

607 SM –TECNICHE AVANZATE DI INDAGINE MICROSCOPICA

ADVANCED MICROSCOPY TECHNIQUES

aa 2024/2025, 1st semester

Aula A8, edificio C11, 9:00-10:00

Agnes Thalhammer
agnes.thalhammer@units.it

Dan Cojoc
cojoc@iom.cnr.it



607 SM –TECNICHE AVANZATE DI INDAGINE MICROSCOPICA

date	lesson/lab	aula	time	docente
23/09/24	intro	Aula A8, C11	9-10	Thalhammer, Cojoc
24/09/24	lesson1	Aula 4B, H2Bis	9-12	Thalhammer
27/09/24	lesson2	Aula 4A, H2Bis	14-16	Cojoc
30/09/24	lesson3	Aula A8, C11	9-10	Thalhammer
01/10/24	lesson4+lab	Sala microscopia F2, C1	9-12	Thalhammer
04/10/24	lesson5	Aula 4A, H2Bis	14-16	Cojoc
07/10/24	lesson6	Aula A8, C11	9-10	Thalhammer
08/10/24	lesson7	Aula 4B, H2Bis	9-12	Thalhammer
14/10/24	lesson8	Aula A8, C11	9-10	Thalhammer
15/10/24	lesson9	Aula 4B, H2Bis	9-12	Thalhammer
18/10/24	lesson10	Aula 4A, H2Bis	14-16	Cojoc



607 SM –TECNICHE AVANZATE DI INDAGINE MICROSCOPICA

date	lesson/lab	aula	time	docente
21/10/24	lesson11	Aula A8, C11	9-10	Thalhammer
22/10/24	lesson12	Aula 4B, H2Bis	9-12	Thalhammer
25/10/24	lesson13	Aula 4A, H2Bis	14-16	Cojoc
28/10/24	lesson14	Aula A8, C11	9-10	Thalhammer
29/10/24	lesson15+lab	Aula 4A, H2Bis	9-12	Thalhammer
05/11/24	lab	CIMA	9-12	Thalhammer
08/11/24	lesson16	Aula 4A, H2Bis	14-16	Cojoc
12/11/24	lesson17	Aula 4B, H2Bis	9-12	Cojoc
15/11/24	lesson18	Aula 4A, H2Bis	14-16	Cojoc
19/11/24	lesson19	Aula 4B, H2Bis	9-12	Cojoc
22/11/24	lesson20	Aula 4A, H2Bis	14-16	Cojoc
	lab			Cojoc



1. How a microscope works

- 1.1. Image formation
- 1.2. Magnification vs resolution
- 1.3. Numerical aperture and working distance
- 1.4. Objectives
- 1.5. Point-spread function and Airy disk
- 1.6. Optical aberrations

2. Contrasting techniques - Lesson + Lab

- 2.1. Brightfield
- 2.2. Darkfield
- 2.3. Phase Contrast
- 2.4. Polarization Contrast
- 2.5. Differential Interference Contrast (DIC)

Agnes Thalhammer
agnes.thalhammer@units.it



3. Fluorescence microscopy

3.1. Fluorescence principle

3.2. Absorption and Emission spectra - Stoke's shift

3.3. The fluorescence microscope –
light sources, filter, dichroic mirror

3.4. Fluorophores

3.5. Staining with fluorophores

3.6. Problems with fluorescence imaging

3.7. Multichannel imaging



4. Confocal, super-resolution and 2-photon microscopy

4.1. TIRF microscopy

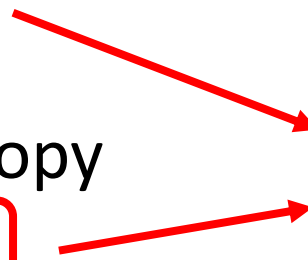
4.2. Confocal microscopy

4.3. 2-photon microscopy

4.4. Superresolution microscopy

4.4.1. SIM microscopy

4.5. FRAP microscopy



dima | Centro
Interdipartimentale
di Microscopia
Avanzata

lab



5. Live-imaging techniques

5.1. Incubation

5.2. The life-imaging microscope

5.3. Contrasting techniques

5.4. Fluorescent labelling of live cells

5.5. Resolution – Speed – Sensitivity

5.6. Examples



6. Quantitative microscopy – Imaging processing and analysis

- 6.1. Digital images
- 6.2. Resolution
- 6.3. Signal-to-noise
- 6.4. Sampling
- 6.5. Quantization

7. Image processing and analysis using ImageJ - Lesson + Lab

- 7.1. Histogram and LUTs
- 7.2. Noise, Filters and Background
- 7.4. ROIs and measurements
- 7.5. Threshold, watershed and particle analysis
- 7.6. Live imaging analysis

Agnes Thalhammer
agnes.thalhammer@units.it



Advanced techniques in optical microscopy:

1. Phase contrast and quantitative phase microscopy – principles and applications (2 h)
2. Super-resolution microscopy (naonsocopy) – principles and techniques
STED – STimulated Emission Depletion, PALM – PhotoActivated Localization Microscopy, MUNFLUX - MINimal fluorescence photon FLUXes microscopy, BALM Binding-Activated Localization Microscopy, PAINT with examples in life science from recent published papers (4 h)
3. Non-linear optical microscopy
- principles and applications (4 h)
4. Acousto-optical microscopy (2 h) principles and applications
5. Optical tweezers microscopy (3 h) principles and applications

Non optical microscopy:

6. X-ray microscopy (4 h) principles and applications

7. AFM microscopy (3 h) principles and applications

8. Electron-microscopy (3 h) principles and applications

Laboratory – 4 h CNR-IOM