

## MICROSCOPY APPLICATIONS FOR IMMUNOLOGICAL RESEARCH

*Lecture and practical Course for students of the PhD program in “Innovation in Immuno-mediated and Hematological Disorders” Sapienza University of Rome*

*Prof. Marcus Thelen & Diego Morone, IRB Bellinzona (CH)*

**Date: 26.03.2025 – 27.03.2025**

**Room: 301 Building D**

**Viale Regina Margherita 295b, Roma**

### Day 1

**10:00 – 14:00 (30 min break) Marcus Thelen / Diego Morone (4h)**

Welcome and course introduction.

Physics light: Light refraction, diffraction, interference resolution, excitation and emission (Jablonski) lifetime and quantum yield. Physics of electron microscopy

General introduction into optics, lenses and objectives (chromatic correction, Plan Apo meanings), long distance and short distance, confocal and bright field microscopy, including microscope essentials, light path, optics, lenses and lens definitions, objectives, transmitted light and fluorescence at microscope (light sources, detector), PSF, crosstalk, computer assisted deconvolution.

**Lunch Break 14:00-15:00**

**15:00-18:00 Marcus Thelen / Diego Morone**

Immunohistochemistry (IHC) sample preparation, staining protocols, and cell migration.

In several groups practical instruction on microscope: hands on wide-field fluorescence microscope (light sources, detectors, filters).

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### Day 2

**9:00 – 13:30 (30 min break) Marcus Thelen / Diego Morone (4h)**

Super resolution Microscopy; Fluorescence Microscopy Application: Fluorescent protein, Tags, Fluorophores  $\text{Ca}^{2+}$ , FRET, FLIM, FRAP, Photoactivation and Photoswitching, 2 photon microscopy. Light sheet microscopy, Introduction to electron microscopy

**Lunch Break 13:30-14:30**

**14:30-15:30 Diego Morone / Marcus Thelen**

Digital images, Image analysis with ImageJ, Open-source softwares for image analysis, 3D analysis (one group in conference room).

**16:00-18:00 Written test**

*Lectures, delivered in dedicated conference rooms, are open to a maximum of 40 students of all current PhD cycles (March 26<sup>th</sup> morning, March 27<sup>th</sup>, all day).*

*Introduction to the wide-field fluorescence microscope will be limited to 15 students divided into small groups (March 26th, afternoon).*

*The course will be held in English.*

*For registration: write to [giovanni.bernardini@uniroma1.it](mailto:giovanni.bernardini@uniroma1.it) before 23.59 of March 15<sup>th</sup> 2025, specifying name, surname, PhD course, PhD cycle and if interested in the practical part.*