CENTURI
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FOR LIVING SYSTEMS

CENTURI Training Course

« A Focus on Imaging »

12-13 & 16-18 April 2018
Luminy Campus

Training Objectives

This training is an introductory course to different microscopy techniques, dedicated to PhD students willing to explore the most advanced and popular techniques used for biological applications.
The course is being held, in Luminy and Joseph Aiguier Campus, in the laboratories LAI, CIML, IBDM, Inmed and IMM. It is composed of **theoretical courses** on the techniques basics and principle and **hands-on practical** in the laboratory in small groups.

By the end of the training the students should have a good understanding of basic optics, wide field microscopy, confocal microscopy, F-techniques (FRAP), electron microscopy, atomic force microscopy, dyes, detectors, single molecule imaging, super-resolution, light sheet microscopy, image processing and quantitative analysis.

Teachers

Aïcha Aouane – IBDM aicha.aouane@univ-amu.fr
Nicolas Brouilly – IBDM nicolas.brouilly@uni-amu.fr
Ignacio Casuso – LAI ignacio.casuso@inserm.fr
Claire Chardès – IBDM claire.Chardès@univ-amu.fr
Leon Espinosa – IMM lespinosa@imm.cnrs.fr
Fabrice Daian – IBDM fabrice.daian@univ-amu.fr
Mathieu Fallet – CIML fallet@ciml.univ-mrs.fr
Yannick Hamon – CIML hamon@ciml.univ-mrs.fr
Artemis Kosta – IMM akosta@imm.cnrs.fr
Hugo Le Guenno – IMM hleguenno@imm.cnrs.fr
Pierre-François Lenne – IBDM pierre-francois.lenne@univ-amu.fr
Sébastien Mailfert – CIML mailfert@ciml.univ-mrs.fr
Cédric Matthews – IBDM cedric.matthews@univ-amu.fr
François Michel – INMED francois.michel@inserm.fr
Pierre Henri Puech – LAI pierre-henri.puech@inserm.fr
Pierre Recouvreux – IBDM pierre.recouvreux@univ-amu.fr
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Length: 5 days
Theoretical courses: 12h30
Practical works: 23h30
Application form: https://goo.gl/forms/fCvI1JMhXY1cFFW83

Requirements

Bring personal computer with spreadsheet software and download the following software:
- **ImageJ**: http://rsb.info.nih.gov/ij/download.html

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Theoretical Courses (TC)

TC 1: Electron Microscopy – Fabrice Richard & Nicolas Brouilly
With electron microscopy one can visualize a sample at the nanoscale resolution.
We will first present the physics and mechanics within an electron microscope. We will then cover the different sample preparation methods and imaging modalities in electron microscopy and their applications.

TC 2: Atomic Force Microscopy – Félix Rico; Ignacio Casuso
Introduction to operation principles and to imaging and force measurement modes.

TC 3: Machine learning – Fabrice Daian
Machine learning and image analysis.

TC 4: General optics and wide field microscopy – Pierre François Lenne
Light mater interaction, diffraction, fluorescence, sources and objective lenses.

TC 5: Dyes and Staining, biological applications – Yannick Hamon
Biological applications and experimental tips enabling to avoid artifacts because of their indiscriminate use. Spectra, physical principles, families of dyes and their physical properties.

TC 6: From sensor to image and introduction to technical obsolescence – Cédric Matthews
From the sensor to the image. Photodetectors: rationale and implementation. Point detectors (PMT, APD) 2D detectors (CCD, CMOS), reading modes. Image formats (open /proprietary access) will be presented, as well as image reading/processing plateforms.

TC 7: Super-resolution and single molecule imaging (theory) – Pierre Recouvreux
The resolution of an optical imaging system is fundamentally limited by diffraction. However, recent technological advances made it possible to «break» the diffraction limit (STED, PALM/STORM,...).
Presentation of these different techniques and the context in which they can be used. Introduction to recent methods to localize and count single fluorescent molecules.

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Practical Work (PW)

PW 1: Electron Microscopy – Nicolas Brouilly, Fabrice Richard, Aïcha Aouane

We will first illustrate the several aspects of sample preparation for transmission and scanning electron microscopy presented during the lecture. We will get you in touch with the electron microscopes we have on the facility and we will cover different kinds of applications for cell biology that we routinely deal with (negative staining, TEM on tissue, SEM and Serial-Block Face SEM).

PW 2: Atomic Force Microscopy (AFM) – Félix Rico, Ignacio Casuso

The AFM can work in different modes and at different length scales. We will image two different samples each using a different AFM mode: contact mode and Force mapping mode. In contact mode we will image purple membrane, a native membrane from an organism from the Dead Sea and rich in bacteriorhodopsin proteins organized in 2D crystals. We will be able to observe individual proteins at nanometer resolution. In PeakForce tapping mode we will image living cells, from the micrometer scale of the whole cell, down to the tens of nanometer of the cell's cytoskeleton. We will discuss about limitations, possible artifacts, sample preparation and the versatility of the AFM technique.

PW 3: Image processing and quantification (Image J workshop) – Pierre Recouvreux

In this session we will work on how to handle images (open, display, manage N-dimensions), understand image quality, calibration and filtering, 2D event counting and segmentation using ImageJ software. Advanced Image J users will work on a case study: how to measure the cortical distribution of a polarity factor in fission yeast cells. We will analyze a large number of cells, imaged in different conditions of illumination, background intensity and protein expression, in order to extract valuable statistical information from the measurements. Requirements: ImageJ and your favorite software for graph creation.

PW 4: Machine learning – Cédric Matthews, Fabrice Daian

The goal of the training is to initiate the first steps of a new way of treating the image using artificial intelligence. The purpose of the course will be to decipher what's behind the concept of Deep Learning as a general approach to tackle hard image analysis problem. Through the presentation and in-depth analysis of one class of architecture (Convolutional Network), we will see how to build such an architecture to solve a hard image segmentation problem. Several examples will be proposed during a practical work in a second time.

PW 5: Image annotation and Omero database – Cédric Matthews

Automatic learning can't work if you don't caracterize your data. The aim of this pratical work is to show how to use Omero database system and to learn how to apply ontology dedicated to biological samples.

PW 6A, 6B: Optical Bench – Sébastien Mailfert (6A), Claire Chardès (6B)

On optical bench: principles of image formation and resolution (Abbe). Acquisition of spectra from different sources (Xenon fluorescence lamp, lasers), spatial spectrum enlargement by diffractive prism, transmission characterization of fluorescence filters, fluorescence detection of solutions, crosstalk, Stokes shift.

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Practical Work (PW)

PW 7 : Structured illumination microscopy – Artemis Kosta

Super-resolution techniques allow us to obtain images with a higher resolution than the diffraction limit. Structure Illumination Microscopy (SIM) is a form of light microscopy that uses one of these techniques. During this session, we will discuss how a SIM microscope works, the advantages and the limitations. We will prepare samples and acquire images with a SIM microscope, we will analyse them to produce a final high-resolution image and we will discuss the results.

PW 8 : Programming with R – Leon Espinosa

Image analysis produces multiparametric datasets (Intensities, positions, sizes, distances, correlations, etc.). The R software is a perfect tool for statistical analysis and graphical representation of these results. In addition, it is possible to directly analyze the images (as matrices) in R or to use R within the image analysis scripts available in the FIJI distribution of ImageJ. We will give examples of these different possibilities. For interested people you can install R or Rstudio with the packages: tidyverse, broom, pracma, EBImage, raster, tiff and an updated version of FIJI.

PW 9A, 9B, 9C: Confocal Microscopy – Sébastien Mailfert (9A), Matthieu Fallet (9B)

How to use spectral imaging for multicolor experiments or auto-fluorescence studies. Reference spectrum, linear unmixing, autofluorescence.

PW 9C, 9D: Confocal Microscopy – Cédric Matthews (9C), François Michel (9D)

How to set-up and tune a confocal microscope. Channel crosstalk, Nyquist-Shannon sampling, photobleaching, gain and offset, laser power vs. Detector gain, scanning speed, resolution.

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Schedule

Thursday April 12th

8h30 - 10h30	**TC 1: Electron Microscopy** N. Brouilly, F. Richard *IBDM ground floor room Nicole le Douarin*		
10h30 - 11h00	***Coffee break***		
11h00 - 12h30	**TC 2: Atomic Force Microscopy** F. Rico *IBDM ground floor room Nicole le Douarin*		
12h30 - 14h00	***Lunch with experts*** *IBDM ground floor, room Nicole le Douarin (meeting point)*		
14h00 - 17h00	***GROUPE 1 & 3*** **PW 1: Electron Microscopy** F. Richard, N. Brouilly *IBDM R-1, room 22-27 ground floor*	***GROUPE 2*** **PW 2: AFM** F. Rico *LAI, Force Microscopy group*	***GROUPE 4*** **PW 2: AFM** I. Casuso *LAI, Force Microscopy group*

Friday April 13th

8h00 - 9h00	**TC 3: Machine learning** Fabrice Daian *IBDM 6th floor room 6031-33*		
9h00 - 12h00	***GROUPE 1*** **PW 2: AFM** F. Rico *LAI, Force Microscopy group*	***GROUPE 2 & 4*** **PW 1: Electron Microscopy** F. Richard, N. Brouilly *IBDM R-1, room 22-27 ground floor*	***GROUPE 3*** **PW 2: AFM** I. Casuso *LAI, Force Microscopy group*
12h00 - 13h00	***Lunch with experts*** *IBDM ground floor, room Nicole le Douarin*		
13h00 - 15h00	***GROUPE 1 & 2*** **PW 3: Workshop imageJ** P. Recouvreux, PH. Puech *IBDM ground floor room Nicole le Douarin*		***GROUPE 3 & 4*** **PW 4: Machine learning** F. Daian, C. Matthews *IBDM 6th floor room 6031-33*
15h00 - 17h00	***GROUPE 1 & 2*** **PW 4: Machine learning** F. Daian, C. Matthews *IBDM 6th floor room 6031-33*		***GROUPE 3 & 4*** **PW 3: Workshop imageJ** P. Recouvreux, PH. Puech *IBDM ground floor room Nicole le Douarin*
17h00 - 18h00	**PW 5: Image annotation and Omero database** C. Matthews *IBDM 6th floor room 6031-33*		

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Monday April 16th	9h00 - 10h30	**TC 4: General Optics and wide field microscopy** P-F. Lenne *IBDM ground floor room Nicole le Douarin*	
	10h30 - 11h00	***Coffee break***	
	11h00 - 12h30	**TC 5: Dyes and staining – Biological applications** Y. Hamon *IBDM ground floor, room Nicole le Douarin*	
	12h30 - 13h30	***Lunch with experts*** *IBDM ground floor room Nicole le Douarin*	
	13h30 - 14h30	**TC 6: From the sensor to the image and Introduction to technical obsolescence** *C. Matthews* *IBDM ground floor, room Nicole le Douarin*	
	14h45 - 16h15	***GROUPE 1 & 2*** **PW 6A : Optical bench - Spectrum** S. Mailfert *CIML, Fougereau Room*	***GROUPE 3 & 4*** **PW 6B : Optical bench - Image formation** C. Chardès *IBDM 8th floor room 8016*
	16h30 - 18h00	***GROUPE 1 & 2*** **PW 6B : Optical bench - Image formation** C. Chardès *IBDM 8th floor room 8016*	***GROUPE 3 & 4*** **PW 6A : Optical bench - Spectrum** S. Mailfert *CIML, Fougereau Room*

Tuesday April 17th	10h00 - 11h30	**TC 7: Super-resolution and Single molecule** P. Recouvreux *IBDM ground floor room Nicole le Douarin*	
	11h30 - 12h30	***Lunch with experts*** *IBDM ground floor room Nicole le Douarin*	
	12h30 - 13h30	***GROUPE 1 & 2*** *Move to CNRS, LCB, Joseph Aiguier*	***GROUPE 3 & 4*** *Move to CNRS, LCB, Joseph Aiguier*
	13h30 - 15h30	***GROUPE 1 & 2*** **PW 7: Structured Illumination Microscopy** A. Kosta, H. Le Guenno *LCB - IMM*	***GROUPE 3 & 4*** **PW 8: Programming with R** L. Espinosa *LCB - IMM*
	16h00 - 18h00	***GROUPE 1 & 2*** **PW 8: Programming with R** L. Espinosa *LCB - IMM*	***GROUPE 3 & 4*** **PW 7: Structured Illumination Microscopy** A. Kosta, H. Le Guenno *LCB - IMM*

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Wednesday April 18th	8h00 - 9h30	**TC 8: Confocal microscopy and advanced techniques** M. Fallet *IBDM ground floor room Nicole le Douarin*			
	9h45 - 12h30	*GROUPE 1* **PW 9A: Spectral Imaging** S. Mailfert *CIML, LSM 880, R-1*	*GROUPE 2* **PW 9B: Spectral Imaging** M. Fallet *CIML, LSM 880, R-1*	*GROUPE 3* **PW 9C: Confocal** C. Matthews *LSM 510* *IBDM*	*GROUPE 4* **PW 9D: Confocal** F. Michel *Confocal LSM 780* *INMED*
	12h30 - 14h00	***Lunch with experts*** *IBDM ground floor, room Nicole le Douarin*			
	14h00 - 17h00	*GROUPE 1* **PW 9D: Confocal** F. Michel *Confocal LSM 780* *INMED*	*GROUPE 2* **PW 9C: Confocal** C. Matthews *LSM 510* *IBDM*	*GROUPE 3* **PW 9A: Spectral Imaging** S. Mailfert *CIML, LSM 880, R-1*	*GROUPE 4* **PW 9B: Spectral Imaging** M. Fallet *CIML, LSM 880, R-1*