

# Nové možnosti rozvoje vzdělávání na Technické univerzitě v Liberci

Specifický cíl A2: Rozvoj v oblasti distanční výuky, online výuky a  
blended learning



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## Image Analysis in a System of NIS Elements

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Národní  
plán  
obnovy



MINISTERSTVO ŠKOLSTVÍ,  
MLÁDEŽE A TĚLOVÝCHOVY

# Image Analysis in a System of NIS Elements

Ing. Monika Vyšanská, Ph.D.



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# Outline

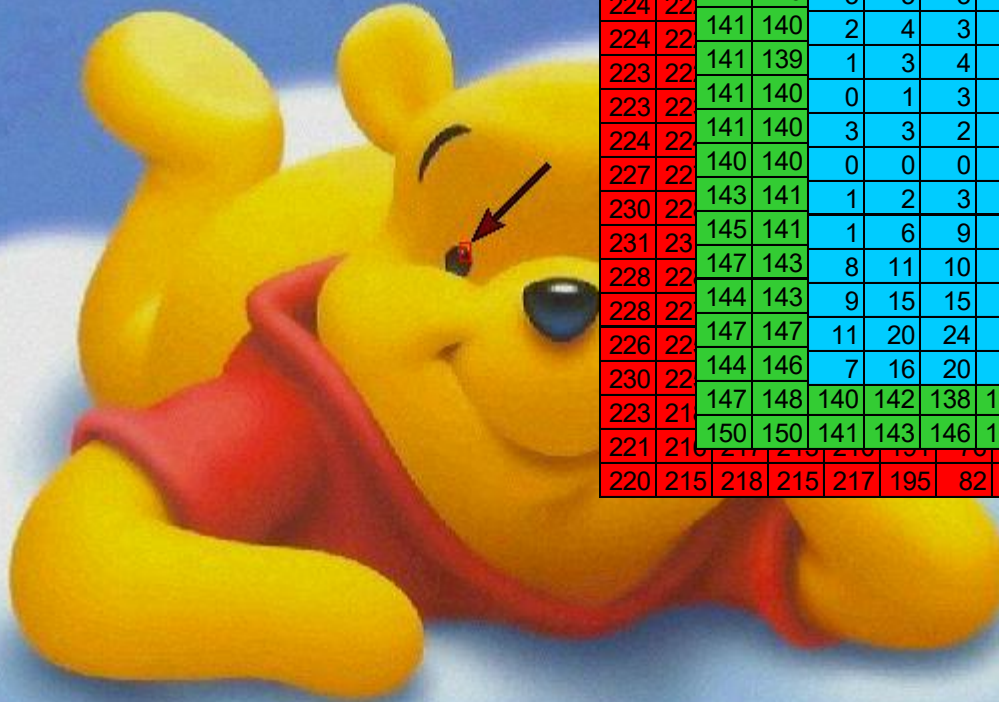
- Image
- Types of Images
- Inputs
- Measurement
  - Calibration
  - Quantitative evaluation of image (what we want to measure - object or field measurements)
  - Evaluation – choice of features, definition of measurement frame, mask and images, which will be accepted for measurement
  - Data presentation
- Macro creation

# Image - discretization

[0,0]

X  
↓

					3	2	2	1	2	1	2	2	4	3
					3	2	3	1	2	1	2	2	3	3
		142	140		3	2	2	1	3	1	2	2	4	4
224	22	142	140		3	2	1	0	3	1	2	2	4	4
224	22	142	140		2	3	3	2	0	1	1	4	5	2
224	22	142	140		2	2	1	0	0	0	0	3	4	2
224	22	142	140		2	3	2	0	0	0	2	5	5	3
224	22	141	140		2	4	2	0	0	2	3	6	5	1
224	22	141	140		3	5	3	0	0	0	3	3	7	6
224	22	141	140		2	4	3	0	1	2	5	2	8	10
223	22	141	139		1	3	4	1	5	6	11	11	22	36
223	22	141	140		0	1	3	2	9	9	12	14	28	50
224	22	141	140		3	3	2	8	8	17	19	23	35	21
227	22	140	140		0	0	0	7	7	18	22	16	10	5
230	22	143	141		1	2	3	11	15	19	25	25	2	4
231	23	145	141		1	6	9	21	21	31	0	0	12	17
228	22	147	143		8	11	10	17	27	27	9	1	18	28
228	22	144	143		9	15	15	26	37	31	16	16	29	40
226	22	147	147		11	20	24	37	43	38	28	23	36	45
230	22	144	146		7	16	20	36	50	39	26	26	26	48
223	21	147	148	140	142	138	119	50	57	52	48			
221	21	150	150	141	143	146	123	52	58	53	51			
220	215	218	215	217	195	82	75	71	73					



# Image - resolution

1024x768



256x768



128x96



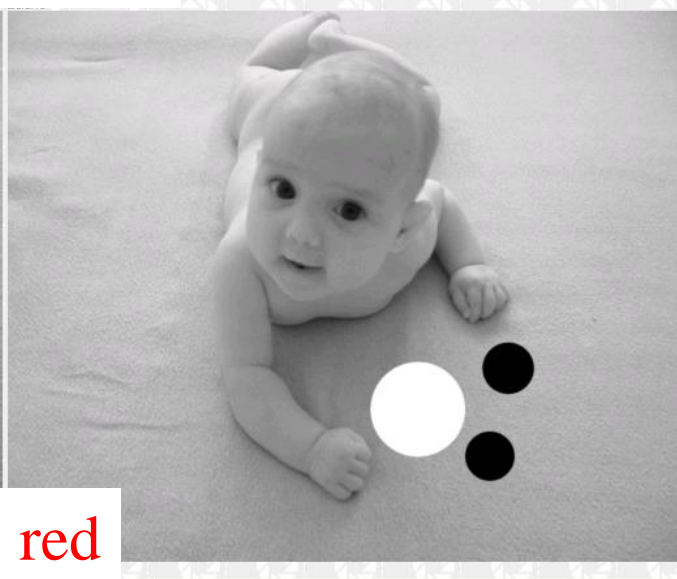
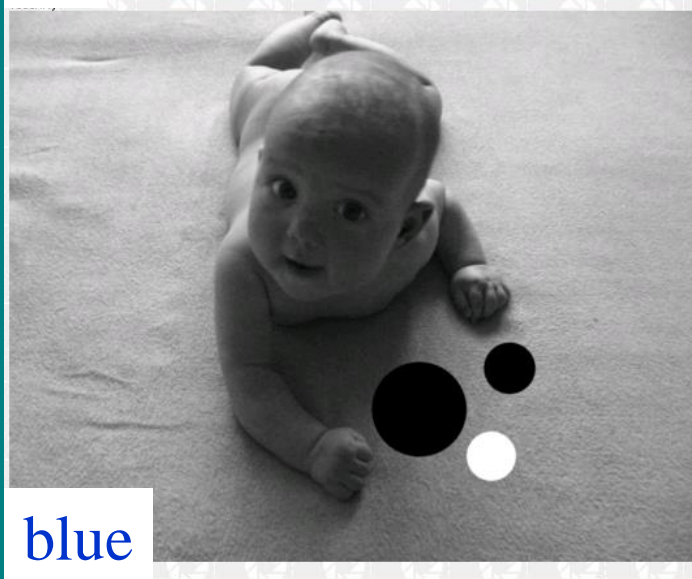
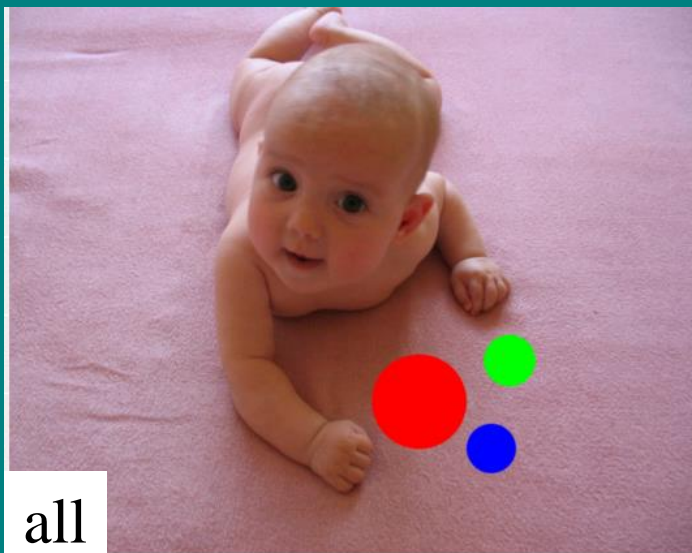
64x48



# Accepted Image Types and their Formats

- **Color (RGB)** — from three components – intensity of red, green and blue. Values of pixels for each component are in range of 0 to 255. For intensity and shade measurement.
- **Gray** — derived images. Values of image points are in range of 0 to 255 – the same for all three components in each image point. Gray image is not own to system of NIS Elements. The system transforms RGB images to Gray images.
- **Binary (BW)** — has two values of pixels: 0 – background, 1 – objects and structures. It is product of segmentation of RGB or gray image. **For shape and size measurement.**
- **\*.tiff, \*.bmp, \*.jpg, \*.lim, \*.jpg2000 (\*.jp2)**

# Color Image - Explanation



# Gray Image - Explanation





# Binary Image - Explanation

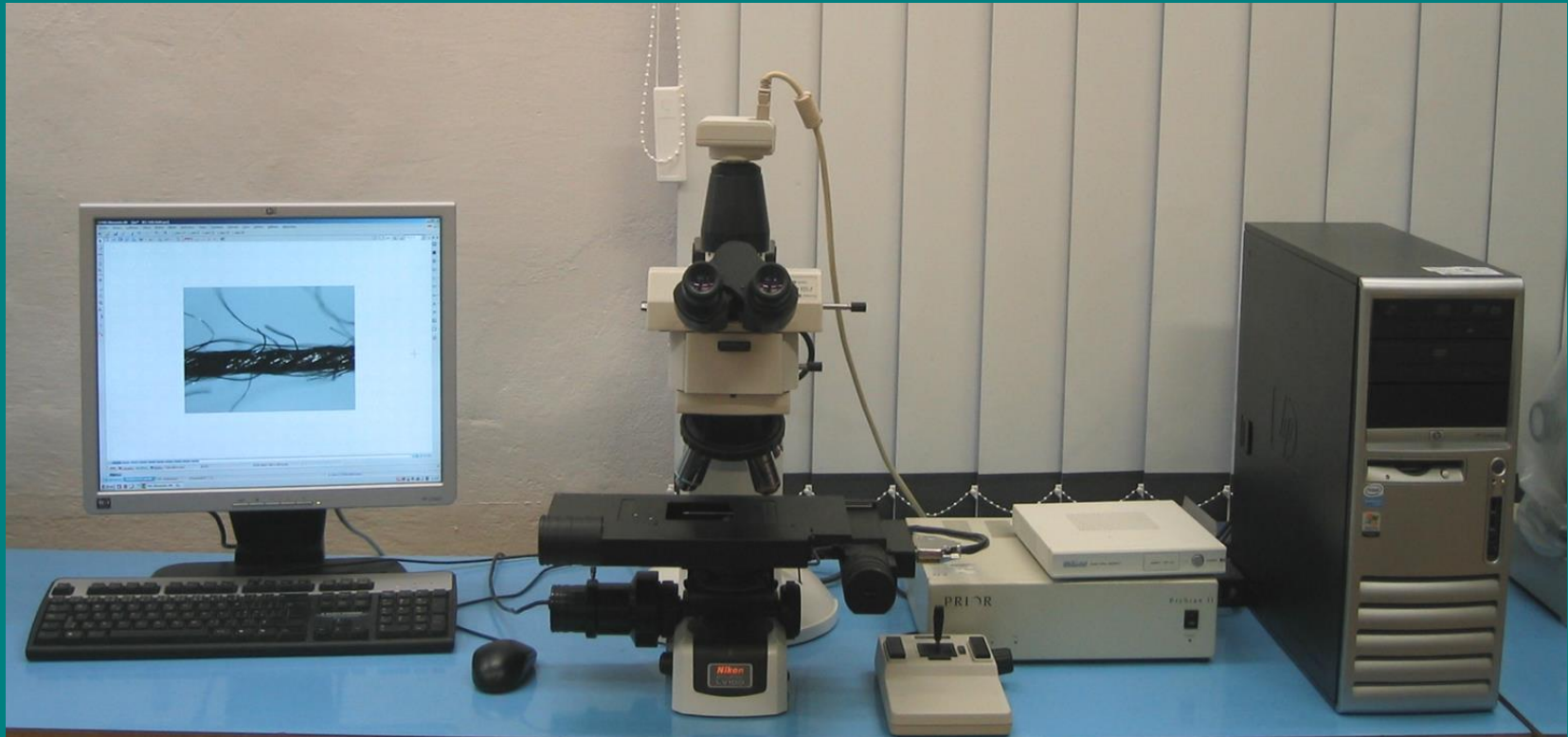


Segmentation (threshold) of gray image on the level of gray image intensity, of:  $\langle 83, 86 \rangle$

# Inputs



# System of Image Analysis



# Screen of NIS Elements System

The screenshot shows the NIS-Elements AR software interface. The main window displays a microscopy image of cells. The interface includes a menu bar (File, Edit, Calibration, Image, Binary, Measure, Reference, Macro, View, Window, Devices, Help), a toolbar, and a right-hand Organizer panel. The Organizer panel lists various layers and tools: Foreground probe, Background probe, Eyepiece scale, Scale, Measurement Frame, Measurement ROI, Profile, Annotation layer, Measurement layer, Binary layer, RGB layer, and Overlay layer. The main image area shows a cluster of cells with a cursor. The status bar at the bottom provides information about the image and the current measurement.

**Opened image** (points to the main image area)

**Main Toolbar** (points to the toolbar)

**Organizer** (points to the right-hand panel)

**Size of image displays on the screen** (points to the image size information in the status bar)

**Summary of accessible calibrations** (points to the top of the main image area)

**Main tool of measurement** (points to the measurement tool icon in the toolbar)

**Arrow** (points to the arrow tool icon in the toolbar)

**Text** (points to the text tool icon in the toolbar)

**Run macro** (points to the run macro icon in the toolbar)

**Close all** (points to the close all icon in the toolbar)

**Set of tool bar** (points to the toolbar area)

**Coordinates of cursor, RGB of actual image** (points to the cursor coordinates in the status bar)

**Components of color image** (points to the RGB color selection in the status bar)

**Image calibration** (points to the calibration information in the status bar)

**Image size** (points to the image size information in the status bar)

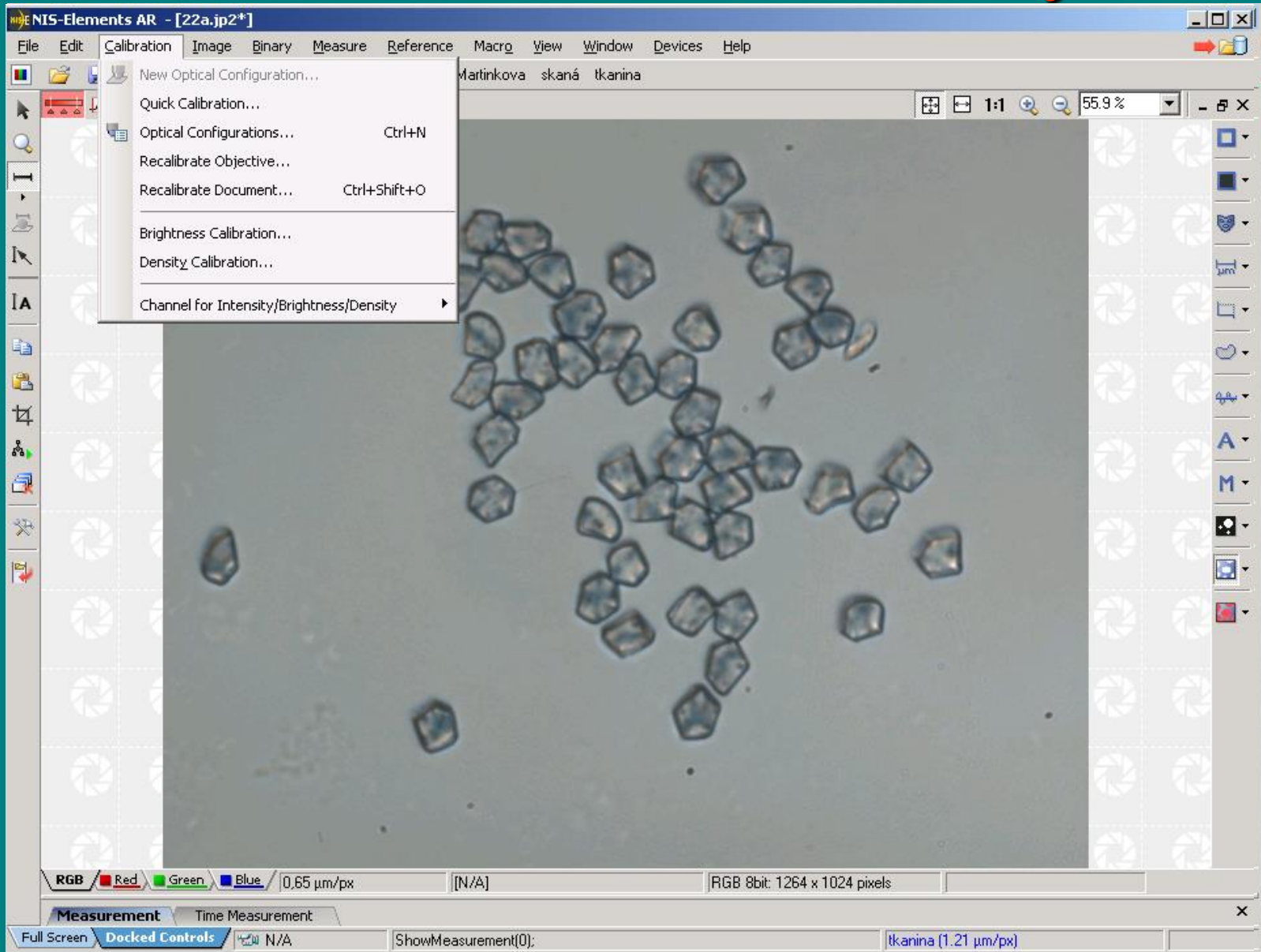
**Data from interactive measurement** (points to the measurement data in the status bar)

**Info about camera** (points to the camera information in the status bar)

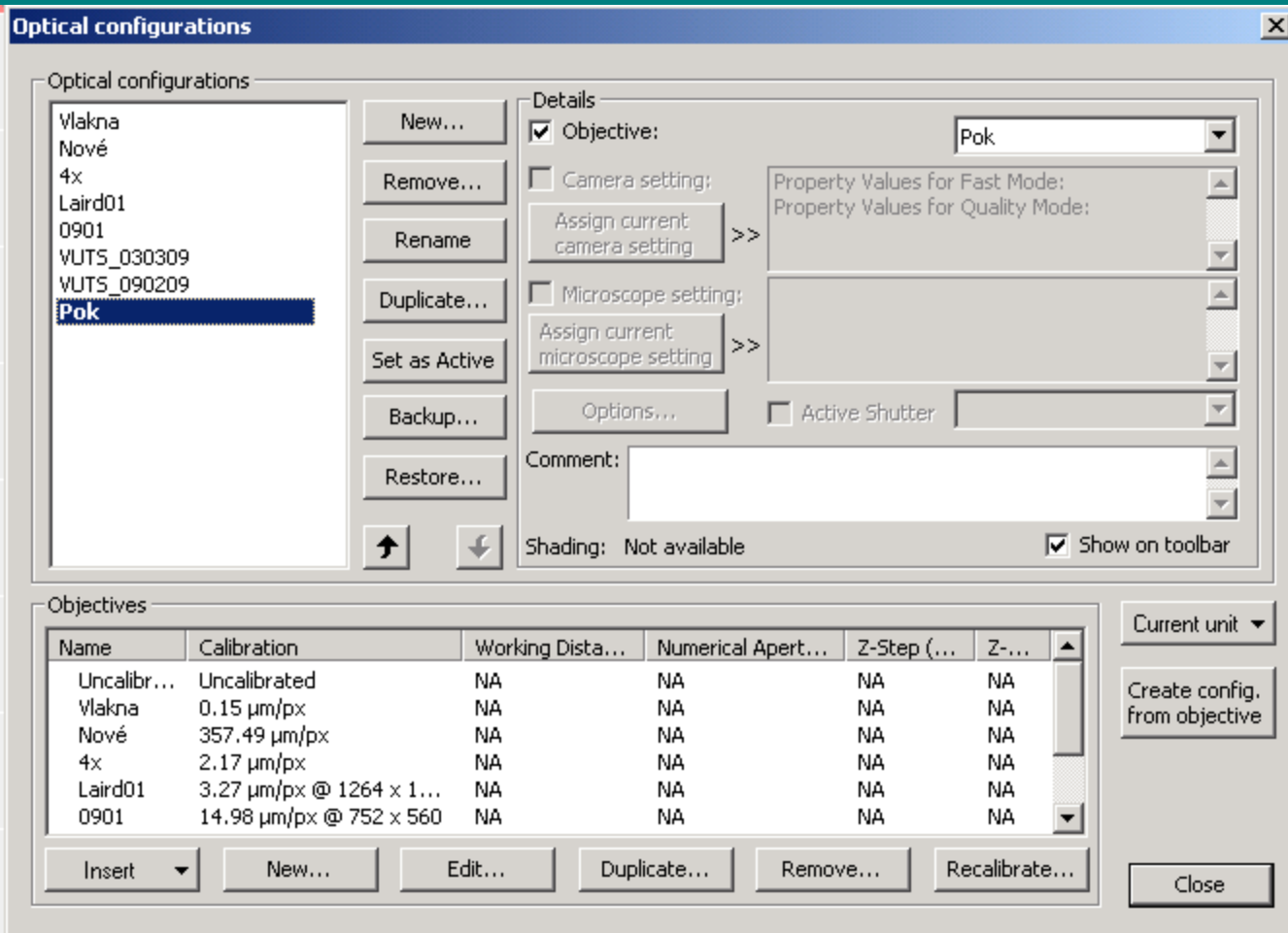
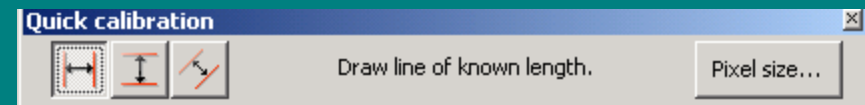
**Executed action** (points to the executed action in the status bar)

**Info about used calibration** (points to the calibration information in the status bar)

# Standard Functions of the System



# Calibration



Optical configurations dialog box. Left pane: List of configurations (Wlakna, Nové, 4x, Laird01, 0901, VUTS\_030309, VUTS\_090209, Pok). Middle pane: Buttons (New..., Remove..., Rename, Duplicate..., Set as Active, Backup..., Restore...). Right pane: Details for 'Pok' objective. Includes checkboxes for Objective, Camera setting, Microscope setting, and Active Shutter. Includes 'Assign current camera/microscope setting' buttons and 'Options...' button. Comment field and 'Show on toolbar' checkbox. Shading: Not available.

Objectives table:

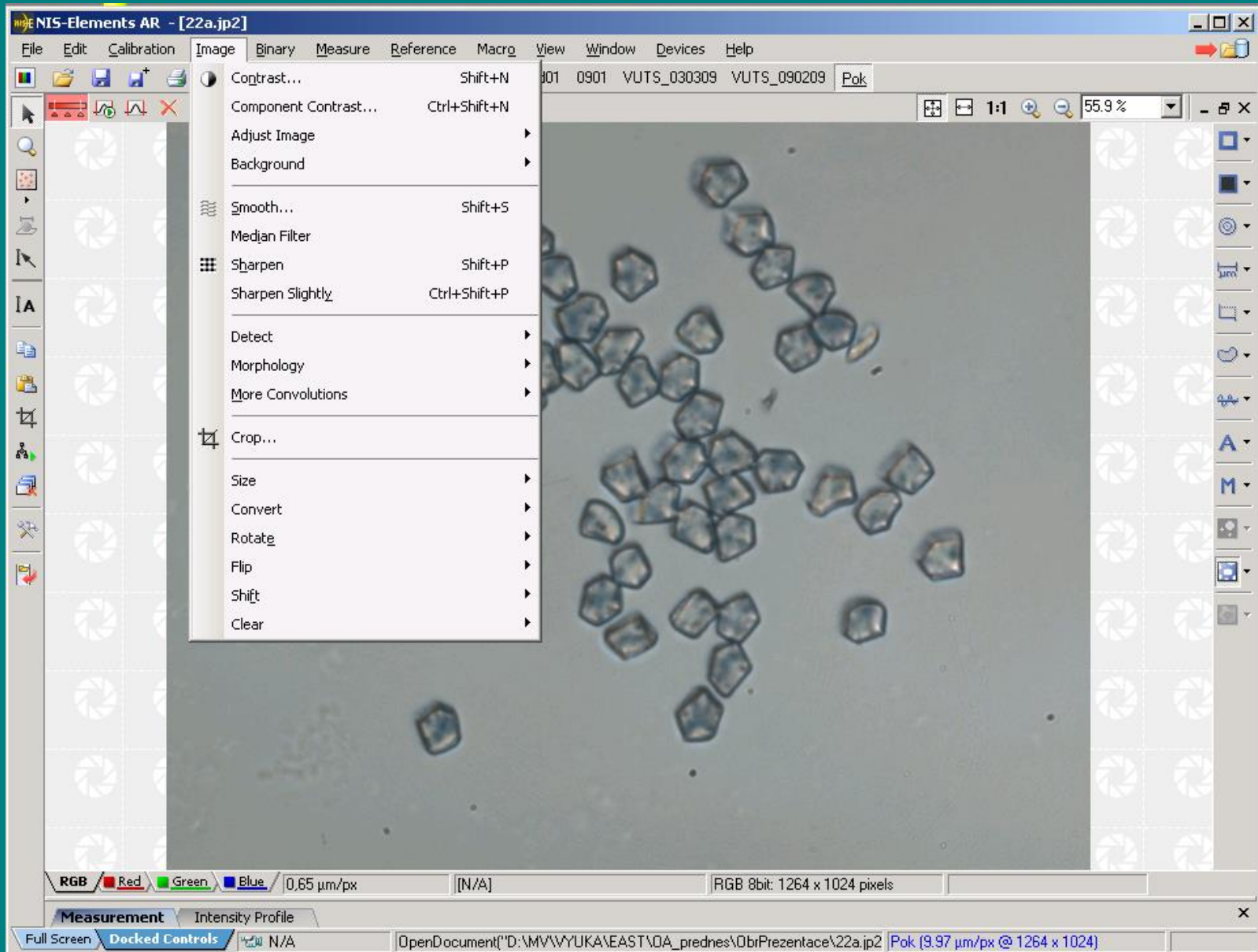
Name	Calibration	Working Dista...	Numerical Apert...	Z-Step (...)	Z-...
Uncalibr...	Uncalibrated	NA	NA	NA	NA
Wlakna	0.15 $\mu\text{m}/\text{px}$	NA	NA	NA	NA
Nové	357.49 $\mu\text{m}/\text{px}$	NA	NA	NA	NA
4x	2.17 $\mu\text{m}/\text{px}$	NA	NA	NA	NA
Laird01	3.27 $\mu\text{m}/\text{px}$ @ 1264 x 1...	NA	NA	NA	NA
0901	14.98 $\mu\text{m}/\text{px}$ @ 752 x 560	NA	NA	NA	NA

Buttons: Insert, New..., Edit..., Duplicate..., Remove..., Recalibrate..., Close.

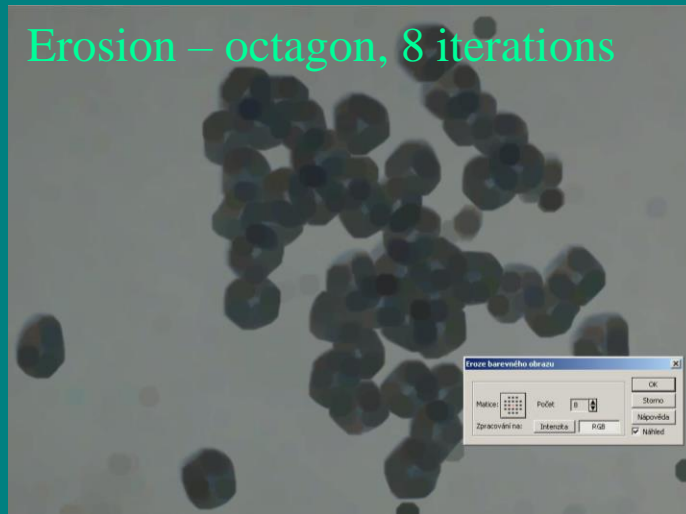
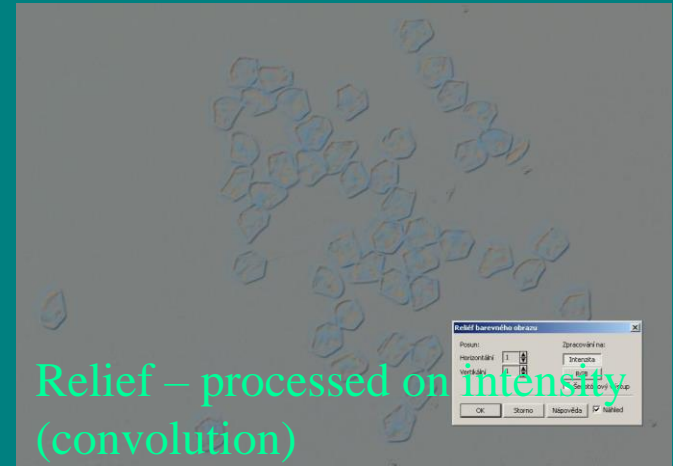
## Calibration

- it assigns real size to the objects
- it is parameter of the NIS Elements and has to be made before the start of measurement

# Image Adjustment – RGB



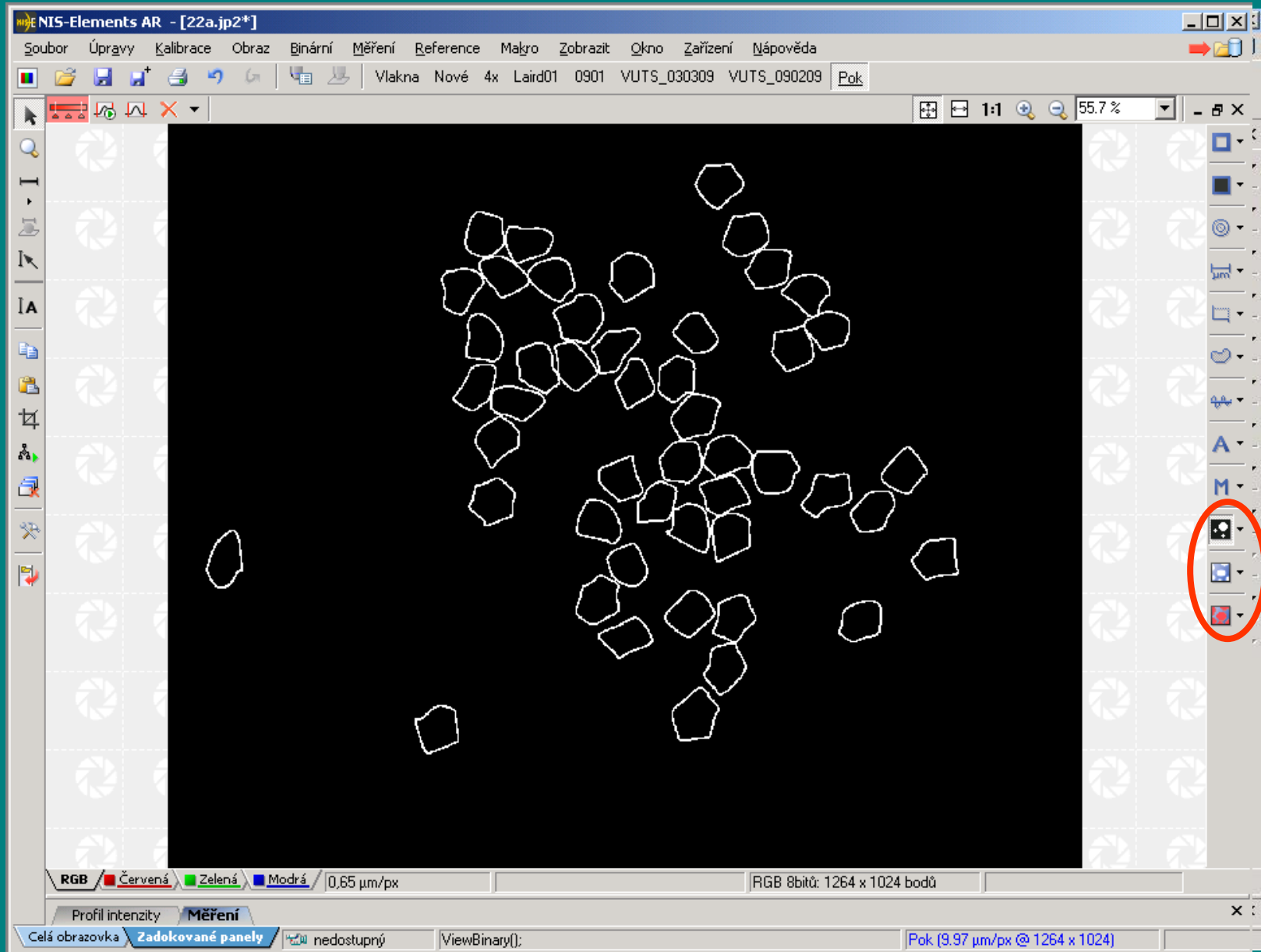
# Morphological Operations – RGB





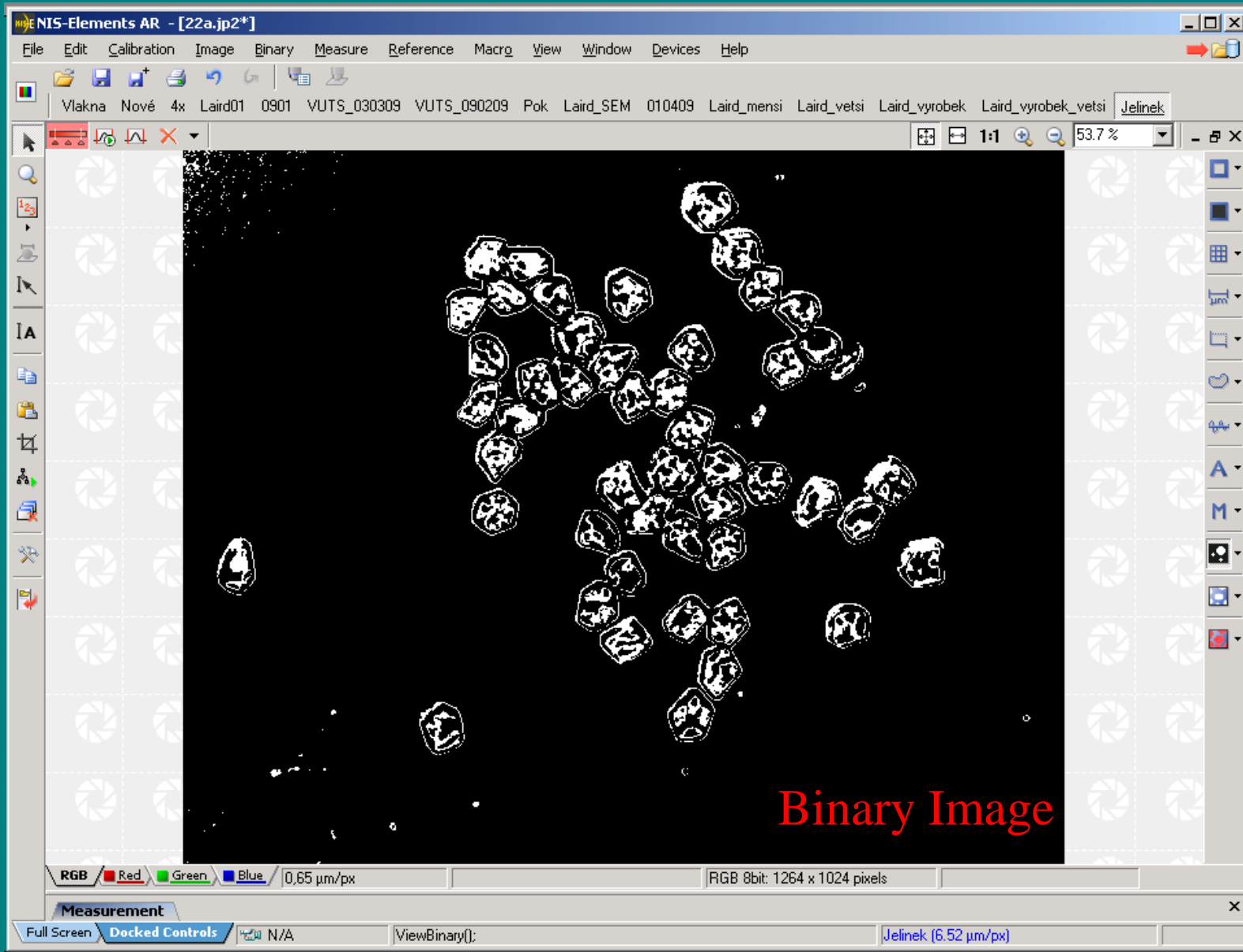
# Image Adjustment - BW

- Subjectively
- Objectively

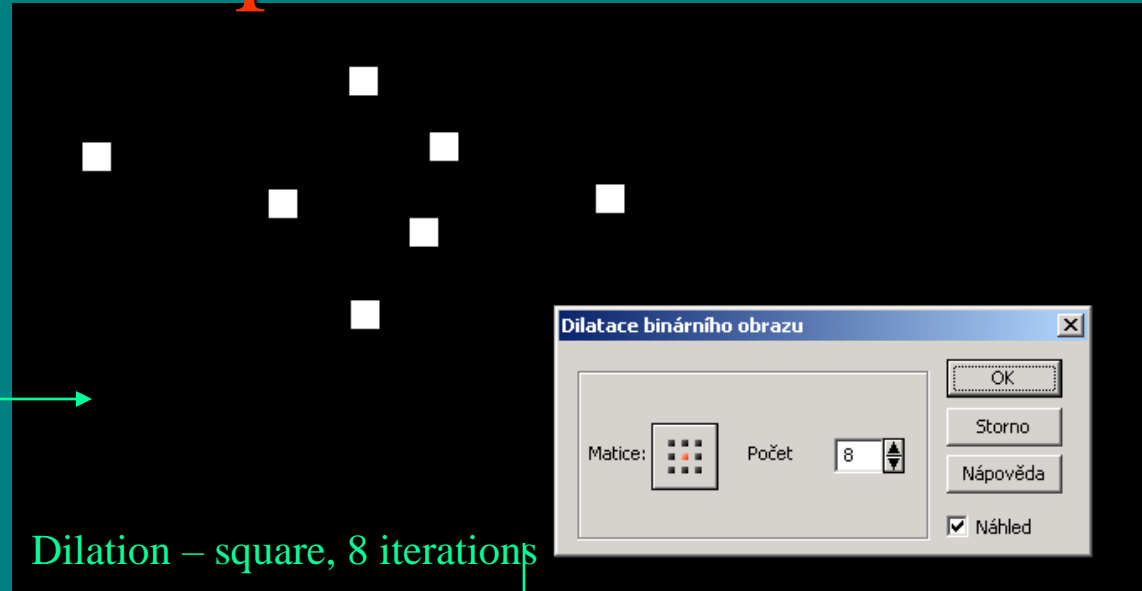
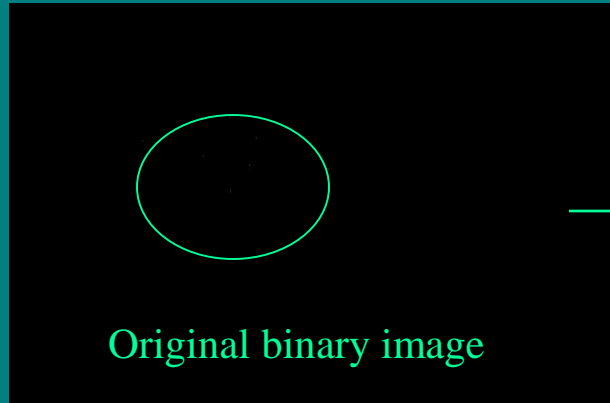


# Image Adjustment - BW

- Subjectively
- Objectively

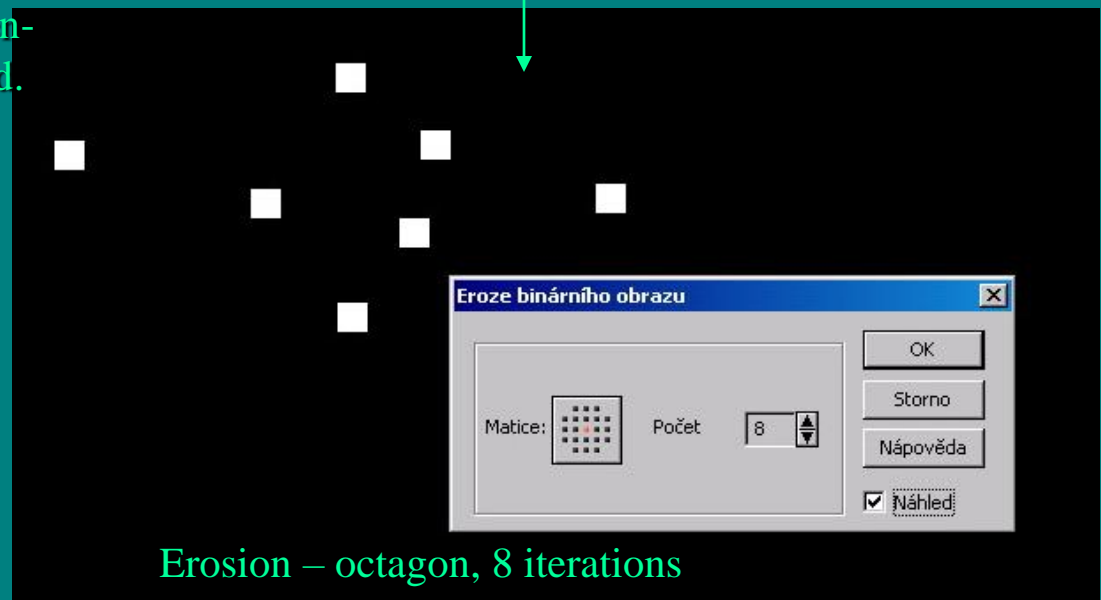


# Morphological Operations – BW

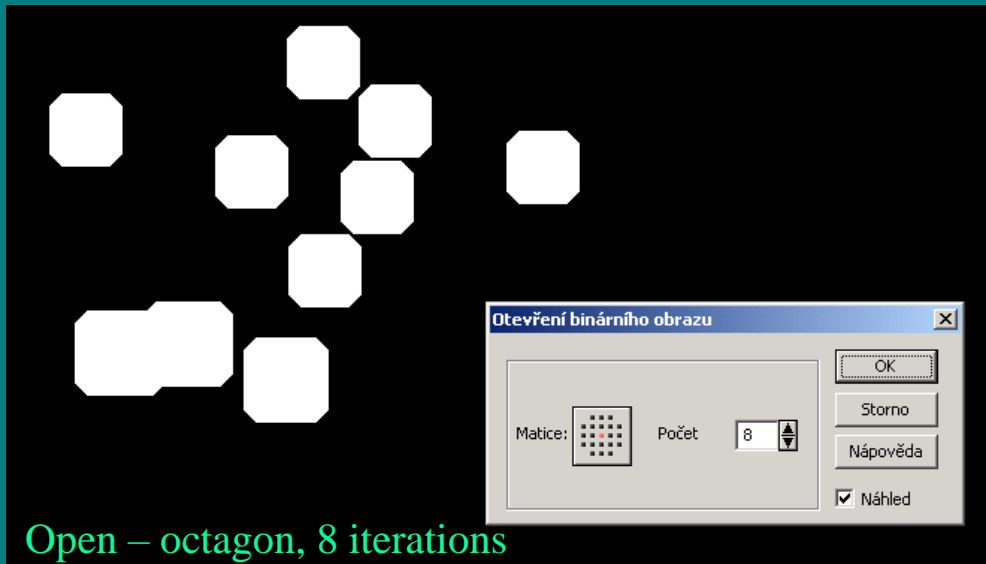


**Dilation** – after dilation the objects are enlarged, the outer layer of objects is added. If the distance between two objects is shorter than the thickness of two layers these objects are connected together.

**Erosion** – after erosion the objects are shrunk, the inner layer of the object is subtracted. If an object is thinner than the thickness of the inner layer this disappears from the image.



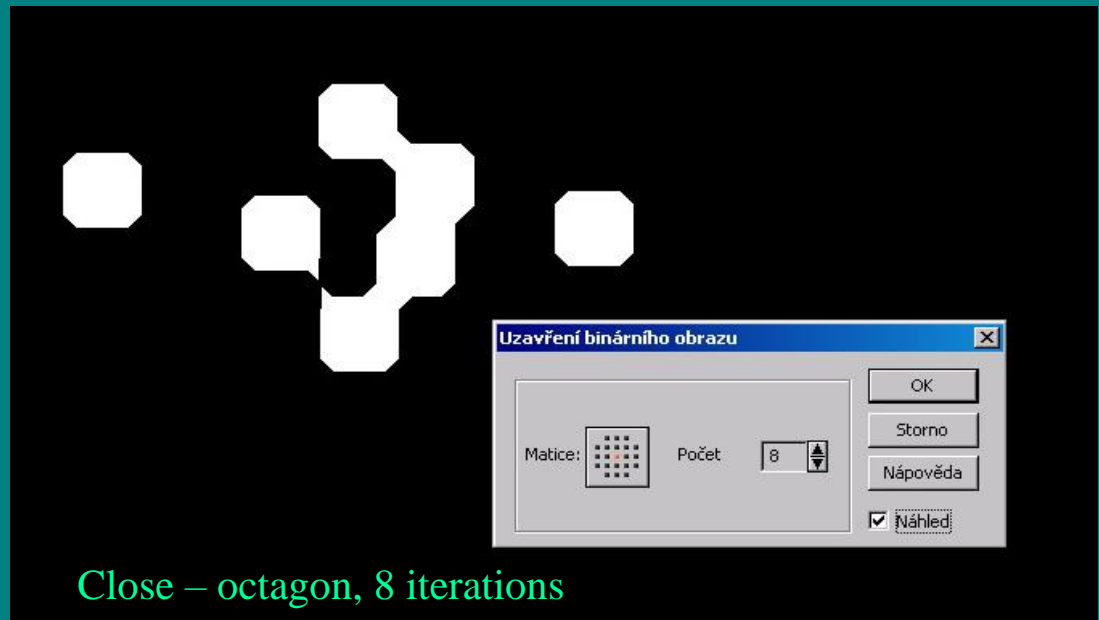
# Open and Close of Binary Image



Open – octagon, 8 iterations

**Open** – the erosion is followed by dilation – the size of objects is not significantly affected. Contours are smoothed, small objects are suppressed and gently connected, particles disconnected.

**Close** – the dilation is followed by erosion – the size of objects is not significantly affected. Contours are smoothed, small holes and small depressions are suppressed. Close objects may be connected together.



Close – octagon, 8 iterations

# Possibilities of Measurement

The screenshot displays the NIS-Elements AR software interface. The main window shows a grayscale image of a biological sample with a grid overlay. The 'Measure' menu is open, listing various measurement options. Two dialog boxes are also visible: 'Object Measurement Setup' and 'Field Measurement Setup'. Red arrows point from the menu items to the dialog boxes, and black arrows point from text labels to the menu items.

**Object and Field measurement**

**Interactive measurement**

**Measure**

- Scan Objects F5
- Single Object... Ctrl+Shift+S
- Trace Object...
- Object Data... Ctrl+F5
- Object Features... Shift+F5
- Restrictions... Alt+F5
- Generate Binary Using Restrictions
- Field F6
- Field Data... Ctrl+F6
- Field Features... Shift+F6
- Length F7
- Area E
- Other Interactive Measurements
- 3D Measurement
- Time measurement
- Volume measurement
- Intensity Profile Shift+F7
- Classifier
- Options...
- Use Measurement Frame
- Measurement Frame...
- Use ROI
- ROI Editor... Ctrl+Shift+R

**Object Measurement Setup**

Features for Measure	Selected for Measure
Area	MaxFeret
FillArea	MinFeret
FillRatio	
EqDiameter	
VolumeEqSphere	
VolumeEqCylinder	
Perimeter	
MeanChord	
Length	
Width	
MaxFeret90	
Circularity	
Elongation	
CentreX	
CentreY	
CentreXpx	
CentreYpx	

**Field Measurement Setup**

Features	Selected for Measurements
NumberObjects	AreaFraction
Area	
MeasuredArea	
Perimeter	
MeanChord	
SurfVolumeRatio	
MeanIntensity	
SumIntensity	
IntensityVariation	
MinIntensity	
MaxIntensity	
MeanRed	

RGB Red Green Blue 0,65 µm/px [N/A] RGB 8bit: 1264 x 1024 pixels

Measurement Full Screen Docked Controls N/A ViewColor(): Jelinek (6.52 µm/px)

# Interactive Measurement

File Edit Calibration Image Binary Measure Reference Macro View Window Devices Help

Vlakna Nové 4x Laird01 0901 VUTS\_030309 VUTS\_090209 Pok Laird\_SEM 010409 Laird\_mensi Laird\_vetsi Laird\_vyrobek Laird\_vyrobek\_vetsi Jelinek

1:1 41.2%

RGB Red Green Blue 0,65 µm/px [N/A] RGB 8bit: 1264 x 1024 pixels

Measurement

Taxonomy: 12 Classes Current Class:F6 Current Count:2 Total Count:49

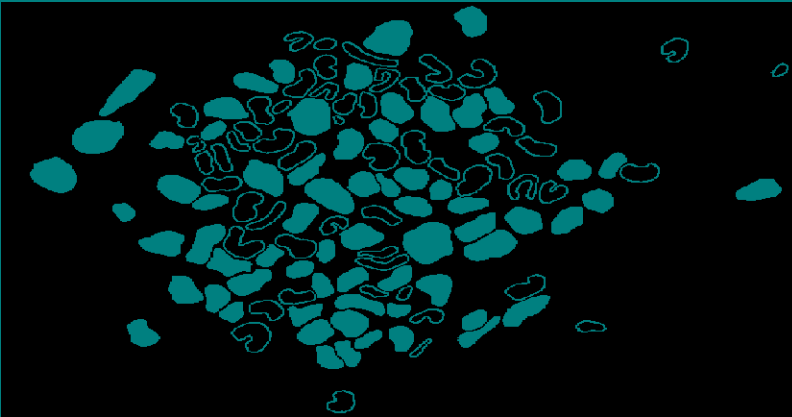
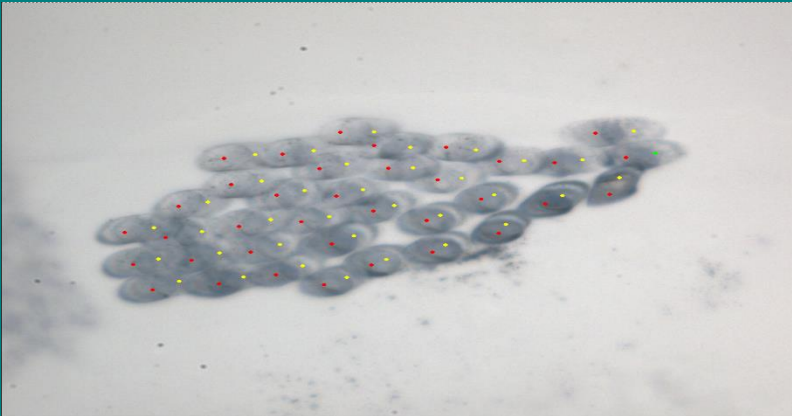
1 +	2 *	3 ■	4 ◆	5 ●	6 ◆	7 □	8 ○	9 ×	10 ⊕
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
16	14	6	5	6	2	0	0	0	0

Use Fn keys

Clear Screen  
Reset  
Menu >

Full Screen Docked Controls N/A UndoNext(); Jelinek (6.52 µm/px)

# Field and Object Measurement



## Object measurement

- Provides user with specific object data such as Length, Area, CentreX/Y, ...

## Field measurement

- Produces information regarding the whole measurement frame area such as AreaFraction, MeanBrightness, DensityVariation, ...

# Measurement — choosing of features

(BW, RGB/BW, object measurement,

field measurement, *object+field measurement*)

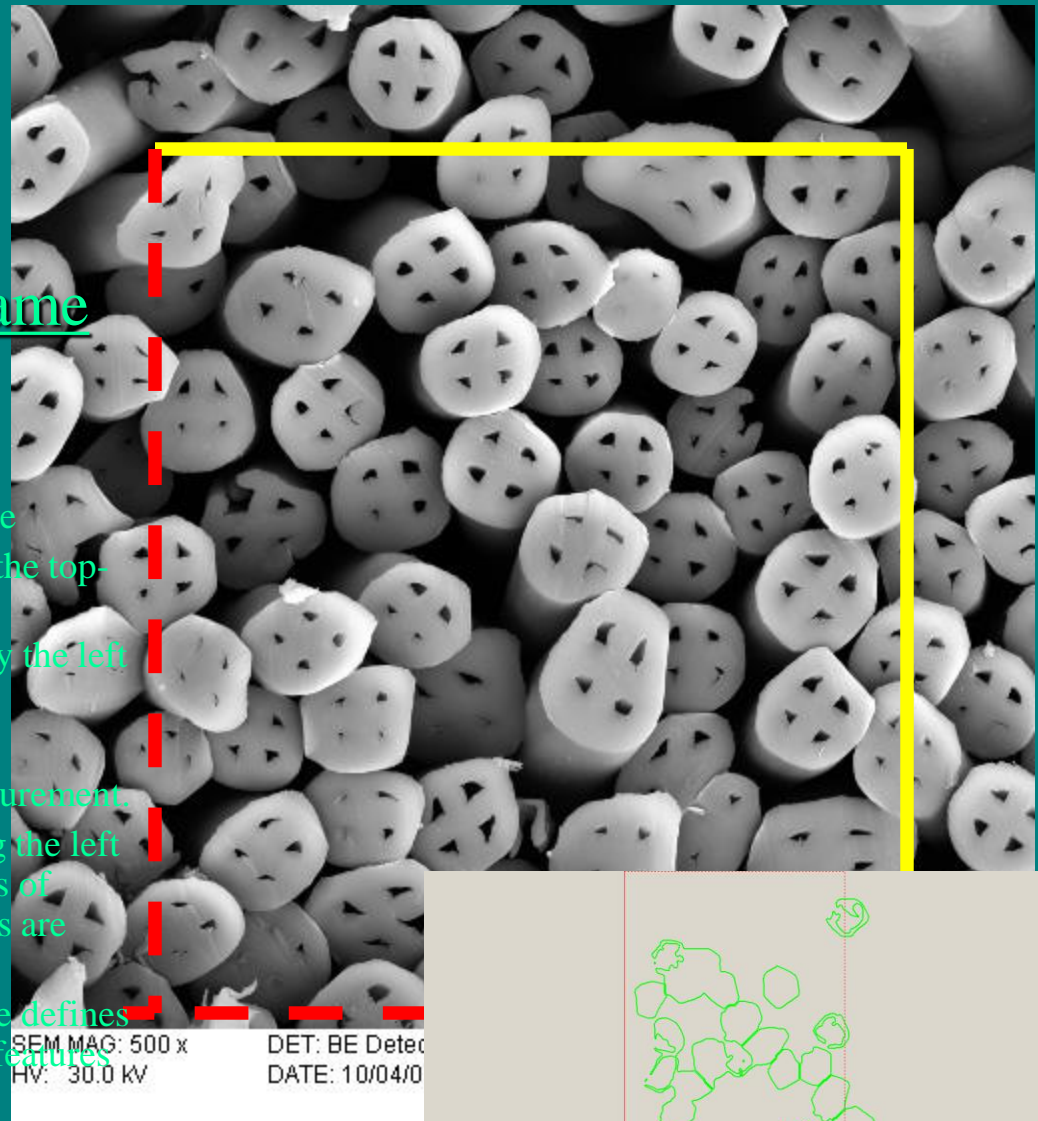
*AcqTime, Area, AreaFraction, BrightVariation, CenterX, CenterY, Circularity, Class, DensityVariation, Elongation, EqDiameter, ExPurity, FillArea, FillRatio, GrayVariation, HueTypical, HueVariation, IntegralDensity, Length, MaxFerret, MaxGray, MeanBlue, MeanBrightness, MeanDensity, MeanGray, MeanGreen, MeanRed, MeanSaturation, MeasuredArea, MinFerret, NumberObjects, Orientation, Perimeter, StartX, StartY, StgPosX, StgPosY, SurfVolumeRatio, Time, VolumeEqCylinder, VolumeEqSphere, Width*



# Measurement

## Definition of Measurement Frame

- Definition of size
  - command Measurement Frame in tool Measure
  - or by key Ctrl+F and type the co-ordinates of the top-left and bottom-right corners
  - or by drag the measurement frame rectangle by the left mouse button.
- Has different meaning for object and field measurement.
- For object measurement – the particles touching the left and bottom lines are excluded from the statistics of objects, particles touching the top and right lines are included.
- For field measurement – the measurement frame defines the rectangular area to which measurements of features are restricted.



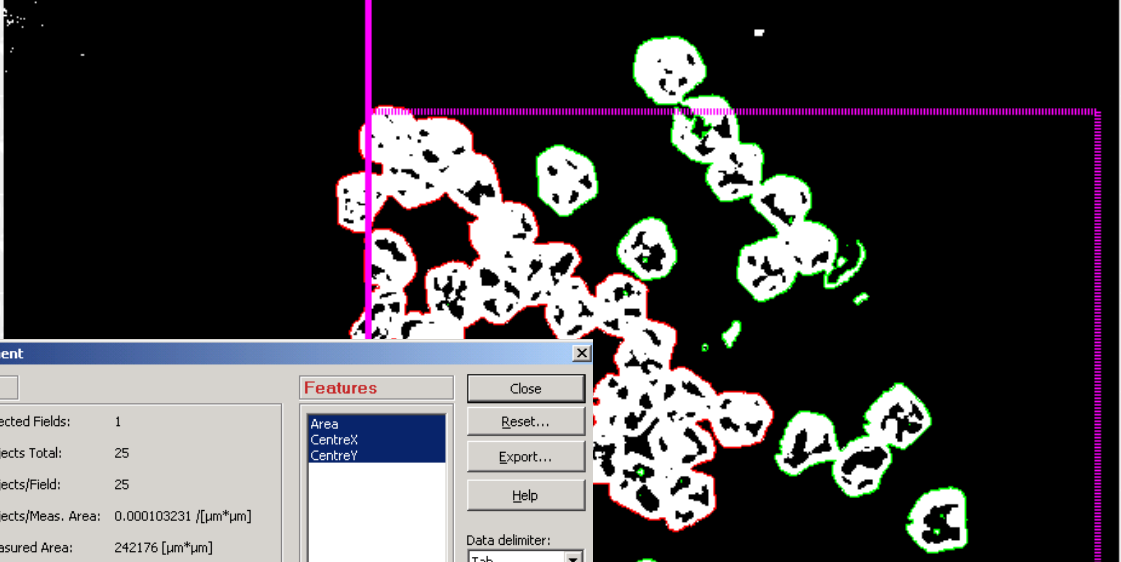
# Measurement – Statistics and Data Presentation

NIS-Elements AR - [22a.jp2\*]

File Edit Calibration Image Binary Measure Reference Macro View Window Devices Help

Vlakna Nové 4x Laird01 0901 VUTS\_030309 VUTS\_090209 Pok Laird\_SEM 010409 Laird\_mensi Laird\_vetsi Laird\_vyrobek Laird\_vyrobek\_vetsi Jelínek

1:1 53.7%



**Object Data Management**

**Fields**

Field / #Objects	Selected Fields:	1
1 / 25	Objects Total:	25
	Objects/Field:	25
	Objects/Meas. Area:	0.000103231 / [μm*μm]
	Measured Area:	242176 [μm*μm]
	Area Fraction:	0.226205

Select All

**Features**

- Area
- CentreX
- CentreY

Close  
Reset...  
Export...  
Help

Data delimiter: Tab  
Decimal separator: Comma

**Statistics**

Feature	Mean	St.Dev	Minimum	Maximum
Area	1034.8	1922.7	0.42772	8619.3
CentreX	542.99	103.68	328.96	695.86
CentreY	307.94	109.66	129.19	509.47

Export...  Mean Values for Every Field

**Distributions**

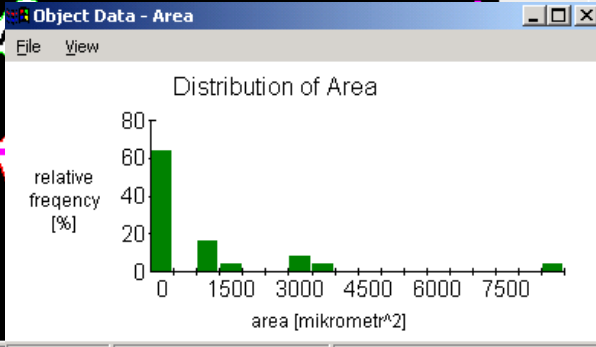
Area

Show Histogram

**Object Data - Area**

File View

Distribution of Area



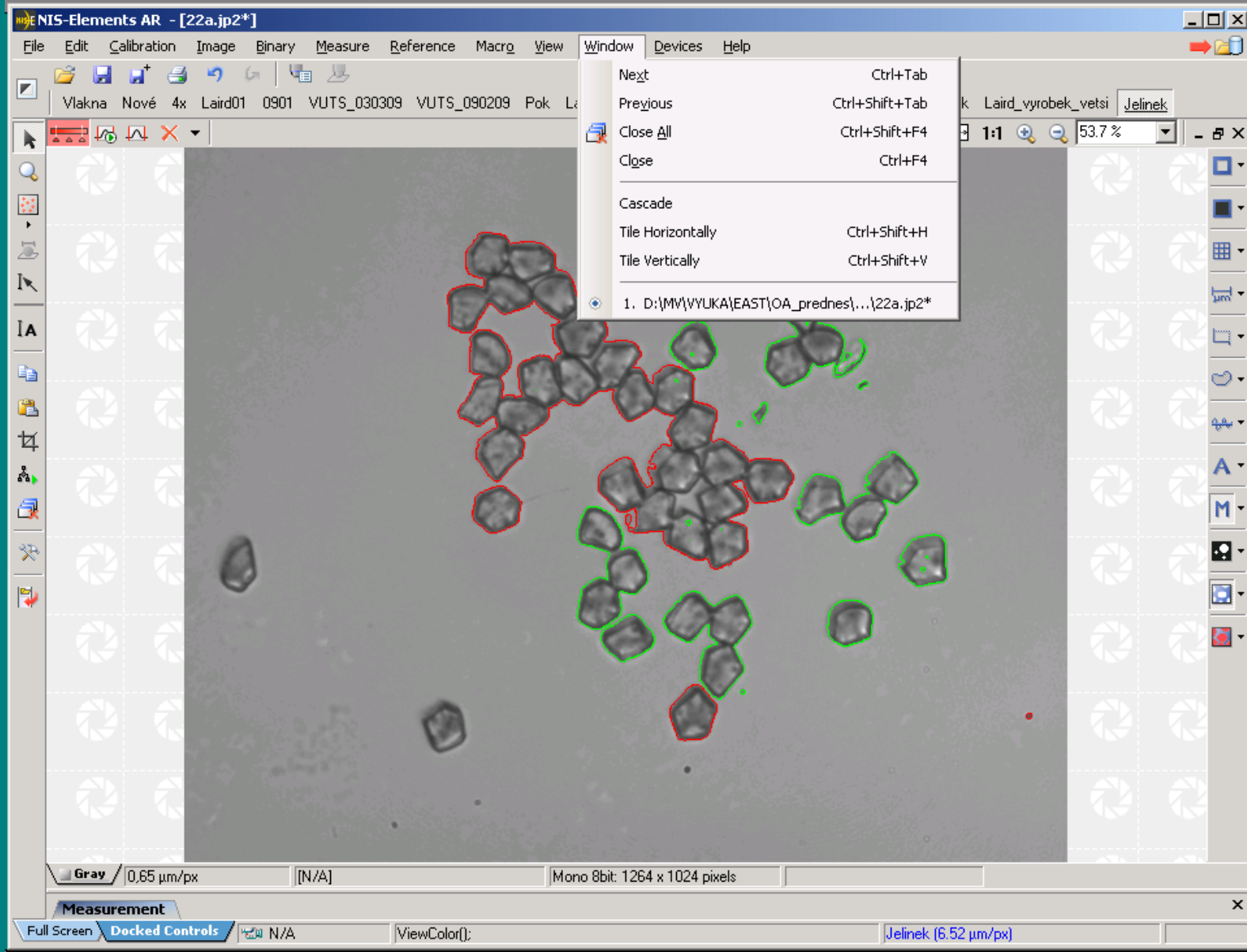
relative frequency [%]

area [mikrometr<sup>2</sup>]

x 1 Num:25 of 25 Range: <unlimited, unlimited> min = 0.43, max = 8619.33, mean =

Full Screen Docked Controls N/A ScanObjects(): Jelínek (6.52 μm/px)

# Further Function of the System



# Macro – Programming in NIS Elements

The screenshot displays the NIS-Elements AR interface. The main window shows a grayscale image of a flower with a white mask. The 'Macro' menu is open, listing various actions and their keyboard shortcuts. A 'Command History' dialog is also open, showing a list of recorded commands. The status bar at the bottom indicates the current image is in Gray mode, 0.65 µm/px, and the macro being executed is 'OpenBinary(4,5)'.

**Macro Menu:**

- Repeat Last (Ctrl+F2)
- History... (F2)
- Command... (Ctrl+F10)
- Write to Port...
- Record... (F3)
- Run (F4)
- Run From...
- New...
- Open... (Ctrl+F8)
- Save As...
- Browse... (Ctrl+Shift+B)
- Edit... (F8)

**Command History Dialog:**

```
OpenBinary(4,5);
_ObjectData();
CloseCurrentDocument(0);
_CloseCurrentDocument();
OpenDocument("E:\MV\WYUKA\EAST\OA_prednes\ObrPrezentace\22a.jp2",0);
ColorToGray();
DefineThreshold(0,0,0,109,109,109,0);
Threshold();
ViewBinary();
FillHoles();
CleanBinary(4,5);
MorphoSeparateObjects(16,4);
OpenBinary(4,5);
```

Commands: 104, Free Memory: 150.7 MB, Free Disk C: 74.4 GB, Free Page File: 537.8 MB

**Status Bar:** Gray | 0.65 µm/px | [N/A] | Mono 8bit: 1264 x 1024 pixels

**Measurement:** Time Measurement

**Bottom Bar:** Full Screen | Docked Controls | N/A | OpenBinary(4,5); | tkanina (1.21 µm/px)

- Recording of made actions,
- Editing in dialog window – Macro,
- Modification of commands from Command History