Monday, November 22, 2021

08:15-08:45 Registration

08:45-09:00 Introductory word

09:00-09:30 Image formation in light microscopy | Ivan Novotny
Spatial frequency, light diffraction, PSF – point spread function, resolution

09:30-10:15 Light microscopy instrumentation | Pavel Krist
Components of microscope: light sources, objectives, fluorescent filters, cubes, principles of detection

coffee break

10:30-11:15 Contrast-enhancing techniques in optical microscopy | Martin Čapek
Köhler illumination, BF/DF, Phase and Nomarski contrast

11:15-11:45 Object visualization in fluorescence microscopy | Pavel Hozak
Methods of biological sample visualization in fluorescence microscopy: immune labeling, expression and practical approaches

lunch

12:45-13:30 Confocal scanning microscope: principles and new trends | Pavel Hozak
Confocal microscope construction and image formation, pinhole – optical sectioning and resolution

13:30-14:00 Spinning disc confocal microscope: principles, advantages | Michaela Blazikova
Image formation in spinning disc microscope, pinhole size and distance, microlenses

coffee break

PRACTICAL PART

14:30-17:30 4x 45 min:

- Confocal Spinning disc (Andor Dragonfly, room 0.171) | Michaela Blazikova
- Confocal Microscope (Leica STED, room 0.174) | Ivan Novotny
- Fluorescence microscope (Leica DM6000, room 0.172) | Jiri Cerny
- Adjusting of Köhler illumination and Phase contrast + DIC (room 0.173) | PRAGOLAB

Tuesday, November 23, 2021

09:00-09:30 Visualization of cell nucleus in super-resolution microscopy | Peter Hoboth
scientific lecture

09:30-10:15 Fluorophores | Jan Sykora
Principles of fluorescence, types of fluorophores

coffee break

10:30-11:15 Super-resolution approaches in light microscopy | Ivan Novotny
Principles and tricks, SMLM, SIM and STED

11:15-11:45 Sample preparation for super-resolution microscopy | Ivan Novotny
Size and thickness of the sample, refractive index mismatch and spherical aberration minimizing, mounting & sealing

Lunch workshop

13:15-14:00 Computative high resolution methods| Michaela Blazikova
Adaptive deconvolution, optical reassignment, SRRF…

coffee break

PRACTICAL PART

14:30-17:30 4x 45 min:

- SIM (OMX, room 0.174) | Michaela Blazikova
- STED (Leica STED, room 0.174) | Ivan Novotny
- Lightning (Leica SP8 - room 0.172) | Davide Basello
- Lattice SIM / PALM (Elyra7, room 0.174) | ZEISS

Wednesday, November 24, 2021

09:00-09:30 Adventure in variety of live-cell imaging | Davide Basello
scientific lecture

09:30-10:00 Introduction to live cell imaging | Ivan Novotny
Essential equipment: plastic and consumables, incubation, gas, autofocus systems, objectives

coffee break

10:15-10:45 Time-resolved live cell imaging I. | Michaela Blazikova
FRAP, FCS

10:45-11:15 Time-resolved live cell imaging II. | Michaela Blazikova
FLIM-FRET

lunch

12:00-12:45 Light Sheet microscopy | Jiri Cerny
Principles of spatially illuminated microscopy, dual-side and multi view acquisition and data processing

12:45-13:30 Quantitative phase microscopy | Martin Capek
Principles of the method, advantages of quantitative phase image in segmentation and analysis

coffee break

PRACTICAL PART

14:00-17:00 4x 45 min:

- Time-resolved: FRAP and FLIM (Leica Stellaris 8, room 0.172) | Michaela Blazikova
- Live cell imaging (Andor Dragonfly, room 0.171) | Ivan Novotny
- Quantitative Phase Microscope (Telight QPi, room 0.175) | Martin Capek
- Light Sheet (Zeiss Z.1, room 0.173) | Jiri Cerny

17:00 Closing remarks

Thursday, November 25, 2021

09:00-09:45 Image formation in transmission electron microscope | Oldřich Benada
Electron microscopy basics, properties of electrons, resolution, wavelength of accelerated electrons, the electrons in an electromagnetic field. electron-electron interaction and analytical electron microscopy, transmission electron microscope – design, image creation, interference, image acquisition in the TEM

09:45-10:30 Scanning electron microscopy | Oldřich Benada
Scanning electron microscope – the construction, signals and image recording (secondary and back-scattered electron imaging), Scanning Transmission Electron Microscopy, SEM image interpretation

coffee break

10:45-11:30 Sample preparation for TEM | Jana Nebesářová
Physical and chemical principles of sample preparation for electron microscopy; chemical methods - fixation, dehydration, infiltration, embedding, preparation of ultrathin sections, contrasting; physical methods - low-temperature processes, microwaves

11:30-12:15 Sample preparation for SEM | Jana Nebesářová
Fixation, dehydration, CPD method, metal coating, freeze fracturing, etching and freeze drying. Sample preparation for cryo-SEM, low vacuum SEM; correlative light-electron microscopy (CLEM), strategies for biological applications; analytical morphomics (morphology, identification, content, functionality)

Lunch

13:15-14:00 Advanced electron microscopy techniques | Vlada Filimoněnko
Ultrastructural immunolabeling (imunogold), volume electron microscopy, cryo electron microscopy, analytical techniques

coffee break

PRACTICAL PART

14:30-17:30 4x 45 min:

- Transmission electron microscopy (JEM-1400FLASH, room 01.152) | Dominik Pinkas
- Scanning electron microscopy (building "U") | Oldřich Benada
- High-pressure freezing, freeze substitution and ultramicrotomy (EM CF labs - rooms 01.155.1., 01.155.2) | Erik Vlčák
- EM immunolabeling: detection, clustering and colocalization in new online software tióol Pattern (lecture room 0.195) | Vlada Filimoněnko

Friday, November 26, 2021

09:00 - 09:45 Introduction to image deconvolution | Ivan Novotný

Principles of image deconvolution, technical aspect of required image quality, contribution of PSF and noise, expected results.

09:45 - 10:30 Optical projection tomography | Martin Čapek

Visualization of big 3D specimens, sample volume reconstruction from physical slices, principles of computed and optical tomography, transmission and fluorescence modes, optical clearing.

coffee break

10:45 - 11:30 Image acquisition by two-photon microscopy | Daniel Hadraba

11:30 – 12:15 Introduction to image processing | Jiří Janáček

Digital coding of multispectral images – formats of image files. Dimensional calibration. ImageJ (FiJi) – widely used freeware for scientific image processing. Basic manipulation with images. Regions of interest. Overlays – scale bar and annotation. Macro language and plugins

Lunch

13:15 – 14:00 Image analysis and visualization in 3D | Jiří Janáček

Quantitative information in images - geometric characteristics of real biological samples and how to estimate them. Visualization of 3D data from biomedical modalities – surface rendering, maximum intensity projection and volume rendering. Visualization cues – movies, lighting, texture, stereopsis, fog, depth color coding etc..

14:00 – 14:45 Preparation of digital images for publication| Oldřich Benada

coffee break

15:00 – 15:30 Recap + Evaluation