# Testi del Syllabus

| | | |
|---|---|---|
| Resp. Did. | **THALHAMMER AGNES** | **Matricola: 038054** |

| | |
|---|---|
| Docenti | **COJOC DANUT ADRIAN, 3 CFU**<br>**THALHAMMER AGNES, 3 CFU** |
| Anno offerta: | **2022/2023** |
| Insegnamento: | **607SM - TECNICHE AVANZATE DI INDAGINE MICROSCOPICA** |
| Corso di studio: | **ME02 - BIOTECNOLOGIE MEDICHE** |
| Anno regolamento: | **2021** |
| CFU: | **6** |
| Settore: | **FIS/03** |
| Tipo Attività: | **C - Affine/Integrativa** |
| Anno corso: | **2** |
| Periodo: | **Primo Semestre** |
| Sede: | **TRIESTE** |

## Testi in italiano

| | |
|---|---|
| **Lingua insegnamento** | Inglese |
| **Contenuti (Dipl.Sup.)** | 1.Optical microscopy: principles<br>1.1.The optical microscope<br>• Image formation; magnification and resolution; diffracted limited resolution;<br>• Optical abberations and image quality;<br>• Digital camera image acquisition (formats, properties, SNR)<br>1.2.Phase imaging techniques<br>• Phase contrast and differential interference contrast (DIC);<br>• Quantitative phase imaging: digital holographic microscopy.<br>1.3. Other techniques to image non-stained samples<br>• Dark field microscopy<br>• Polarization microscopy<br>1.4. Fluorescence microscopy<br>• Epifluorescence basics; confocal; two photons;<br>• Super-resolution techniques: STED. PALM, MULTIFLUX. SIM<br>• Other techniques: FRET, FRAP, FLIM<br><br>2. Optical microscopy: applications in cellular biology and biotechnology<br>2.1. White light microscopy applications<br>• bright field, dark field, contrast, objectives, sample preparation<br>2.2. Immunofluorescence protocols and fluorescence microscopy applications<br>• fluorophores, multichannel fluorescence labelling, sample preparation, fixing, blocking, bleaching, quenching, antifade agents<br>• laboratory: white light and fluorescent microscopes (2 h)<br>2.3. Confocal, superresolution and multiphoton microscopy applications<br>• sample preparation, thickness, penetration depth, resolution, bleaching, multichannel imaging<br>• choosing the right microscopy technique<br>• laboratory: confocal, SIM superresolution and multiphoton microscopes (4 h) |

2.4. Live imaging microscopy applications
• sample preparation, labelling, perfusion, temperature, humidity, oxygenation, osmolarity, phototoxicity, resolution-speed-sensitivity
• calcium-, voltage- and pH-sensitive dyes
2.5. Digital image processing and analysis
• acquisition, saving
• image display options (LUTs, brightness, contrast, filters, denoising, z-stack projections, overlay)
• quantitative microscopy (grey values, histogram, threshold, binary image, masks, intensity profile, particle analysis, colocalization, 3D reconstruction, time series analysis)

3. Vibrational spectrocopy and microscopy
• Infrared IR , RAMAN

4. Electronic mciroscopy and X-ray microscopy
• SEM and TEM
• Synchrotron radiation and phase contrast and fluorescence X ray microscopy
• Free electron laser and coherent imaging
• Electron microscopy,X-ray microscopy vs optical microscopy

5. Scanning probe microscopy
• Atomic Force Microscopy (AFM)),
• Scanning Tunneling Microscopy (STM);
• Near field scanning optical microscopy (SNOM)

6. Contact less manipulation techniques at molecular and cellular level
• Optical Tweezers (OT) and scissors
• Magnetic and acoustic tweezers

7. Biomechanics at the single molecule and single cell level
• Force spectroscopy: AFM vs Optical tweezers
• Mechanical properties – physiology, diagnosis

8. Laboratory: Optical Tweezers; Digital Holographic Microscopy (4 h)

**Testi di riferimento**

All the material presented and discussed in class will be made available to students. Some arguments are partly taken from the following textbooks:
Guy Cox, Optical Imaging Techniques in Cell Biology, Taylor and Francis
Jacobs, Huang and Kwon, Introduction to Cell Mechanics and Mechanobiology, Garland Science
Murphy, D. B. and Davidson, M. W. (2012) References, in Fundamentals of Light Microscopy and Electronic Imaging, Second Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118382905.refs
O'Farrell, M. (2006) Basic Light Microscopy, in Cell Biology Protocols (eds J. R. Harris, J. Graham and D. Rickwood), John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/0470033487.ch1
(2013) Fluorescence Microscopy, in Fluorescence Microscopy: From Principles to Biological Applications (ed U. Kubitscheck), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. doi: 10.1002/9783527671595.ch3

**Obiettivi formativi**

The main objectives of this course are:
1) Knowledge and understanding: Students are provided with a basic knowledge of microscope techniques with emphasis on the recently developed methodologies.
2) Applying knowledge and understanding: The practical and theoretical lectures aim at explaining the optimal usage of different microscopes. The students will be able to choose the microscopic technique that best allows the study of a given biological problem in terms of spatial resolution, temporal dynamics, interaction and sample damage and spectral and compositional chemical information, mechanical and morphological structure.

**Prerequisiti**

The student should have basic knowledge of physics and cell biology.

| | |
|---|---|
| **Metodi didattici** | Frontal lessons with aid of presentations in electronic format. Optical microscopy laboratory.<br>Any changes these indications, which may become necessary to ensure the application of safety protocols related to the COVID19 emergency, will be communicated on the Department's and Degree Course websites and Lecture course Moodle page. |
| **Altre informazioni** | The detailed program and the ppt presentations used to support teaching will be available on Moodle2 website of the course |
| **Modalità di verifica dell'apprendimento** | The assessment of the students' learning will take place with a written test on Moodle using multiple choice questions and some open questions distributed on the main topics presented. The written exam will produce a note up to 30 cum laude, a result under 18/30 will be considered as not passed. |
| **Programma esteso** | 1.Optical microscopy: principles<br>1.1.The optical microscope<br>• Image formation; magnification and resolution; diffracted limited resolution;<br>• Optical abberations and image quality;<br>• Digital camera image acquisition (formats, properties, SNR)<br>1.2.Phase imaging techniques<br>• Phase contrast and differential interference contrast (DIC);<br>• Quantitative phase imaging: digital holographic microscopy.<br>1.3. Other techniques to image non-stained samples<br>• Dark field microscopy<br>• Polarization microscopy<br>1.4. Fluorescence microscopy<br>• Epifluorescence basics; confocal; two photons;<br>• Super-resolution techniques: STED. PALM, MULTIFLUX. SIM<br>• Other techniques: FRET, FRAP, FLIM<br><br>2. Optical microscopy: applications in cellular biology and biotechnology<br>2.1. White light microscopy applications<br>• bright field, dark field, contrast, objectives, sample preparation<br>2.2. Immunofluorescence protocols and fluorescence microscopy applications<br>• fluorophores, multichannel fluorescence labelling, sample preparation, fixing, blocking, bleaching, quenching, antifade agents<br>• laboratory: white light and fluorescent microscopes (2 h)<br>2.3. Confocal, superresolution and multiphoton microscopy applications<br>• sample preparation, thickness, penetration depth, resolution, bleaching, multichannel imaging<br>• choosing the right microscopy technique<br>• laboratory: confocal, SIM superresolution and multiphoton microscopes (4 h)<br>2.4. Live imaging microscopy applications<br>• sample preparation, labelling, perfusion, temperature, humidity, oxygenation, osmolarity, phototoxicity, resolution-speed-sensitivity<br>• calcium-, voltage- and pH-sensitive dyes<br>2.5. Digital image processing and analysis<br>• acquisition, saving<br>• image display options (LUTs, brightness, contrast, filters, denoising, z-stack projections, overlay)<br>• quantitative microscopy (grey values, histogram, threshold, binary image, masks, intensity profile, particle analysis, colocalization, 3D reconstruction, time series analysis)<br><br>3. Vibrational spectrocopy and microscopy<br>• Infrared IR , RAMAN<br><br>4. Electronic mciroscopy and X-ray microscopy<br>• SEM and TEM<br>• Synchrotron radiation and phase contrast and fluorescence X ray microscopy<br>• Free electron laser and coherent imaging<br>• Electron microscopy,X-ray microscopy vs optical microscopy |

| | |
|---|---|
| | 5. Scanning probe microscopy<br>• Atomic Force Microscopy (AFM)),<br>• Scanning Tunneling Microscopy (STM);<br>• Near field scanning optical microscopy (SNOM)<br><br>6. Contact less manipulation techniques at molecular and cellular level<br>• Optical Tweezers (OT) and scissors<br>• Magnetic and acoustic tweezers<br><br>7. Biomechanics at the single molecule and single cell level<br>• Force spectroscopy: AFM vs Optical tweezers<br>• Mechanical properties – physiology, diagnosis<br><br>8. Laboratory: Optical Tweezers; Digital Holographic Microscopy (4 h) |
| **Obiettivi Agenda 2030 per lo sviluppo sostenibile** | |

## Obiettivi per lo sviluppo sostenibile

| Codice | Descrizione |
|---|---|
| 3 | Salute e benessere |
| 9 | Industria, innovazione e infrastrutture |

## Testi in inglese

| | |
|---|---|
| | English |
| | 1.Optical microscopy: principles<br>1.1.The optical microscope<br>• Image formation; magnification and resolution; diffracted limited resolution;<br>• Optical abberations and image quality;<br>• Digital camera image acquisition (formats, properties, SNR)<br>1.2.Phase imaging techniques<br>• Phase contrast and differential interference contrast (DIC);<br>• Quantitative phase imaging: digital holographic microscopy.<br>1.3. Other techniques to image non-stained samples<br>• Dark field microscopy<br>• Polarization microscopy<br>1.4. Fluorescence microscopy<br>• Epifluorescence basics; confocal; two photons;<br>• Super-resolution techniques: STED. PALM, MULTIFLUX. SIM<br>• Other techniques: FRET, FRAP, FLIM<br><br>2. Optical microscopy: applications in cellular biology and biotechnology<br>2.1. White light microscopy applications<br>• bright field, dark field, contrast, objectives, sample preparation<br>2.2. Immunofluorescence protocols and fluorescence microscopy applications<br>• fluorophores, multichannel fluorescence labelling, sample preparation, fixing, blocking, bleaching, quenching, antifade agents<br>• laboratory: white light and fluorescent microscopes (2 h)<br>2.3. Confocal, superresolution and multiphoton microscopy applications<br>• sample preparation, thickness, penetration depth, resolution, bleaching, multichannel imaging<br>• choosing the right microscopy technique<br>• laboratory: confocal, SIM superresolution and multiphoton microscopes (4 h) |

2.4. Live imaging microscopy applications
• sample preparation, labelling, perfusion, temperature, humidity, oxygenation, osmolarity, phototoxicity, resolution-speed-sensitivity
• calcium-, voltage- and pH-sensitive dyes
2.5. Digital image processing and analysis
• acquisition, saving
• image display options (LUTs, brightness, contrast, filters, denoising, z-stack projections, overlay)
• quantitative microscopy (grey values, histogram, threshold, binary image, masks, intensity profile, particle analysis, colocalization, 3D reconstruction, time series analysis)

3. Vibrational spectrocopy and microscopy
• Infrared IR , RAMAN

4. Electronic mciroscopy and X-ray microscopy
• SEM and TEM
• Synchrotron radiation and phase contrast and fluorescence X ray microscopy
• Free electron laser and coherent imaging
• Electron microscopy,X-ray microscopy vs optical microscopy

5. Scanning probe microscopy
• Atomic Force Microscopy (AFM)),
• Scanning Tunneling Microscopy (STM);
• Near field scanning optical microscopy (SNOM)

6. Contact less manipulation techniques at molecular and cellular level
• Optical Tweezers (OT) and scissors
• Magnetic and acoustic tweezers

7. Biomechanics at the single molecule and single cell level
• Force spectroscopy: AFM vs Optical tweezers
• Mechanical properties – physiology, diagnosis

8. Laboratory: Optical Tweezers; Digital Holographic Microscopy (4 h)

All the material presented and discussed in class will be made available to students. Some arguments are partly taken from the following textbooks:
Guy Cox, Optical Imaging Techniques in Cell Biology, Taylor and Francis
Jacobs, Huang and Kwon, Introduction to Cell Mechanics and Mechanobiology, Garland Science
Murphy, D. B. and Davidson, M. W. (2012) References, in Fundamentals of Light Microscopy and Electronic Imaging, Second Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118382905.refs
O'Farrell, M. (2006) Basic Light Microscopy, in Cell Biology Protocols (eds J. R. Harris, J. Graham and D. Rickwood), John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/0470033487.ch1
(2013) Fluorescence Microscopy, in Fluorescence Microscopy: From Principles to Biological Applications (ed U. Kubitscheck), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. doi: 10.1002/9783527671595.ch3

The main objectives of this course are:
1) Knowledge and understanding: Students are provided with a basic knowledge of microscope techniques with emphasis on the recently developed methodologies.
2) Applying knowledge and understanding: The practical and theoretical lectures aim at explaining the optimal usage of different microscopes. The students will be able to choose the microscopic technique that best allows the study of a given biological problem in terms of spatial resolution, temporal dynamics, interaction and sample damage and spectral and compositional chemical information, mechanical and morphological structure.

The student should have basic knowledge of physics and cell biology.

Frontal lessons with aid of presentations in electronic format. Optical microscopy laboratory.
Any changes these indications, which may become necessary to ensure the application of safety protocols related to the COVID19 emergency, will be communicated on the Department's and Degree Course websites and Lecture course Moodle page.

The detailed program and the ppt presentations used to support teaching will be available on Moodle2 website of the course

---

The assessment of the students' learning will take place with a written test on Moodle using multiple choice questions and some open questions distributed on the main topics presented. The written exam will produce a note up to 30 cum laude, a result under 18/30 will be considered as not passed.

1.Optical microscopy: principles
1.1.The optical microscope
• Image formation; magnification and resolution; diffracted limited resolution;
• Optical abberations and image quality;
• Digital camera image acquisition (formats, properties, SNR)
1.2.Phase imaging techniques
• Phase contrast and differential interference contrast (DIC);
• Quantitative phase imaging: digital holographic microscopy.
1.3. Other techniques to image non-stained samples
• Dark field microscopy
• Polarization microscopy
1.4. Fluorescence microscopy
• Epifluorescence basics; confocal; two photons;
• Super-resolution techniques: STED. PALM, MULTIFLUX. SIM
• Other techniques: FRET, FRAP, FLIM

2. Optical microscopy: applications in cellular biology and biotechnology
2.1. White light microscopy applications
• bright field, dark field, contrast, objectives, sample preparation
2.2. Immunofluorescence protocols and fluorescence microscopy applications
• fluorophores, multichannel fluorescence labelling, sample preparation, fixing, blocking, bleaching, quenching, antifade agents
• laboratory: white light and fluorescent microscopes (2 h)
2.3. Confocal, superresolution and multiphoton microscopy applications
• sample preparation, thickness, penetration depth, resolution, bleaching, multichannel imaging
• choosing the right microscopy technique
• laboratory: confocal, SIM superresolution and multiphoton microscopes (4 h)
2.4. Live imaging microscopy applications
• sample preparation, labelling, perfusion, temperature, humidity, oxygenation, osmolarity, phototoxicity, resolution-speed-sensitivity
• calcium-, voltage- and pH-sensitive dyes
2.5. Digital image processing and analysis
• acquisition, saving
• image display options (LUTs, brightness, contrast, filters, denoising, z-stack projections, overlay)
• quantitative microscopy (grey values, histogram, threshold, binary image, masks, intensity profile, particle analysis, colocalization, 3D reconstruction, time series analysis)

3. Vibrational spectrocopy and microscopy
• Infrared IR , RAMAN

4. Electronic mciroscopy and X-ray microscopy
• SEM and TEM
• Synchrotron radiation and phase contrast and fluorescence X ray microscopy
• Free electron laser and coherent imaging
• Electron microscopy,X-ray microscopy vs optical microscopy

5. Scanning probe microscopy
• Atomic Force Microscopy (AFM)),
• Scanning Tunneling Microscopy  (STM);
• Near field scanning optical microscopy (SNOM)

6. Contact less manipulation techniques at molecular and cellular level
• Optical Tweezers (OT) and scissors
• Magnetic and acoustic tweezers

7. Biomechanics at the single molecule and single cell level
• Force spectroscopy: AFM vs Optical tweezers
• Mechanical properties – physiology, diagnosis

8. Laboratory: Optical Tweezers; Digital Holographic Microscopy (4 h)

## Obiettivi per lo sviluppo sostenibile

| Codice | Descrizione |
|---|---|
| 3 | Good health and well-being |
| 9 | Industries, innovation and infrastructure |