# Welcome to "Physics of Biomedical Microscopy"

This is the course PM of the course SK2500, "Physics of Biomedical Microscopy", a 6.0 credit course on microscopy.

It is also the course PM of the course SK2501, " "Physics of Biomedical Microscopy, extended course", a 7.5 credit course which is identical to SK2500, but contains a seminar at the end of the course. The seminar will be scheduled once the course starts.

It is also the homepage of the PhD courses FSK3500/FSK3501. Lectures and labs, seminar and exam are identical to SK2500/SK2501. PhD students also need to complete a small project, preferable related to their PhD project.

The course consists of lectures, labs and written exam. For SK2501 there is also the seminar. For FSK3500/3501 there is also a project.

The course literature is two compendia written by Kjell Carlsson. They can be found on the course Canvas page.

Course responsible and teachers are Anna Burvall and Ilaria Testa.

# To pass the course

To pass the course, you should:

- complete all four <u>labs</u>
- complete a <u>lab report</u> on lab 4 and reach pass level
- pass the <u>exam</u>
- For SK2501 and FSK2501, complete and pass the seminar
- For FSK3500 and FSK3501, complete a project including presentation and report

The result on the exam determines the grade on the course.

### **Intended learning outcomes**

After completing the course the student should be able to:

- adjust the illumination system to obtain optimal performance in transmission microscopy.
- select a suitable light source and optical filters, and correctly adjust the illumination system for fluorescence microscopy.
- select a suitable objective (correction, immersion etc) for various types of microscopic investigations.
- select a suitable contrast method (phase contrast, DIC, fluorescence, darkfield etc) and correctly use this technique to obtain high-quality images.
- the expected image quality regarding resolution and signal-to-noise ratio for different practical imaging situations.
- understand and be able to describe the physical limitations for microscope performance concerning resolution and signal-to-noise ratio.

- describe performance for different types of microscopes by using (and in some simple cases calculating) optical transfer functions.
- select a suitable sampling density for digital image recording in microscopy.
- do computer processing of microscopic images to visualise three-dimensional structures.
- perform quantitative measurements in microscopic images using a computer.
- extract relevant information from a scientific publication and present this in the form of a seminar.

# Lectures

Lecture schedule, autumn 2017

Lecture	Contents	Compendium
	Basics of light microscopy	
1	Imaging ray-path and illumination ray- path	<i>LM</i> : sect. 1.1 – 1.2, App. I
	Aberrations, objective types, magnification, numerical aperture (IT)	
2	Contrast techniques:	
	Absorption, fluorescence, phase contrast, DIC, dark-field (IT)	<i>LM</i> : sect. 1.5
3	Fundamentals of radiometry and photometry, microscope photometry,	<i>LM</i> : sect. 1.6, App. IV
	detectors, noise (IT)	<i>IP</i> : p. 5-16
4	Resolution, mathematical representation of the imaging process (AB)	<i>IP</i> : p. 17-23
		<i>LM</i> : р. 12-14
5	The Fourier transform and its interpretation, the optical transfer function OTF (AB)	<i>IP</i> : p. 23-29

6	OTF for diffraction-limited optics, 2- dimensional Fourier transforms, 2D OTF, OTF for detectors (AB)	<i>IP</i> : p. 29-47 <i>LM</i> : sect. 1.3
7	OTF for an imaging chain, sampling and aliasing, reconstruction calculations, multidimensional sampling (AB)	<i>IP</i> : p. 47-64
8	Coherent imaging in microscopy, role of condenser numerical aperture (AB)	<i>LM</i> : sect. 1.4
9	Introduction to confocal microscopy, (AB)	<i>LM</i> : sect. 2.1
10	Imaging properties of confocal microscopy (AB)	<i>LM</i> : sect. 2.2
11	Confocal microscopy: Limitations and errors, multi-channel detection (AB)	<i>LM</i> : sect. 2.3-2.4
12	Super resolution fluorescence microscopy, Stimulated emission depletion microscopy (IT)	<i>LM</i> : sect. 3
13	Super resolution fluorescence microscopy, Single Molecules based microscopy (IT)	LM: sect. 3
14	Problem solving (IT)	

*Literature references: LM* = Light Microscopy compendium, *IP* = Imaging Physics compendium

*Lecturers*: AB = Anna Burvall, IT = Ilaria Testa

# Labs

Each student will perform four 4-hour laboratory exercises. The contents are given in the table below. A suitable schedule will be worked out at the start of the course.

#### Lab # Contents

- Build your own microscope with Koehler illumination
  Practical use of research microscopes in different imaging modes
  Confocal microscopy #1
- 4 Confocal microscopy #2

Course laboratory: Roslagstullsbacken 21, level 2, see map at the bottom of the page.

Sign-up for labs will start at 13.00 on Tuesday November 5th.

# Please note that the lab sessions start at 8:00 and 13:00, not quarter past like the lectures!

A report on labs 3 and 4 should be handed in (see "Assignments" in the white side bar) by Jan 11th.

# Read the introductory parts and literature references (if any) before the lab session. In some cases there are problems that you should look at before the lab session.

### Exam

Written examination (TEN1; 4 credits). 5 hours, 6 problems. Max. 10 points/problem.

Grading:

- 53-60p = A
- 45-52p = B
- 37-44p = C
- 30-36p = D
- 25-29p = Fx
- < 25p = F.

Students who receive grade Fx can, after completing a supplementary examination, receive grade E (pass).

Times and locations:

• Wed. Jan. 14, 2020, 14-19, FB55

The exam sets the grade on the course. Other tasks such as labs must also be complete before the entire course is reported into ladok.

Old exams are available.

#### Aids allowed at the written examination:

- Compendium "Imaging Physics," will be handed out. (Chap. 19, Problems and solutions, and appendices 1-5 and 7 are not included. Appendices 6 and 8 are included.)
- Compendium "Light Microscopy" (will be handed out).
- Extracts from Mathematics Handbook Beta (chapter 13, Fourier series & transforms; will be handed out).
- Pocket calculator.
- Normal aids such as pens/pencils, eraser, ruler, food.

No "pure" math problems will be given at the written examination, but the student may be required to calculate simple Fourier transforms in order to solve problems dealing with imaging physics.

Bringing something to eat and drink is strongly recommended, as the exam lasts for 5 hours.