

Jiří Janáček



# Introduction to image processing



Microscopy methods in biomedicine, October 17<sup>th</sup>, 2025

1

## Imaging

Light, electrons, X-rays  
Microscopes, tomographs

3

## Analysis

Measurement -  
Geometrical and/or optical  
characteristics

2

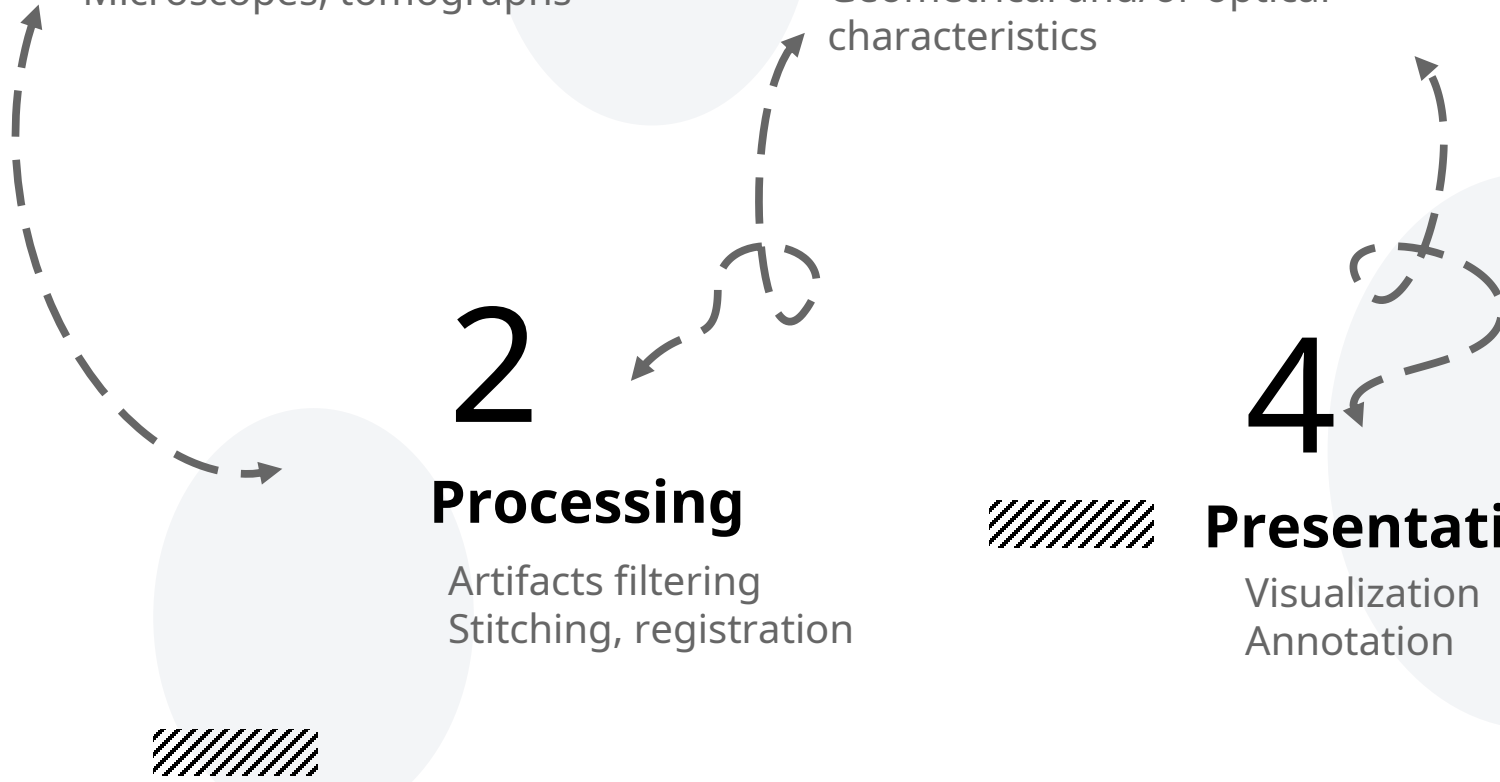
## Processing

Artifacts filtering  
Stitching, registration

4

## Presentation

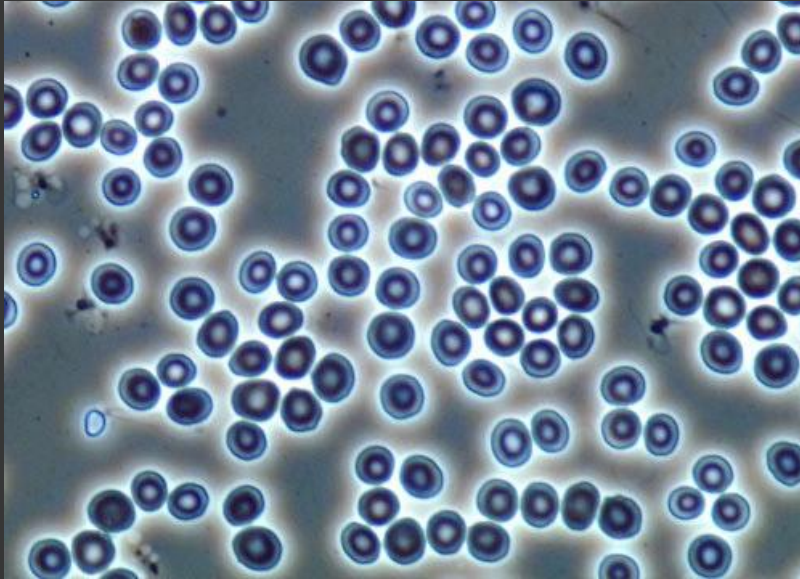
Visualization  
Annotation



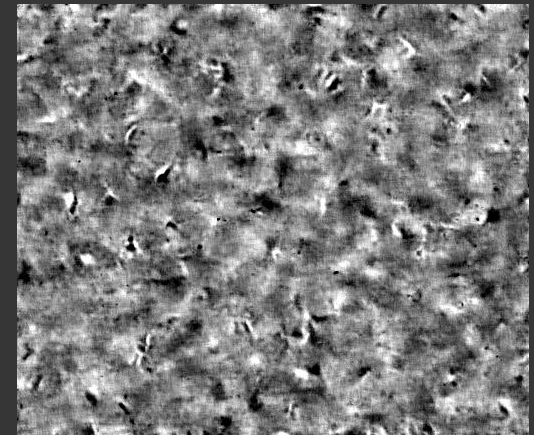
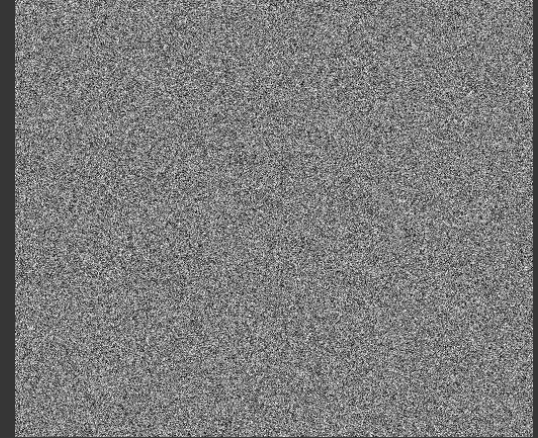
# Microscopic images

- The images are results of sample selection and preparation, microscopic modality, digitization and processing
- They contain **information** and **artifacts**
- Interpretation of images by observer depends on human visual system and experience.

# Artifacts in microscopic modalities and image processing

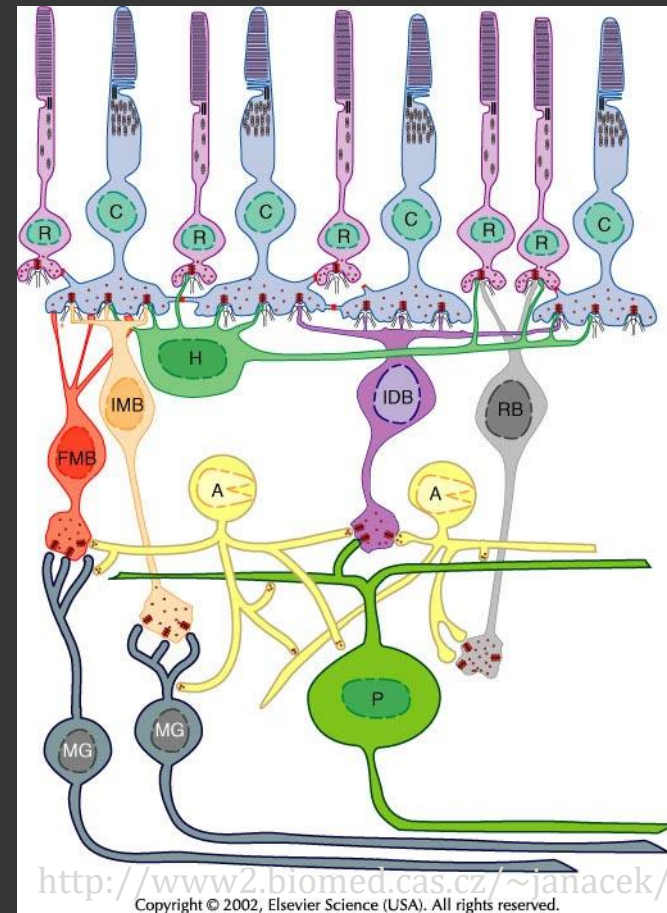
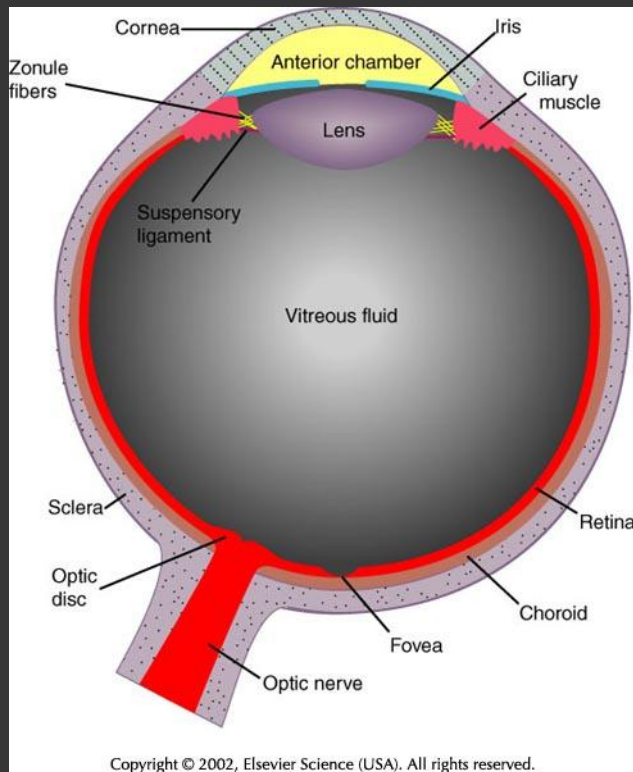


- Erythrocytes, phase contrast
- AI “improvement” of noise:



# Visual perception of images

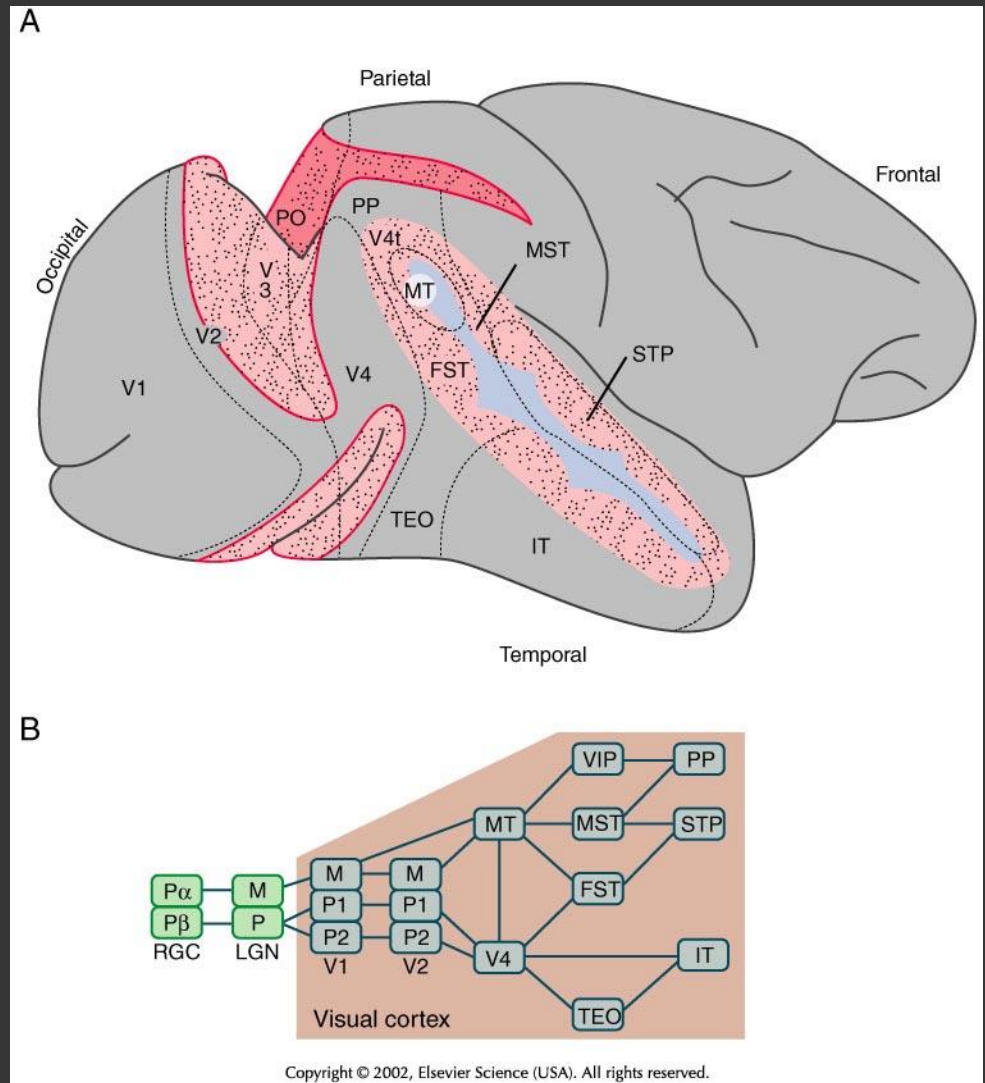
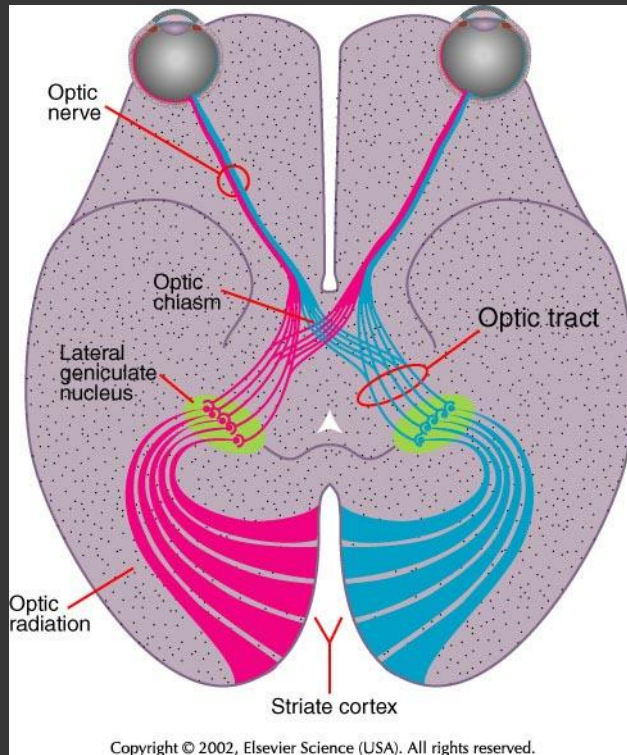
- Object recognition by visual cues is a complex process involving retina ...





# Image visual perception

- ...and visual cortex



# Visual cues for detection of objects

- Color, texture (contrast w.r.t. background)
- Boundary
- Size, shape
- Continuous movement or change

# Automated processing and analysis

- Purpose: Extraction of information, artifacts removal.
- Automated processing and analysis can be reproducible and less biased.
- Automation saves labor and time.



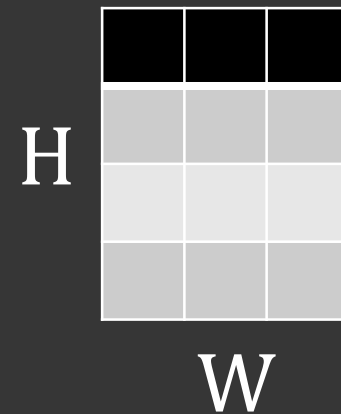
# Sources of digital images

Digital cameras (CMOS, CCD, cooled, electron multiplying - EMCCD) connected to microscope  
Scanning microscope (laser scanning confocal, SEM, AFM)

Connected to PC (grabber card, USB, wifi...)  
Setting sensitivity to **cover the value range** (bits, signed), (White ballance), **dimensional calibration**.

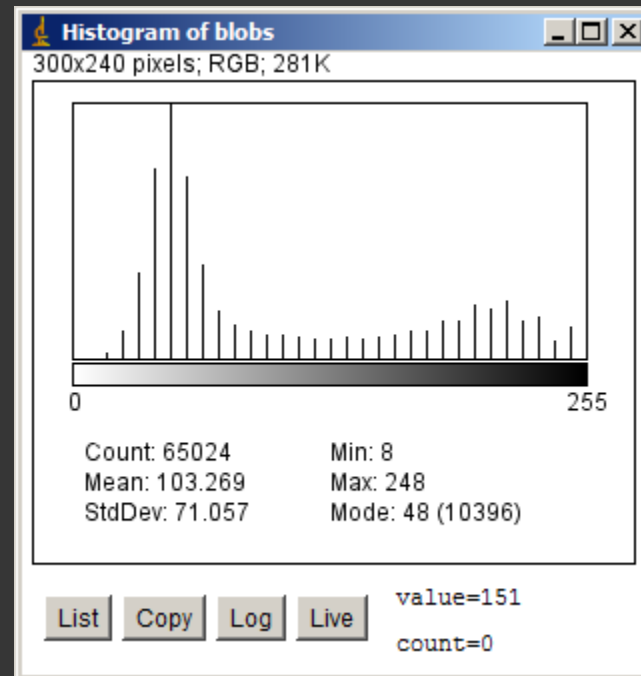
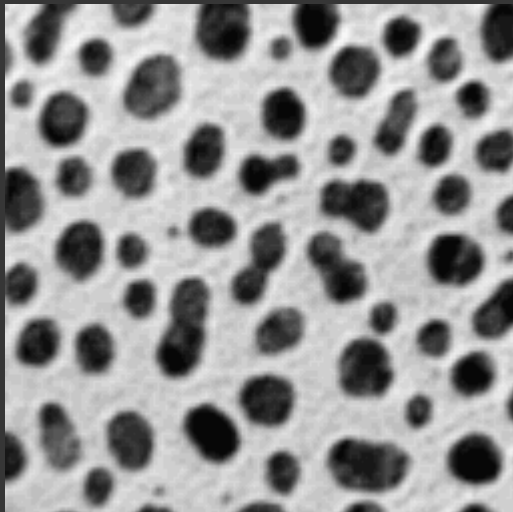
# Digital images of real objects, mathematical definition

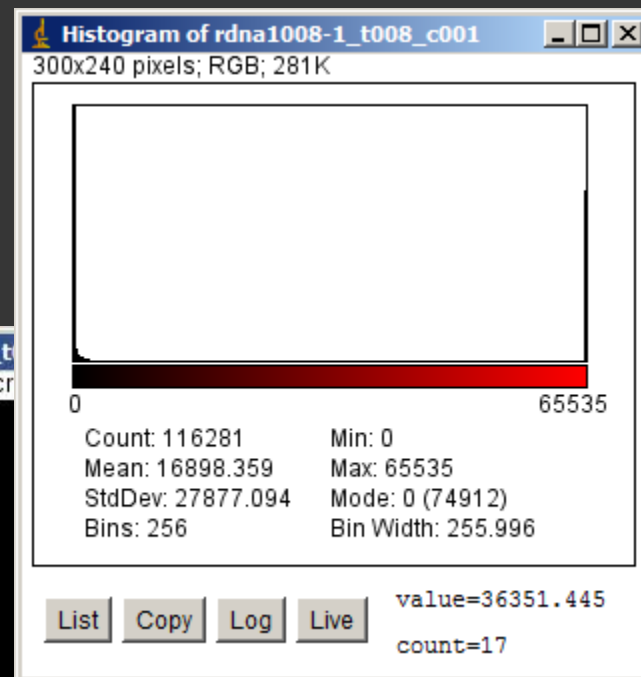
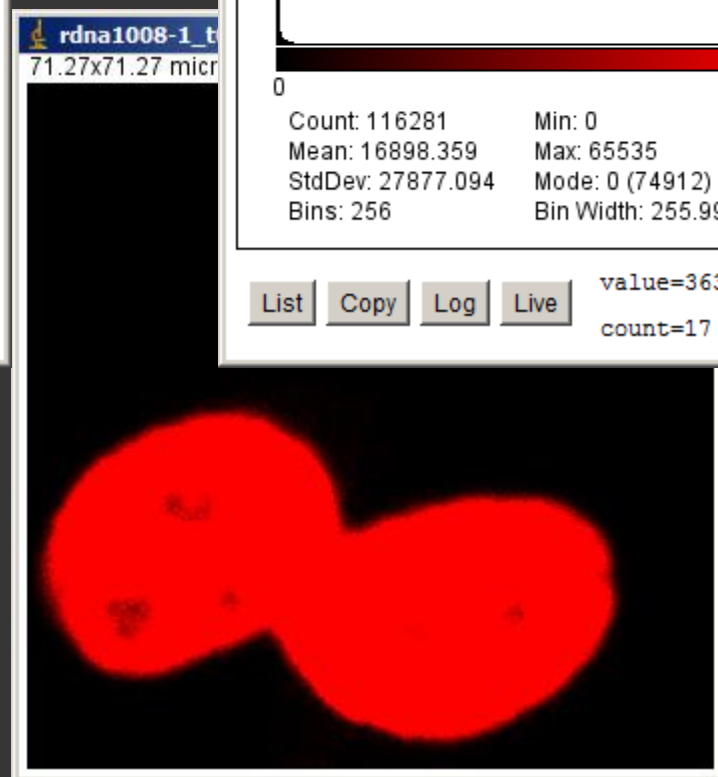
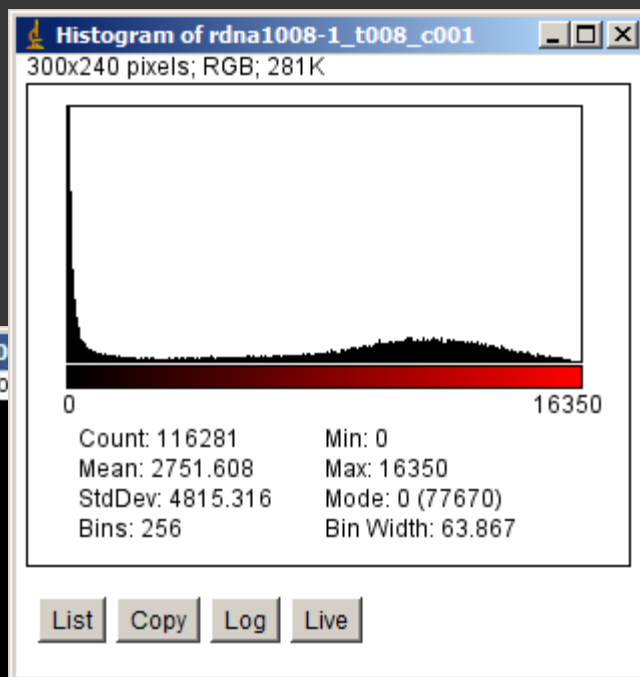
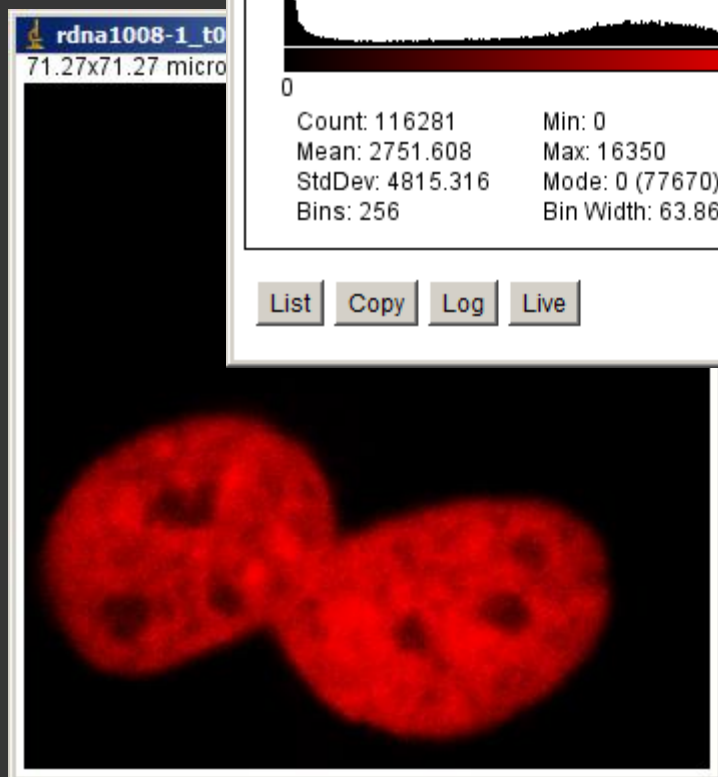
- Matrix of  $W \times H$  pixels
- Pixel value (format)
  - 1 or more numbers
  - 8, 16, 32 bits each
  - Signed or unsigned
- Pixel size, calibration



# Histogram of values (i.e. covering the value range)

- WB image “Blobs”

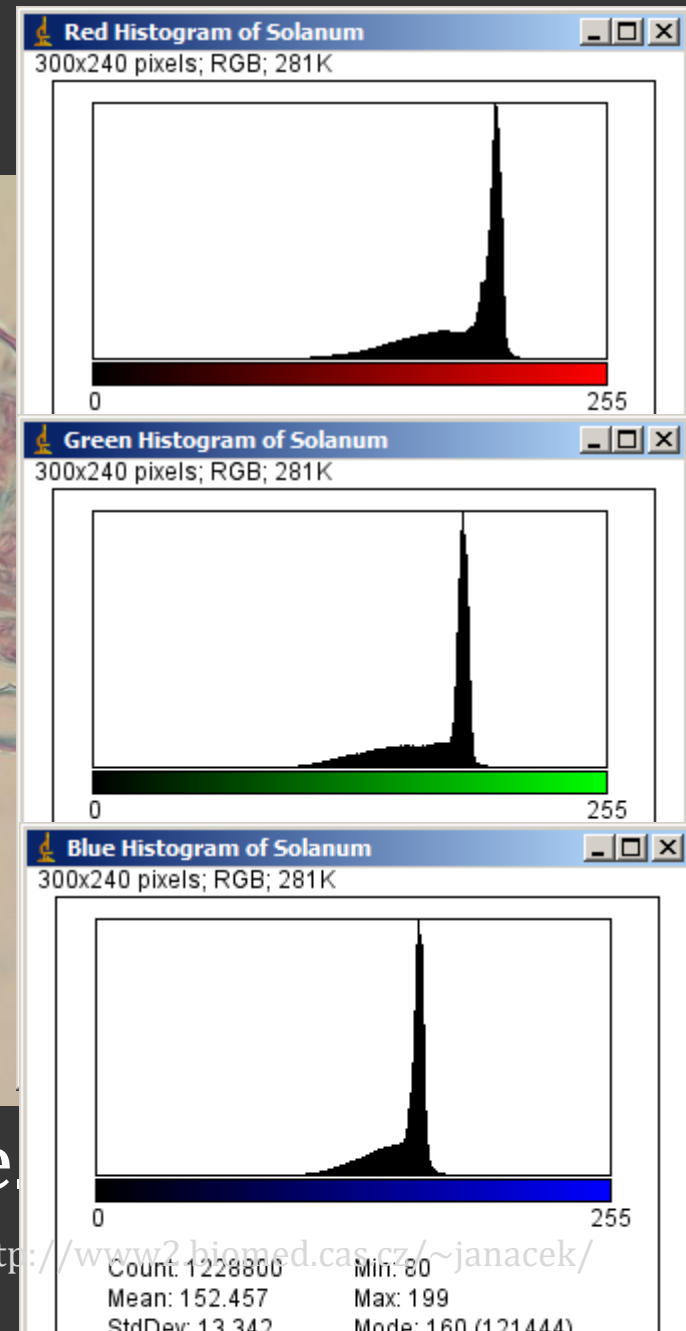




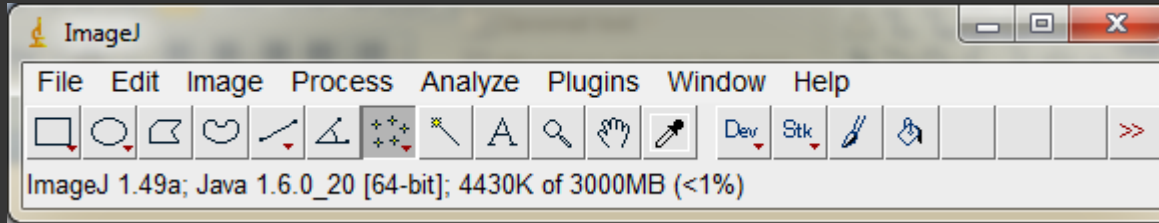
# Color histogram



- W. Balance, dynamic range



# ImageJ free program

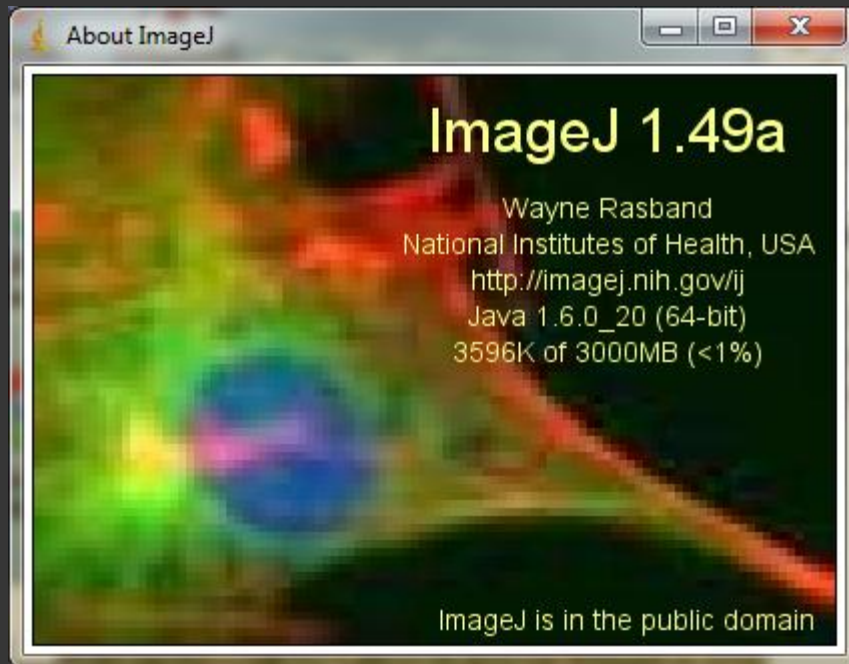


Window:  
Menu  
Toolbar  
Information

- Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA.
- FiJI is just ImageJ -  
<https://imagej.net/software/fiji/>



# Web, documents, support

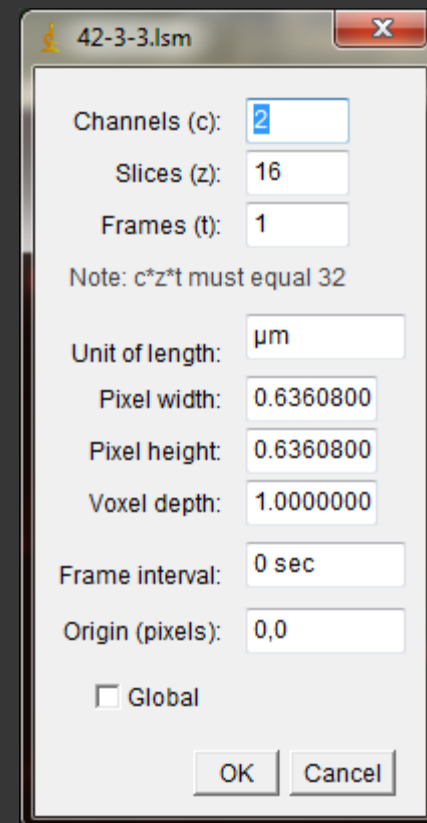
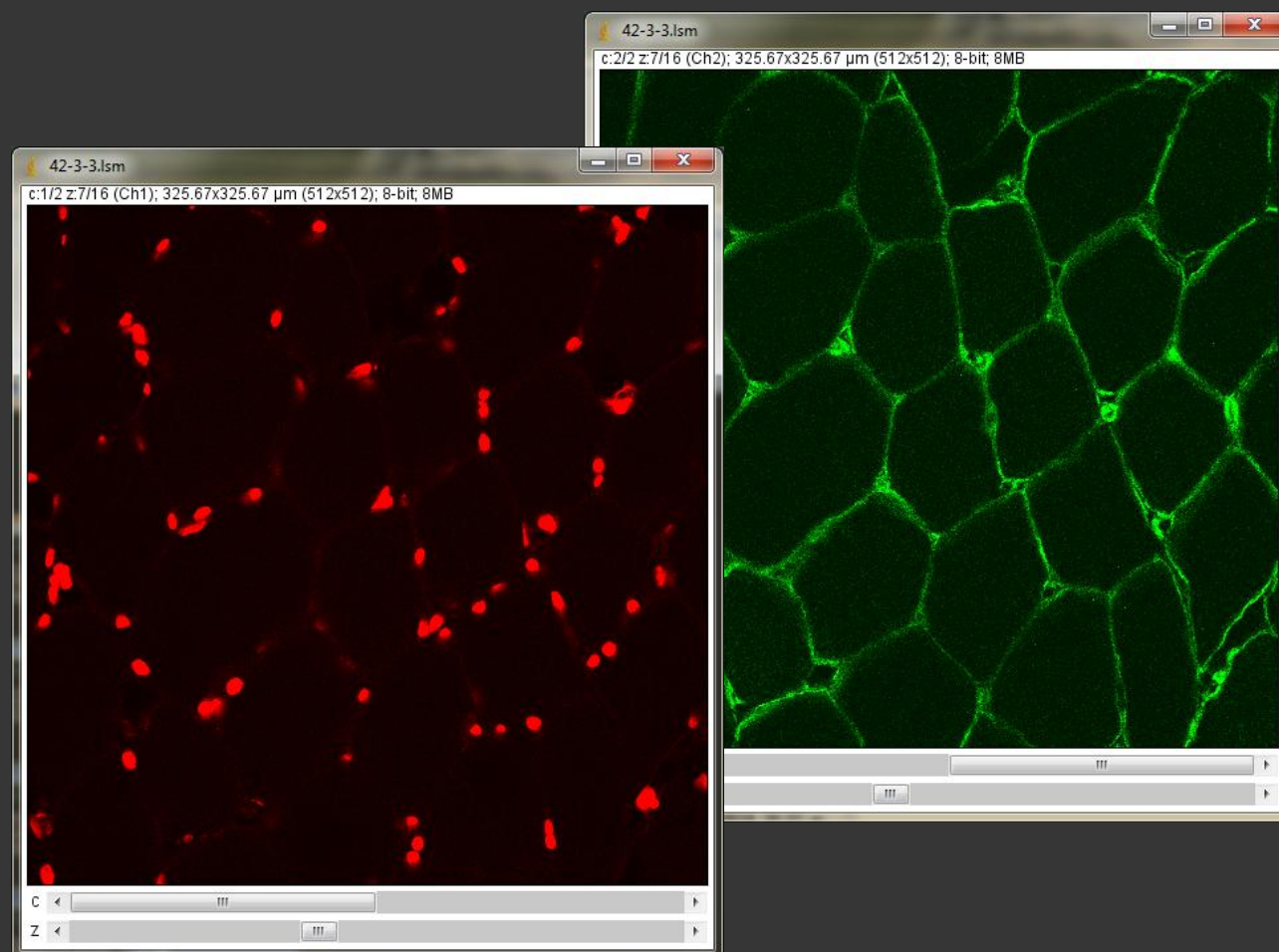


- <https://imagej.net/ij/>
- Download: ImageJ User Guide
- Textbook: Image Processing with ImageJ

# Image formats

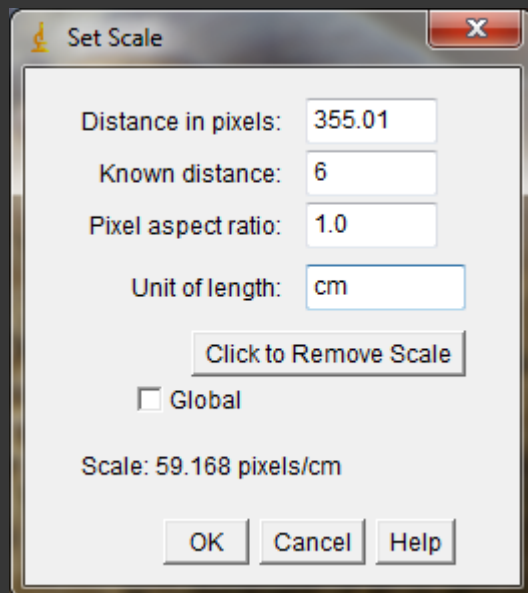
- Basic: 8-bit, 16-bit, 32-bit TIFF, GIF, JPEG, BMP and "raw" input
- 3D or video: stacks, sequences and multiTIFFs input
- Microscopic and biomedical: BioFormats LOCI (biophotonics research laboratory at the University of Wisconsin-Madison). DICOM, Leica, Zeiss, Nikon, Olympus etc.

# LSM Zeiss



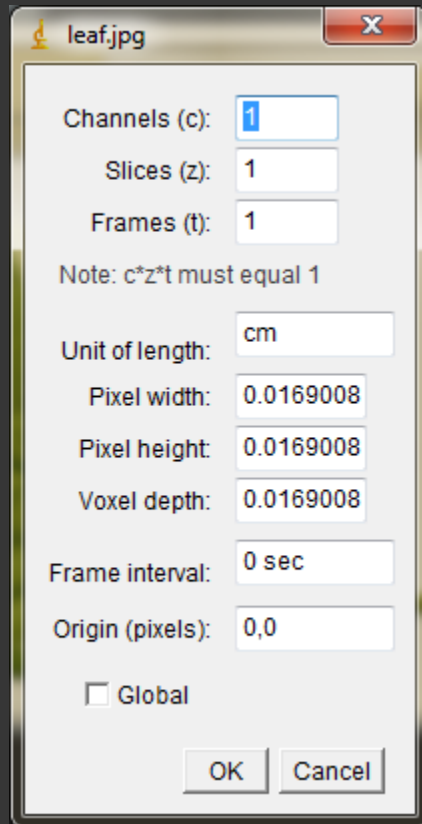
# Calibration

- Analysis>Set scale



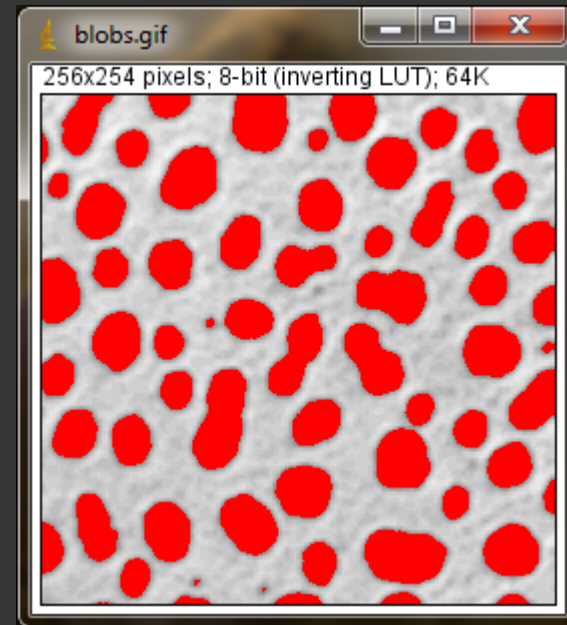
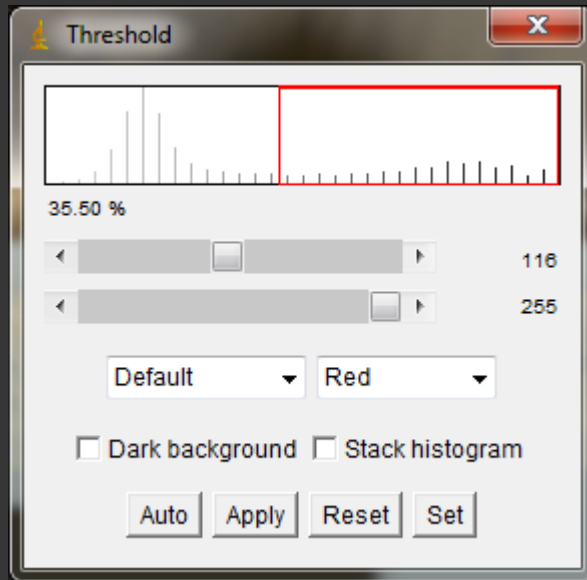
# Calibration

- Image>Properties



# Segmentation

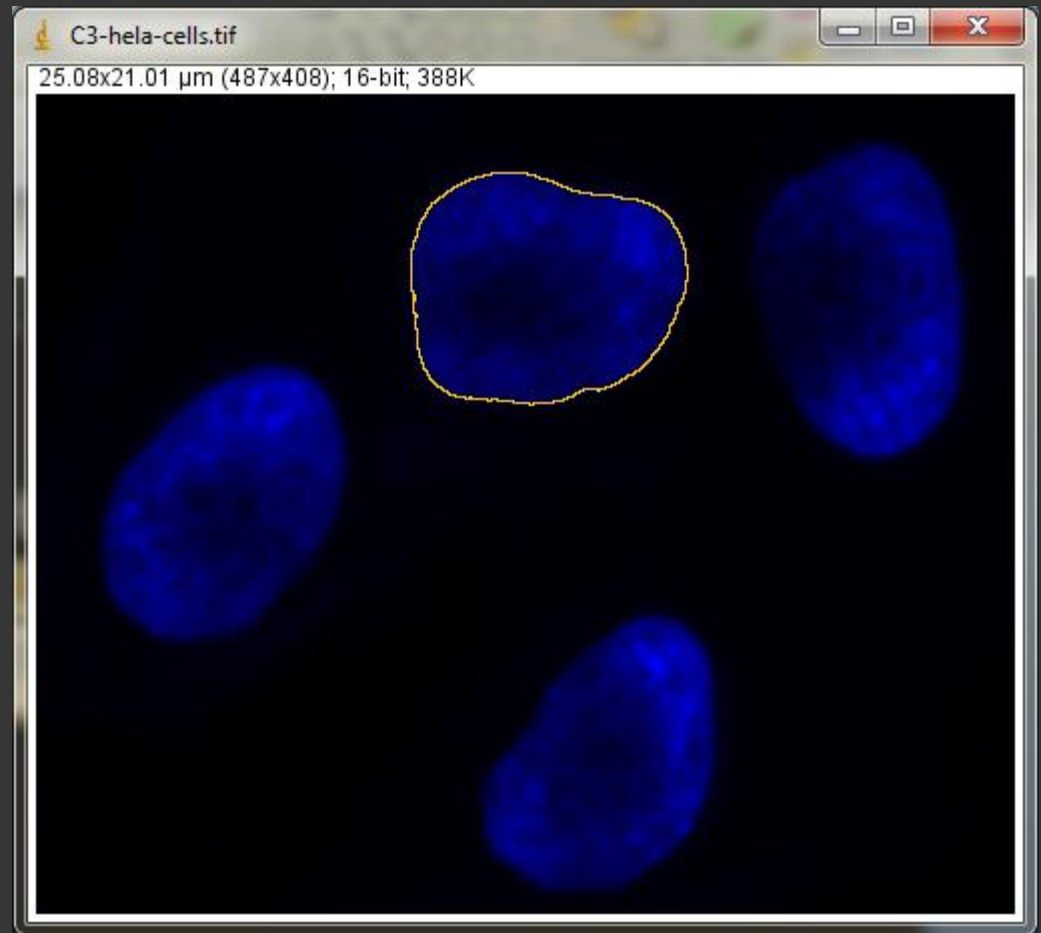
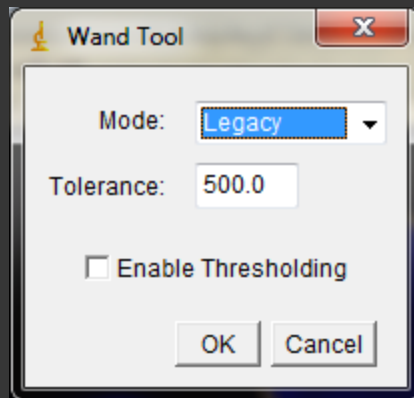
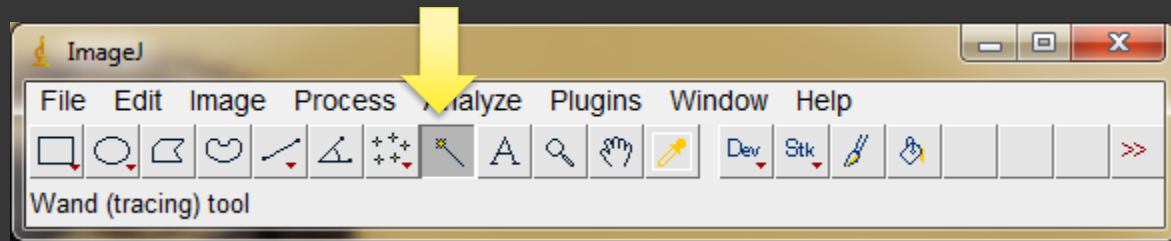
- Thresholding (Image->Adjust->Threshold)





# Segmentation

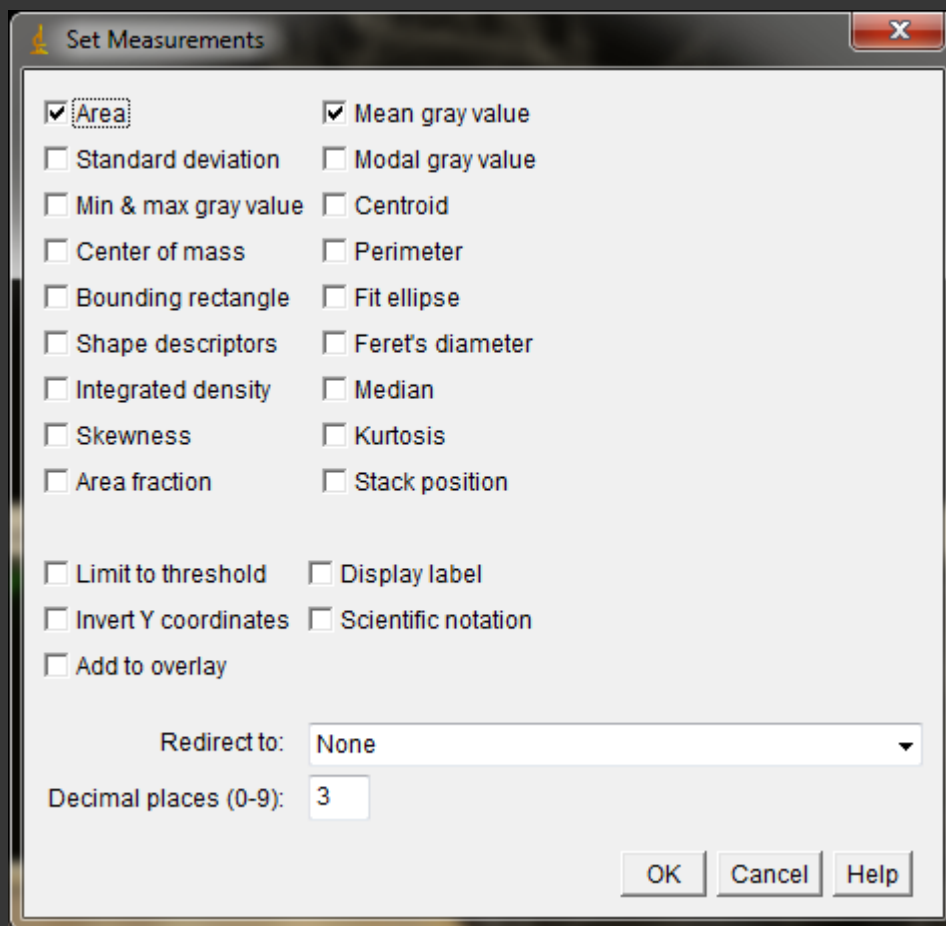
- Interactive selection (Wand tool)



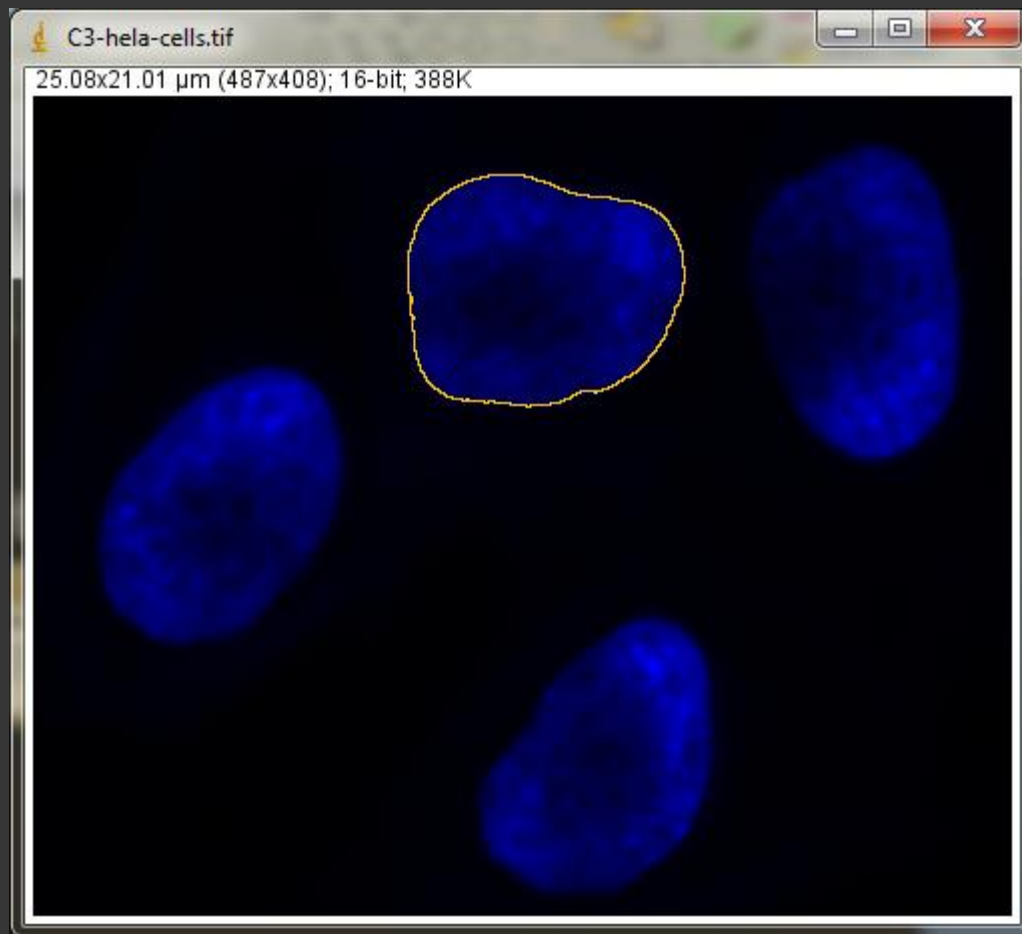
# 2D Analysis (menu Analyze)

- Set measurements (what will be measured)
- Measure (ROI = selection, summary value) or Analyze particles (objects in binary image)

# Set measurement (which parameters)



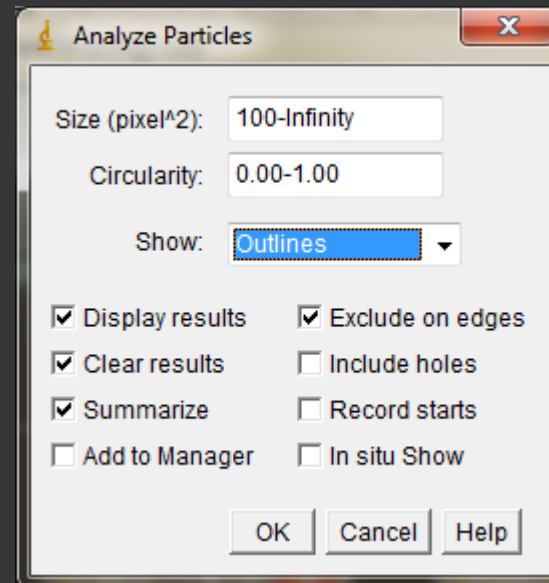
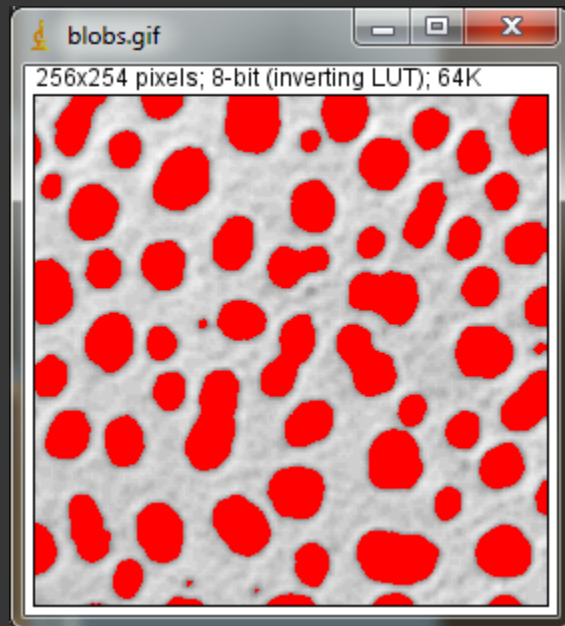
# ROI (= selection) measurement



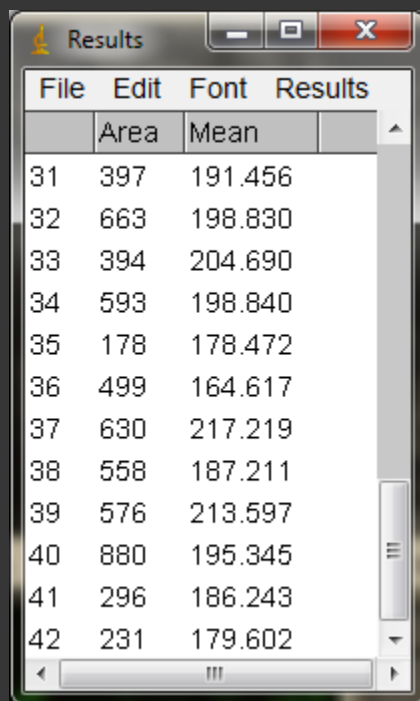
A screenshot of a "Results" window, which is a small dialog box with a title bar containing minimize, maximize, and close buttons. It contains a table with measurement data for the selected ROI. The table has a menu bar with "File", "Edit", "Font", and "Results". The table itself has two columns: "Area" and "Mean". The first row of data shows the values "34.125" and "1121.591" for the selected ROI, which is identified by the number "1" in the first column.

	Area	Mean
1	34.125	1121.591

# Objects measurement

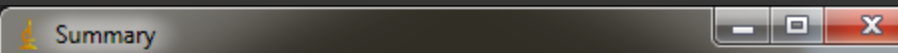


# Objects measurement



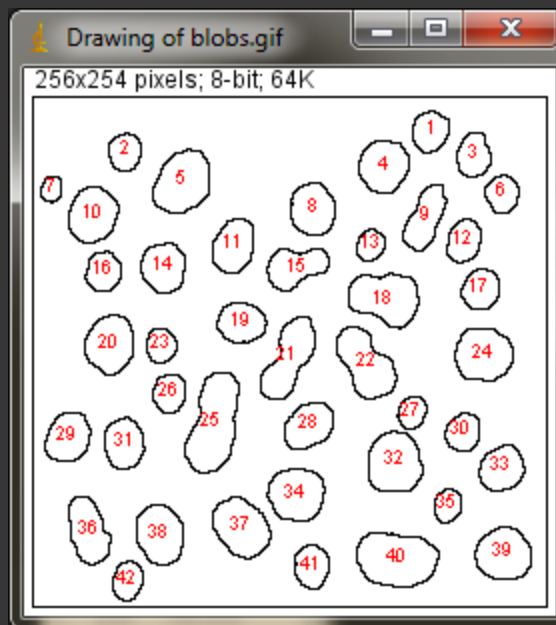
Results

File	Edit	Font	Results
	Area	Mean	
31	397	191.456	
32	663	198.830	
33	394	204.690	
34	593	198.840	
35	178	178.472	
36	499	164.617	
37	630	217.219	
38	558	187.211	
39	576	213.597	
40	880	195.345	
41	296	186.243	
42	231	179.602	



Summary

File	Edit	Font			
Slice	Count	Total Area	Average Size	%Area	Mean
blobs.gif	42	18238.000	434.238	28.048	189.236

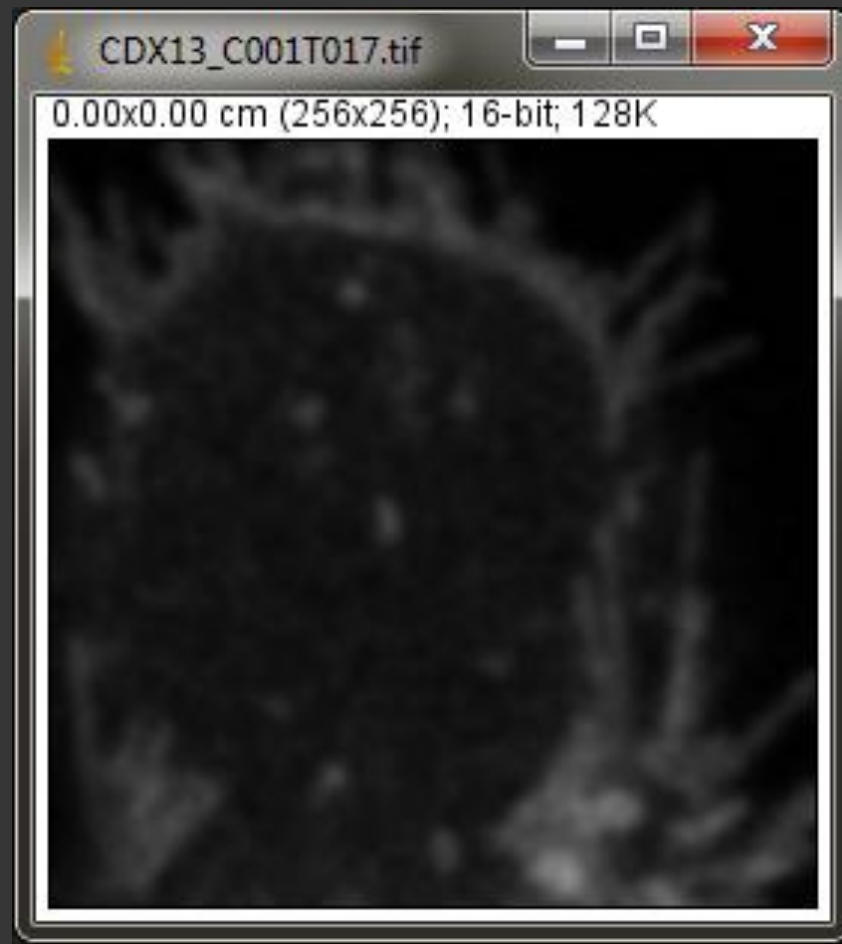
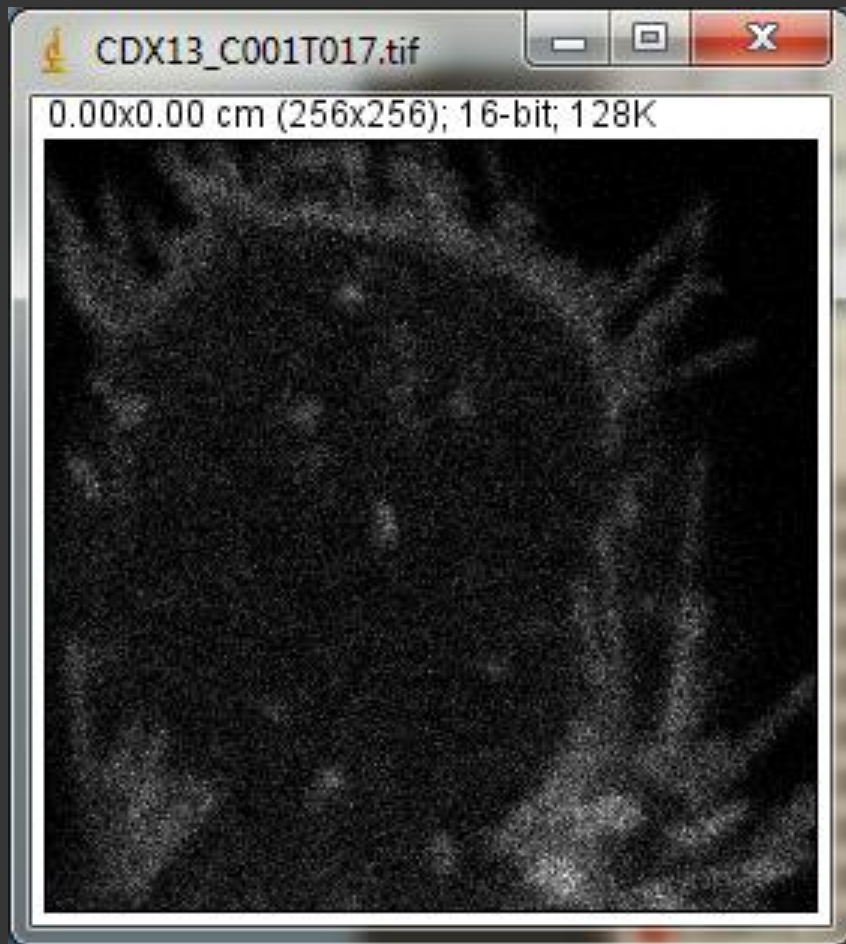




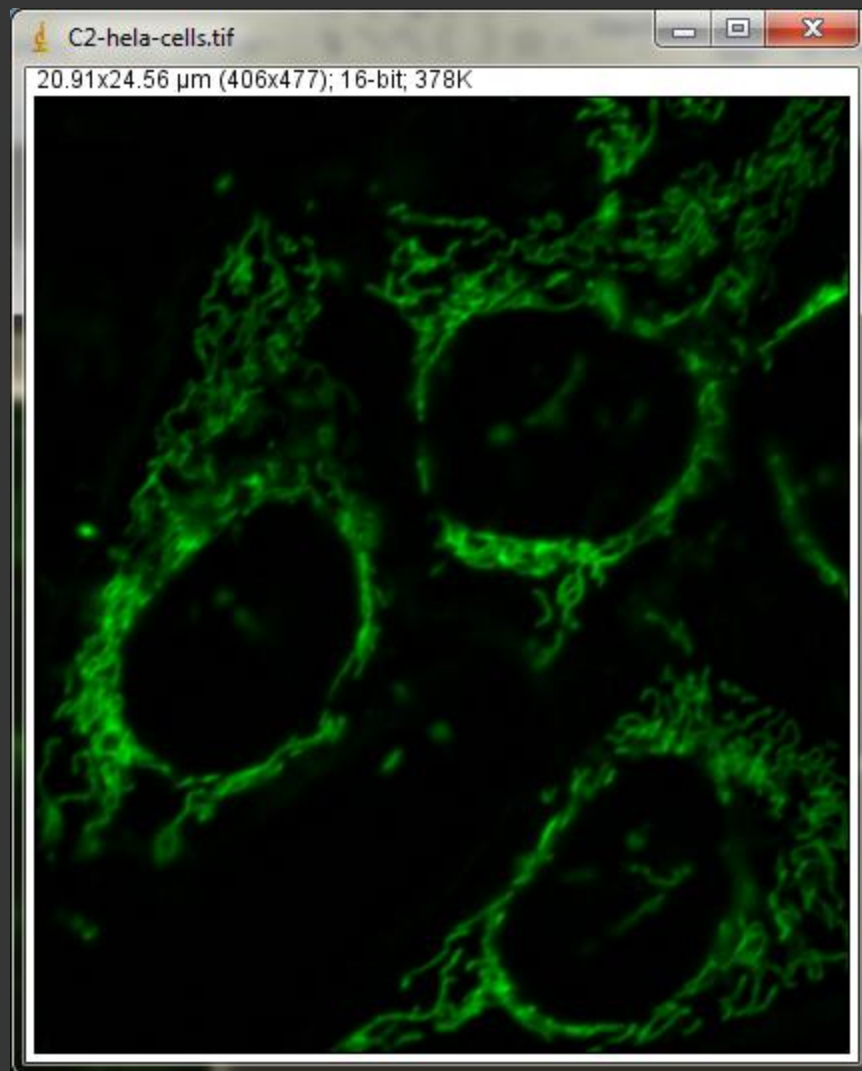
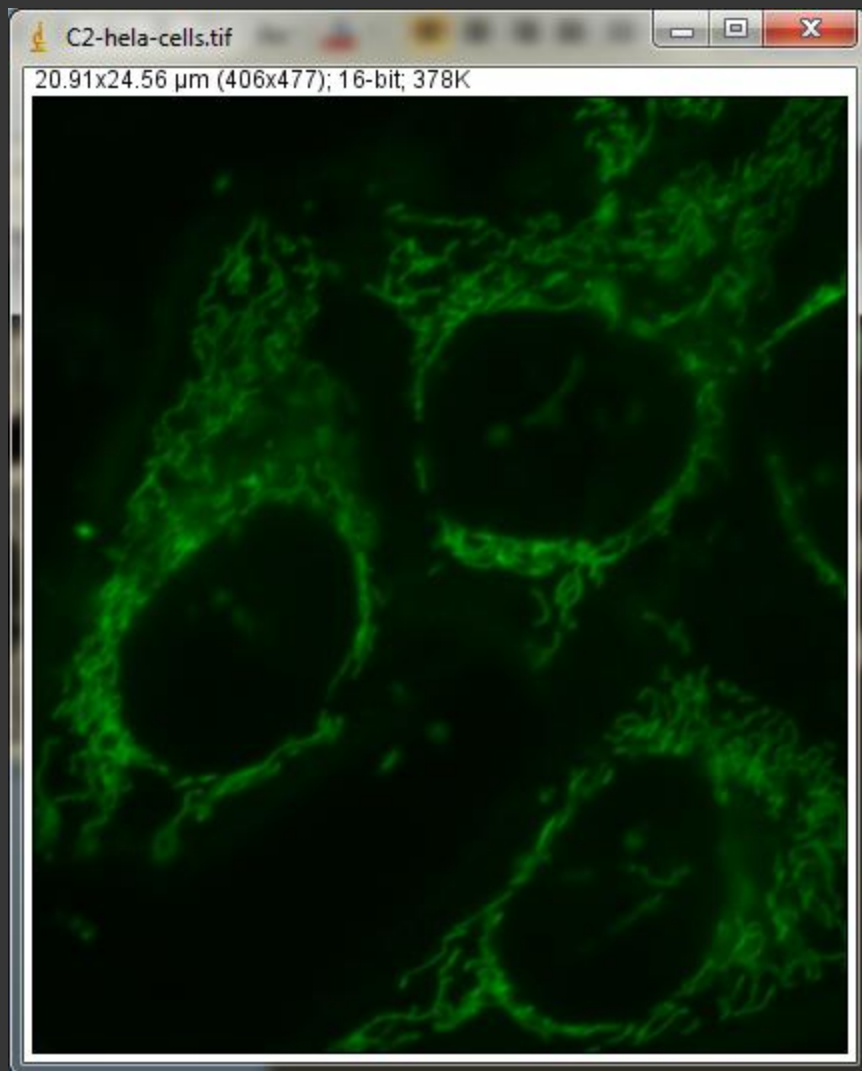
# Filtering images (menu Process ) to remove noise and enhance objects

- 2D Gaussian, median, morphological (minimum, maximum), subtract background.
- 3D (izotropic voxel) Gaussian, median, morphological .

# Noise suppression by Gaussian



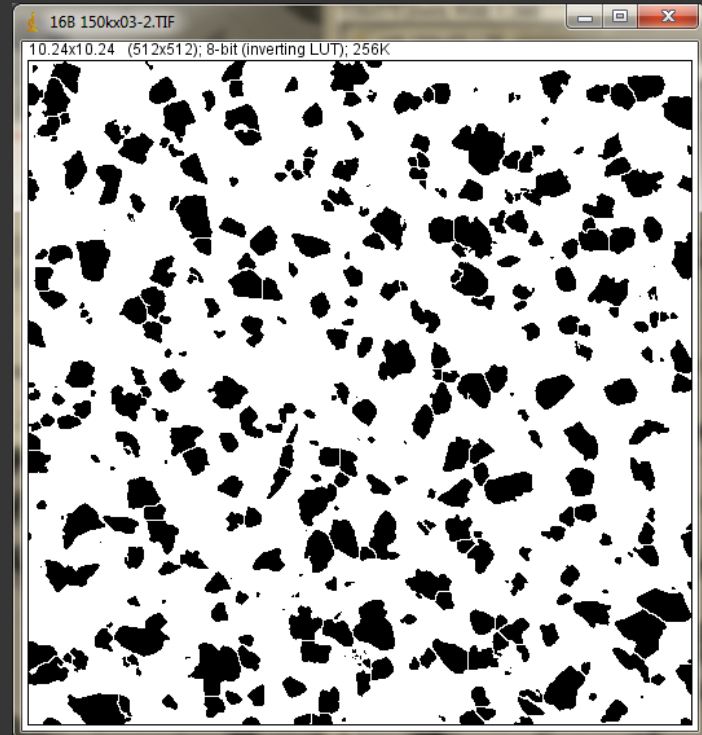
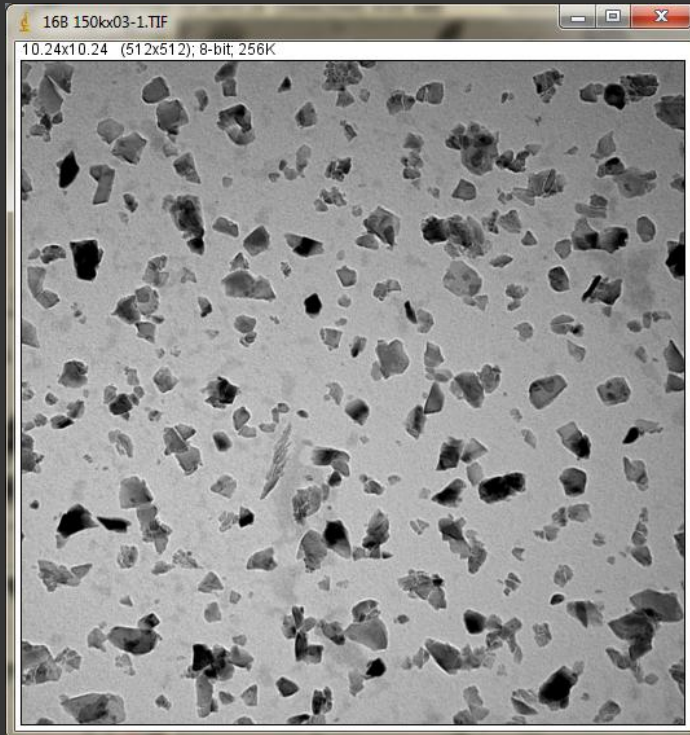
# Background removal



# Analysis - standard procedure

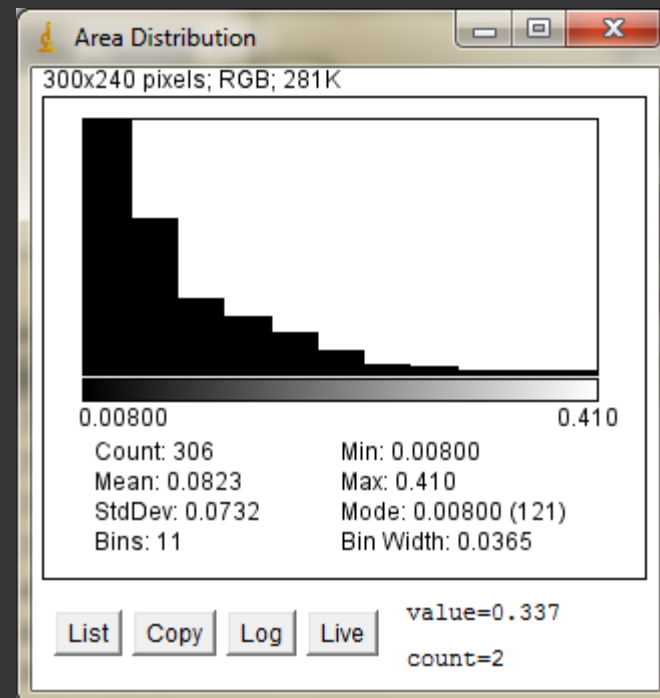
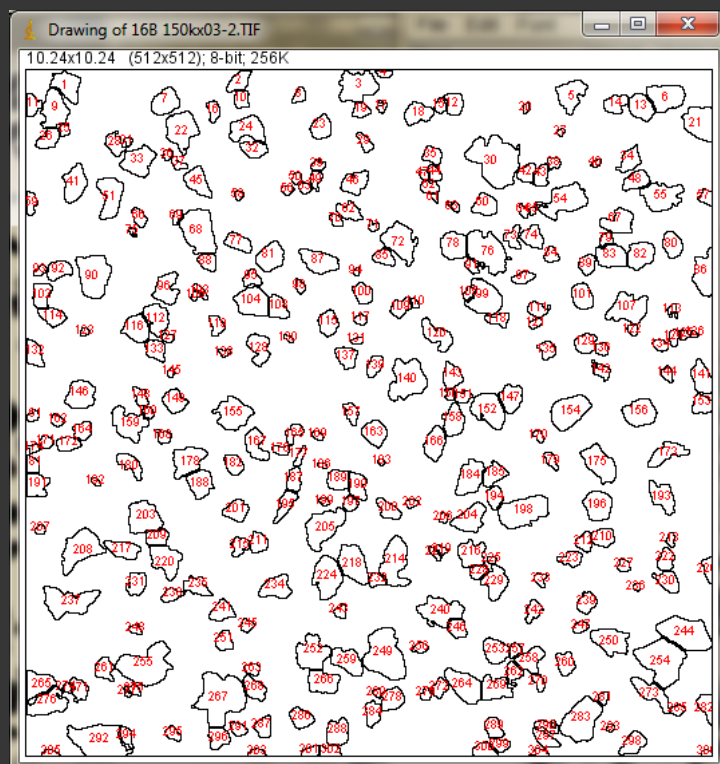
- 1 Noise suppression
- 2 Background removal
- 3 Segmentation
- 4 Measurement

# Example: Nanodiamonds (dr. Řehoř ÚOCHB) preprocessing



- Scale, smooth, subtract background, threshold, watershed object separation

# Analysis



- Analyze particles



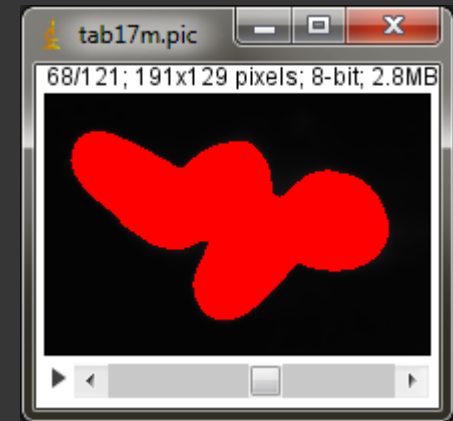
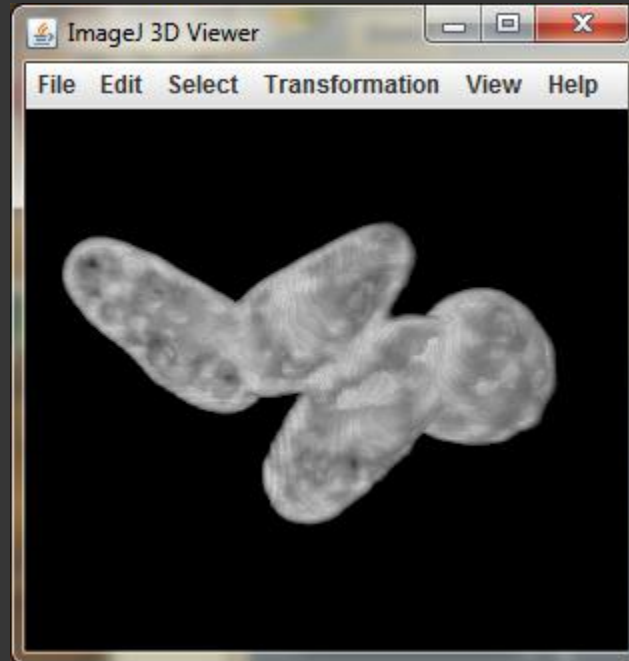
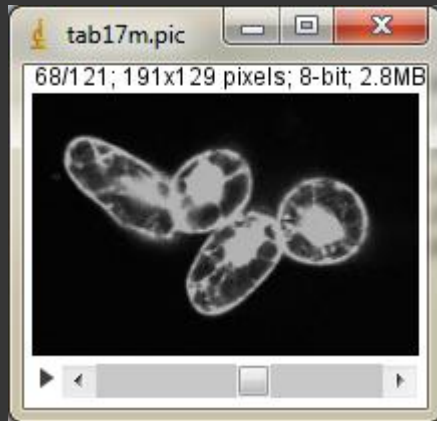
# Auxillary functions

- Conversion of pixel formats, splitting and merging of color channels, brightness and contrast adjustment, resampling (scale), crop, filling – color inpainting ...
- Working with Stacks (XYZtc), animation – for 3D images and video processing and analysis.

# Plugins – extensions of ImageJ

- Dedicated algorithms and tools
- 3D visualization and analysis
- Fiji: instalation ImageJ2 + Plugins
- Plugins in language JAVA can be added to ImageJ, developed in Eclipse IDE
- Modification of examples or similar plugins
- Biomat plugins + source

# 3D processing and visualization



- Gaussian3D, threshold

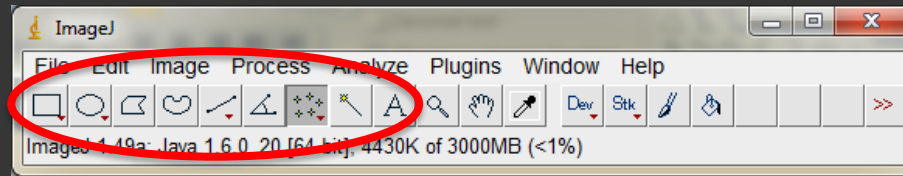
# Macro

- Automatization of processing of large amount of images (that can not be processed at once as stack).
- Recording interactive processing (Macro->Record) and/or editation of macro code in ImageJ Macro Language.

# Macro sets, install macros

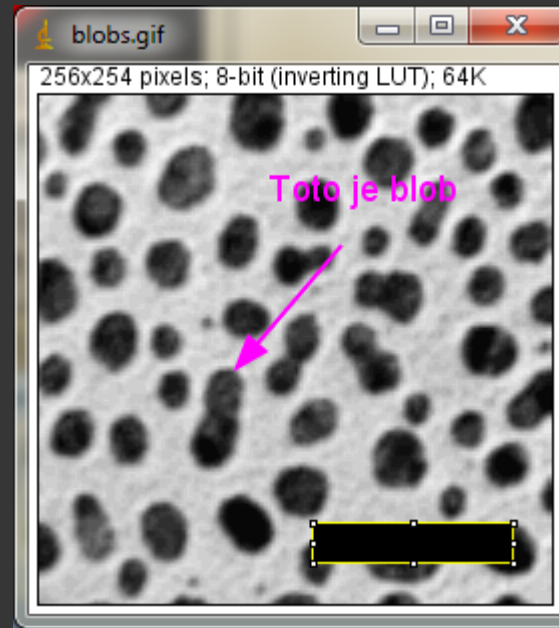
- macro "Process ND" {
- run("Scale...", "x=0.25 y=0.25 z=1.0 width=512 height=512 interpolation=None average process create title=nd-1");
- run("Smooth", "stack");
- run("Subtract Background...", "rolling=20 light sliding stack");
- setOption("BlackBackground", false);
- run("Threshold...");
- }
- macro "Measure ND" {
- run("Convert to Mask", "stack");
- run("Watershed", "stack");
- run("Analyze Particles...", "size=20-Infinity pixel show=Outlines display clear summarize");
- }

# Region of interest, „selection“



- Object created using tools (polygons, lines, points, arrows, text) or by Edit→Selection
- ROI localize action of some processes
- Stored in vector overlay and used as nondestructive annotation without pixelization
- Saved with image in TIFF format

# Decorations



- Scale – rectangle ROI, Edit->fill
- Overlay – arrow, text, ctrl B
- Color: options->color



# Image processing ethics

- Analysis shall not be biased by our expectation.
- No processing is allowed in figures for publications (except mosaicing, uniform histogram stretching) and the images should be rather representative, than nice.
- Any processing shall be well documented in the publication and the source data shall be available.

# References to important topics

- Image perception:

<http://www.drjohnruss.com/downloads/seing.pdf>