Program

BASIC LEVEL

1.1 Principles of Optical Microscopy – Cesare Covino *(University San Raffaele, Milano)*
An optical microscope is a type of microscope which uses visible light as an illumination source and a system of lenses to magnify and reveal microscopic features. From its, this instrument has opened a new world and a new way for scientists to explore the mysteries of living matter. Understanding how the microscope works and the concepts of resolution and contrast, is crucial for getting the best performances from our instruments for the correct interpretation of results.

1.2 Resolution and Sampling – Dario Parazzoli *(IFOM, Milano)*
An image is worth a thousand words. This is true but we need to acquire it in a proper way to be faithful to reality. Sampling is key for a correct interpretation of the event we are interested in.

1.3 Contrast Techniques in Transmitted Light Microscopy - Spartaco Santi *(CNR, Bologna)*
Contrast in an image is essential to distinguish features in achieving high-quality images. The different contrast techniques in brightfield microscopy will be described: phase contrast, differential interference contrast (DIC), darkfield illumination and Kölher illumination.

1.4 Fluorescence Microscopy and Photochemistry – Chiara Peres *(CNR, Bologna)*
Fluorescence microscopy is an optical imaging technique that revolutionized observation of organic and inorganic substances. We discuss the principles of fluorescence and photochemistry, main properties of fluorochromes, fundamental components and operating principles of a fluorescence microscope.

1.5 Confocal and Spinning Disk Microscopy – Chiara Cordiglieri *(INGM, Milano)*
Confocal microscopy is the fundamental technique which enables multiple fluorescence imaging of complex biological samples via optical sectioning. Different methodologies have been developed over the years, including confocal laser scanning and spinning-disk confocal microscopy, to accomplish multi-labelling multi-dimensional imaging, going far beyond the visible in biology morphology, structure, and functions.

1.6 History of Microscopy – Alessandro Gambardella *(Rizzoli, Bologna)*
The History of Microscopy, from antiquity to the present day, is an exciting journey through surprising intuitions and discoveries, bringing us face to face with the personalities who shaped the development of this discipline.

1.7 Live Imaging – Cesare Covino *(San Raffaele, Milano)*
Time-Lapse Microscopy of living cells is a powerful way to monitor cellular processes that occur over an extended time. There are two main aspects of live imaging that make it essential for modern research. An ever-increasing number of investigations uses live-cell imaging techniques to obtain dynamic information about cellular and tissue functions. Because of these advantages, live-cell imaging has become a fundamental analytical tool in biomedical research.

1.8 Stereomicroscopy – Andrea Giacomini *(EVIDENT Europe GmbH)*
In this module I am going to introduce stereomicroscopy, from its invention to its current applications. What's a stereomicroscope? How does it work? Why has it been invented? How many types of stereomicroscopes exist? When should I use a stereomicroscope? What are the different components of a stereomicroscope?

1.9 Digital Imaging and Sensor Technologies – Spartaco Santi *(CNR, Bologna)*
The role of the microscopy cameras in imaging experiments. Parameters: sensitivity, field of view, imaging speed, resolution, dynamic range. The main sources of noise: photon (Poisson) noise, readout noise and thermal (dark) noise. Main camera technologies: interline CCD, EMCCD and sCMOS.

1.10 Image Processing and Deconvolution – Emanuele Martini *(IFOM, Milano)*
This module will introduce the main concepts and topics of bioimage processing for visualization, analysis, image enhancement, and deconvolution for recovery from degradation processes.

Program

ADVANCED LEVEL

2.1 Structured Illumination Microscopy (SIM) – Mario Faretta *(IEO, Milano)*

The Structured Illumination Microscope is one of the first implementations of super resolution microscopy and provides an excellent tool for the fixed and living samples observation. We will focus on its working principles to understand the advantages and limitations of this technology.

2.2 Total Internal Reflection Fluorescence Microscopy – Dario Parazzoli *(IFOM, Milano)*

TIRFM is an elegant optical technique for selective imaging of fluorescent molecules in an extremely thin axial region. Thanks to the unique properties of and induced evanescent wave, TIRFM is a fundamental tool in live cell imaging such as vesicles membrane trafficking, in single molecule detection experiments and it is an important player if associated to different super resolution techniques such as PALM, STORM and SIM.

2.3 2D and 3D Stochastic Optical Reconstruction Microscopy – Mario Faretta *(IEO, Milano)*

Single Molecule Localization Microscopy approaches revolutionized the classical imaging approaches by looking at fluorescent molecules one by one. SMLM provides data able to describe structures at the levels of 10-20 nm. After exploring the founding principles, we will examine pros and cons and then the latest developments that allow accessing the sub-nanometre scale.

2.4 Two-Photon Excitation (2PE) Microscopy – Francesca Cella Zanacchi *(University of Pisa)*

TPE became an election tool for deep and tissue imaging in the biomedical and biophysical context. When combined with other advanced fluorescence imaging techniques TPE provides improved imaging capabilities and performance. In this lesson we will both explain the basics of the two-photon absorption process and highlight how this technique can be successfully combined with 3D imaging methods and super-resolution microscopy.

2.5 Light-Sheet Microscopy – Zeno Lavagnino *(IFOM, Milano)*

Live, volumetric imaging is key to understanding complex processes in biology. In this framework, light-sheet microscopy is starting to become the golden standard. In this module we will discuss the principle behind the technique, and review some of its applications.

2.6 Electron and Scanning Probe Microscopy - Alessandro Gambardella *(Rizzoli, Bologna)*

Beyond optical methods, the electron and scanning probe techniques brought microscopy into a new era where the "small" word has revealed its astonishing complexity and variety.

2.7 Stimulated Emission Depletion (STED) Microscopy – Marco Castello and Paolo Bianchini *(IIT, Genova)*

In this module, an overview of STED optical microscopy, a technique that surpasses the diffraction limit, will be provided. The fundamentals of this technique, the underlying physical principles enabling super-resolution, and the necessary imaging conditions will be explained. Practical examples on optimizing performance under such conditions will be presented. Finally, a practical demonstration will showcase a laboratory imaging session utilizing a custom instrument.

2.8 Label-Free Microscope – Alberto Diaspro *(IIT, University of Genova)*

Label-free microscopy is a cutting-edge imaging technique that revolutionizes the field of microscopy by eliminating the need for fluorescent or chemical dyes to visualize biological samples. This innovative approach allows for non-invasive and real-time imaging of specimens, providing valuable insights into their structure, composition, and dynamics.
Mueller Matrix Microscopy is an advanced imaging technique that combines polarized light microscopy with the Mueller matrix formalism to provide quantitative information about the optical properties of samples.

2.9 Second Harmonic Generation Microscopy – Chiara Peres *(CNR, Bologna)*
SHG Microscopy is a non-linear optical imaging technique used to visualize and quantify events deep in tissue with a label free method. Here, we discuss the principles of SHG and the main properties of harmonophores, the concept of light polarization, the SHG main characteristics and how SHG works at the molecular and macromolecular level. We also discuss a simple version of SHG setup, and two imaging implementations based on the SHG principle.

2.10 Macromolecule Dynamics by Single Molecule Fluorescence Microscopy
Davide Mazza *(San Raffaele, Milano)*
Measuring the dynamics of macromolecules can provide fundamental information about their interactions and their functions. In this lesson I will describe how advanced fluorescence microscopy allows us to probe these dynamics, down to single-molecule resolution.

2.11 Expansion Microscopy – Paolo Bianchini and Chantal Usai *(IIT, Genova)*
Expansion microscopy (ExM) is a novel super-resolution microscopy method that enables nano-scale imaging of biological samples using conventional microscopes. In ExM, the specimen is physically magnified inside a swellable polymer network. This course will explain the technique and its validation.

2.12 The Intelligent Microscope – Alberto Diaspro *(IIT, University of Genova)*
The concept of the intelligent microscope represents the advancement of optical microscopy by incorporating the latest technologies and techniques. Alberto Diaspro discusses the idea of an intelligent microscope, inspired by Federico Chezy's collection of images from the 1610s, which marked a shift from textual descriptions to visual representations of the real world. The combination of AI and multiple microscopes amplifies the power and potential of microscopy.

2.13 Holotomographic Microscopy – Spartaco Santi *(CNR, Bologna)*
This presentation highlights the progress in Holotomographic Microscopy, allowing 3D imaging of live cells without labels. It surpasses 2D imaging constraints by employing polytomography for authentic 3D cell images through refractive index. The method quantifies cell structure and traits, offering swift capture, high detail, and minimal phototoxicity.