Julien Colombelli

Advanced Digital Microscopy Core Facility

In the past two decades the advent of fluorescent proteins has revolutionised the role of light microscopy in the biological and medical sciences. Digital imaging now goes beyond morphological studies by offering scientists ways to study the functions of proteins, cells and tissues. The applications of fluorescence illumination and observation constantly expand, and together with the most recent developments in laser technology, modern optical microscopes have tremendously diversified to address research of increasing complexity and specificity. State-of-the-art fluorescence microscopy is rapidly growing on several fronts: high resolution multidimensional live imaging, full volume and deep penetration imaging, high-content imaging, time-resolved protein dynamics, laser manipulation of living systems, image processing and analysis, and super resolution.

The Advanced Digital Microscopy Core Facility offers access and support to state-of-the-art instruments, from automated conventional and spectral confocal microscopy to emerging techniques for cell manipulation and imaging. Inaugurated in January 2009 in a newly constructed laboratory optimised for microscopy, the facility offers scientists open access to the instruments necessary to perform all the steps of digital imaging, from sample preparation through image acquisition up to image analysis and interpretation. The facility puts emphasis on offering customised and adapted solutions for optical imaging and image analysis by combining instruments, developing emerging technologies and tailoring custom image analysis to each scientific project.

Since its inauguration, the facility has set up an automatic web-based platform for open access to instruments and for the management of user access and interventions. In one year, it has registered nearly 200 users accross IRB Barcelona and the Barcelona Science Park, for a total usage of nearly 14,000 hours on six advanced systems and six routine microscopes. Confocal microscopy reached an average use of 8 to 15 hours per day, depending on the system.

Since January 2009, the facility has set up new technologies at IRB Barcelona and the Barcelona Science Park. To diversify and improve the various possibilities of multidimensional confocal microscopy, two new technologies were implemented: spinning disk confocal microscopy, which offers fast 3D live imaging of multiple labels in cells and organisms; and multiphoton microscopy, tailored to our most advanced confocal microscope, where an ultrashort pulsed laser now allows excitation of fluorophores deeper into organisms and tissues to increase depth of observation and also to decrease phototoxicity through thick samples.

High content imaging is now possible at IRB Barcelona with a new fully automated widefield microscope that records whole plate and full slide preparations, with highly stable fluorescence excitation for a reliable statistical analysis of multispectral fluorescence images. High contrast surface imaging is now also possible with TIRF microscopy, which offers imaging of the thinnest optical layer (<100nm) at glass interfaces, for cellular dynamics (membrane, cytoskeleton, ...) or single molecule dynamics.

Laser manipulation made a step forward with the implementation of a custom laser-based platform to perform combined laser nanosurgery and FRAP. The system performs cell ablation, subcellular compartments surgery, single filaments nanodissection, organ manipulation, etc... With a tailored spinning disk unit, the system offers cell and developmental biologists access to critical biophysical experiments to study multicellular and intracellular dynamics.

Imaging processing and analysis is emphasized at ADM where access is given to image analysis workstations on which commercial and custom software solutions are available to IRB Barcelona and PCB scientists. In October 2009, ADM hosted an image processing workshop organised by Bitplane, the European Imaris User Group Meeting 2009, which gathered 30 scientists at IRB Barcelona to learn and improve their skills in 3D image processing.

Services for IRB Barcelona researchers

Visitor lab

Our installations offer access to equipment for on site experiments to scientists and external visitors. Live samples can be stored in cell culture incubators, access to hoods, centrifuge, fridge and freezers are also given to allow sample preparation and manipulation pre- and post- acquisition.

Spectral confocal microscopy

We offer three spectral confocal microscope systems, two inverted and one upright, for 3D to 5D imaging (x,y,z,t and up to 5 spectral channels), *in vivo* imaging (*ie* FRET) and photobleaching experiments (*ie* FRAP). All systems are equipped with

405nm excitation for photoactivation, and environmental control of temperature and CO_2 to allow live imaging.

Spinning disk confocal
Our Andor spinning disk unit offers fast and sensitive imaging (up to 10 frames per second) with optical sectioning for *in vivo* live imaging with two laser lines (488nm, 561nm) and EMCCD technology.

Multiphoton microscopy
A tunable (710-990nm) ultrashort pulsed Ti:Sapphire is tailored to our SP5 confocal microscope to perform multiphoton imaging and imaging is performed with non-descanned external detectors. Among the applications available: Live and fixed imaging deep in embryos and tissues, Second Harmonic Generation.

Total internal reflection fluorescence microscopy is implemented on our automated widefield CellR microscope, to perform high contrast fluorescence imaging at glass surfaces with an axial extent around 100nm, with two laser lines (488nm, 561nm).

High content 'screening' and long-term live imaging
A fully automated inverted microscope, ScanR CellR from Olympus, offers highly stable fluorescence imaging for quantification of cellular assays on live samples, fixed preparations, multi-well plates, and multi-slides. An advanced incubation chamber precisely controls CO_2 and temperature for long-term imaging (from 1 hour to 1 week imaging).

Laser-based manipulation of living cells and organisms
Several lasers are coupled and controlled with x,y scanners to perform laser manipulation of fluorescent cells. A pulsed UV laser can be precisely scanned to perform laser ablation of cells and subcellular components, DNA damage, microdissection. The laser can also be used to perform correlative microscopy by inscribing the cell location inside the glass sample holder. A second laser can be scanned to perform photobleaching in wide field fluorescence mode.

Research Group Members

Core Facility Manager:
Julien Colombelli

Research Officers:
Lídia Bardia, Anna Lladó

The combination of laser nanosurgery and photobleaching gives access to unique cellular dynamics experiments where the distribution of fluorescent proteins can be manipulated and further analysed. For instance, DNA damage is performed within nuclei, and the dynamics of the recruited proteins can be quantified with subsequent FRAP. The system is also equipped with a spinning disk module for optical sectioning in thick samples.

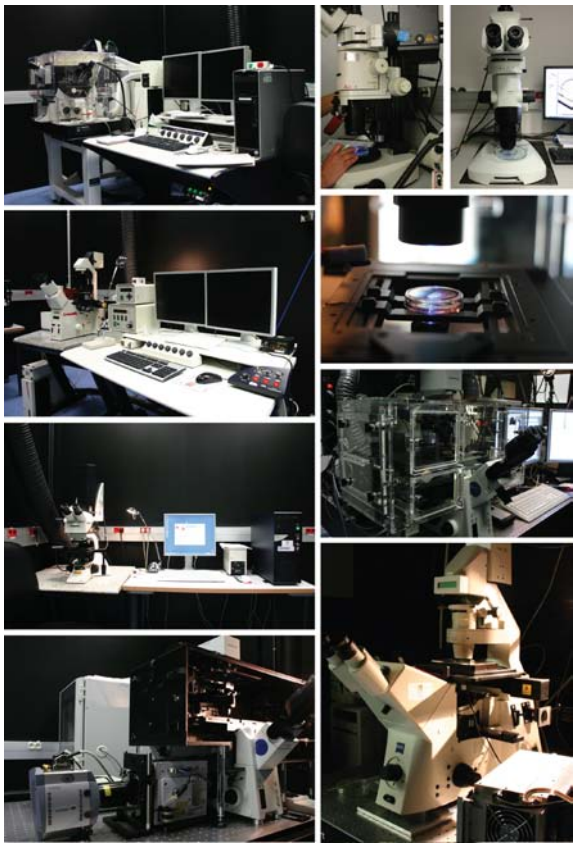

Figure 1. ***Overview of some advanced microscopy systems at ADM.*** *(From left to right and top to bottom): SP5 multiphoton spectral confocal, fluorescent and reflection stereoscope and macroscope, SP2 spectral confocal, live cell imaging, SPE confocal microscope, high-throughput automated widefield system with TIRF, spinning disk with EMCCD camera, laser nanosurgery and FRAP for live cells and organisms.*

Microdissector for fixed sample isolation

Implemented on an inverted microscope, a pulsed laser system from Olympus-MMI serves as a microscopic knife to dissect around subpopulations of cells within a flat sample preparation, *eg* a histology section. The isolated subpopulations can be transferred by laser expulsion into a recipient for further biochemical analysis (*ie*, PCR, etc...).

Fluorescence and reflection stereoscopy and macroscopy

A stereoscope and a macroscope offer low magnification and stereoscopic view from 0.6x to over 20x. Time sequences in fluorescence and in 3D can be performed, as well as automated reconstruction of in focus information through large volumes.

Conventional epifluorescence

Available on four microscopes, three upright and one inverted, widefield fluroescence microscopy is available with CCD cameras. Transmission and reflection microscopy contrast are also available with color camera to perform phase contrast, DIC (Differential Interference Contrast, or Normasky), color imaging of histological preparations.

Image processing

Two image processing workstations are freely available for data processing, visualization and interpretation, and are equipped with Imaris and other software packages. ADM also invests efforts in developing custom software applications for post acquisition image analysis, adapted to the needs of each users, typically giving the possibility to overcome limitations of comercial packages and focus on specific quantification tasks or automation of computing routines to process large datasets.

Scientific output

Publications

Solon J, Kaya-Copur A, Colombelli J and Brunner D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell*, **137**(7), 1331-42 (2009)

Other references

Colombelli J, Besser A, Kress H, Reynaud EG, Girard P, Caussinus E, Haselmann U, Small JV, Schwarz US and Stelzer EHK. Mechanosensing in actin stress fibers revealed by a close correlation between force and protein localization. *J Cell Sci*, **122**, 1665-79 (2009)

Timinszky G, Till S, Hassa PO, Hothorn M, Kustacher G, Nijmeijer B, Colombelli J, Altmeyer M, Stelzer EHK, Scheffzek K, Hottiger MO and Ladurner AG. A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nat Struct Mol Biol*, **16**, 923-29 (2009)

Collaborations

Advanced manufacturing technologies for microscopy
CIM Foundation-UPC (Barcelona, Spain)

Cellular mechanics during Drosophila *morphogenesis*
Jerome Solon, Centre for Genomic Regulation (Barcelona, Spain)

Microscopy development
European Molecular Laboratory & EMBLEM GmbH (Heidelberg, Germany); Olympus Soft Imaging Solution (Munich, Germany); Izasa SA (Barcelona, Spain)