DEPARTMENT OF MEDICINE, HUDDINGE

H7F2870, Microscopy: Improve your Imaging Skills - from Sample Preparation to Image Analysis, 6 credits (hec)

Mikroskopi: förbättra dina kunskaper om imaging - från provberedning till bildanalys, 6 högskolepoäng

Third-cycle level / Forskarnivå

Approval

This syllabus was approved by The Committee for Doctoral Education on 2024-09-02, and was last revised on 2025-09-18. The revised course syllabus is valid from spring semester 2026.

Responsible department

Department of Medicine, Huddinge, Faculty of Medicine

Prerequisite courses, or equivalent

The applicants must:

a) have an active microscopy project involving imaging of a fluorescent sample, started minimum 3 months prior to the start of the course;

b) be able to prepare their sample and bring it to the course;

c) In their lab or local facility, have been trained on and have regularly used a fluorescence microscope able to acquire images of fluorescent samples;

d) have access to that microscope during the course (some assignments require submitting new images);

e) set aside time so they can be fully committed to the course for 3 weeks as well as the equivalent of a few days of work before the course (including preparing their samples).

Researchers who have not yet used microscopy or do not have an active microscopy project are advised to get trained at their local imaging facility, actively acquire images for at least 3 months then apply to the LCI course next year.

Purpose & Intended learning outcomes

Purpose

The aim of this course is to enable PhD students and researchers who have recently acquired images of fluorescent samples but feel insecure about their microscopy skills and knowledge, to become proficient in designing and performing microscopy for their OWN project.

The course is NOT aimed at training people to use the LCI facility microscopes. The focus is instead on enabling the students to acquire enough theoretical and practical knowledge to

1) assess and if needed improve the preparation of their OWN sample to enable extraction of reliable and meaningful data,

2) assess and if needed improve the imaging settings in their OWN software and on their OWN microscope, available in their lab/facility,

3) make their scientific question compatible with extracting data from fluorescence images.

The aim is to provide to the course students the tools needed to acquire on ANY wide field, confocal or light sheet microscope, images of their samples that reliably answer their scientific question.

Intended learning outcomes

At the end of the course, the participants should be able to:

1- Explain how their own microscope works and argue why this is the most suitable type of microscope to answer their scientific question, or why a different type of microscope would be more adequate.

2- Justify why the objective they use is adequate to answer their scientific question or why another objective would work better.

3- Calculate if the pixel size in their images fulfils the Nyquist sampling theorem, explain why this is appropriate for their experiment or which sampling settings would work better.

4- Identify typical pitfalls that make microscopy data unreliable (saturation, bleedthrough, undersampling), explain why they are a problem and which settings can be adjusted to avoid them.

5- Assess how their own sample corresponds to/deviates from a perfect sample for light microscopy, justify why they are preparing their sample the way they do, and infer how they can improve it.

6- Formulate their scientific question in terms of which metrics in the image need to be measured and which image resolution is required to enable this measurement.

Course content

Sample preparation, choice of microscope, objective and settings for acquisition, image format and management, image processing for data extraction, formulation of the scientific question, preparation of figures and text for publication, ethics.

The course is designed so that, throughout the course, the students apply all the points above to their OWN project, sample and equipment. The students never use the microscopes at our facility.

Aside from the points described in the Learning Outcomes, the participants will be able to learn

the following:

- The differences between wide field, confocal and light sheet microscopes as well as the different types of confocal microscopes
- How to pick the best combination of fluorophores for their own sample on their own microscope
- How to find the sample without bleaching it
- How to adjust the condenser for proper transmitted light imaging
- How to set the following microscope parameters: resolution, pixel size, averaging, scan speed, illumination power, detector gain and offset, camera readout rate, exposure time and binning
- Many practical tricks about fixation, mounting and handling of their sample in a way that is optimal for imaging
- Many personalized tips on how to improve the imaging of their own sample on their own microscope (through the workshop where we will image their own sample)
- How to deal with the challenges of imaging fluorescent volumes
- What hardware or software autofocus, spectral detector, resonance scanner are used for
- Where to get help to create an image analysis pipeline for their own images and scientific question
- The ethics of handling scientific images for publication
- How to easily assemble a figure for publications

Forms of teaching and learning

Lectures, videos, workshops, group discussions, project presentations, quizzes, assignments and portfolio.

Language of instruction

The course is given in English

Grading scale

Pass (G) /Fail (U)

Compulsory components & forms of assessment

Compulsory components

Attendance to all sessions is compulsory. Any absence must be reported to the course leader in advance by e-mail. Absence from any part of the course is generally not accepted but could in exceptional cases be compensated by a written additional assignment to ensure the learning outcomes of the day have been reached. If it is not possible to compensate, the student will be given the chance to complete the course by attending the missing sessions the following year.

Forms of assessment

The final mark (pass or fail) will depend on the way the students demonstrate in their portfolio assignments that they have reached the Learning Outcomes.

Course literature

Reference literature

Handbook of Biological confocal microscopy, James Pawley Springer Editions 2006 (available in PDF format from the KI library)

Other information

Replacing H2F2870.