PROCESSING AND ANALYSIS OF MICROSCOPIC IMAGES IN BIOMEDICINE (PAMIB)

4-8 October 2021

4th OCTOBER – MONDAY

09:00 - 09:45 **Digital image formation,** Michaela Blažíková, theoretical lecture

Lecture introduces you into formation of digital images, basic principles of cameras, image discretization. It will tell you what pixels, pixel size, bit depth, magnification and field of vision are. Luminescence, intensity and color will be described as well.

Keywords: image formation, pixel, intensity.

09:50 - 10:35 **Digital image terminology,** Ivan Novotný, theoretical lecture

The lecture will explain basic terms in the field of digital image processing, e.g., resolution and sampling using explanation of Nyquist theorem, histogram and its reading, image depth (8/16/24/32 bit), noise, LUT.

Keywords: resolution, sampling, histogram.

10:55 - 11:40 **Digital image terminology (continuation) / Introduction into Fiji - Part1,** Ivan Novotný / Michaela Blažíková, theoretical lecture / practicals

What is Fiji useful for? What is a multipage image? Where do we have colors coded? Grayscale vs. RGB; channels vs. stacks, hyperstacks.

Aims: We will do Fiji installation and solve update issues, demonstrate file opening/file type setting/bit depth setting/file saving, contrast/brightness adjusting/image resizing. Then we will work with channels, will split and merge images, define channel color, learn image stacks/hyperstacks, something about image dimensionality and setting image properties.

Requirements: laptop with Fiji, Java up to date
Keywords: file type, image adjustment, stack, hyperstack, multipage image, LUT.
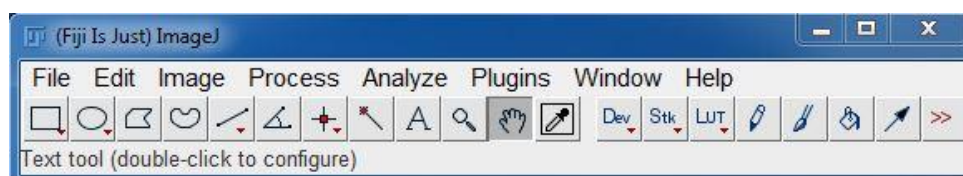
11:45 - 12:30 **Introduction into Fiji - Part2,** Michaela Blažíková, practicals

Aim: In this practical we will continue with our work in Fiji and will learn basics of image manipulation.

12:30 - 13:30   **LUNCH**

13:30 - 14:15   **Introduction into Fiji – Part3**, Michaela Blažíková, practicals

Aims: We will pay attention to thresholding, image filtering, enhancement and denoising. We will create animations, demonstrate orthogonal views, montages, RGB image manipulation, MIP, Z-Project.

Requirements: laptop with Fiji, Java up to date
Keywords: thresholding, image filtering, denoising, orthogonal views.

14:20 - 15:05   **Image analysis in Fiji**, Michaela Blažíková, practicals

Aims: We will examine several simple image analysis tools available in Fiji. Using examples, we will learn how to work with ROI manager, how to use profile plots. We will use thresholding to measure characteristics of objects in the image, find edges and maxima in the image. We will count particles in 2D and 3D, perform 2D image registration, create a kymograph and learn how to use plugins.

Requirements:
- laptop with Fiji
- image data
- both images and plugins can be downloaded from the course webpage

Keywords: ROI, thresholding, image registration.

15:10 - 15:55   **Fiji: Stand-alone practical tasks,** Michaela Blažíková, practicals

Aims: The participants will work on selected simple image processing and analysis tasks on their own, using Fiji software.

Requirements:
- laptop with Fiji
- image data
- both images and plugins can be downloaded from the course webpage

Keywords: hyperstack, image adjustment, ROI, thresholding.

5th OCTOBER – TUESDAY

09:00 - 09:45   **Image acquisition conditions and deconvolution**, Ivan Novotný, theoretical lecture

The image acquisition and requirements for successful deconvolution will be described together with description of various microscope types and their specificity. Quality of images regarding deconvolution results will be discussed as well.

Keywords: widefield fluorescent microscope, confocal microscope, STED, point spread function (PSF), z-stack, SNR.

09:50 - 10:35 **Huygens: Image deconvolution I**, Ivan Novotný, practicals

10:55 - 11:40 **Huygens: Image deconvolution II**, Ivan Novotný, practicals

11:45 - 12:30 **Huygens: Image deconvolution III (stand-alone practical tasks)**, Ivan Novotný, practicals

Aims: Practical deconvolution of various microscopic data. Image metadata and image parameters for the deconvolution. Parameters of the deconvolution – SNR, iterations, quality threshold.

Requirements:
- laptop with Huygens Professional Trial
- image data can be downloaded from the course webpage

Keywords: Image metadata, image parameters, deconvolution, Huygens, SNR.

12:30 - 13:30 **LUNCH**

13:30 - 14:15 **Segmentation methods**, Martin Čapek, theoretical lecture

We will define what segmentation is, will classify segmentation methods and will describe individual basic approaches, like region growing and splitting, Watershed transform, thresholding, Hough transform, model-based comparisons, border areas, active contours, and others. We will show how to use Fiji for solution of segmentation tasks. Practical examples from biomedical practice – using region growing for fusion of CT and MRI data sets, line segmentation for stitching medical images will be exemplified a well.

Keywords: segmentation, object finding, thresholding.

14:20 - 15:05 **Fiji: Using segmentation for detection of structures in various microscopic images**, Martin Čapek, practicals

Aims: 1. To do simple segmentation tasks using a micro CT image of a tooth; 2. To detect circular objects – sections through an embryonic mouse intestine – in a confocal image using the Hough transform; 3. To automatically count number of red blood cells in a phase-contrast image by the Watershed transform; 4. To detect erythrocytes in a DIC (differential interference contrast) image using template matching; 5. To remove air bubbles in 3D data acquired by an optical projection tomography microscope (OPT) by using Segmentation Editor plugin.

Keywords: Hough transform, region growing, Watershed transform.

15:10 - 15:55 **Fiji: Artificial Intelligence (AI) Approaches to image segmentation**, Martin Čapek, practicals

Aim: By using various plugins in Fiji that incorporate techniques of artificial intelligence we will segment objects of interest in microscopic images.

Requirements:
- laptop with Fiji installed
- image data
- installed plugins
- both images and plugins can be downloaded from the course web page

6th OCTOBER – WEDNESDAY

09:00 - 09:45 **I am watching you or what it means „tracking"**, Michaela Blažíková, theoretical lecture

Very fast development of light microscopy methods allows us to collect large amount of dynamic data. We are not only able to localize organelles or protein complexes but also individual molecules in time. In this lecture we will focus on methods used to determine the parameters of the movement of intracellular organelles and protein complexes, specifically their velocity and trajectory. Together with changes in organelle sizes and changes in fluorescence, the resulting information represents an output that uniquely characterizes the specific process. Examples from projects that enabled us to clarify character of movement and interactions of intra-nuclear proteins, localization of proteins into cell membranes and dynamics of endoplasmic reticulum will be presented.

Keywords: tracking, trajectory, localization.

09:50 - 10:35 **Fiji: Tracking – practicals**, Michaela Blažíková, practicals

Aims: To track objects in the image during the time lapse experiment using several Fiji plugins. The participants will obtain several time series with moving objects and areas. Using Fiji plugins, we will track the objects in time, characterize their movement using velocity and trajectory, and compare the results.

Requirements:
- laptop with Fiji
- image data
- both images and plugins can be downloaded from the course webpage
- *MosaicToolSuite* from update site

**Keywords:** tracking, movement characterization, localization.

10:55 - 11:40 **Evaluation of colocalization in microscopic images**, Martin Čapek, theoretical lecture

This lecture explains conditions for getting images suitable for colocalization, with image pre-processing, and describes basic colocalization approaches, like Pearson's correlation coefficient, Manders' coefficients, Costes' methods, Van Steensel' approach. Object based colocalization, free programs and Fiji plugins available for image colocalization will be discussed as well.

Keywords: colocalization, image similarity, Pearson's correlation coefficient.

11:45 - 12:30 **Fiji: Evaluation of colocalization in various microscopic images**, Martin Čapek, practicals

The **goal** of this exercise is to evaluate the colocalization in high quality image data using *JACoP* plugin (*J*ust *A*nother *Co*localization *P*lugin) and to analyze low quality noise images and data with bleed through using *Coloc 2* plugin. Images of a dimeric protein stained with greed and red dyes, respectively, and images of cells stained by GFP and DAPI will be used as examples.
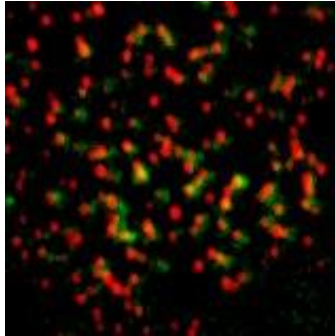
Requirements:
- laptop with Fiji
- image data
- installed plugin - *jacop_.jar*
- both images and the plugin can be downloaded from the course web page

Keywords: low quality microscopic data, colocalization, *JACoP*, *Coloc 2*.

12:30 - 13:30   **LUNCH**

13:30 - 14:15   **Pattern: Evaluation of colocalization in EM data**, Vlada Filimonenko, practicals (demo)

Aims: The practicals include the introduction into the topic of the quantitative evaluation of the spatial dependencies and associations in point patterns. One of the fields of application is analysis of immunolabeling in transmission electron microscopy (TEM) images. TEM labelling shares the molecular mechanisms with the immunolabeling technique in fluorescent microscopy (FM), however, the method of detection is different. TEM visualization technique requires the presence of the metal nanoparticles, which are conjugated with the antibodies, resulting in point labelling pattern. Our recently developed free online software tool "Pattern" allows for semi-automated detection of gold particles in EM images and statistical evaluation of their clustering, colocalization and mapping of significantly labelled areas. This part will be presented in the form of a demonstration. Instructions and sample images for testing of Pattern tool will be provided following the presentation.
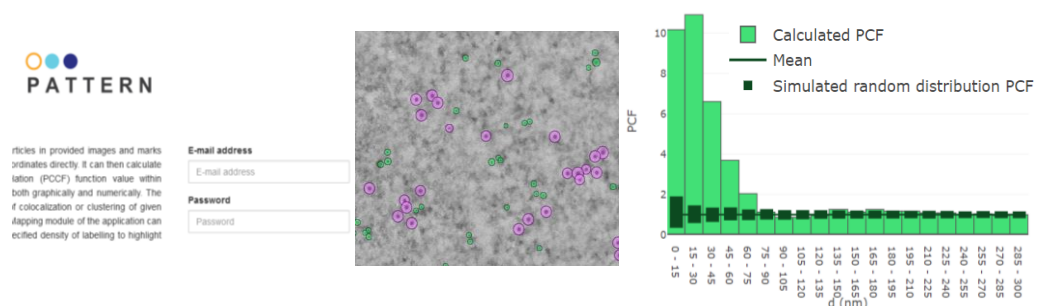
14:20 - 16:30   **Fiji: Macros - Introduction into IJM language**, Martin Čapek, theoretical lecture with demos

The lecture will give you basics of ImageJ Macro (IJM) language. This includes knowledge of variables, operators, conditional and looping statements, strings, debugging macros, built-in macro functions, which is necessary for creating your own macros.

14:20 - 16:30   **Fiji: Using macros for data processing and analysis**, Martin Čapek, practicals

Aims: Introduction into macro recorder and macro editor. Creating macros for segmentation and counting nuclei in multiple images by using macros; the automatic processing of open images.

Requirements:
- laptop with **Fiji**
- **image data and macros** can be downloaded from the course webpage

7th OCTOBER – THURSDAY

09:00 - 09:45   **3D and 4D image visualization and analysis in Imaris**, Daniel Reisen, BITPLANE (www.bitplane.com), theoretical lecture

Imaris includes a set of key tools, which cater for the needs of researchers in live cell imaging and, in particular, developmental biology, but also in Neurobiology. Furthermore, it addresses the ability to work on large datasets. The available tools include:
- Visualization of terabyte multidimensional data sets
- Detection, tracking and analysis of cells and organelles
- Tracking of cell division, lineage analysis
- Rotational and translational drift correction
- Angle measurements
- Neuron and spine analysis
- Colocalization studies
- A wide range of plugins (XTensions) and advanced interactive plotting for results exploration and comparison between samples

09:50 - 10:35   **Imaris: Examples of interactive image analysis and visualization (using cloud computers)**, Daniel Reisen, practicals

Aims: Introduction into the basic functionalities for experiment data management, visualization and segmentation of the 3D and 4D microscopy datasets. Multiple volume rendering and modes, clipping planes, cross-section slices, animations and snapshots will be demonstrated:
- User interface - Arena/Surpass/Vantage
- Surface detection and extraction of statistical data
- Spots detection and extraction of statistical data
- Tracking examples

- Colocalization
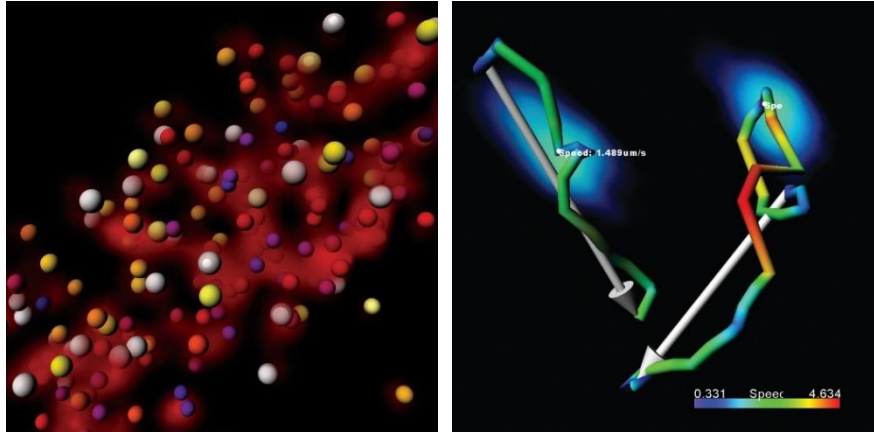
10:55 - 11:40 **FRAP data analysis**, Michaela Efenberková, theoretical lecture

One of the very important and also very fast microscopic methods for analysis of protein dynamics in a specific cell region is FRAP (fluorescence recovery after photobleaching). It is based on the principle that we are able to bleach the fluorescence of selected fluorescence proteins by their targeted high intensity illumination. In the illuminated region no recovery of the fluorescence is assumed, therefore, the fluorescence that starts to appear in the photobleached region results from proteins that come from unbleached areas. According to properties of the curves describing the fluorescence recovery we are able to deduce the amount of mobility of the specific protein. In this lecture the FRAP method and its modifications will be presented and especially possibilities of its evaluation will be discussed.

Keywords: photobleaching, FRAP, FRAP parameters, mobility.

11:45 - 12:30 **Fiji: FRAP data analysis**, Michaela Efenberková, practicals

Aims: To determine the mobility of small nuclear ribonucleoprotein particles (snRNP) that move around and interact inside the cell nucleus using FRAP (fluorescence recovery after photobleaching). The participants will obtain time series of fluorescence images with snRNP-GFP. A complete FRAP experiment, i.e. the situation before photobleaching, closely after photobleaching and in a few time points during the recovery will be provided. The aim of this task is to evaluate the presence of the signal in both bleached and reference areas and to determine the representation of mobile and immobile protein fraction. We will also examine several ways how to determine other FRAP curve parameters. At the end we will compare the results.

Requirements:
- laptop with Fiji
- image data
- script http://fiji.sc/Analyze_FRAP_movies_with_a_Jython_script
- both images and the script can be downloaded from the course webpage

Keywords: FRAP, curve fitting, curve parameters, mobility.

12:30 - 13:30 **LUNCH**

13:30 - 14:15   **3D image processing and geometrical modelling**, Jiří Janáček, theoretical lecture

Main topics of the talk are the processing of 3D biomedical images and visualization of the spatial data. Detection of capillaries in confocal images and construction of 3D models from tomographic data or physical sections will be presented.
- Data sources: CLSM, MRI, tomography, registration of physical slices.
- Voxel size determination, correction of sample shrinkage.
- Filtration, segmentation and isosurface detection.
- Measurement of volume, surface area and length in 3D.
- 3D visualization: volume and surface rendering; visual cues: stereoscopy, motion, shading and texture.

Keywords: 3D reconstruction, triangulated isosurface.

14:20 - 15:05   **Fiji: Image filtration / Morphological image processing and analysis**, Jiří Janáček, practicals

Aims: Basic microscopy image processing and segmentation with Fiji using linear filtration and mathematical morphology filters. Important features on 2D images (thick objects, fibers, dots) and on 3D images (thick objects, surfaces, fibers, dots). Properties of image noise.

- Gaussian filter and its action on noisy image features.
- Morphological operations (erosion, dilation, opening, closing, volume opening) for artefacts removal.
- Rolling ball and Lipschitz filter for background removal.
- Stack contrast adjustment.
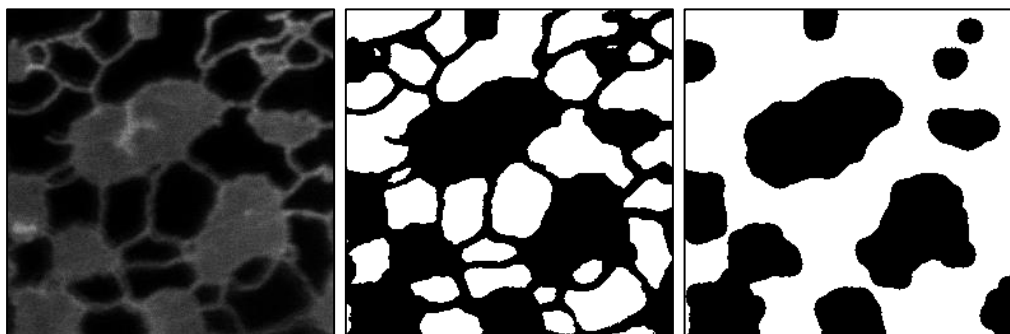
Requirements:
- laptop with Fiji
- both images (retic/ 04_10_04_latrunkulin_500nM_lat25min_5_ch00.tif, capillaries_brain.tif) and the plugin (*biomat.jar*) can be downloaded from the course webpage

Keywords: mathematical morphology, image noise, image filtration, Gaussian filter.

15:10 - 15:55 **Stereological methods and measurement**, Barbora Radochová, theoretical lecture

This is an introductory lecture into stereology and sampling. Several methods used in stereology will be explained together with the principle of systematic uniform random (SUR) sampling. Stereological methods can be used for estimating geometrical features like volume, length, surface area and number of particles. You will learn at least one method for each type of feature.
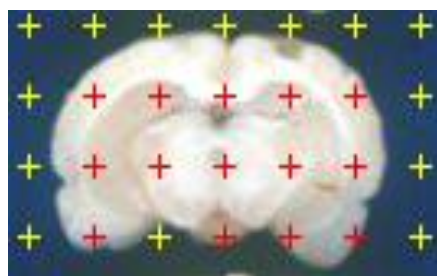
Keywords: stereology, volume, surface area, length, number.

8th OCTOBER – FRIDAY

09:00 - 12:30 **Estimation of volume and surface (Point Grid, Cavalieri's principle and Fakir)**,
Barbora Radochová, practicals in two parallel separated groups

Aims: We will learn how to estimate volume using Point grid method and Cavalieri´s principle on 2D data (images of a rat´s brain) in FIJI (Grid Tool). We will then use this method also on 3D data (series of optical sections from an isolated islet of Langerhans). We will pay attention to random sampling of the sections. We will then use Fakir method (Fakir sw) to estimate both volume and surface area of the same islet of Langerhans and compare the results. At the end we will try in practice the principle of systematic uniform random (SUR) sampling on real carrot – we will estimate its volume by point grid method and compare it with carrot´s real volume. The real volume of carrot will be found with the help of graduated cylinder.

Requirements:
- laptop with **Fiji** and installed *Fakir* sw, **mouse with a wheel button**
- **image data** – rat´s brain, islet of Langerhans
- carrot, graduated cylinder, razor blade, worksheets, point counting grid (these will be distributed)

Keywords: point counting, Cavalieri's principle, SUR, Fakir, real volume

Estimation of length and particle numbers (Slicer, Disector), Barbora Radochová,
practicals in two parallel separated groups

Aims: We will learn how to estimate length in 3D space using Slicer method on 3D data (capillaries in a rat´s brain). We will revise the principles of particle counting in 2D (Gundersen) and 3D (Disector) space. Then we will use Disector plugin in FIJI to estimate the density of particles in the series of optical sections).

Requirements:

- laptop with **FIJI** (Windows 7 or newer) with installed *Disector* plugin
- *Slicer* sw
- **image data** – 3D stacks of images (capillaries and particles)

Keywords: slicer, disector, length, particle counting.

09:00 - 12:30   **3D analysis: Scale setting, 3D image filtration and measurement in Fiji**, Jiří Janáček, practicals in two separated groups

Aims: To learn basic skills in 3D image processing. Measurement of tobacco cell volume and measurement of length of capillary system in skeletal muscle.

1. Correction of the light attenuation.
2. Filtering out the noise by Gaussian, image thresholding.
3. Watershed operation for separation of objects.
4. Volume, surface area and length measurement in 3D image.

Requirements:

- laptop with *Fiji*, plugin *MorphoLibJ* (from *IJBP-plugins* update site), plugin *Biomat* (from *Biomat* update site)
- **image data** (testball.pic, tab18m.pic, capillaries_brain.tif, batcochlea.tif) - can be downloaded from the course web page

Keywords: Gaussian filtering, mathematical morphology.
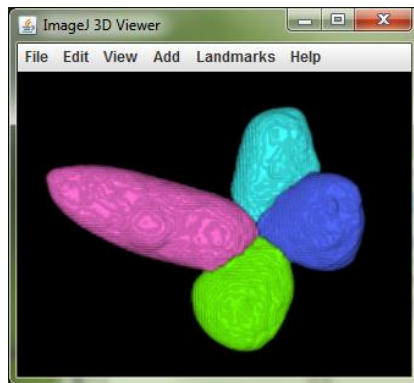
Triangulated surfaces reconstruction, Jiří Janáček, practicals in two separated groups
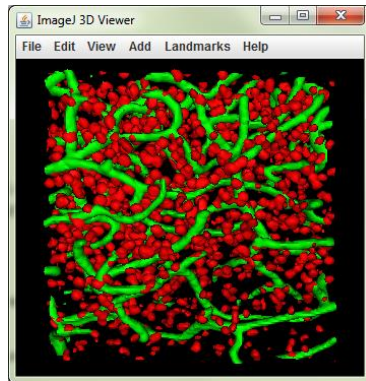
Aims: To visualize tobacco cell and brain capillaries with cell nuclei from CLSM images.

1. Isosurface detection and export.
2. Animation of 3D models.

Requirements:

- laptop with *Fiji*
- **image data** (tab17m.tif, capillaries_brain.tif) - can be downloaded from the course web page

Keywords: isosurface detection.

12.30 - 13.00 INFORMAL LUNCH WITH PIZZA

13.00 - 14.00 FINAL COURSE EVALUATION AND CERTIFICATE HANDOVER