1. Annotation

Course Description

Overview of current imaging research techniques in basic biomedical research. Various applications in neurobiology, cancer biology and preclinical studies of novel and emerging advanced microscopy technologies. Analysis of experiments and research described in recent scientific papers. The introduction of the course also includes core mathematics and optics. The course will outline and compare different optical microscopy techniques and super-resolution imaging in biomedical research. Topics also include clearing agents and techniques, optical imaging of brain activity in vivo using genetically encoded probes, immediate early gene mapping, intravital imaging, applications for functional analyses of neuronal circuits. The course aims to teach students to understand basic principles of the current imaging techniques, microscope design, and image formation. The course will also offer laboratory practice in sample preparation, confocal imaging, and image analysis. Students will learn how to choose the most appropriate imaging method for their own research project.

Course Prerequisites

Course requires BS level knowledge of general physics, molecular, cellular biology and neurobiology.

2. Structure and Content

Course Academic Level

Master-level

Number of ECTS credits

6
# Core Physics and Mathematics

1. Background physics including wave behavior, optics and nuclear physics. Aberrations.

2. Core mathematics, Fourier analysis.

3. Fundamental principles of microscope design and image formation.
   3.1 Microscope design, Kohler illumination, objectives, numerical aperture, image formation.
   3.2 Bright field, dark field, phase contrast, differential interference contrast (DIC), fluorescence microscopy.
   3.3 Digital cameras, image recording, signal to noise ratio.

4. Resolution
   4.1 Rayleigh and Abbey definitions of resolution
   4.2 Resolution and numerical aperture

# Microscopy in Biology - Imaging of Biological Systems

1. Confocal microscopy

2. Two-photon microscopy

3. Light-sheet microscopy

4. Photo-activatable imaging, protein dynamics in living cells

5. Total internal reflection fluorescence microscopy TIRF

6. Förster resonance energy transfer FRET

7. Fluorescence lifetime imaging FLIM

8. Fluorescence recovery after photobleaching FRAP

9. Total Internal Reflection Microscopy (TIRF)

10. Super resolution imaging
   10.1 Stimulated emission depletion STED microscopy and related techniques
   10.2 Stochastic optical reconstruction microscopy STORM
   10.3 Photoactivated localization microscopy

11. Airyscan

12. Light-sheet microscopy

13. Choosing the right microscopy technique for the research project

### Nanoscale Imaging and Probing

1. Nanoscale imaging and probing - Electron Microscopy
2. Nanoscale imaging and probing - Scanning Probe Microscopy
3. Image Analysis with open source software

### Imaging in Neurobiology

1. Clarity and other clearing agents and techniques
2. Optical imaging of brain activity in vivo using genetically encoded probes.
   2.1 Calcium imaging
3. Immediate early gene mapping
4. Intravital imaging – experimental setup and applications

### 3. Assignments

<table>
<thead>
<tr>
<th>Assignment Type</th>
<th>Assignment Summary</th>
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</thead>
<tbody>
<tr>
<td>Project</td>
<td>As homework, research topics related to the course are given for careful studying during the week before the seminar. Students prepare 15 mins Powerpoint presentations and present it in front of class</td>
</tr>
<tr>
<td>Test/Quiz</td>
<td>15-mins short written exercises are given at the beginning of each lecture</td>
</tr>
<tr>
<td>Team Project</td>
<td>The final project</td>
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<tr>
<td>Report</td>
<td>Each student is required to prepare a report on the laboratory part of the course</td>
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### 4. Grading

<table>
<thead>
<tr>
<th>Type of Assessment</th>
<th>Grade Structure</th>
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<tbody>
<tr>
<td>Graded</td>
<td>Activity Type</td>
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<tr>
<td></td>
<td>Projects</td>
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<td></td>
<td>Test/Quiz</td>
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<td></td>
<td>Final Project</td>
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<td></td>
<td>Lab Reports</td>
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<td></td>
<td>Midterm Exam</td>
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<td>Final Exam</td>
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**Grading Scale**

- A: 85
- B: 75
- C: 65
5. Basic Information

Attendance Requirements | Optional with Exceptions

Maximum Number of Students | Overall: 15
| Per Group (for seminars and labs): 5

Course Stream | Science, Technology and Engineering (STE)

Course Delivery Frequency | Every year

Students of Which Programs do You Recommend to Consider this Course as an Elective?

<table>
<thead>
<tr>
<th>Masters Programs</th>
<th>PhD Programs</th>
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<tbody>
<tr>
<td>Biotechnology</td>
<td>Life Sciences</td>
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<tr>
<td>Photonics and Quantum Materials</td>
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Course Tags | Physics, Biotechnology

6. Textbooks and Internet Resources

**Required Textbooks**

<table>
<thead>
<tr>
<th>Title</th>
<th>ISBN-13 (or ISBN-10)</th>
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**Recommended Textbooks**

<table>
<thead>
<tr>
<th>Title</th>
<th>ISBN-13 (or ISBN-10)</th>
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<tbody>
<tr>
<td>Optical Imaging Techniques in Cell Biology, Guy Cox. Second Edition</td>
<td>9781439848258</td>
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**Web-resources (links)**

<table>
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<tr>
<td><a href="http://www.zeiss-campus.magnet.fsu.edu/">www.zeiss-campus.magnet.fsu.edu/</a></td>
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7. Facilities

**Software**

ImageJ, ZEN Software, CellProfiler 3.0
Equipment

Imaging lab.

Labs for Education

Imaging Lab

8. Learning Outcomes

Knowledge

Modern techniques and methods in microscopy and imaging
Design of experimental research using imaging techniques

Skill

Ability to read and analyze scientific literature in broad area of imaging applications in basic biomedical research
Ability to critically assess data presented in the literature
Ability to present data in English and discuss experimental data.

Experience

Choosing the right microscopy technique for the research project

Do you want to specify outcomes in another framework?

Knowledge-Skill-Experience is good enough

9. Assessment Criteria

Select Assignment 1 Type

Project

Input Example(s) of Assignment 1 (preferable)

1. Array tomography
2. Iterative expansion microscopy
3. Optical transfer function
4. Structured Illumination Microscopy
5. Imaging CRISPR
6. Deep tissue imaging
7. Whole-body and whole-organ clearing reagents
8. Fluorescent protein indicators
9. Bioluminescence in preclinical research
10. Small microscopes for deep brain imaging
11. Brainbow
12. Calcium imaging in neurons
13. Two-photon excitation applications in neuroscience
1. Describe the properties and applications of fluorescent proteins and fluorescent dyes. Describe the differences.

2. Draw an image of an arrow placed vertically at the focal plane \( f \) of a thin convex lens.

3. Imagine you are working in a new cellular neurobiology lab studying the functions of individual neurons and their interactions. Please choose from the following microscopes those you would prefer to purchase:
   - confocal microscope with Airyscan
   - conventional upright microscope
   - two-photon microscope
   - super-resolution microscope (based on SIM technology).
   Explain why.

10. Additional Notes

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