DEPARTMENT OF CLINICAL NEUROSCIENCE

K8F2348, Functional Fluorescence Microscopy Imaging (fFMI) in Biomedical Research, 3 credits (hec)

Funktionell Fluorescens Mikroscopi Avbildning (fFMA) i biomedicinsk forskning, 3 högskolepoäng

Third-cycle level / Forskarnivå

Approval

This syllabus was approved by the The Committee for Doctoral Education on 2023-10-31, and was last revised on 2024-02-01. The revised course syllabus is valid from autumn semester 2024.

Responsible department

Department of Clinical Neuroscience, Faculty of Medicine

Prerequisite courses, or equivalent

No prerequisite courses, or equivalent, demanded for this course.

Purpose & Intended learning outcomes

Purpose

This is a basic course on advanced fluorescence microscopy imaging and correlation spectroscopy techniques for quantitative characterization of molecular transport and interactions in live cells. The purpose of the course is to give an introduction of the underlying physicochemical principles, hands-on training and an overview of applications of these specialized techniques in biomedical research. At the end of the course, the student will have hands-on experience with live-cell imaging and specialized fluorescence microscopy and correlation spectroscopy techniques. The course is suitable for doctoral students lacking training in mathematics, physics, or optical engineering who want to apply these techniques in their research.

Intended learning outcomes

1. Use fundamental aspects of molecular structure to explain light-matter interactions and the emission of fluorescence. Use this knowledge to discuss fluorescent properties of different types

of fluorophores.

2. Compare the buildup of different types of fluorescence microscopes. Identify different optical elements and explain their function.

3. Assess the strengths and weaknesses of different fluorescence labeling procedures and justify the choice in relation to the biological question studied.

4. Explain the theoretical background behind specialized fluorescence microscopy based methods for studying molecular interactions in live cells. Discuss pros and cons in relation to the biological question studied.

5. Explain the theoretical background behind different super-resolution microscopy techniques. Discuss pros and cons in relation to the biological question studied.

6. Assess the adequateness of the fluorescence microscopy based technique used in the scientific literature concerned. Evaluate pros and cons in relation to the biological question studied.

7. Select an appropriate fluorescence microscopy based technique for a biological question of interest, justify the choice and specify instrumental requirements.

Course content

Fluorescence microscopy and associated techniques are indispensable research tools for investigating molecular mechanisms of biological processes. Versatility of fluorescence microscopy based techniques comes from the possibility to characterize fluorescence emission by spatial position, intensity, wavelength, lifetime and polarization. In addition, fluorescence microscopy and correlation spectroscopy based techniques allow us to quantitatively study the cellular dynamics of molecules and the kinetics of their interaction with high spatio-temporal resolution and ultimate, single-molecule sensitivity. These techniques bring new biological insight at an unprecedented rate and are of crucial importance for the development of life sciences.

The course covers the following topics. Luminescence and the nature of light (Fluorescence, Phosphorescence, Light scattering). Fluorescent markers and their photo-physical properties (organic fluorescent dyes for covalent conjugation (Rhodamine 6G, Alexa dyes, Cyanine dyes); intrinsically Fluorescent Proteins (Aequorea victoria (GFP, YFP), Discosoma coral (DsRFP) and Montipora (Keima) families); selectively binding dyes (e.g. DAPI, Hoechst 33342, DraQ 5, DiI); quantum dots). Instrumentation for Confocal Laser Scanning Microscopy (CLSM): light sources, optical elements, objectives, detectors, read-out devices. Quantization and sensitivity in fluorescence imaging (instrumental sensitivity, method sensitivity, absolute sensitivity). Factors affecting quantitative accuracy. Point Spread Function. Spatially resolved fluorescence imaging: multi-photon excitation, Total Internal Reflection Fluorescence (TIRF) Microscopy, Single Plane Illumination Microscopy (SPIM), Super-resolution techniques (STORM, PALM and STED). Fluorescence based methods for studying molecular diffusion and interactions in live cells (FRAP, FRET, FLIM, FCS, FCCS, ICS). Image analysis techniques for quantitative characterization (CellProfiler).

Forms of teaching and learning

The course includes lectures, laboratory hands-on sessions, demonstrations, discussion sessions,

group journal club presentation and discussion session, quizzes for self-testing, and short written assignments.

Language of instruction

The course is given in English

Grading scale

Pass (G) /Fail (U)

Compulsory components & forms of assessment

Compulsory components

All sessions are compulsory. Please report any absence to the course organizers in advance by e-mail. Absence from any part of the course (lectures, laboratory sessions, discussion sessions and exam) is generally not accepted but could in special cases be compensated by an individually tailored additional module and a special written examination organized by the course committee.

Forms of assessment

The final assignment consists of a project report (5-10 pages presentation in PowerPoint) and an oral presentation of the project report (10 min + 5 min for Q & A).

Course literature

Recommended literature

Selected chapters from:
Joseph R. Lakowicz, Principles of Fluorescence Spectroscopy, Springer, 2006.
Pawley, James (Ed.) Handbook of Biological Confocal Microscopy, Springer, 3rd edition, 2006.

On-line virtual microscopy interactive tutorials:
http://www.olympusconfocal.com/java/index.html
https://www.microscopyu.com/tutorials
http://zeiss-campus.magnet.fsu.edu/tutorials/index.html
http://bitesizebio.com/category/technical-channels/microscopy-imaging/
http://fcsxpert.com/classroom/

Handouts:
Scientific papers with related methodology.