

**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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# Principles of image capture in the NIS Elements image analysis system

Ing. Bc Monika Vyšanská, PhD.



Financováno  
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Národní  
plán  
obnovy



MINISTERSTVO ŠKOLSTVÍ,  
MLÁDEŽE A TĚLOVÝCHOVY

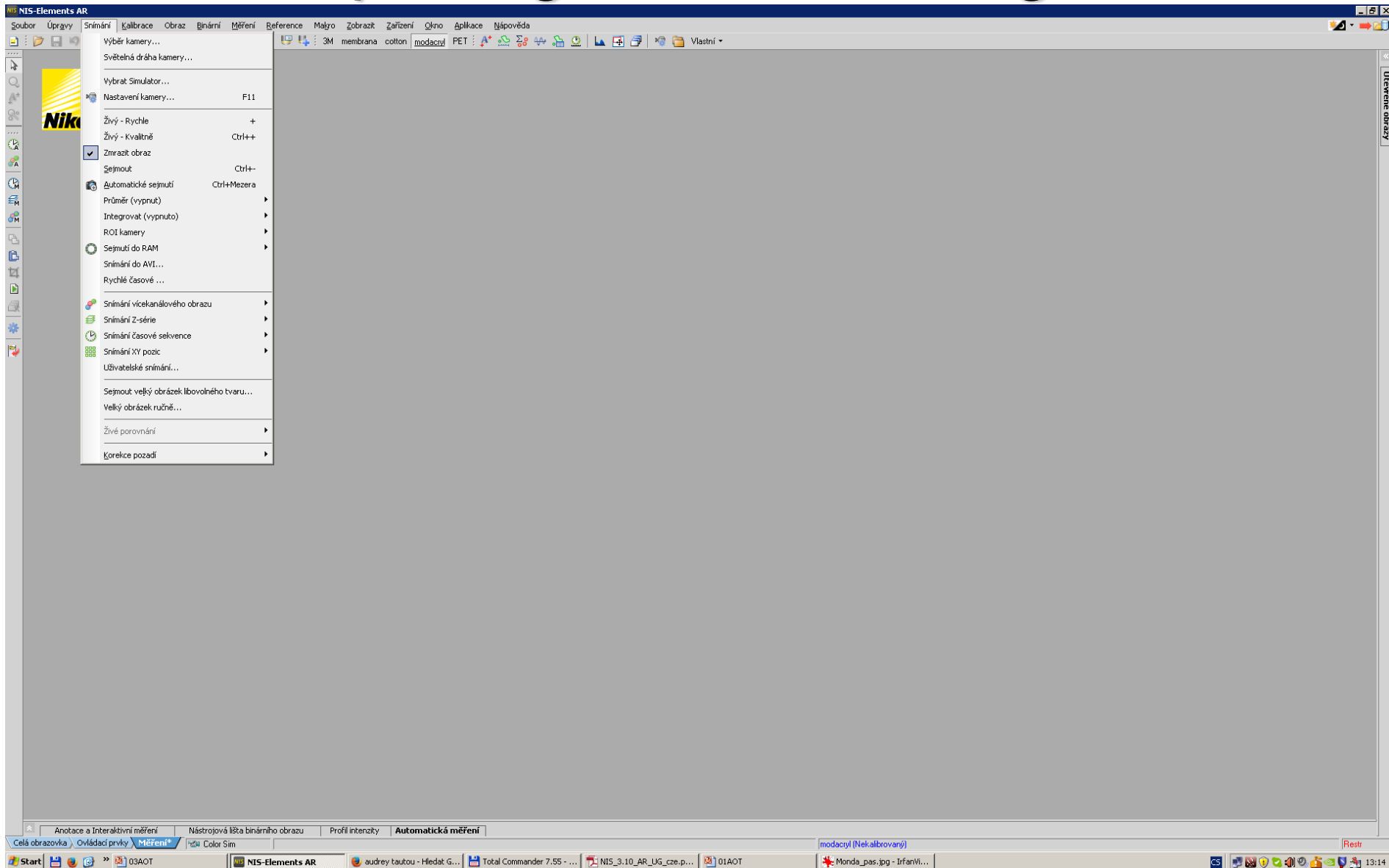
# **Principles of image capture in the NIS Elements image analysis system - image capture, ND2 capture (temporal, x, y, z), RAM capture.**

Ing. Monika Vyšanská, PhD.

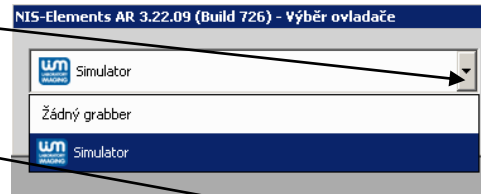
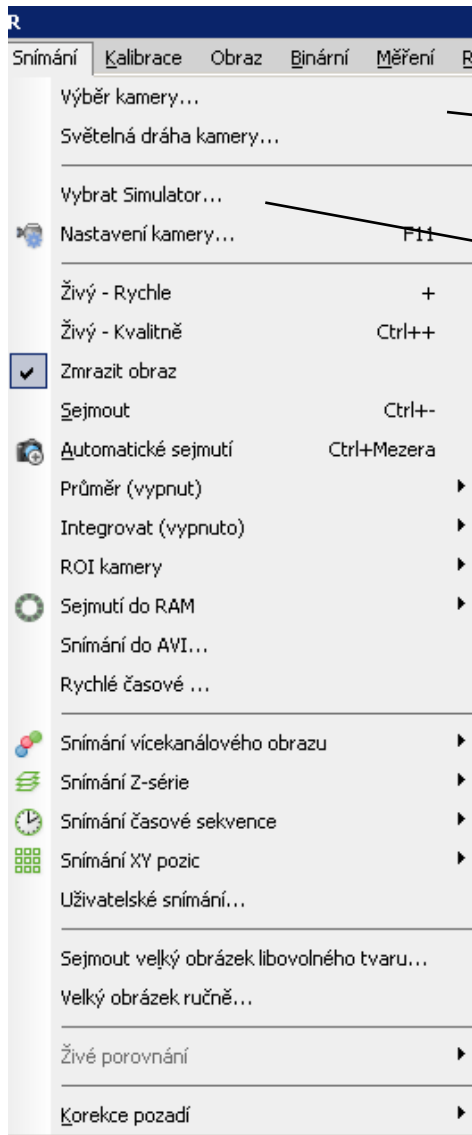
# Capturing individual images [1]

- If the HASP (hardware) key does not support scanning, the "Scanning" item will not appear in the main menu at all!
- Turn on the camera and all connected devices and launch NIS-Elements AR™. Select the appropriate camera driver.
- In the application, display the [Camera Settings] control window ([Capture > Camera Settings]).
- Switch the camera to [Live-Fast] mode and adjust the camera parameters (especially the exposure) to get a clear and distinct image.
- Focus on the sample.
- Switch the camera to [Live-quality] mode and adjust the settings, especially [exposure time] and [resolution]. This mode will be used each time an image is captured.
- Switch back to [Live-Fast] mode, which is more suitable for smooth operation.
- Take a picture using the button in the horizontal toolbar, or use [Take > Take].
- The downloaded image will automatically open as a new document named "Downloaded".

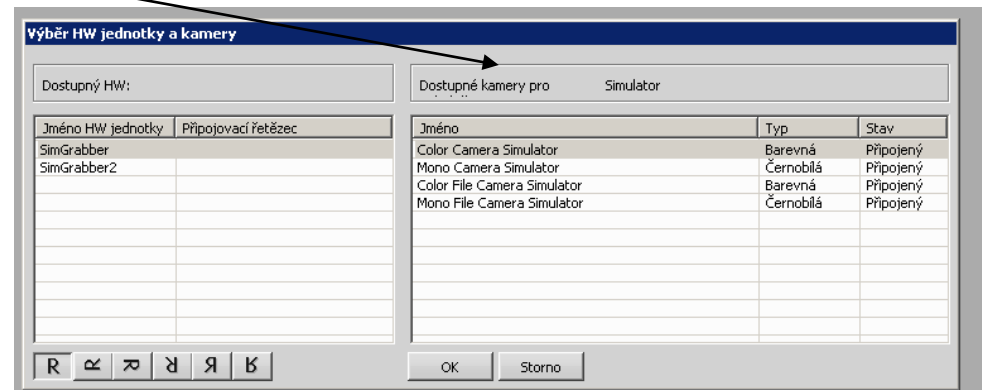
# Capturing individual images



# Capturing individual images – simulator



Usage: e.g. when creating (debugging) when a camera is not available.



# Sensing - camera light path, live, frozen image

The screenshot displays the NIS-Elements AR software interface. The main window shows a camera menu on the left with options like 'Živý - Rychle' (Live - Fast) and 'Živý - Kvalitně' (Live - High Quality). A red circle highlights these options. A dialog box titled 'Světelná dráha kamery' (Camera Light Path) is open, showing settings for 'Automaticky přepni na port:' (Automatically switch to port:), 'Dual/Quad View' status, 'Aktivace Dual/Quad View se provádí v okně opt. konfigurací' (Dual/Quad View activation is performed in the optical configuration window), 'Výchozí aktivní clona:' (Default active aperture:), 'Převracení čočky:' (Lens flip: 1.00x), and 'Úhel kamery:' (Camera angle: 0°). The 'OK' and 'Storno' buttons are visible at the bottom of the dialog.

**Světelná dráha kamery**

Automaticky přepni na port:

'Dual/Quad View' je připojený

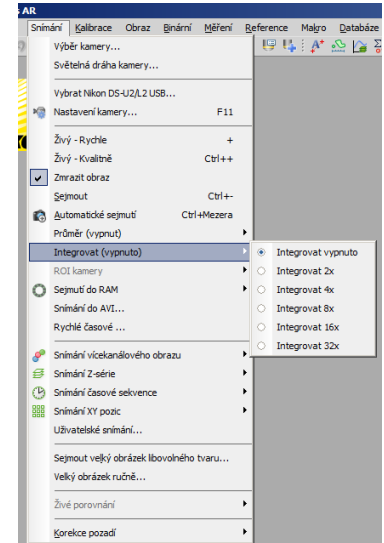
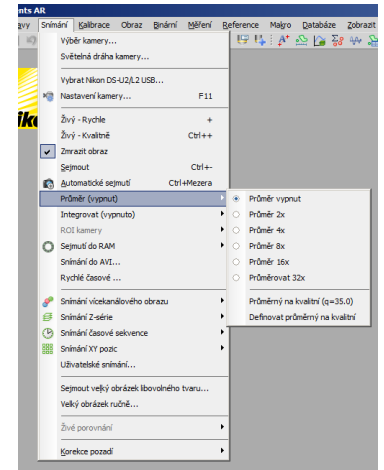
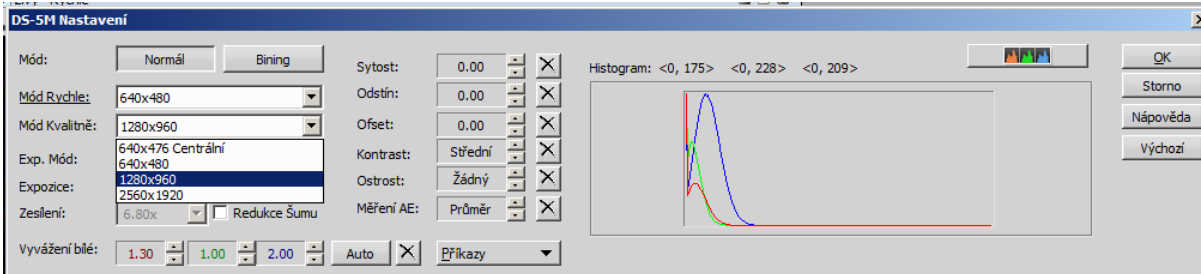
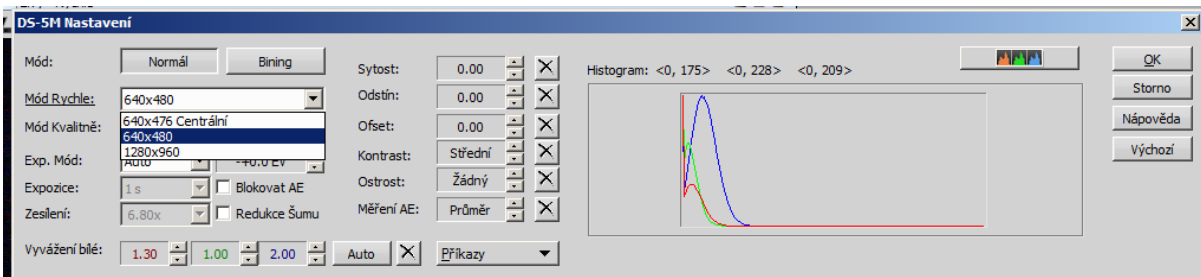
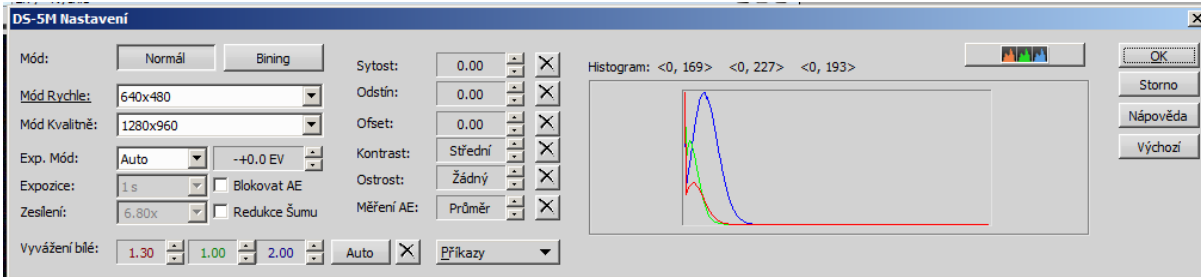
Aktivace Dual/Quad View se provádí v okně opt. konfigurací

Výchozí aktivní clona:

Převracení čočky:  x

Úhel kamery:  °

# Shooting - Camera Setup



# Shooting - Camera Setup [1]

- *Format Fast (Focusing) / Quality (Shooting)*
  - Usually cameras have different resolutions. NIS-Elements AR™ allows you to create two resolution presets - [Fast] and [Quality]. The formats can vary in image size and frame rate (frames per second - fps). The higher the resolution, the lower the frame rate can be achieved. The available resolutions depend on the type of camera.
- *Binning*
  - Binning mode noticeably improves the sensitivity of the camera chip by integrating several elements (pixels) together. This results in lower resolution and faster scanning speeds. For example, [binning x4] adds the signal from a 4x4 element chip area into a single pixel of the resulting image.
- *Exposure mode*
  - Exposure mode sets how the exposure time is calculated. Automatic and manual modes are available:
  - [Auto Exposure] - Calculates the optimal combination of [exposure time] and [gain] to achieve the optimal exposure of the image. Image quality is a priority, so longer exposure times are preferred. Normally, you trigger auto exposure only once by pressing the [Auto Exposure] button, or continuously by checking the appropriate check box.
  - [Manual Exposure] - The user manually specifies both [Exposure Time] and [Gain].
- *Exposure*
  - If you are using [Manual Exposure] mode, this option controls the exposure time. This is the time for the signal to accumulate on the camera chip between two consecutive frames. Increasing the exposure time increases the brightness and quality of the image (less gain means less noise), but decreases the shooting speed.



# Shooting - Camera Setup [1]

- *Amplification*
  - Increasing the gain will increase the brightness of the image, increase the shooting speed (by reducing the exposure time), but reduce the image quality (the higher the gain, the more noise).
- *Averaging*
  - Averaging is a technique commonly used to reduce noise in an image. This method can be used to average different numbers (2, 4, 8, etc.) of adjacent frames. Averaging, of course, multiplies the exposure time required to obtain a single image.
- *Offset*
  - Adjusts the brightness of the image. It is a constant (positive or negative) that changes the values of all pixels in the image. A negative offset value can be used in fluorescence experiments, where a suitable setting will create a continuous black background and thus improve (along with amplifying or adjusting the lighting) the contrast of the scene.
- *Contrast*
  - It affects the lighting dynamics in the image. The option offers several modes for different scene lighting.
- *Gamma*
  - Setting the gamma parameter will provide non-linear brightness adjustment in the image. For  $\gamma < 1$ , the dark parts of the image are highlighted, whereas for  $\gamma > 1$ , the bright parts of the image are highlighted.

# Shooting - Camera Setup [1]

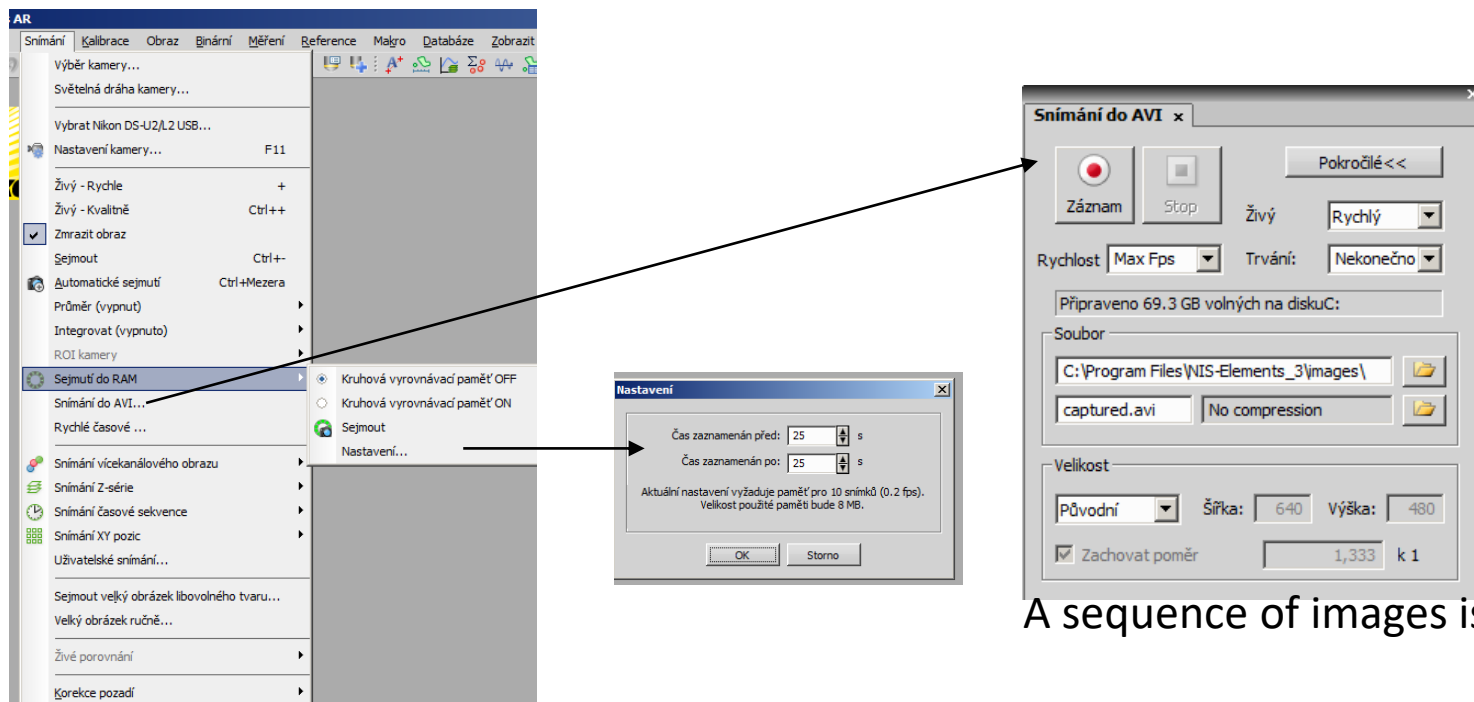
- *AE compensation*
  - In automatic exposure modes, AE compensation affects how the optimal exposure settings (exposure time and gain) are calculated. Compensation values are expressed in Exposure Values (EV). Setting compensation to +1.0 EV doubles the brightness of the image (i.e. doubles the exposure time or gain).
- *AE lock*
  - This option locks the current auto exposure settings (exposure time and gain).
- *Sharpness*
  - Some camera settings allow sharpness control, which affects how sharp the edges appear in the image.
- *Shade*
  - The tint shifts all colors of the image along the displayable color gamut to one side or the other.
- *Saturation*
  - Saturation affects the display of colours. Setting saturation to 0 produces a gray image.
- *White balance*
  - Typically, colour cameras have tools to adjust the red, green and blue gain separately. This is used to remove colour cast from white areas.
- *Auto White Balance (AWB)*
  - The AWB button performs automatic white balance. Adjusts the red, green and blue components of the image to produce a neutral white color.

# Capture - capture to RAM, AVI

The Snapshot to RAM technique allows sequential recording of processes that take place in tens of milliseconds. In order to track such fast processes, you first need a sufficiently powerful (fast) camera.

The RAM sensing technique uses part of the RAM to continuously store a kind of history of the live image. When the dedicated memory is full, its contents are overwritten from the beginning, so that this history is always available. Based on the expected duration of the observed action, choose the size of this memory (in seconds) [1].

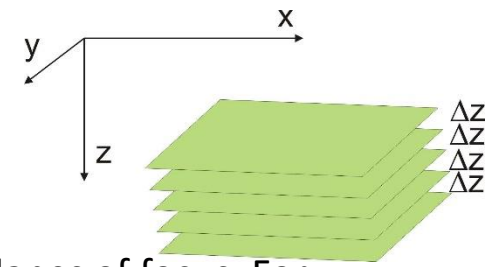
AVI capture is a simple way to record processes in real time. The result is a \*.avi video file that can be played with a standard video player, so you don't need NIS-Elements AR™ to view the result.



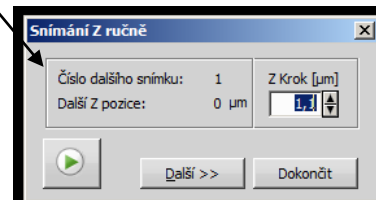
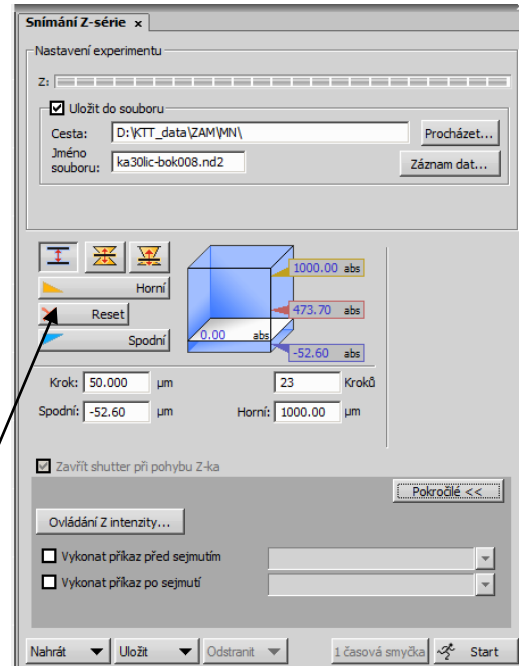
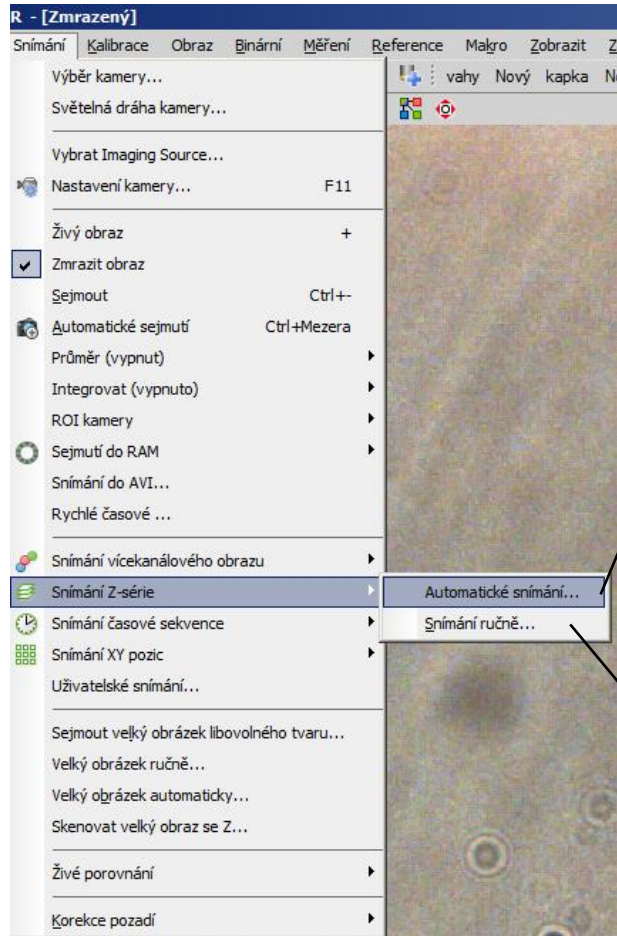
A sequence of images is captured to \*.avi.

A sequence of images is captured into RAM.  
EXA\_04

# Capturing 3D images - data



The series is created by taking multiple images of the sample in different planes of focus. For example, such a Z-series can be used to create a 3D model of a sample [1].



# SAMPLE OF THE REAL SERIES

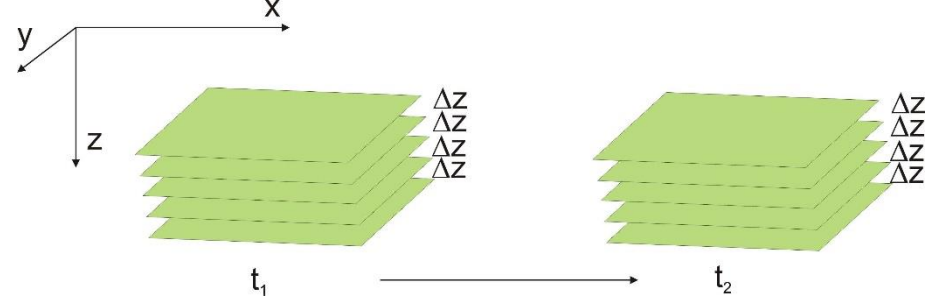
PJ yarn with a nominal fineness of 20 tex and a nominal twist of 720 [1/m] - Saunova, O. - BP, 2013



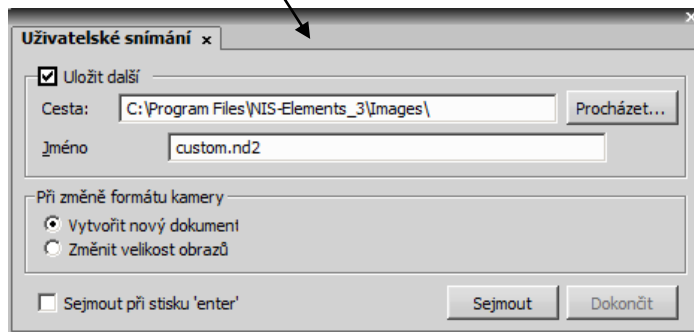
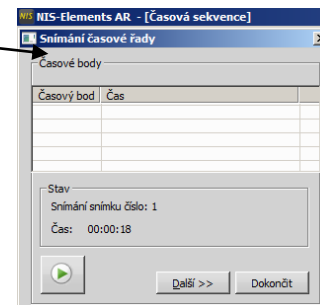
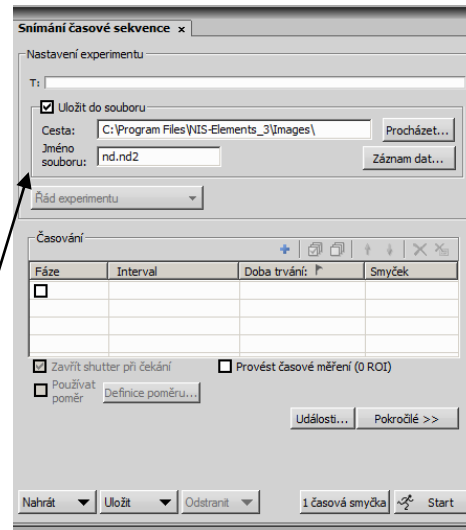
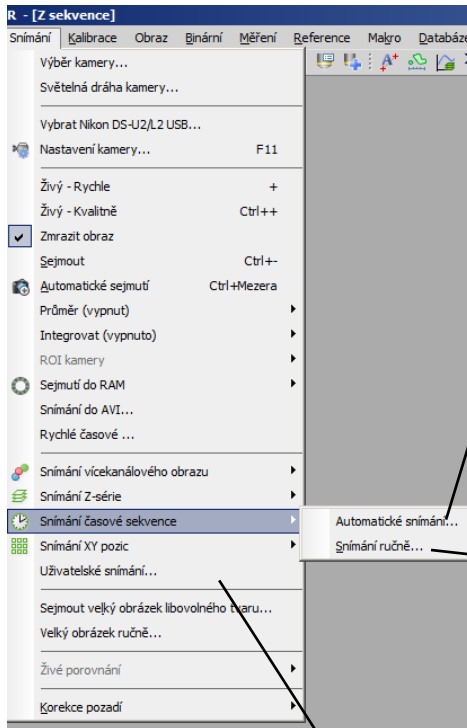
IMAGES FROM DIFFERENT DEPTHS OF FIELD

FINAL IMAGE  
THROUGH THE FUNCTION OF  
**EDF – extended depth focus**

# Capturing 4D images – data



A sequence of images is captured over a period of time to create a time-lapse document [1].



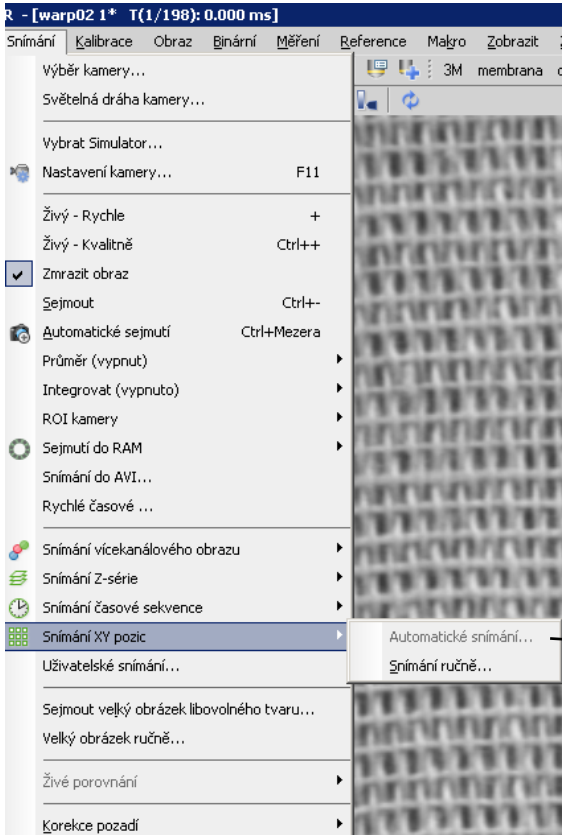
# SHOW OF THE REAL $t$ SERIES \*.avi mimo \*.ppt

Info for each frame in the time sequence, generally nd2 sequence

Property	Value
Čas (1/198):	0s
Doba trvání:	278,2s
Požadovaný čas:	0,0000003s
Prům. rozdíli:	1,412s
Min. rozdíli:	1,394s
Max. rozdíli:	2,199s
Casových kroků/s:	0,71
FPS souhrnné:	0,71

Software interface details: NIS-Elements AR - [warp02 1\* T(1/198): 0.000 ms], 173.0% zoom, RGB 8bitů: 752 x 560 bodů [N/A], Automatická měření, ND\_ConvertFinish(Handle, "C:\DODUCEM\1\Monika\LOCALS\1\Temp\NIS149.tmp"), modacnyl (Nel.albvorany)

# Multi-point sensing [1]



Images can be captured from different areas of the sample. This functionality is only available when a motorized XY(Z) stage is connected. Then any array of XY positions can be defined for scanning. If desired, the Z dimension can be included or coordinates can be saved for later use. The definition of the XY position array can be saved in a file for later use. To start the scan, use the command [Capture > Capture XY Positions > Auto Capture].

A screenshot of a dialog box titled 'Body'. It contains a table with columns for 'Jméno bodu' (Point Name), 'X [mm]', 'Y [mm]', 'Z [μm]', and 'PFS'. The table has three rows of data, each with a checked checkbox in the first column. The first row has a right-pointing arrow next to the point name. Below the table are several checkboxes and buttons: 'Zahrnout Z' (checked), 'Relativní XY' (unchecked), 'Optimalizovat', 'Nahrát...', 'Uložit...', and 'WP...'.

Jméno bodu	X [mm]	Y [mm]	Z [μm]	PFS
<input checked="" type="checkbox"/> #1 ->	0.000	0.000	0.03	Posunout vš N/A
<input checked="" type="checkbox"/> #2	50.000	17.000	0.03	N/A
<input checked="" type="checkbox"/> #3	50.000	17.000	4.12	N/A
<input type="checkbox"/>				

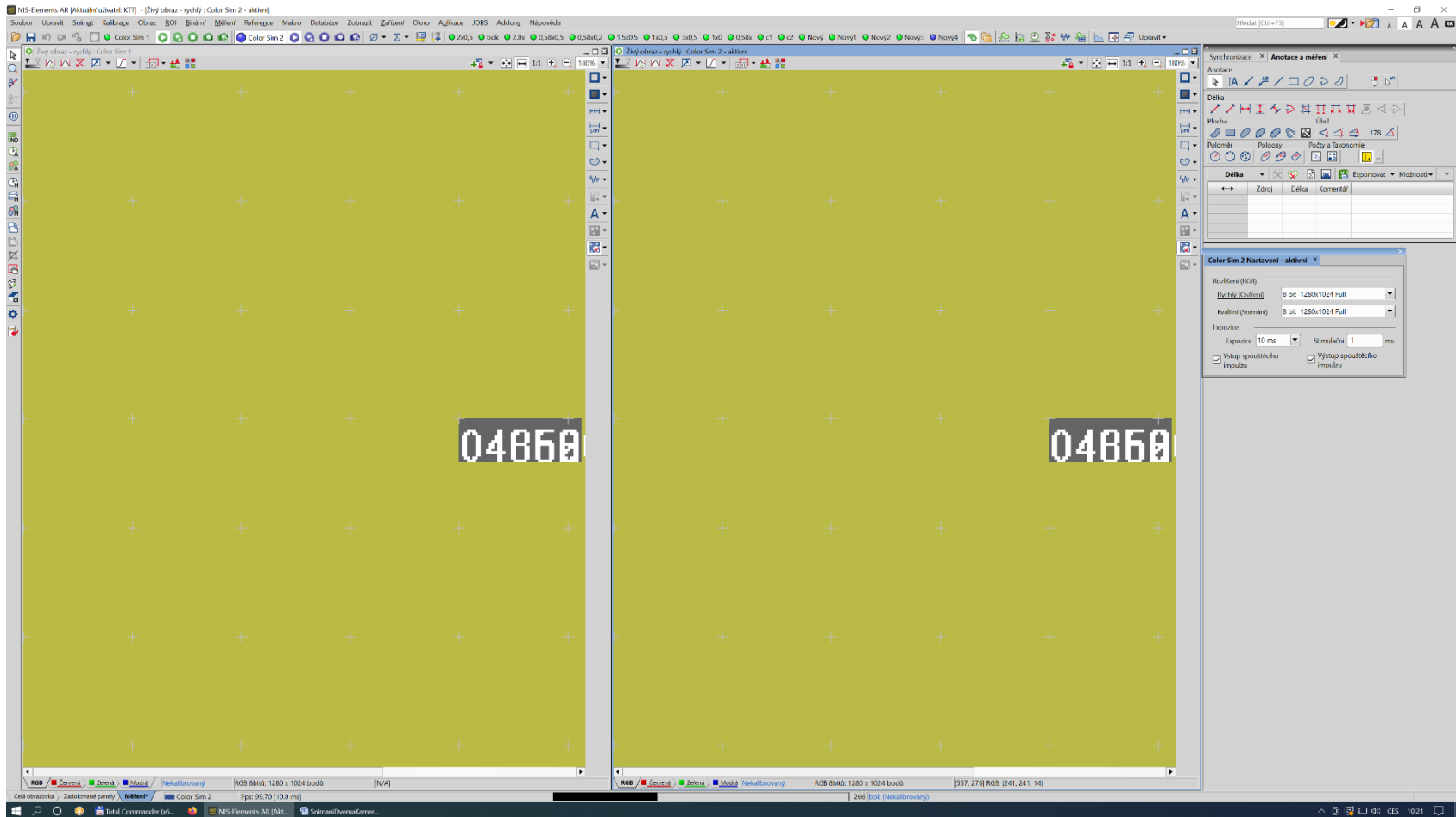
The user defines the positions by moving the motorised table.

With the [Relative XY] item, all coordinates will be calculated as relative to the specified reference position. The reference point is marked with a cross in the table.



# Simultaneous capture with two cameras

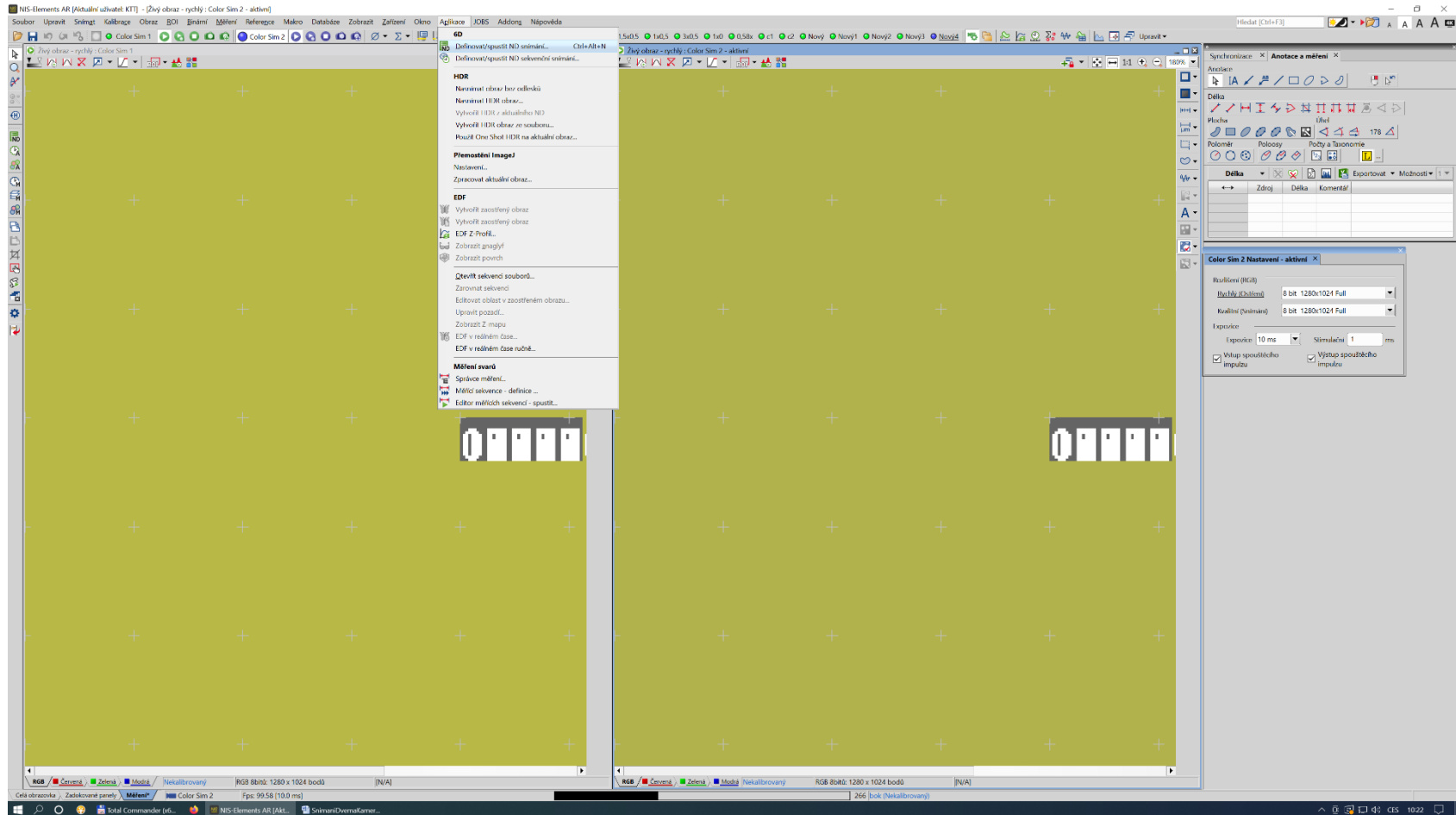
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



It is necessary to have both cameras active.

# Simultaneous capture with two cameras

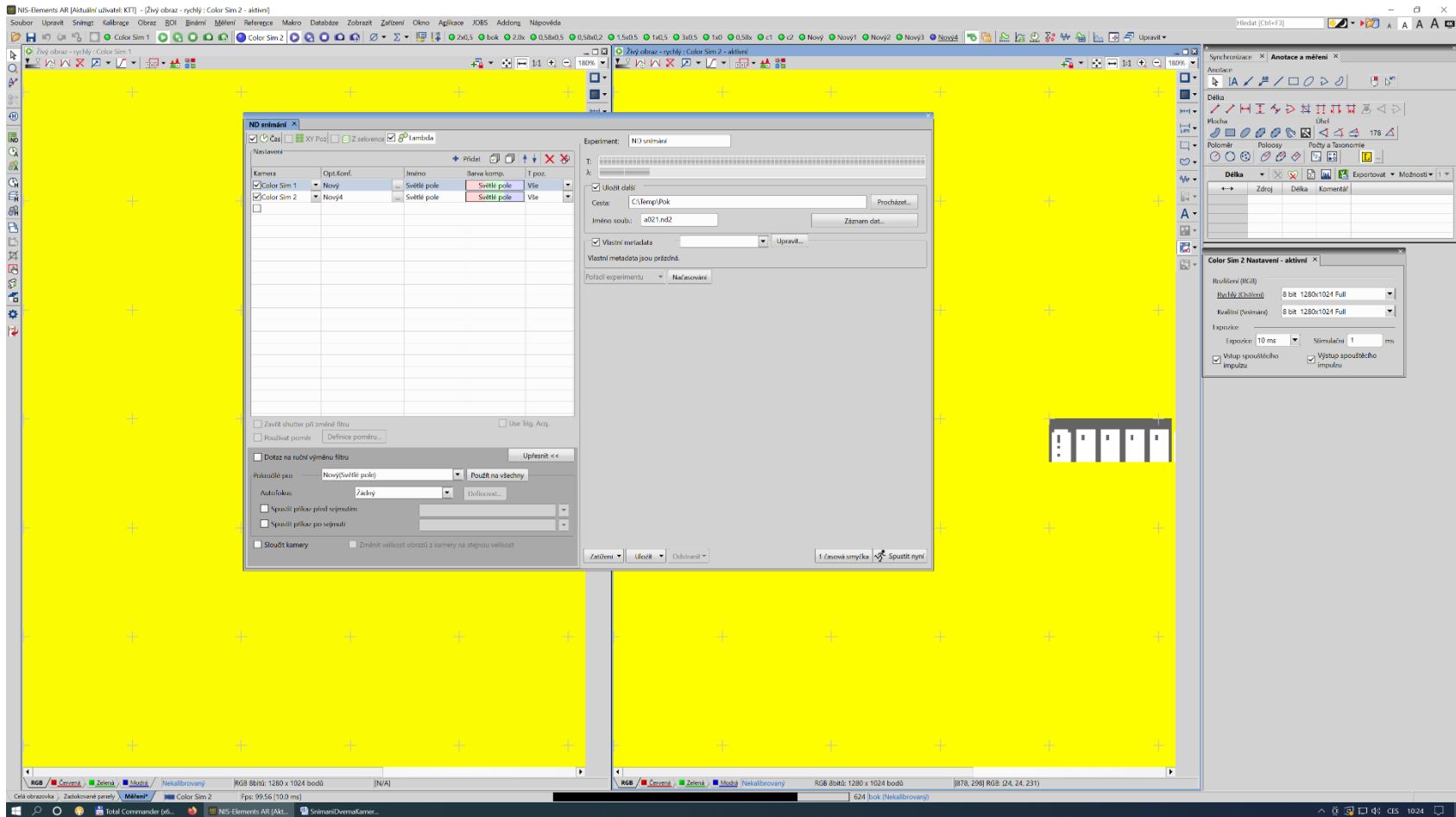
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



We used Applications - 6D - Define/trigger ND capture...

# Simultaneous capture with two cameras

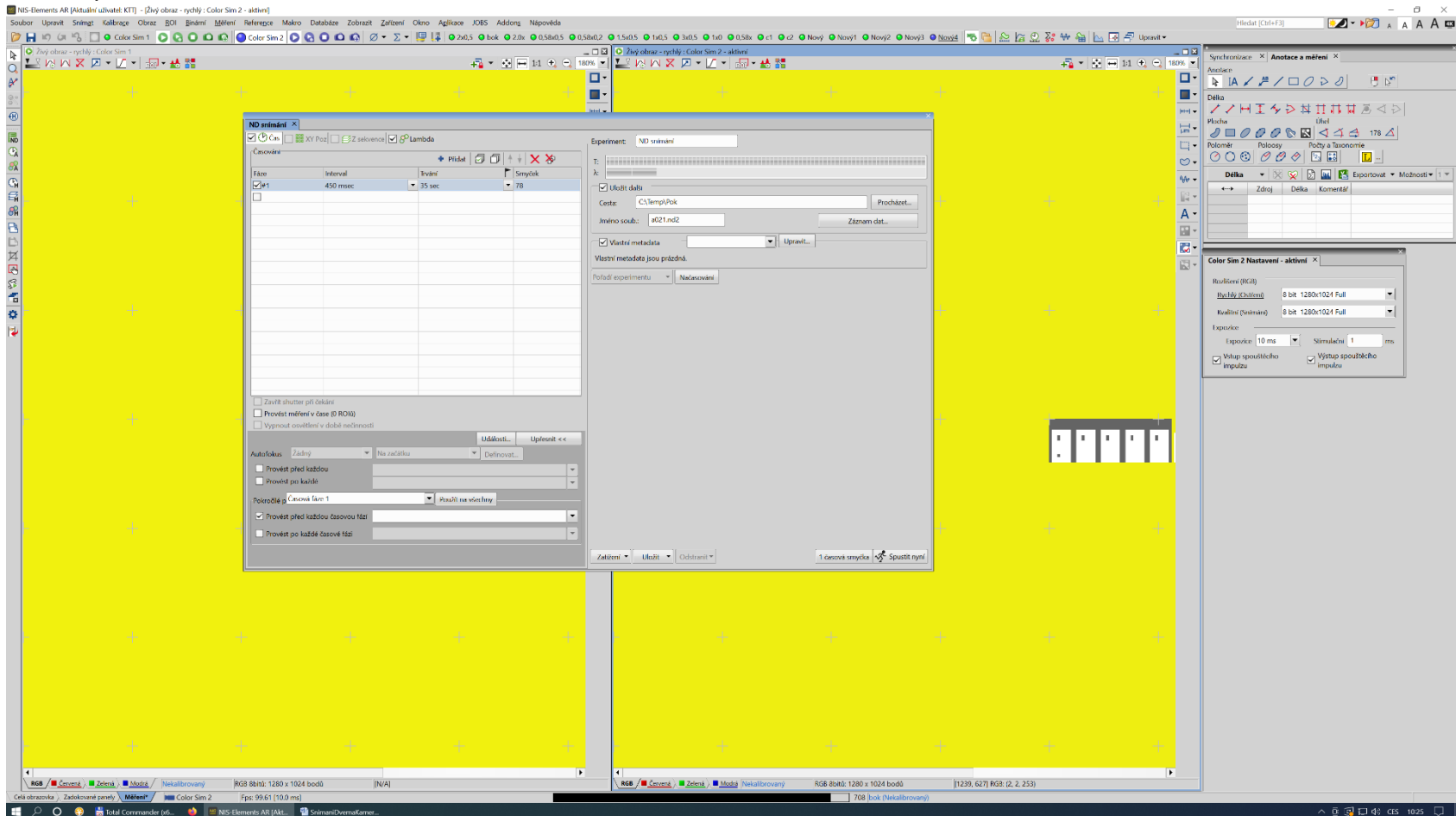
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



We selected two active cameras in the wavelength range for sensing and assigned them an optical configuration.

# Simultaneous capture with two cameras

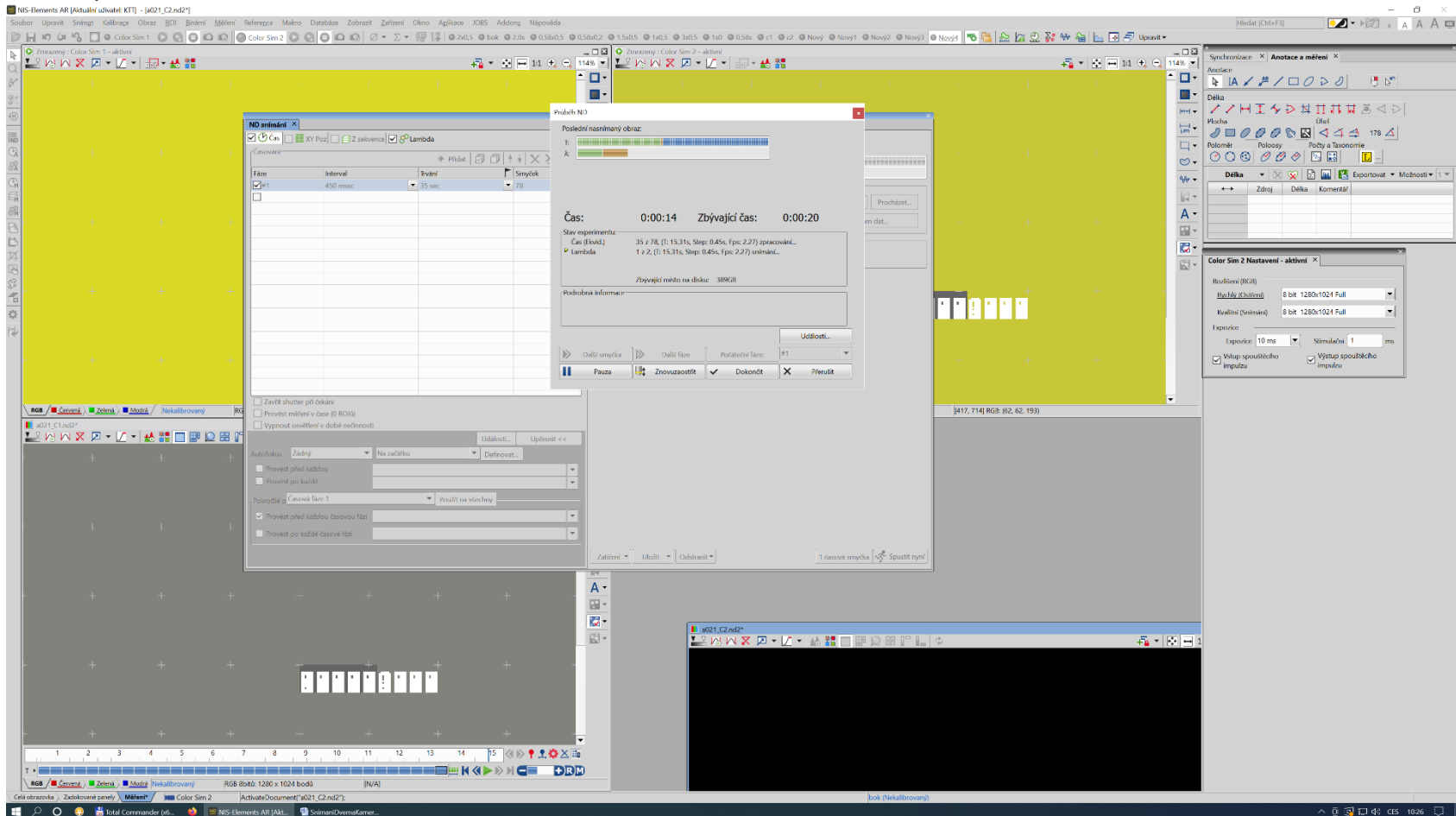
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



We have selected the appropriate time for scanning (estimated from the pre-test).

# Simultaneous capture with two cameras

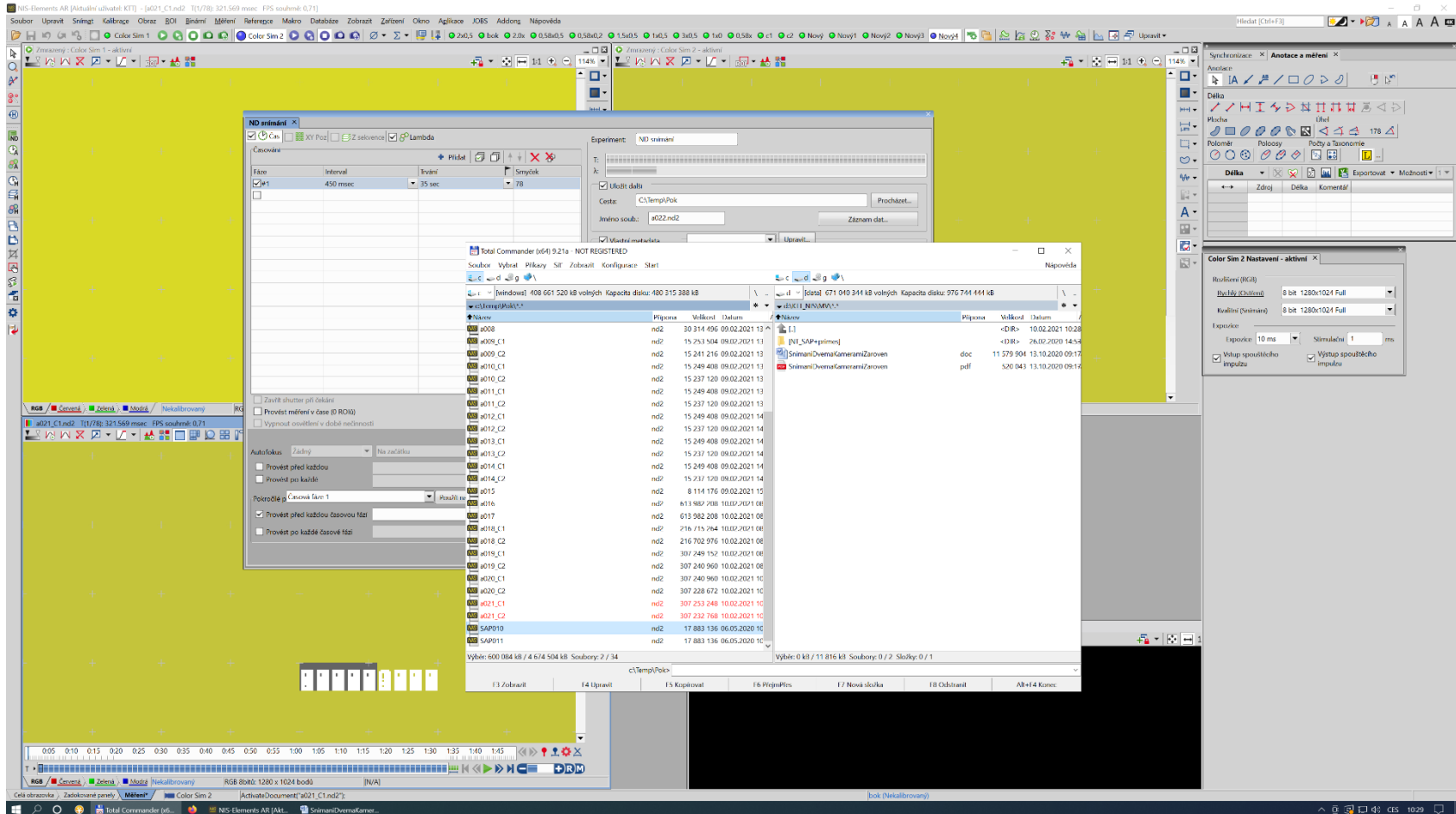
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



Once triggered, both cameras are scanned simultaneously for a given time.

# Simultaneous capture with two cameras

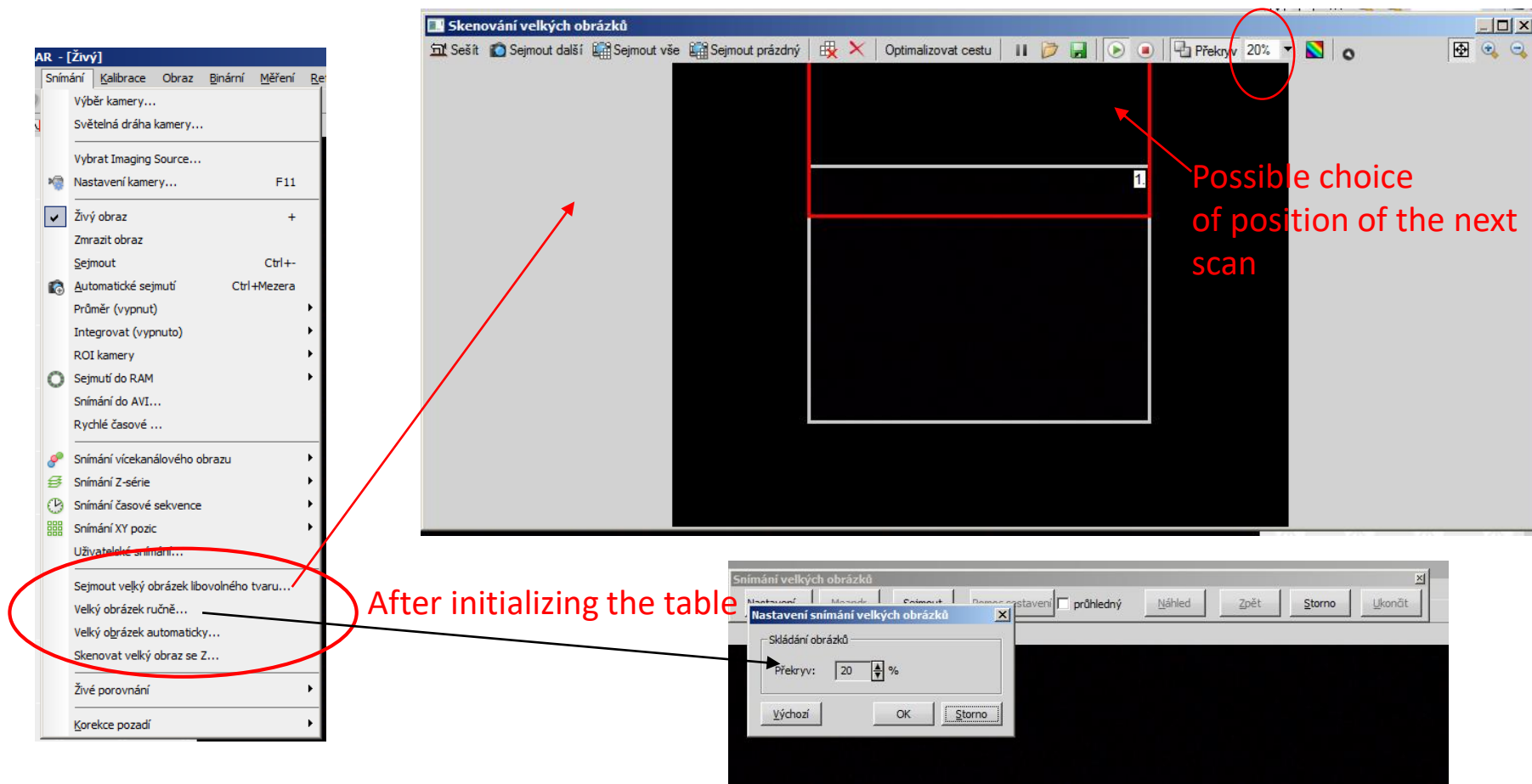
(demonstration on simulators, SAP capture demonstration outside this presentation in \*.avi)



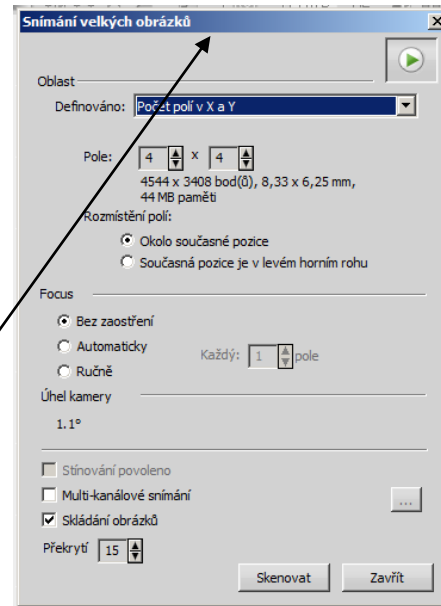
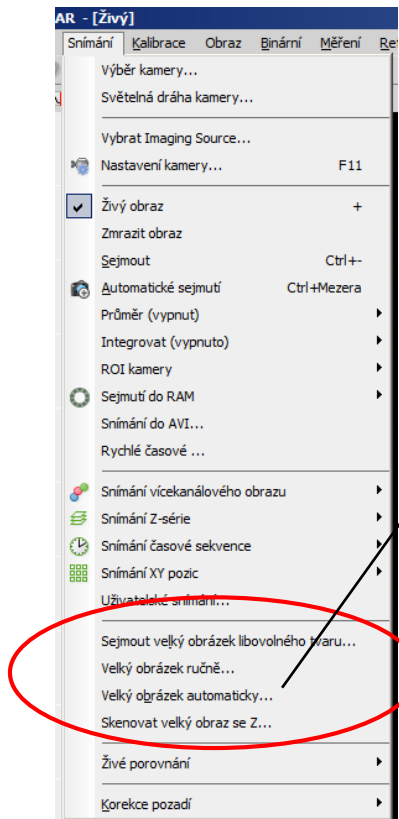
The results are stored in two nd sequences labeled ...\_C1.nd2, ...C2.nd2.

# Capturing large images

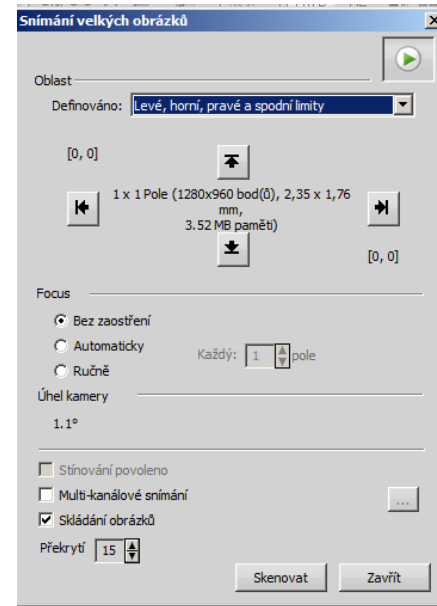
The [Large Image] tab is used to capture large images in a combined ND experiment. It is necessary to have a motorized table. When started, the system takes several images in sequence and combines them together to get one large image [1].



# Capturing large images



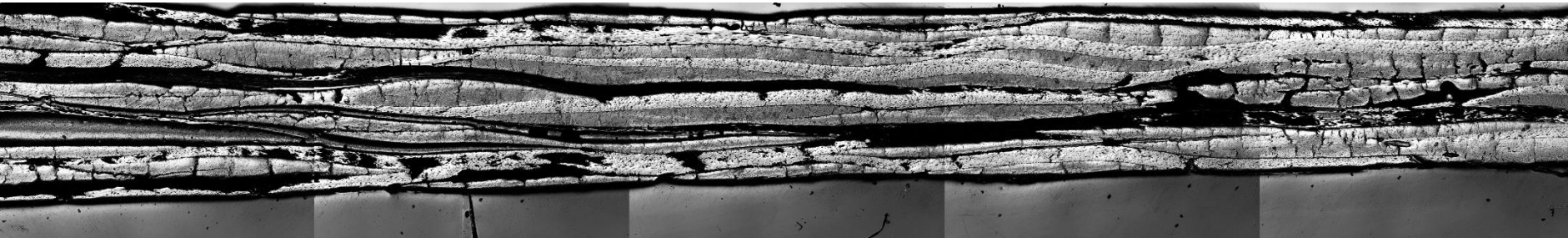
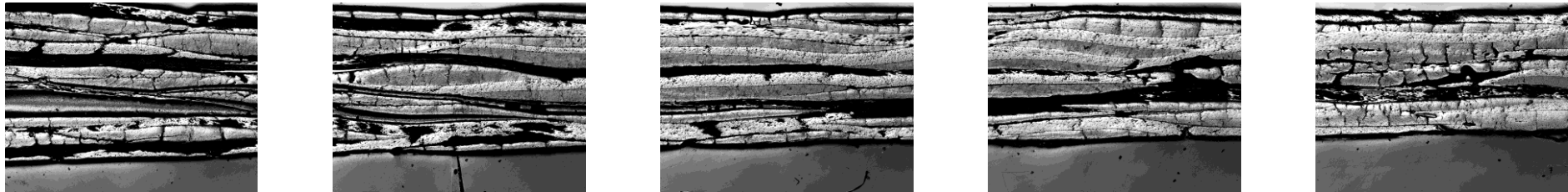
Defining an area by field size



Defining the area by x, y coordinates of the rectangle



# LARGE COMPOSITE IMAGE - composite (5% overlap)



Müller, M.: Porosity of carbon composite, semester thesis, EAS, 2013

# Combination of N dimensions/dimensions

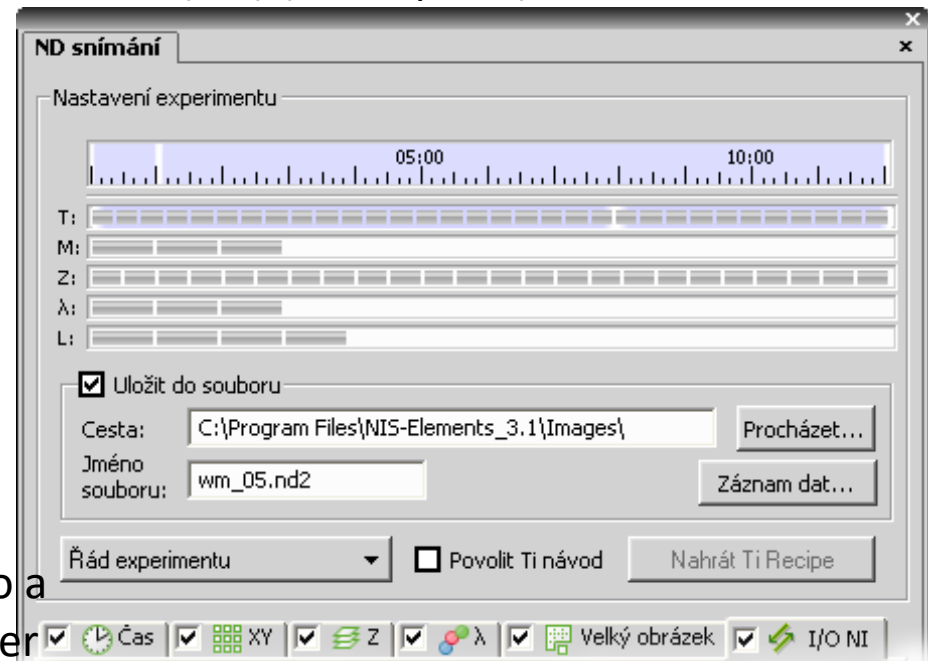
## [1]

By capturing multiple dimensions at once, you end up with a multi-dimensional document.

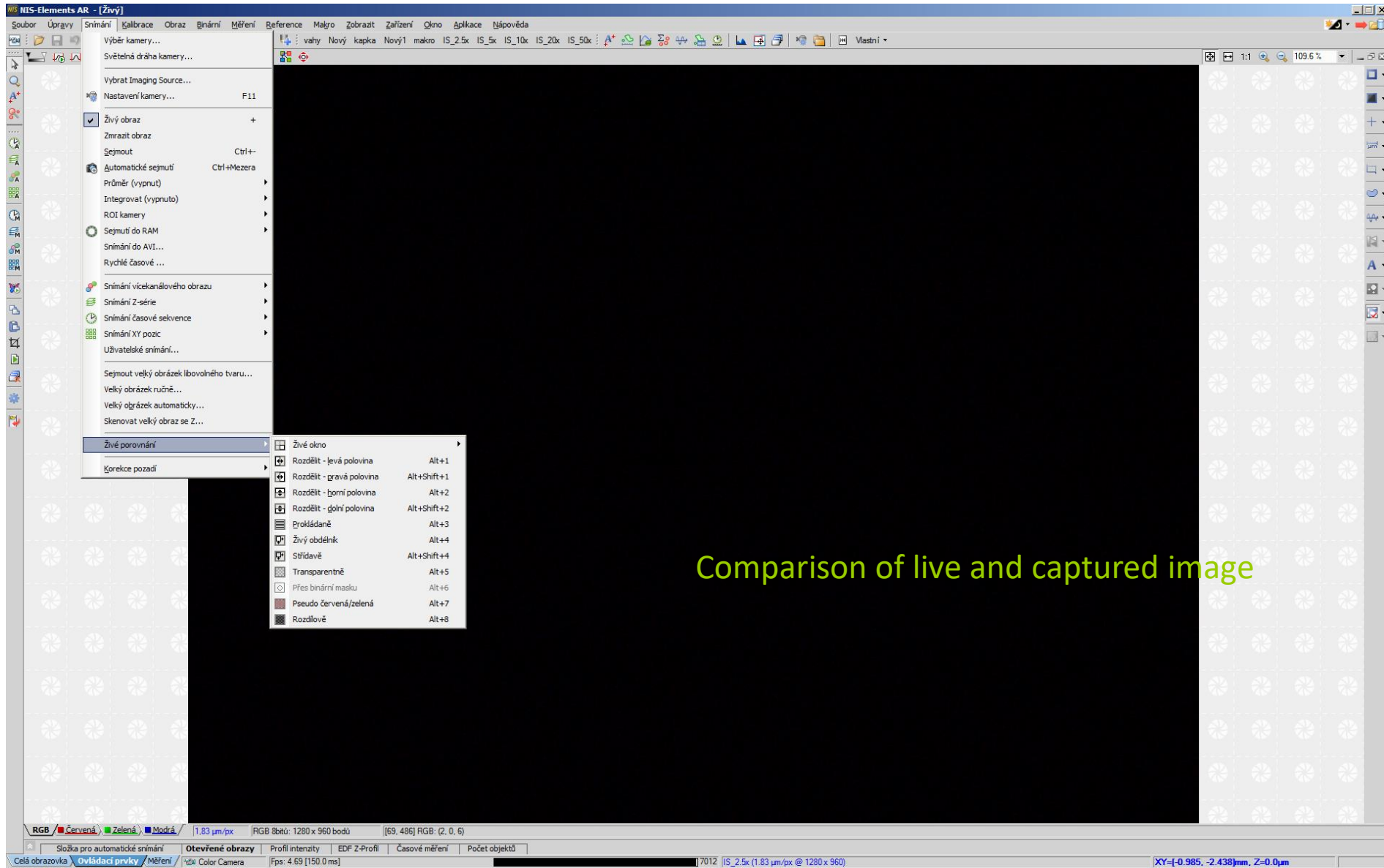
- Run [Applications > ND > Define/Run Experiment (ND) (-6D- option)].
- The following control appears:

Select one or more dimensions (tabs) to be included in the resulting ND2 file. Above the tabs is a preview of the nd2 file structure.

- Press the [Experiment Order] button and select the order of the dimensions to be scanned.
- Set the scanning parameters for each dimension.
- Choose whether to save the ND directly to a file or to keep it in memory and save it later after it is finished. If saving to file is selected, set the path and file name.

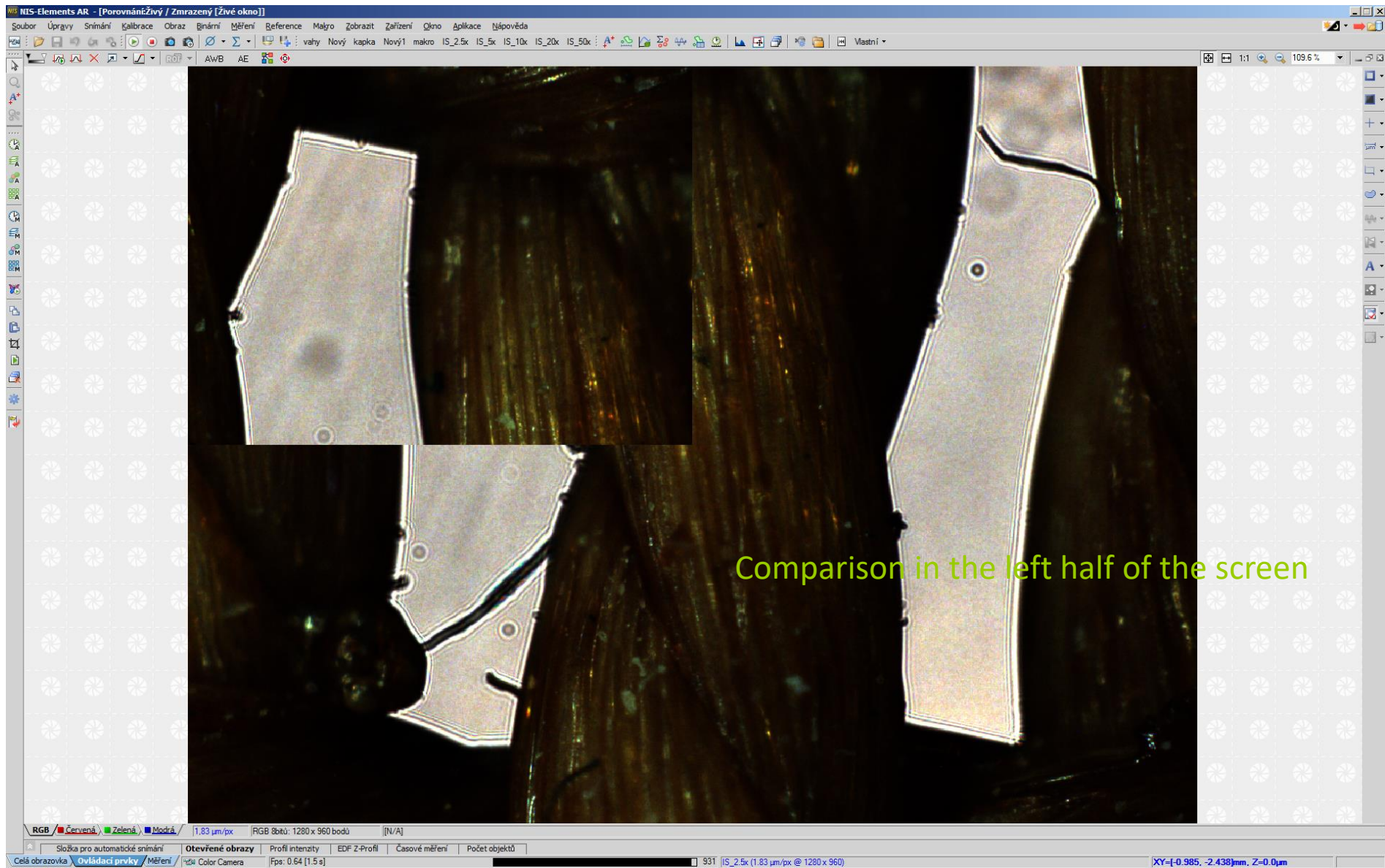


# Live x captured image comparison

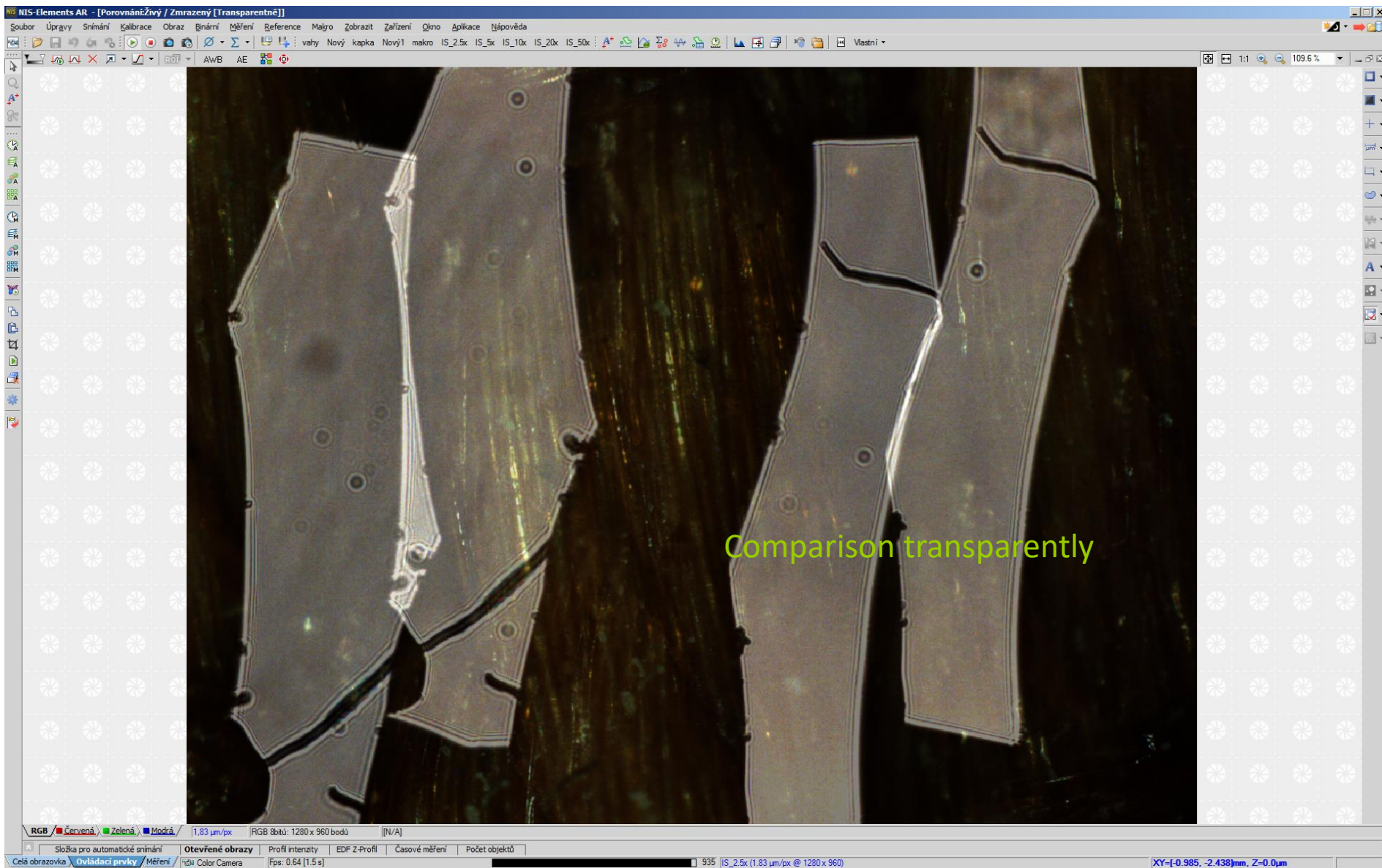


Comparison of live and captured image

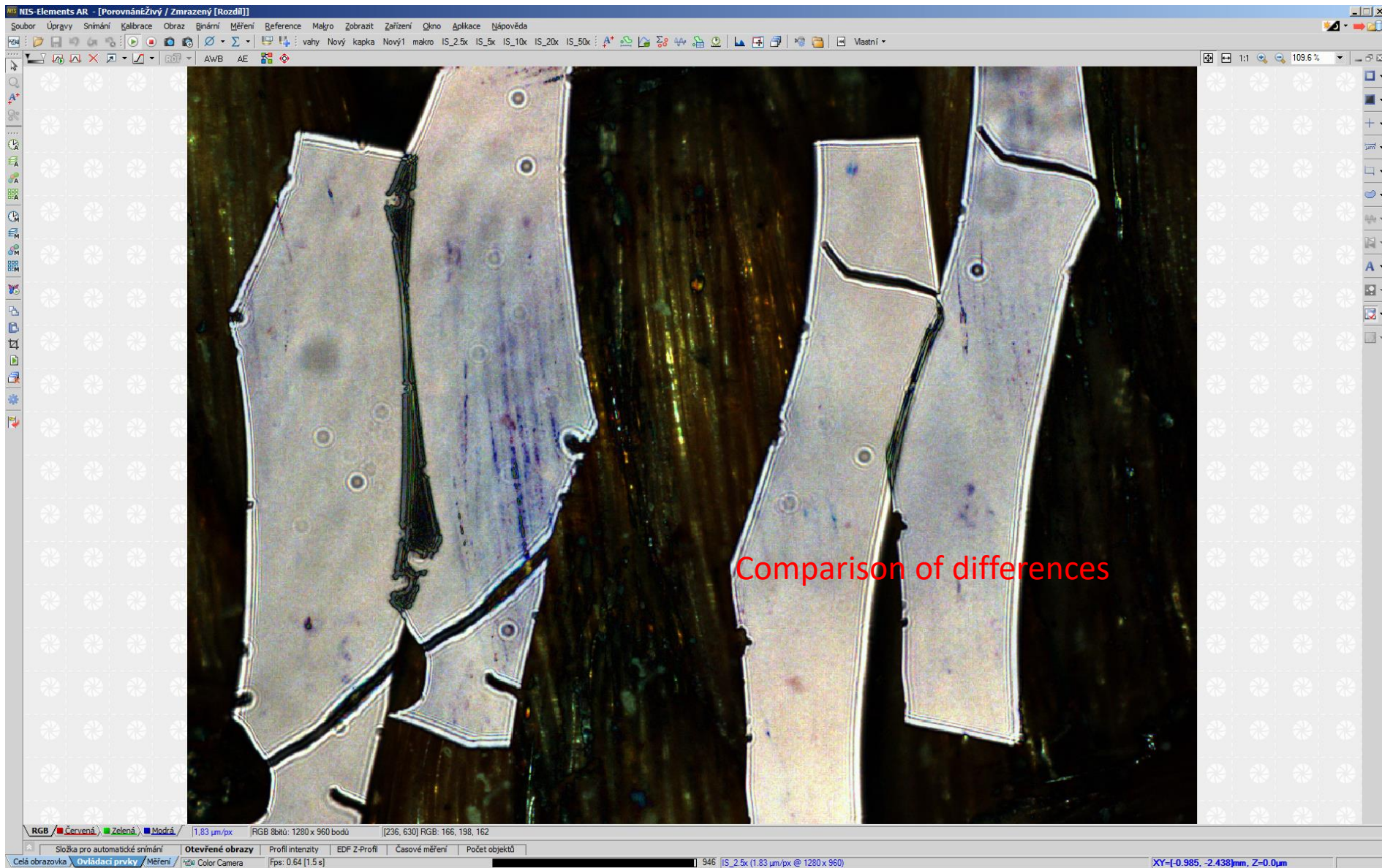
# Live x captured image comparison



# Live x captured image comparison



# Live x captured image comparison



# Background correction

The screenshot displays the NIS-Elements AR interface with a central microscopy image. On the left, the 'Korekce pozadí' (Background Correction) menu is open, showing options like 'Vypnutá korekce pozadí' (Background correction off) and 'Zapnutá korekce pozadí' (Background correction on). A sub-menu is also visible, listing actions such as 'Snímání korekčního obrazu...' (Acquire correction image...), 'Použít jako korekční obraz...' (Use as correction image...), 'Použít stínovou korekci na aktuálním obraze' (Use shadow correction on current image), 'Smazat korekční obrázky' (Delete correction images), and 'Zobrazit korekční obraz' (Show correction image).

The 'Sejmout stínový obraz' (Acquire shadow image) dialog box is open, showing correction options: 'Korekce' (Correction) with 'Multiplikativní (pro světelnou mikroskopii)' (Multiplicative (for light microscopy)) selected, and 'Subtraktivní (pro fluorescenci)' (Subtractive (for fluorescence)). Other options include 'Spline interpolace, vyhlazení: 10' (Spline interpolation, smoothing) and 'Udržet střední intenzitu obrazu' (Keep average image intensity). The 'Optická konfigurace' (Optical configuration) is set to 'Není' (None). A red circle highlights the instruction: 'Nasnímejte několik prázdných scén stisknutím tlačítka Další. Poté opusťte toto okno pomocí tlačítka Dokončit.' (Acquire several empty scenes by clicking the Next button. Then exit this window using the Done button.) Below this, it says 'Budete snímat první obrázek' (You will acquire the first image) and has buttons for 'Další >>' (Next >>), 'Dokončit' (Done), and 'Zrušit' (Cancel).

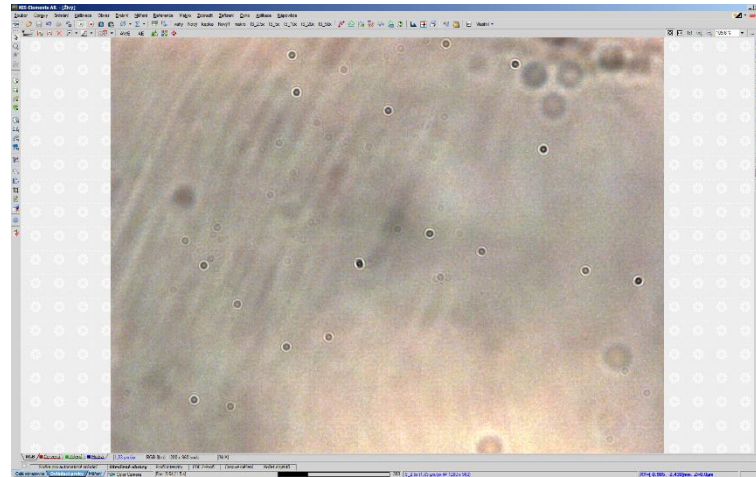
The 'Nastavit stínovou korekci' (Set shadow correction) dialog box is also open, showing 'Podílová korekce' (Ratio correction) selected over 'Rozdílová korekce' (Difference correction). It includes 'OK', 'Storno' (Cancel), and 'Nápověda' (Help) buttons. A note at the bottom states: 'Podílová korekce se obvykle používá pro světlé pole, rozdílová korekce pro fluorescenci' (Ratio correction is usually used for bright fields, difference correction for fluorescence).

Text annotations on the image include: 'Then follows, see sample below...' with an arrow pointing to the 'Snímání korekčního obrazu...' option, and 'The image is saved as a correction' with an arrow pointing to the 'Dokončit' button in the 'Sejmout stínový obraz' dialog.

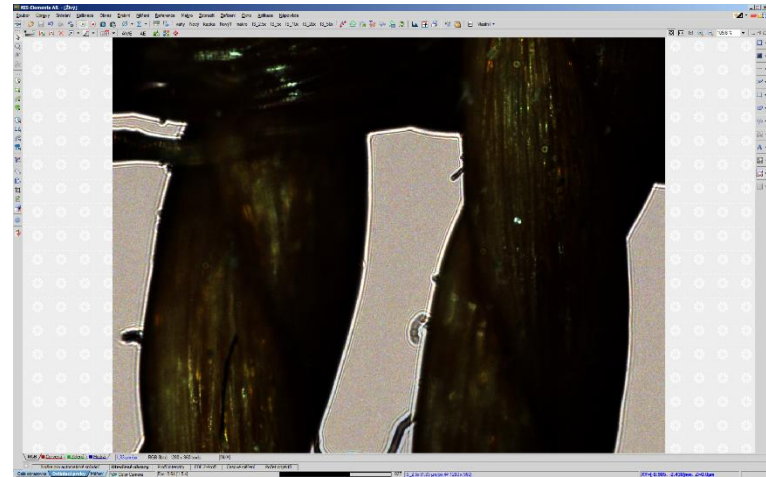
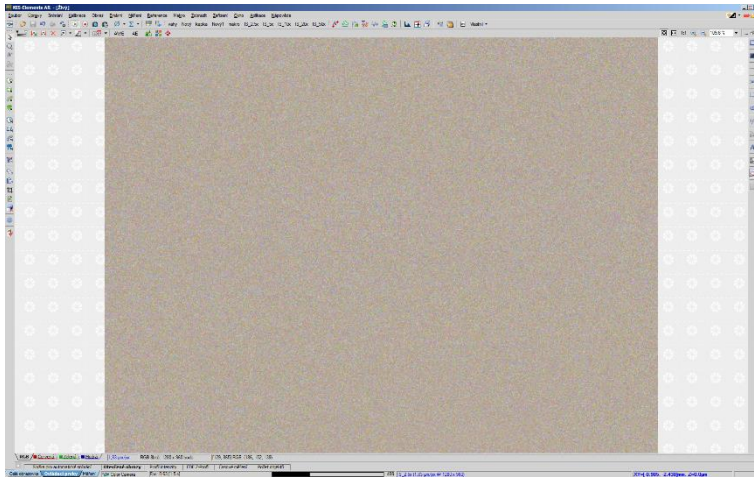
The software status bar at the bottom shows: 'Celá obrazovka Ovládací prvky / Měření', 'Otevřené obrázky', 'Profil intenzity EDF Z-Profil Časové měření Počet objektů', '1,83 µm/px [RGB 8bit: 1280 x 960 bodů] [(N/A)]', 'IS\_2.5x (1.83 µm/px @ 1280 x 960)', and 'XY=[-0.985, -2.438]mm, Z=0.0µm'.

# Background correction

BEFORE BACKGROUND CORRECTION



AFTER BACKGROUND CORRECTION





# References used

[1] manual of NIS-Elements, verze 3.10, 2010, Laboratory Imaging