 2009, staff at the Facility plan to set up new microscopy techniques to enhance certain features of confocal microscopy and to focus on fluorescence laser manipulation.

To increase fluorescence imaging speed, a spinning disk equipped with a sensitive electron-multiplying (EM) camera will be set up. Higher sensitivity will reduce phototoxicity and increase acquisition speed to favour live confocal imaging of fast events. The system also offers multipositioning and temperature control for the simultaneous imaging of multiple samples.

A multiphoton contrast will be set up on the current Leica SP5 spectral confocal in order to achieve deeper imaging with reduced out-of-focus phototoxicity.

A new Olympus MVX10 fluorescence macroscope with motorised components will become available for flexible imaging and manipulation at low magnification.

An Olympus Total Internal Reflection Fluorescence (TIRF) Microscope will be set up to allow high quality imaging at the interface with glass surfaces. TIRF typically provides high contrast fluorescence images of 100nm axial depth to image membrane and cytoskeletal dynamics, single molecules, adhesions, etc. The system is also equipped with multipositioning and incubation chamber to allow high-throughput live data acquisition.

A laser-based manipulation platform is being established to combine techniques like laser nanosurgery, fluorescence recovery after photobleaching (FRAP), fluorescence photoactivation and automated imaging. The platform will be useful in many fields of applications like rapid molecular dynamics, cytoskeletal dynamics, diffusion and transport-based protein exchanges, force and morphogenesis in cells and organisms, DNA damage, neurobiology, etc.

## SCIENTIFIC OUTPUT

### Collaborations

*Development of laser-based microscopy techniques*
Ernst Stelzer and Light Microscopy Group, European Molecular Biology Laboratory (Heidelberg, Germany)