31st March – Monday

9:00 - 9:45 **Digital image formation and terminology,** *Michaela Blažíková*, lecture

The lecture introduces you to the formation of digital images, basic principles of cameras, and image discretization. It will explain what pixels, pixel size, resolution, bit depth, magnification, field of vision, histogram, LUT are.

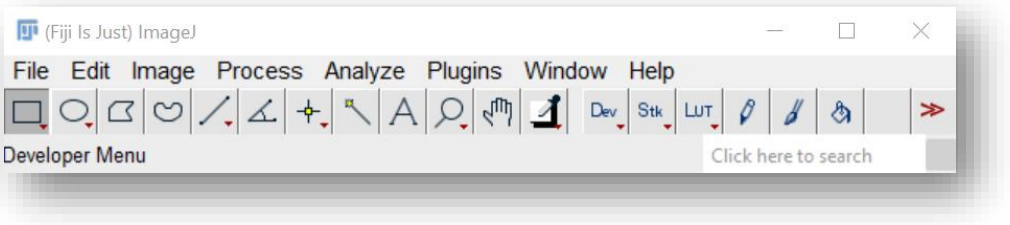
Keywords: image formation, pixel, intensity, resolution, sampling.

9:50 - 10:35 **Introduction into Fiji - Part1,** *Jan Valečka*, 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, 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.



10:55 - 11:40 **Introduction into Fiji - Part2,** *Jan Valečka*, practicals

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

11:45 - 12:30 **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.

12:30 - 13:30 **Lunch**

13:30 - 14:15 **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.

14:20 - 15:05 **Guidelines for processing and presenting microscopy images in scientific publications**, *Jan Valečka*, lecture

Aims: This theoretical lecture will provide specific guidelines for showing microscopy images in presentations and how to process them for scientific publications, what adjustments are allowed and what are not allowed.

15:10 - 15:55 **NIS Elements: Demo of simple analyses and advanced automatized tasks,** *Michaela Blažíková,* demo with practicals

Aims: This demo will introduce the NIS Elements software, the type of analysis and image processing tasks that can be performed in it. The participants will learn how to build up simple workflows.

1st April – Tuesday

9:00 - 9:45 **Image acquisition conditions and deconvolution**, *Ivan Novotný*, 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.

9:50 - 10:35 **Huygens: Image deconvolution I**, *Ivan Novotný*, practicals

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

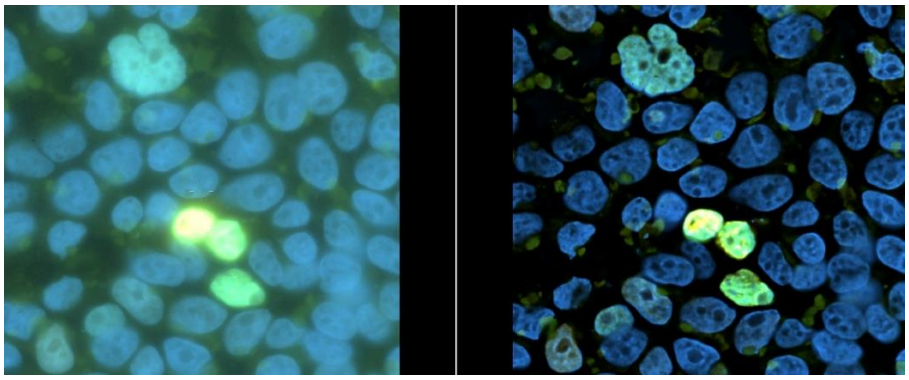
11:45 - 12:30 **Huygens: Image deconvolution III (stand-alone practical tasks)**, *Ivan Novotný*, practicals

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
- 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*, lecture
We will define what segmentation is, classify segmentation methods and will describe individual basic approaches, like region growing and splitting, Watershed transform, thresholding, model-based comparisons, active contours, and others. We will show how to use Fiji for solution of segmentation tasks.

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 automatically count number of red blood cells in a phase-contrast image by the Watershed transform; 3. To detect erythrocytes in a DIC (differential interference contrast) image using template matching; 4. Simple segmentation using Level Sets; 5. To remove air bubbles in 3D data acquired by an optical projection tomography microscope (OPT) by using Segmentation Editor plugin.

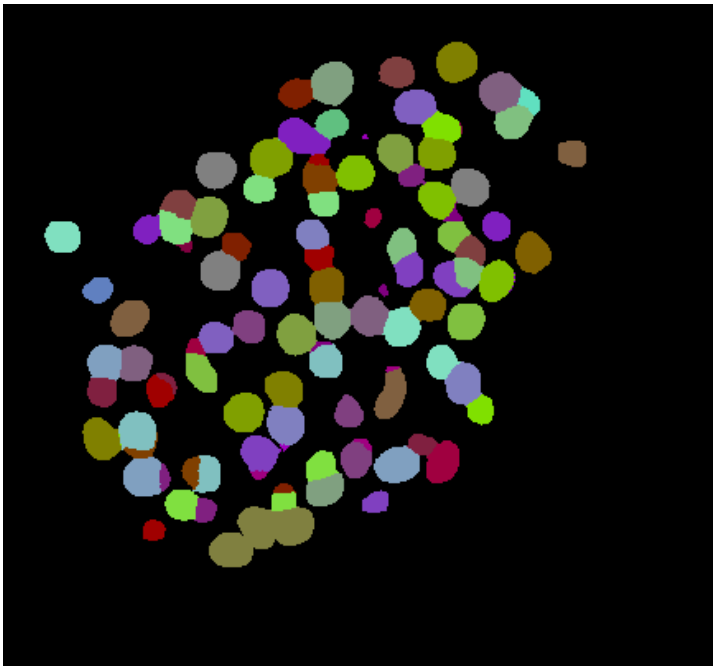
Keywords: region growing, Watershed transform.

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

Aim: By using plugins in Fiji that incorporate techniques of artificial intelligence – Trainable Segmentation Weka, StarDist – 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



2nd April – Wednesday

9:00 - 9:45 **Tracking – principles and algorithms**, *Michaela Blažíková*, lecture

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.

9:50 - 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

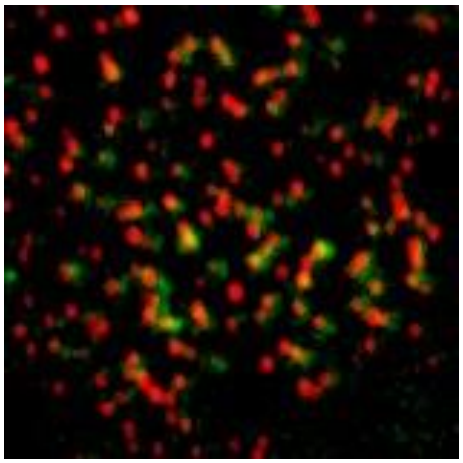
Keywords: tracking, movement characterization, localization.

10:55 - 11:40 **Evaluation of colocalization in microscopic images**, *Martin Čapek*, 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 *BIOP 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 green and red dyes, respectively, and images of cells stained by GFP and DAPI will be used as examples.

Requirements:

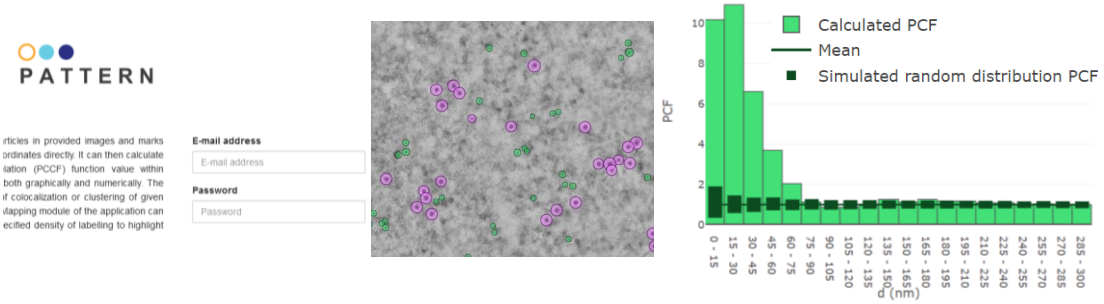
- laptop with Fiji
- image data
- plugins from the **PTBIOP** update site installed
- both images and the plugin can be downloaded from the course web page

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 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 - 15:05 **Fiji: Macros - Introduction into IJM language**, *Jan Valečka*, lecture with demos

The lecture will give you the 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.

15:10 - 15:55 **Fiji: Using macros for data processing and analysis**, *Jan Valečka*, 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

3rd April – Thursday

9:00 - 9:45 **3D and 4D image visualization and analysis in Imaris**,
Oxford Instruments, https://imaris.oxinst.com/, **distant online lecture**

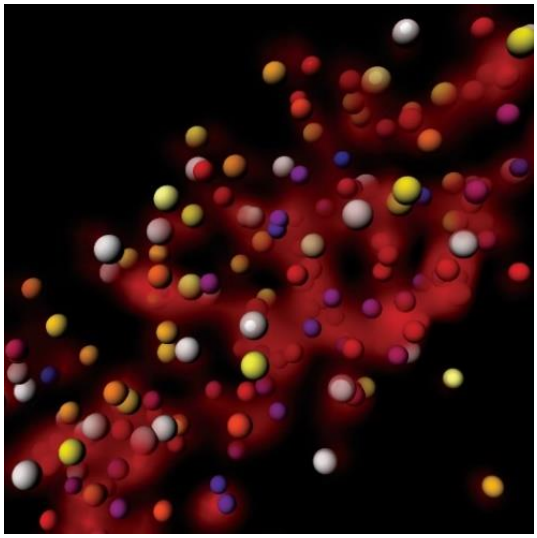
Imaris includes a set of key tools, which cater for the needs of researchers in live cell imaging and developmental biology, but also in Neurobiology. Furthermore, it addresses the ability to work on large datasets. The available tools include:

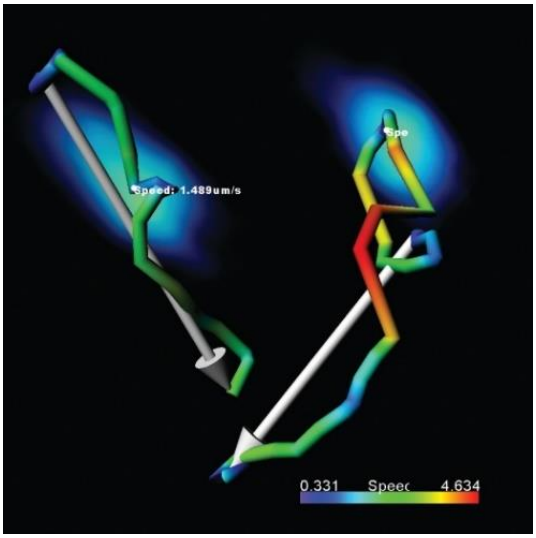
- Visualization of terabyte multidimensional data sets
- Detection, tracking and analysis of cells and organelles
- Tracking of cell division, lineage analysis
- Rotational and translational drift correction
- Angle measurements
- Neuron and spine analysis
- Colocalization studies
- A wide range of plugins (XTensions) and advanced interactive plotting for results exploration and comparison between samples

9:50 - 10:35 **Imaris: Examples of interactive image analysis and visualization (using cloud computers)**, Oxford Instruments, https://imaris.oxinst.com/,
online practicals using cloud computers

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 Blažíková*, lecture

One of the very important and 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 can 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 can 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 Blažíková*, 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.

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*, 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.

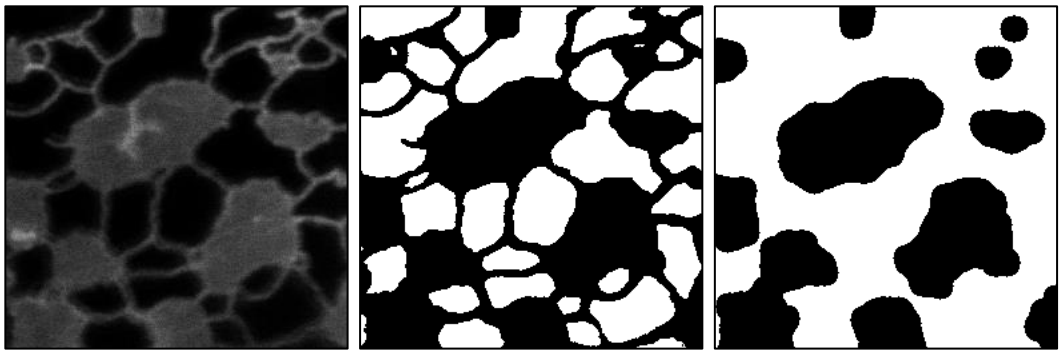
- Data sources: CLSM, MRI, tomography, registration of physical slices.
- Voxel size determination, correction of sample shrinkage.
- Filtration, segmentation and isosurface detection.
- Measurement of volume, surface area and length in 3D.
- 3D visualization: volume and surface rendering; visual cues: 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.



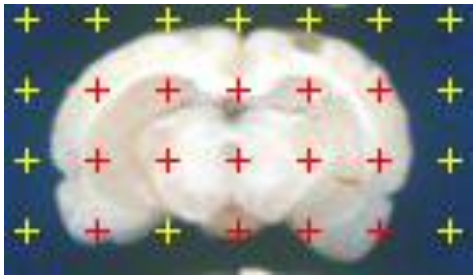
Requirements:

- laptop with Fiji
- both images (retic/ 04_10_04_latrunkulin_500nM_lat25min_5_ch00.tif, capillaries_brain.tif)
- plugin ***Biomat*** (from ***Biomat*** update site)

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

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

This is an introductory lecture on 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.

4th April – Friday

9:00 - 12:30 **Estimation of volume and surface area (Point Grid, Cavalieri's principle and Fakir)**, *Barbora Radochová*, **practicals in two parallel separated groups**

Aims: We will learn how to estimate: 1) volume of a rat´s brain using Point grid method and Cavalieri´s principle in FIJI (Grid Tool); 2) volume of a real carrot using the principle of systematic uniform random (SUR) sampling; 3) volume and surface area of an isolated pancreatic islet using Fakir method (Fakir sw).

Requirements:

- laptop with **Fiji** and ***Fakir*** sw, **mouse with a wheel button**
- **image data** – rat´s brain sections, islet of Langerhans (3D stack of images)
- carrot, graduated cylinder, razor blade, worksheets, point counting grid (these will be distributed)

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

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

Aims: We will learn how to estimate: 1) length of capillaries in a rat´s brain using Slicer method (Slicer sw); 2) number of cells in a rat´s brain using Disector method in FIJI (plugin Disector).

Requirements:

- laptop with ***Slicer*** sw and FIJI with installed ***Disector*** plugin
- **image data** – capillaries and cells (3D stacks of images)

Keywords: Slicer, Disector, particle counting

9:00 - 12:30 **3D analysis: Scale setting, 3D image filtration and measurement in Fiji**, *Jiří Janáček*, **practicals in two separated groups**

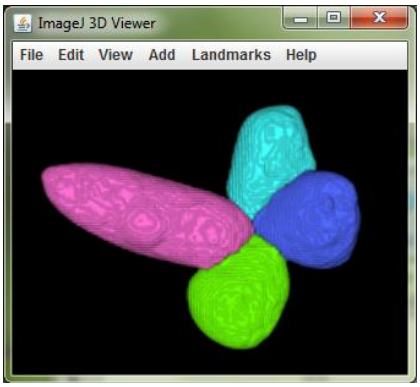
Aims: To learn basic skills in 3D image processing. Measurement of tobacco cell volume and measurement of length of capillary system in skeletal muscle.

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**

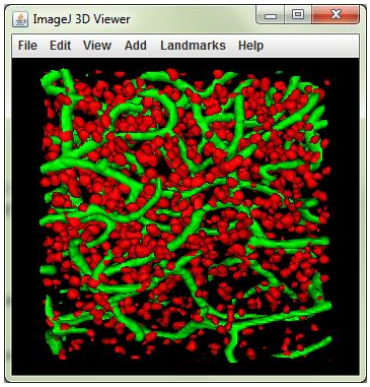
Aims: To visualize tobacco cells and brain capillaries with cell nuclei from CLSM images.

1. Iso-surface 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: iso-surface detection.



12.30-13.00	**Final course evaluation and Certificates handover**
13.00-14.00	**Informal lunch**