

# **Different and Unusual Technologies in Pathology Evaluation**

AnaPath

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AnaPath GmbH, Switzerland

# Hard Material Technique

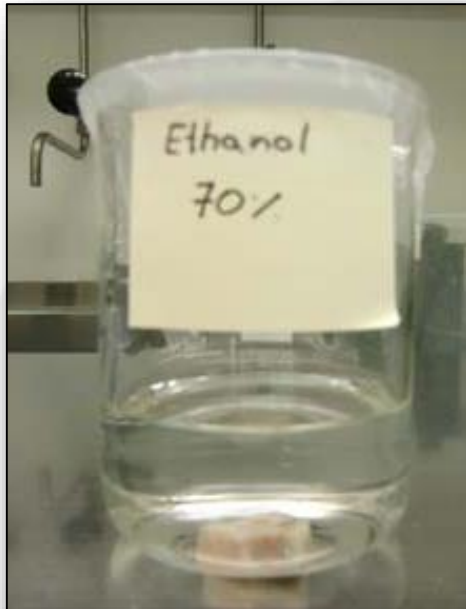


- Diamond Saw

- Fixed for saw cut



# Embedding



**Dehdration in  
ethanole**

**Dehydration  
under Vacuum**

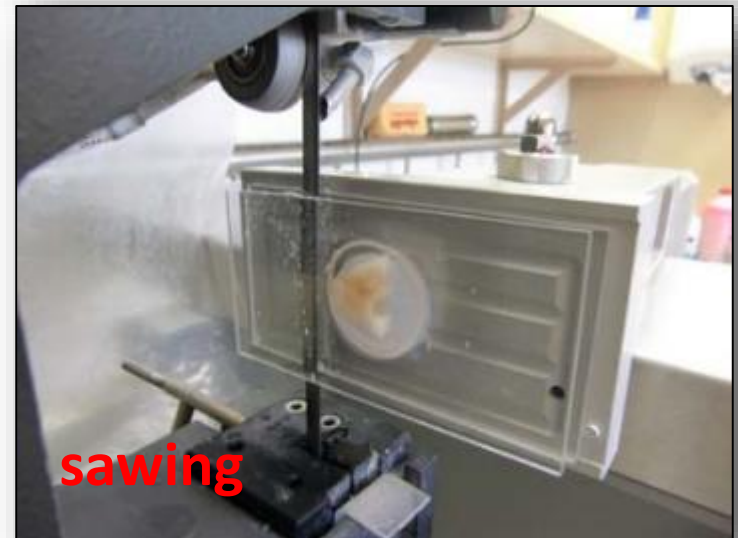
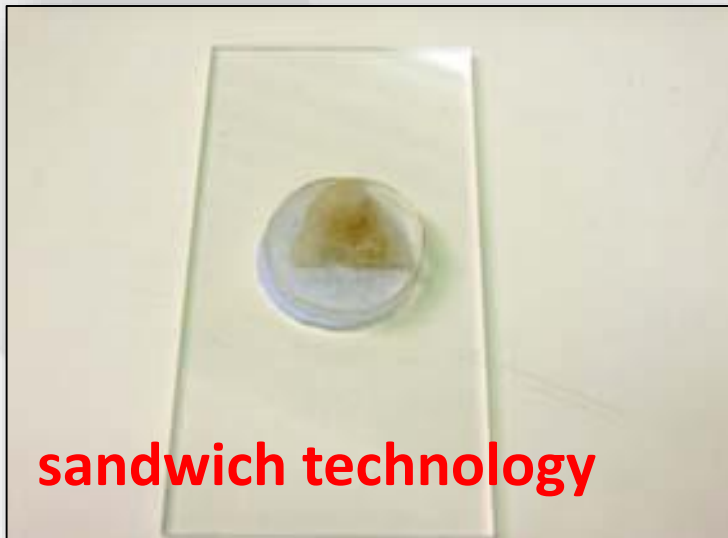
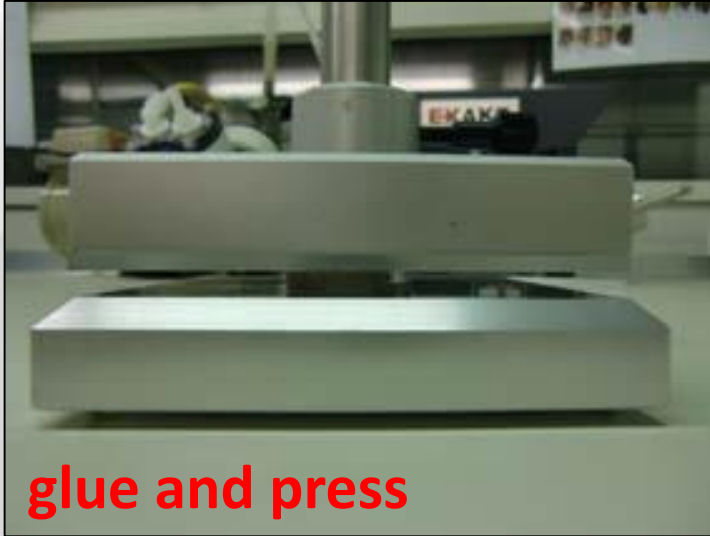


**MMA Solution**

**Samples in jar for  
MMA infiltration  
and polymeri-  
sation**

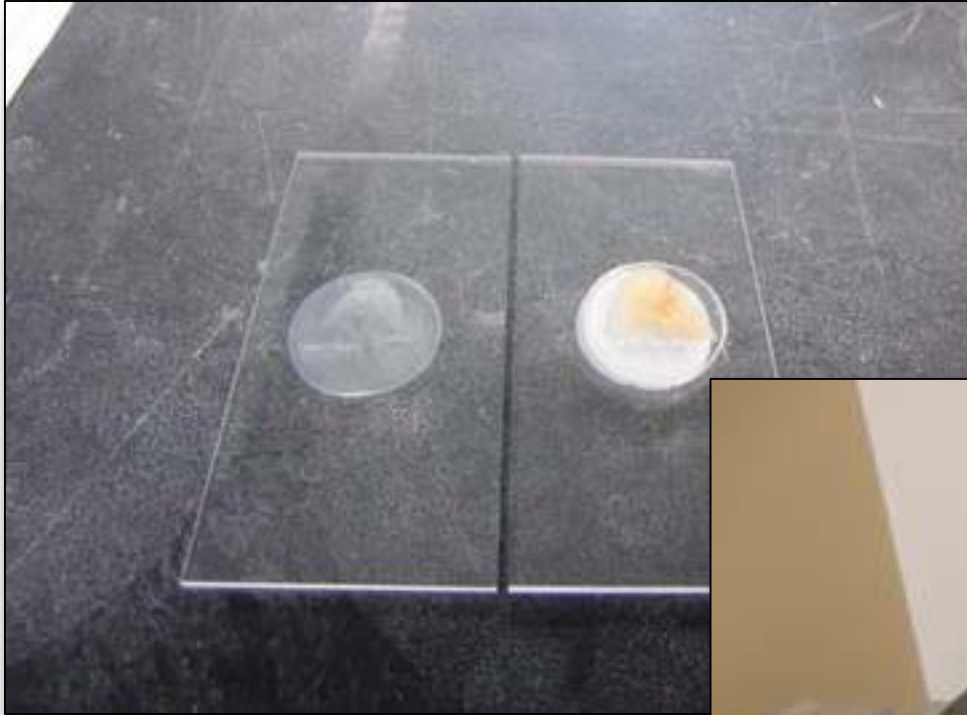


# Block preparation

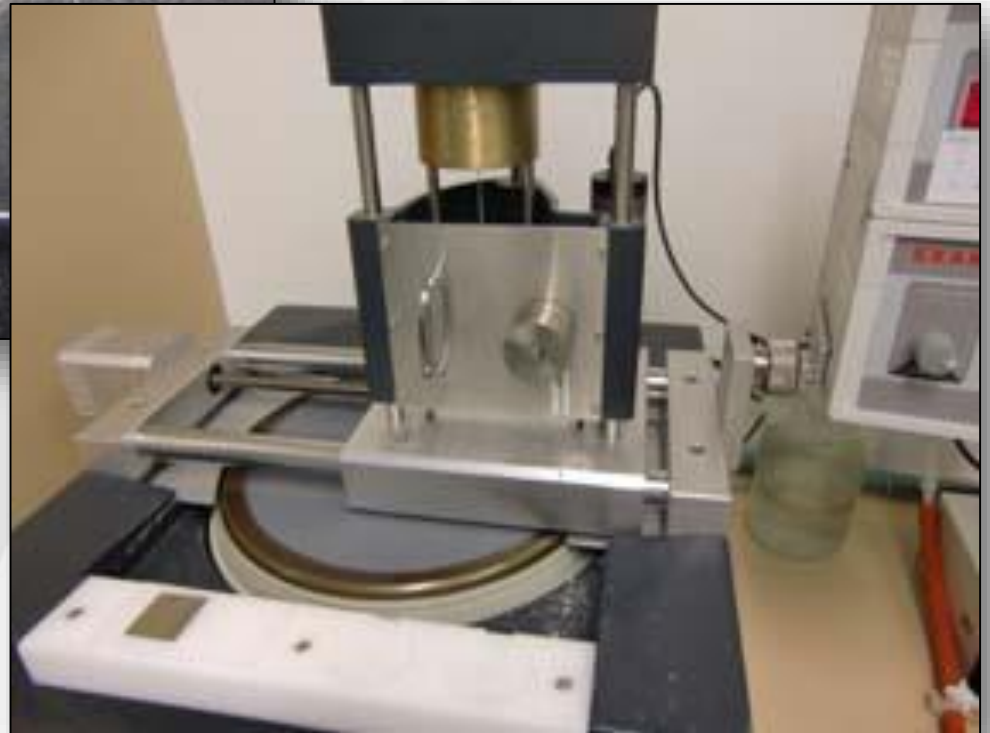


# Saw Cut

cut and block after sawing



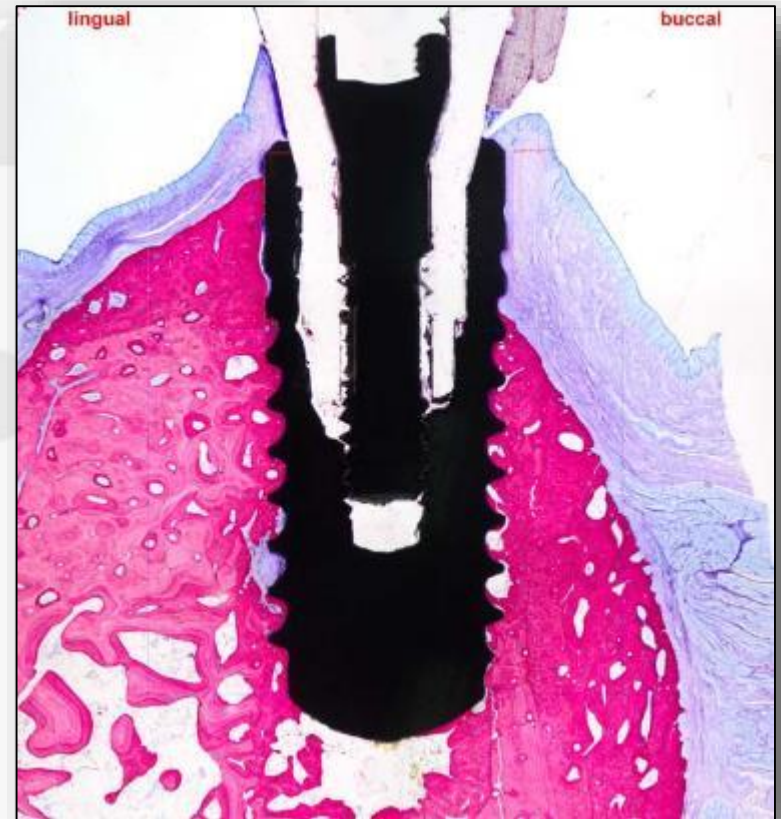
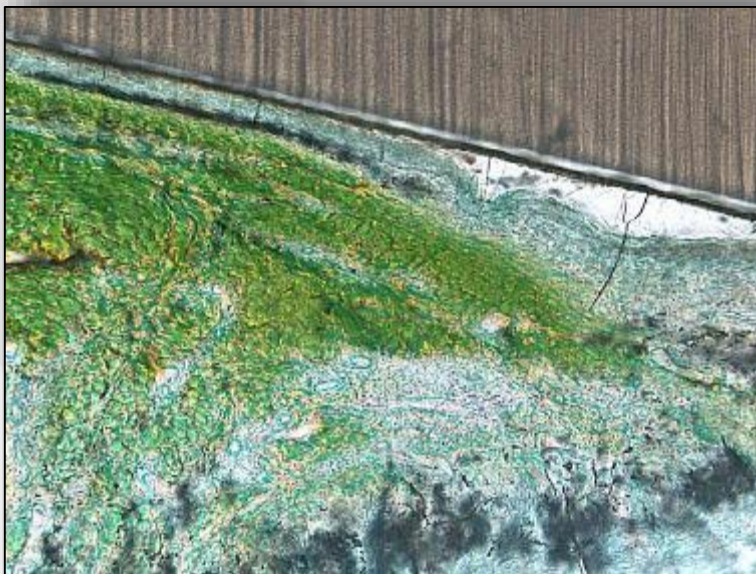
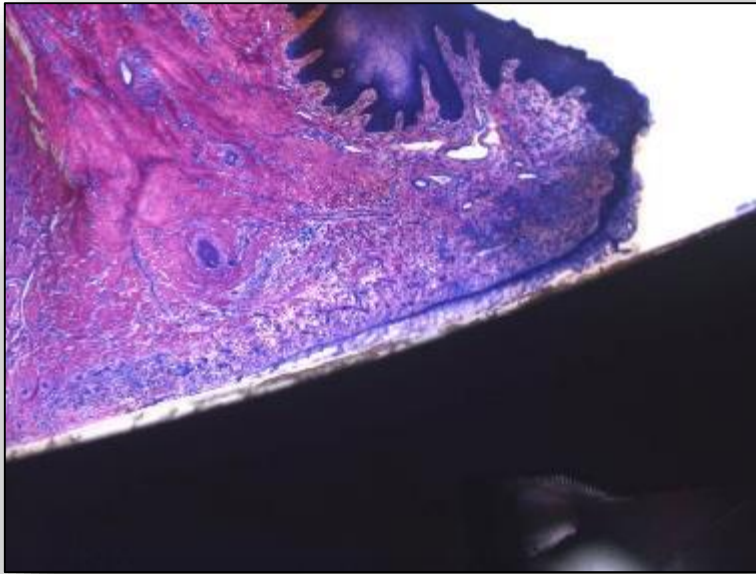
grinding



# Polish, Stain and Cover Slip

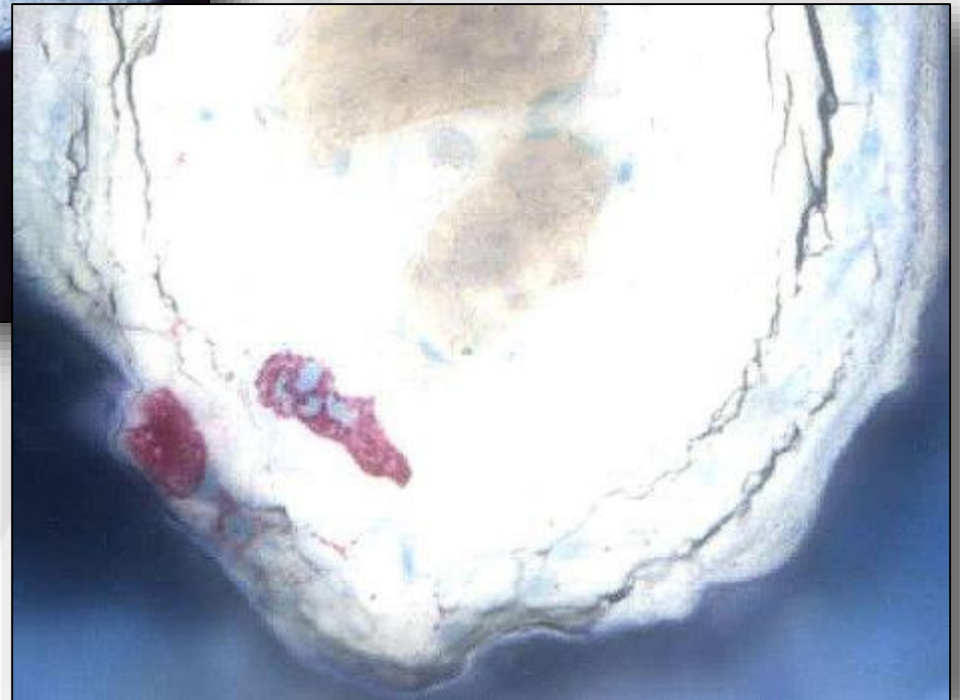
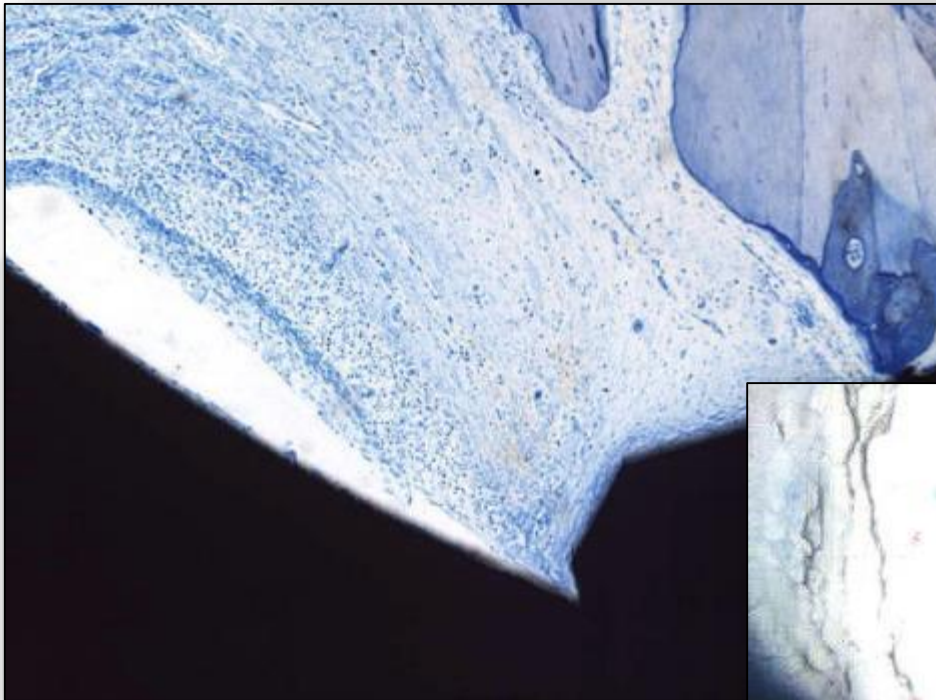


# Special Stains: Hard Material



# Special Stains: Toluidine Blue, Trap

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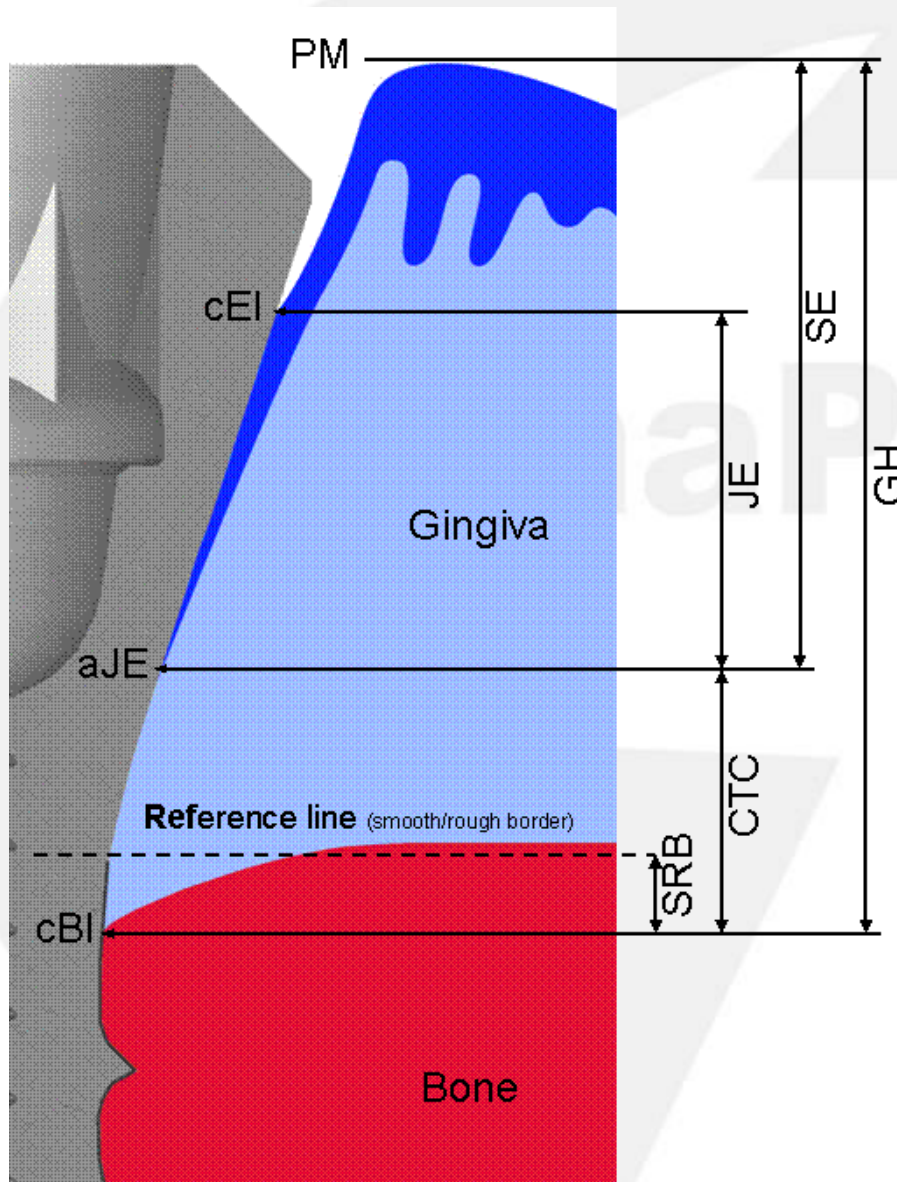


# Hard Material: Overview

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# Image Analysis: Designs



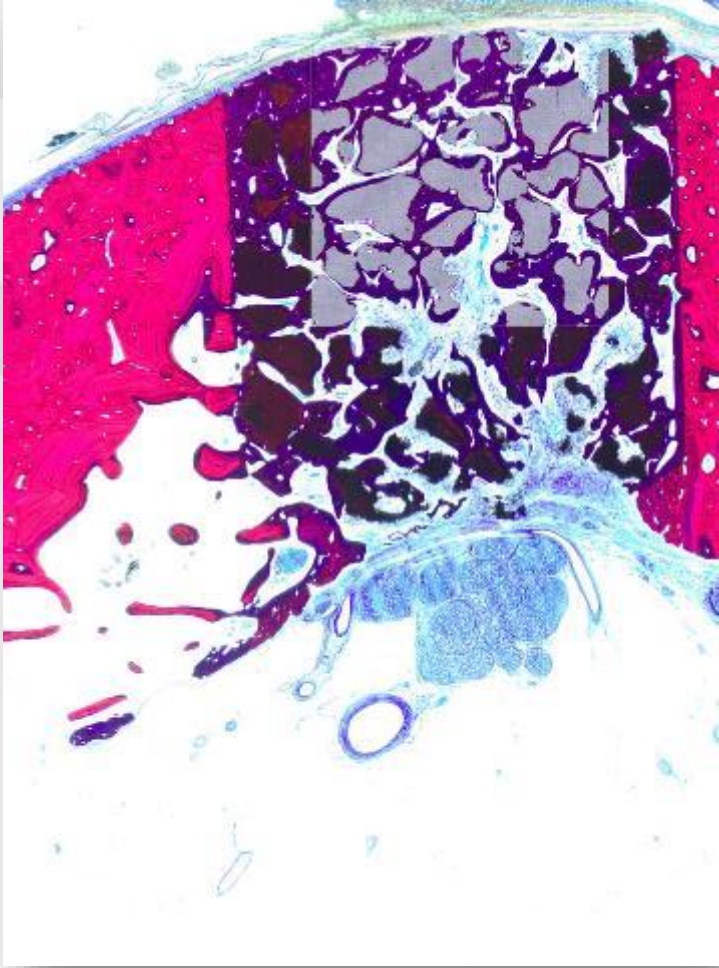
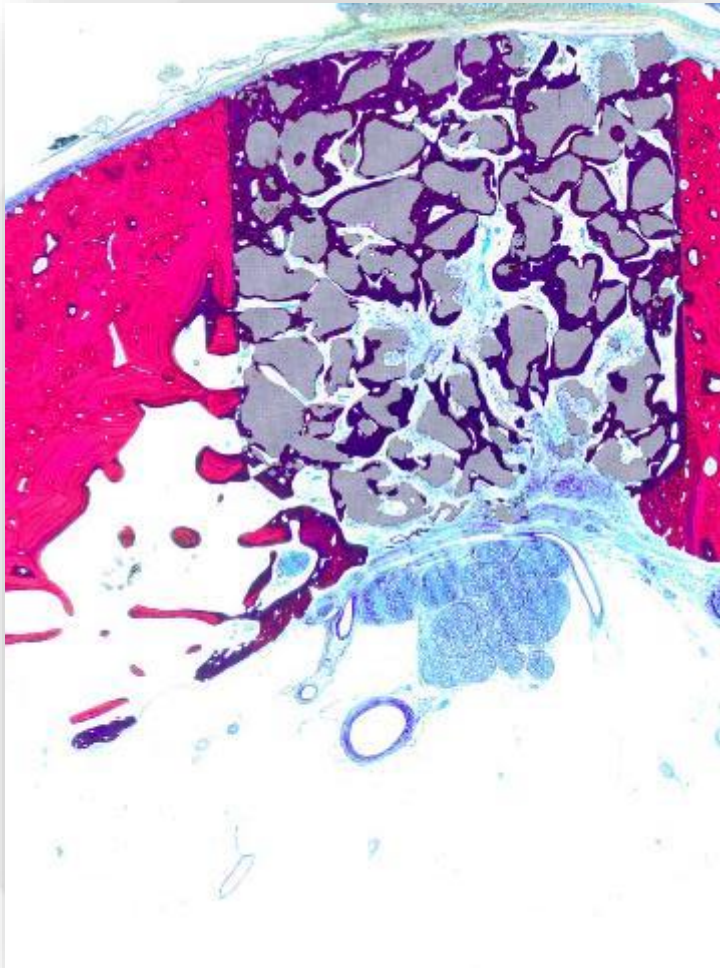
## Landmarks;

- PM: marginal portion of the **peri-implant mucosa**
- cEI: most **coronal epithelium to implant contact**
- aJE: **apic. Limit of long junctional epithelium**
- Ref: **Smooth/rough boarder (machined/SLA)**
- cBI: most **coronal bone to implant contact**

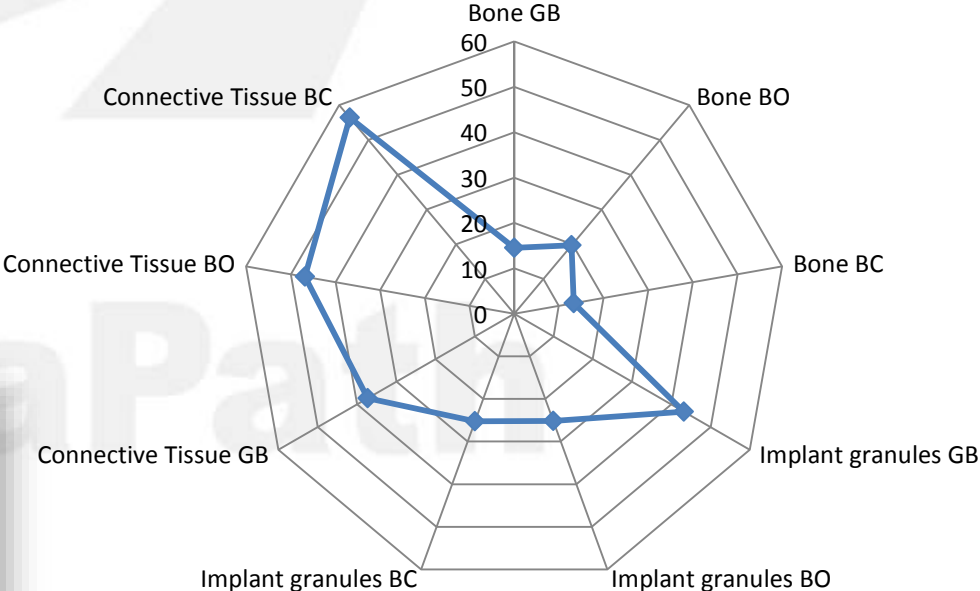
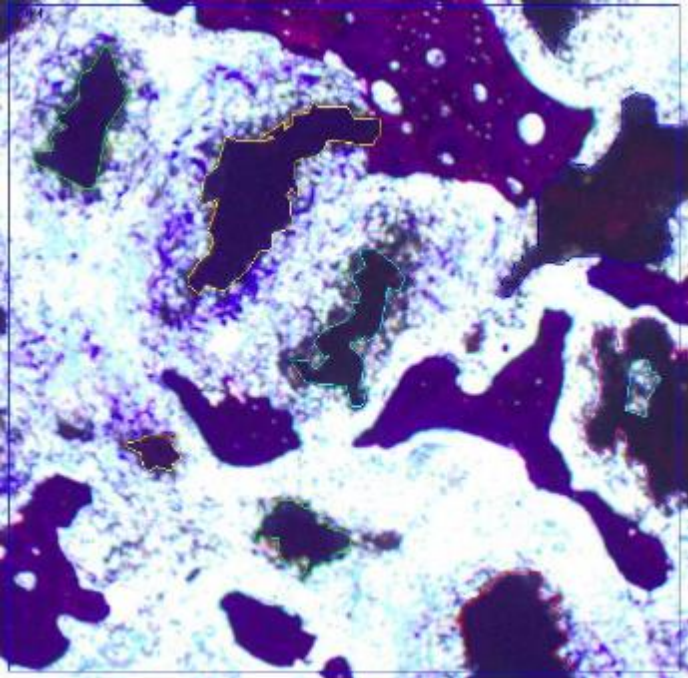
## Lengths:

- SE: **Sulcus Epithelium**
- JE: **long junctional epithelium**
- CTC: **connective tissue contact**
- GH: **gingival height**

# Image Analysis: ROI Selection

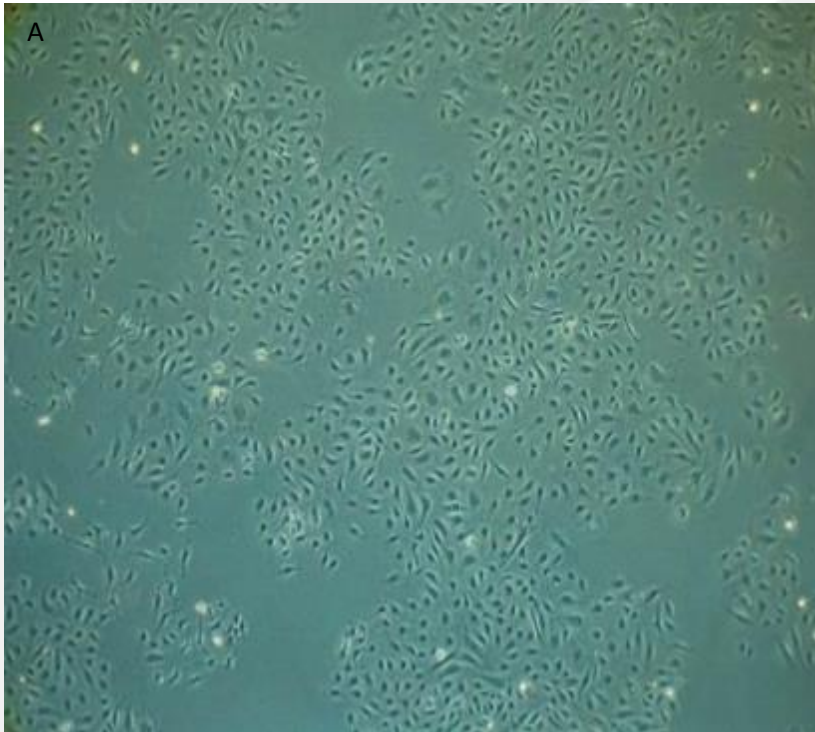


# Image Analysis: Documentation

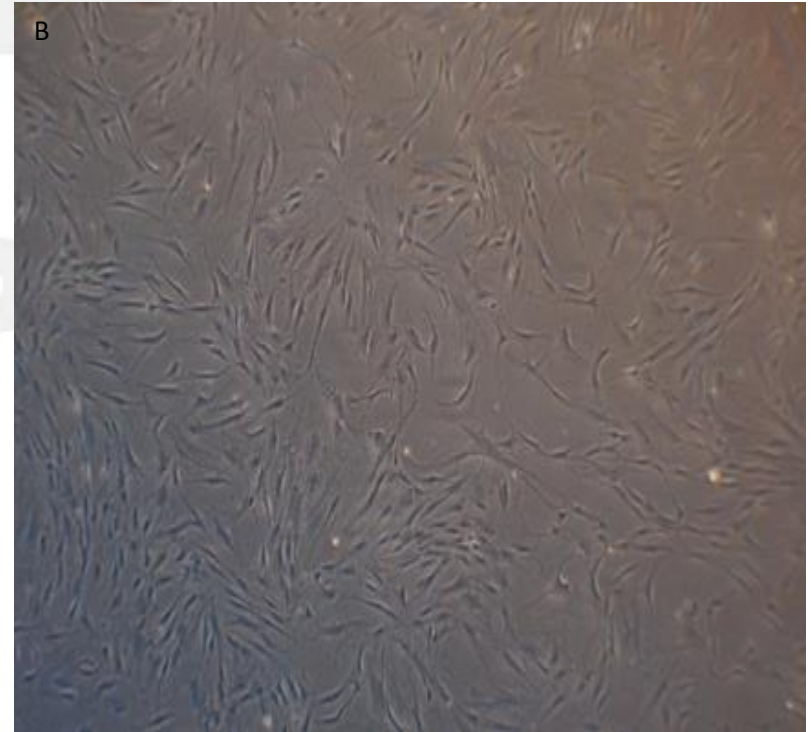


# Engineered Tissues

## Morphological Characterization of the Cell Cultures by Light Microscopy



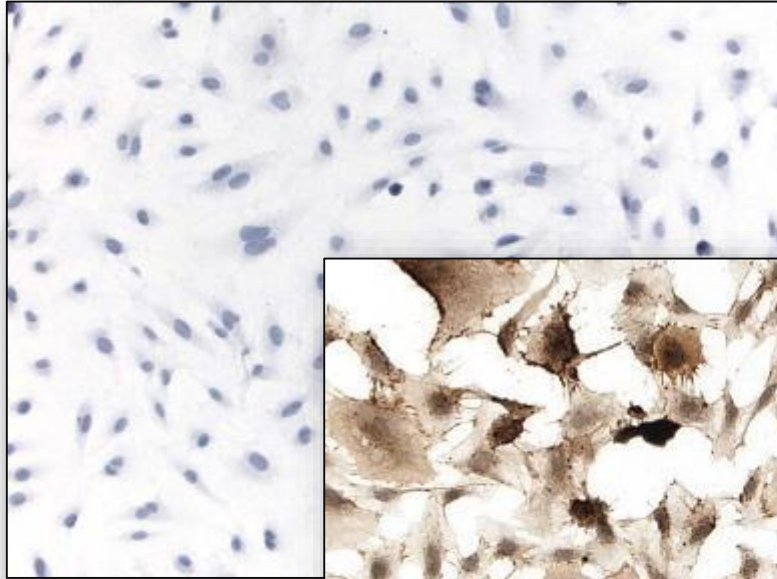
**Endothelial Cells with Cobblestone Appearance**



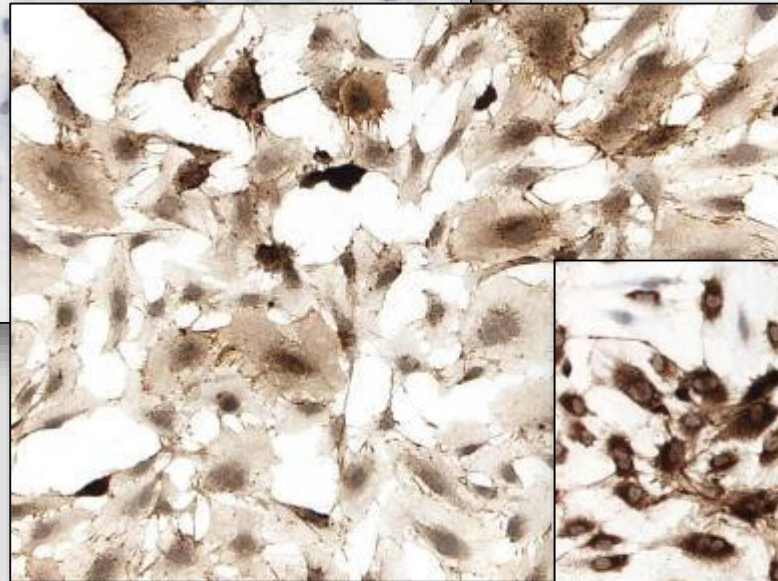
**Fibroblasts with Spindle Shape Appearance**

Weber K, Stenger R: Engineered heart valve grafts. . Classic Examples in Toxicologic Pathology (4th Edition) Eds: Drommer W, Karbe E, Germann PG, 4th Edition, ISBN 978-3-9814653-0-3, 2011

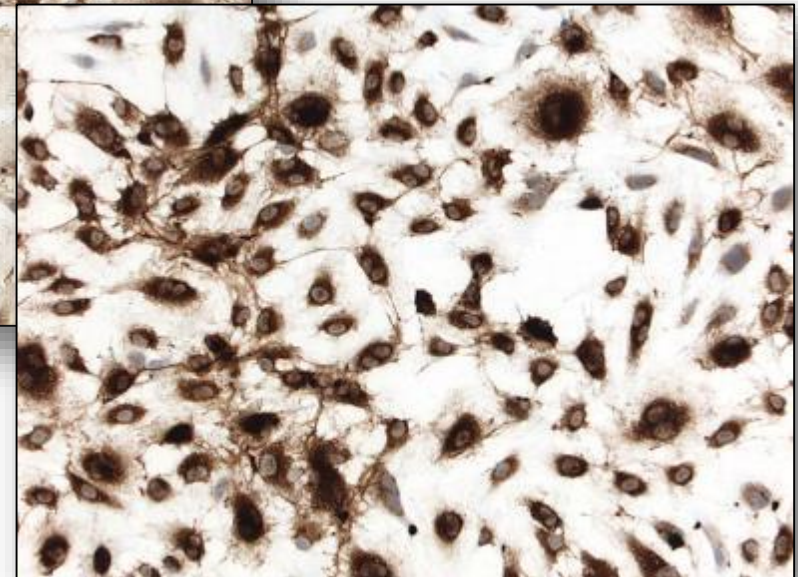
# Engineered Tissues: Quality Check during Manufacturing Process: Endothelia



**$\alpha$ -Smooth Muscle Actin**



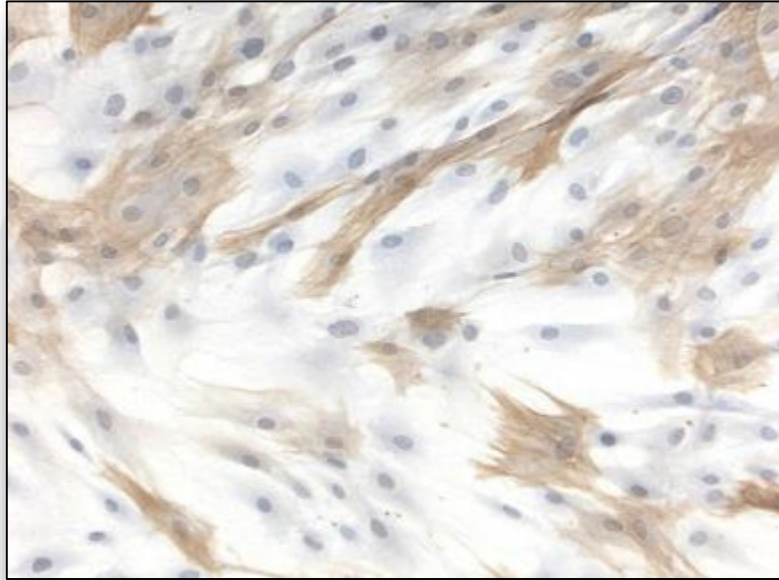
**CD31**



**IHC on cultured cells  
in culture chambers**

**vWF**

# Engineered Tissues: Quality Check during Manufacturing Process: Fibroblasts



**$\alpha$ -Smooth Muscle Actin**

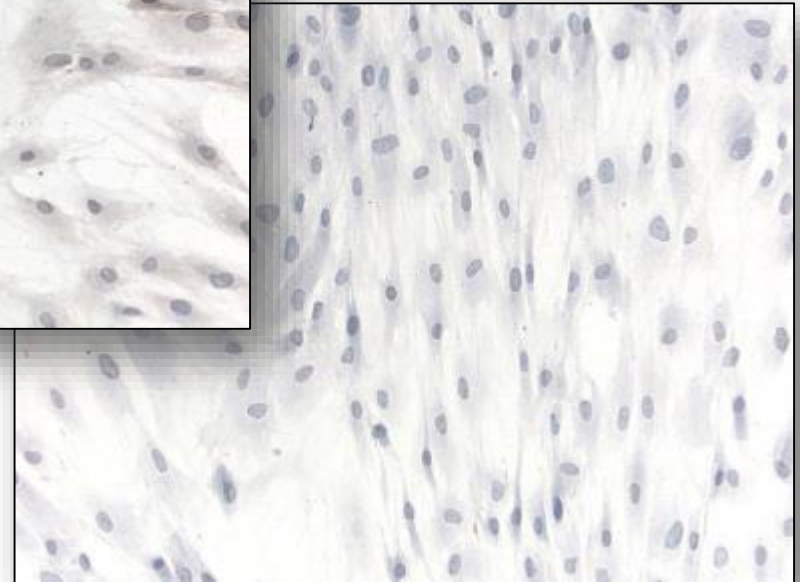


**CD31**

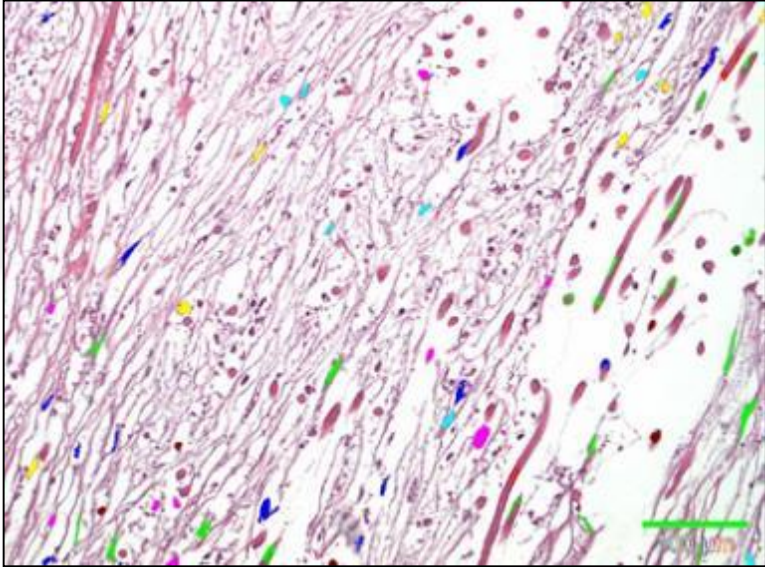
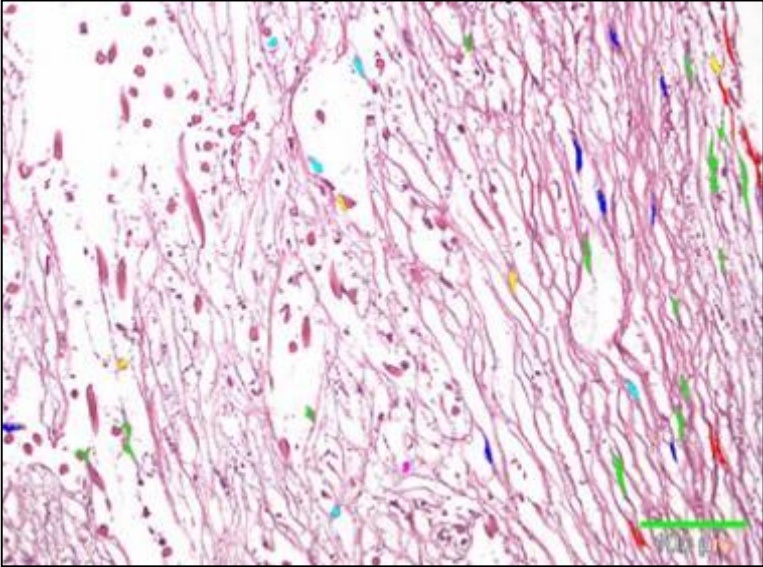
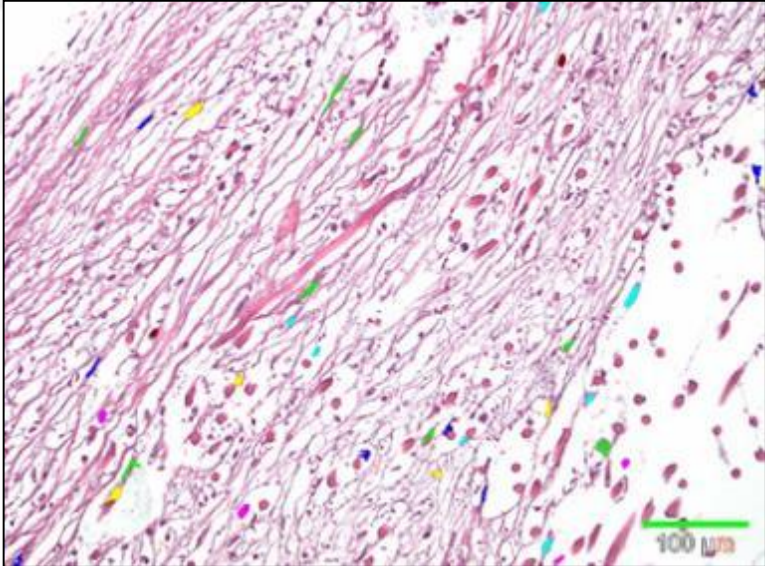
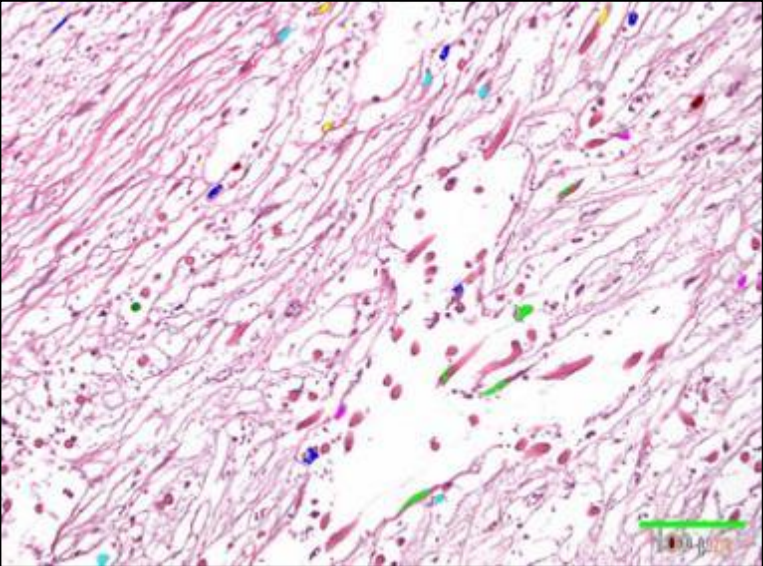


**vWF**

**IHC on cultured cells  
in culture chambers**



# Particle Analysis: ATMP, Determination of Cell Count per Weight Unit (Tissue)





# Resulting amount of nuclei by particle analysis on 4 sections (732 x 543 $\mu\text{m}$ ) per sample

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mean of 21505  $\pm$  7009 (Minimum: 15455, Maximum: 31628) cells/ $\text{mm}^3$

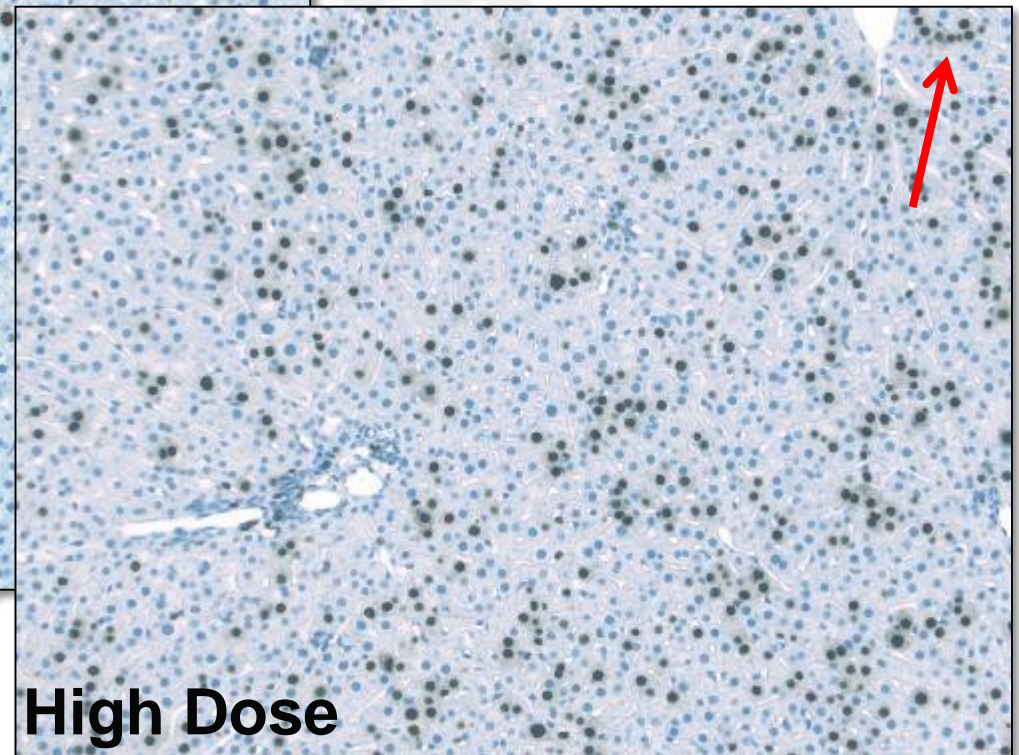
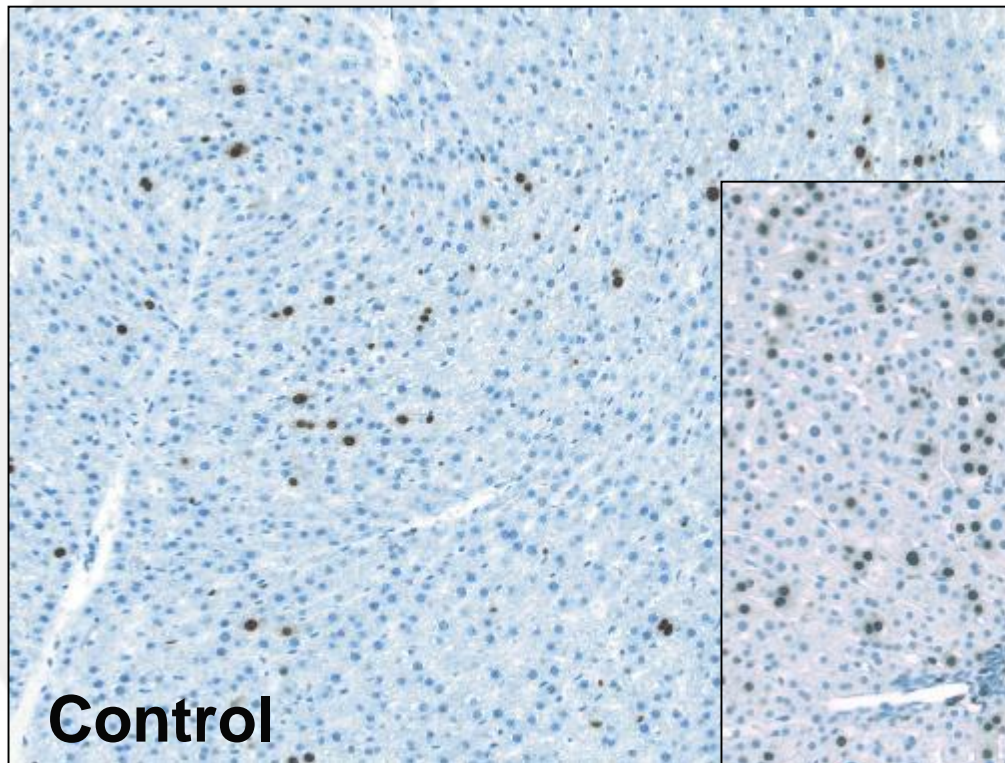
| Sample Number              | T01 VS07 | T01 VS09 | T01 VS11 |
|----------------------------|----------|----------|----------|
| Mean cells / $\text{mm}^3$ | 3858     | 3774     | 6793     |
| Sample Number              | T02 VS07 | T02 VS09 | T02 VS09 |
| Mean cells / $\text{mm}^3$ | 3606     | 4696     | 7380     |

The total area of measurement is equivalent with an area of 397476  $\mu\text{m}^2$  at an approximate thickness of 3  $\mu\text{m}$ , i.e. 1192328  $\mu\text{m}^3 = 0.01192428 \text{ mm}^3$ .

# Mitosis: Particle Analysis

Increased staining index with cell proliferation markers in hepatocytes

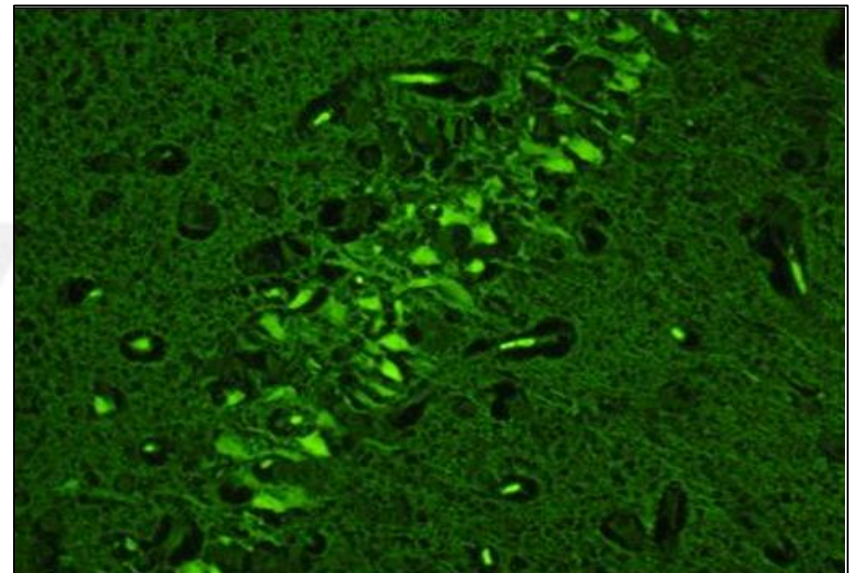
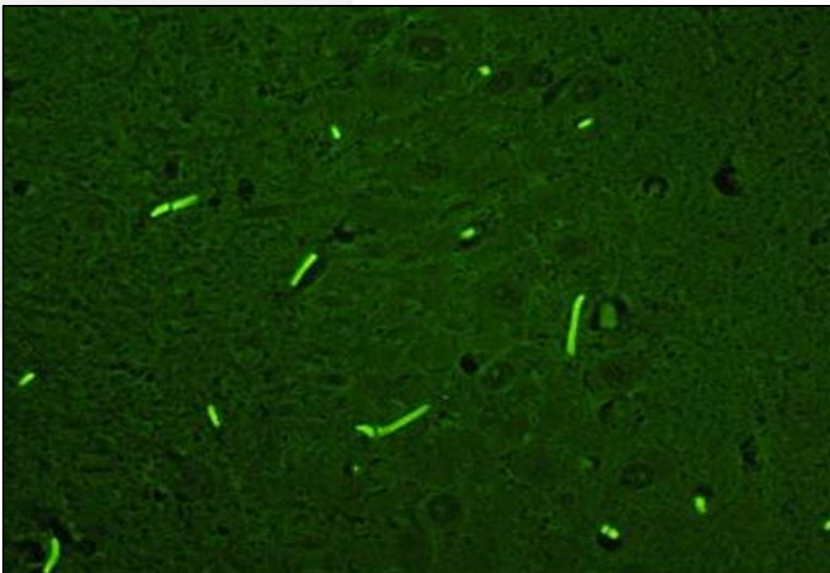
Counting positive nuclei and total cell numbers



# Neuropathology: Detection of Degeneration

## Fluor Jade

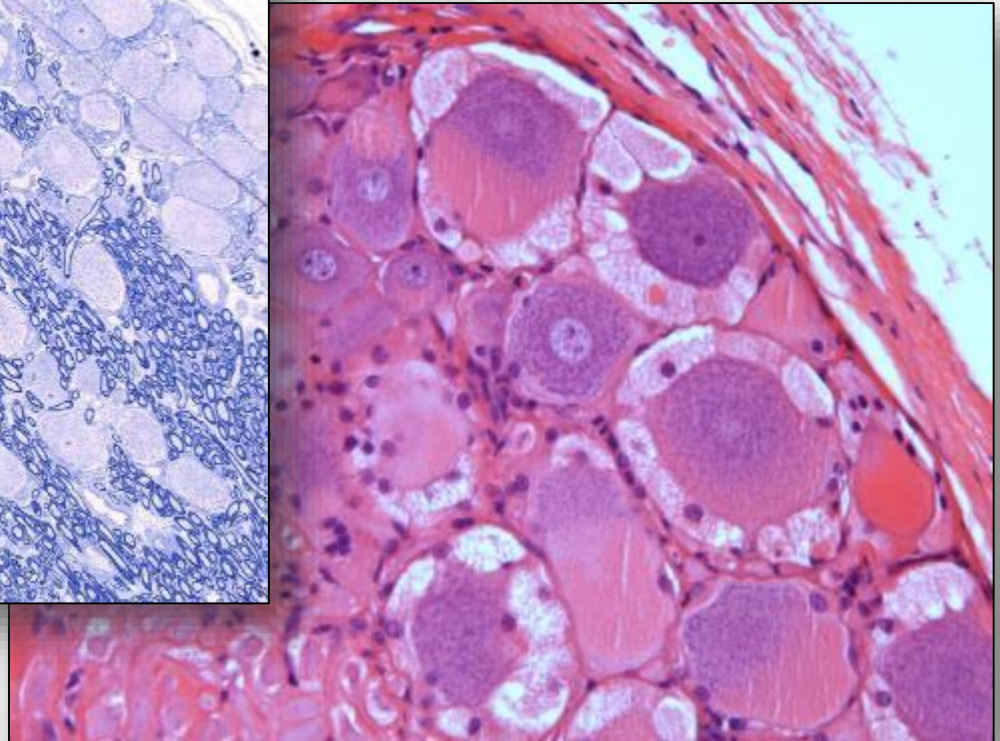
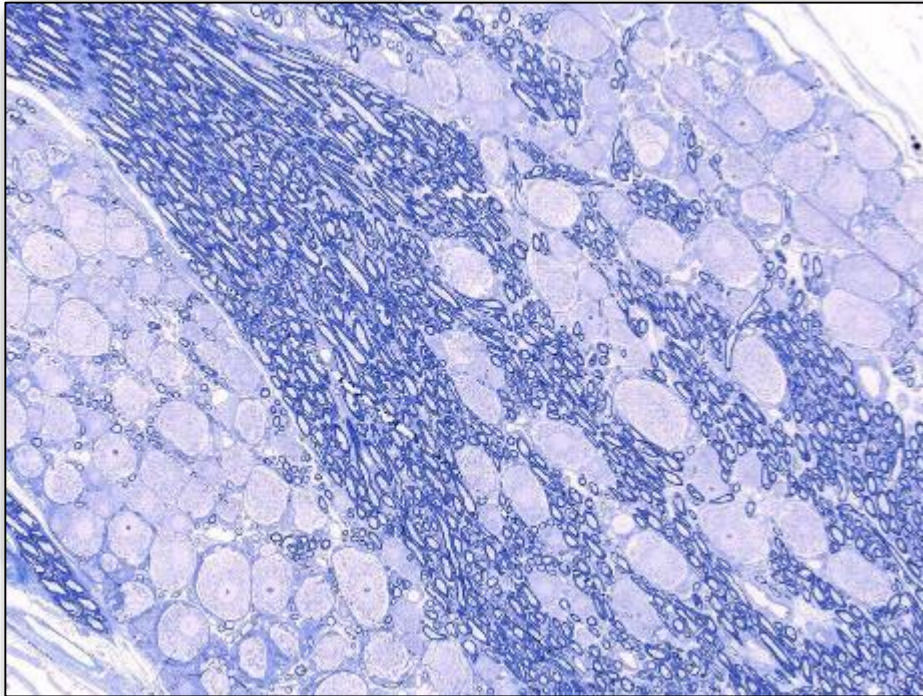
- **Control:**
  - pyramidal neurons are dark
  - Yellow spots by fluorescence erythrocytes
- **Treated:**
  - necrotic pyramidal neurons are bright yellow



# Neuropathology: Neurotoxicity

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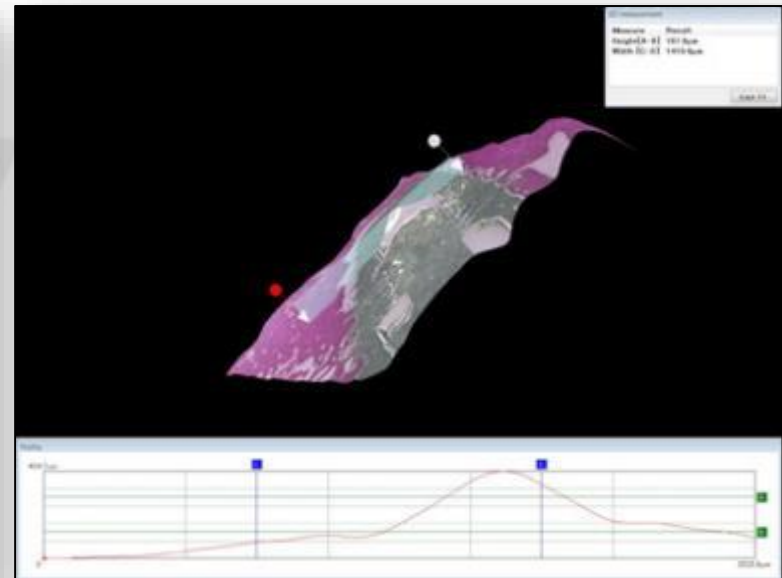
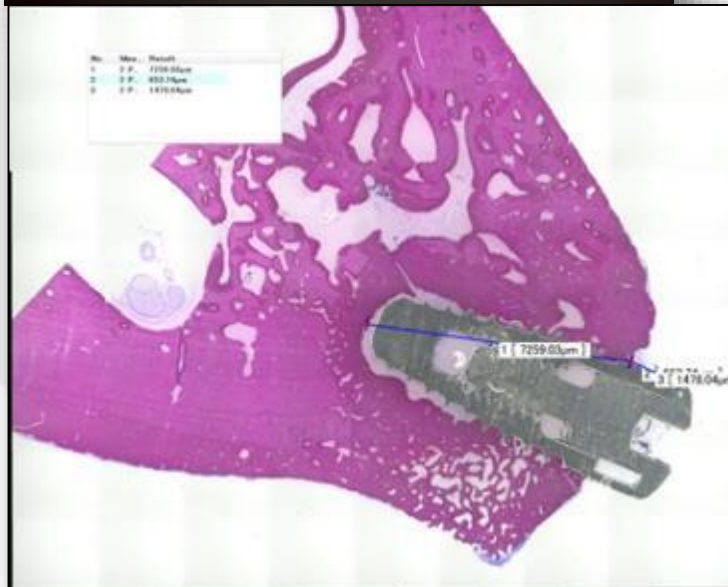
- **Semithin**



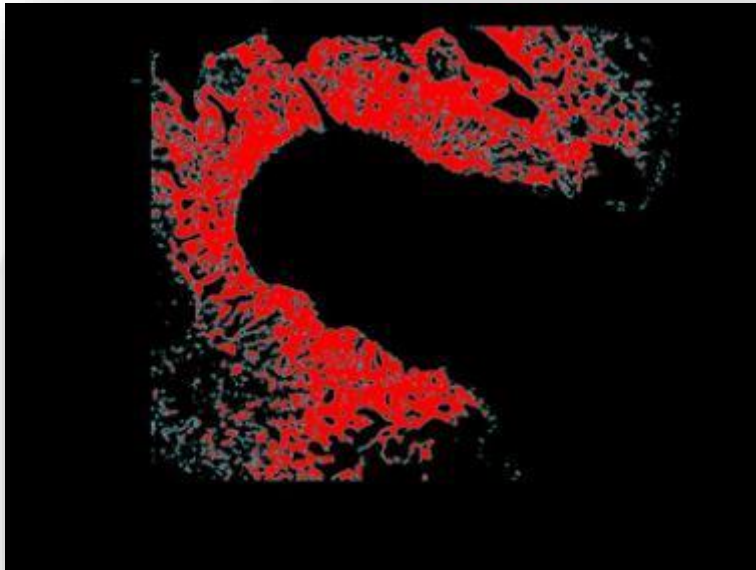
# Digital Microscopy: VHX2000 (Keyence)



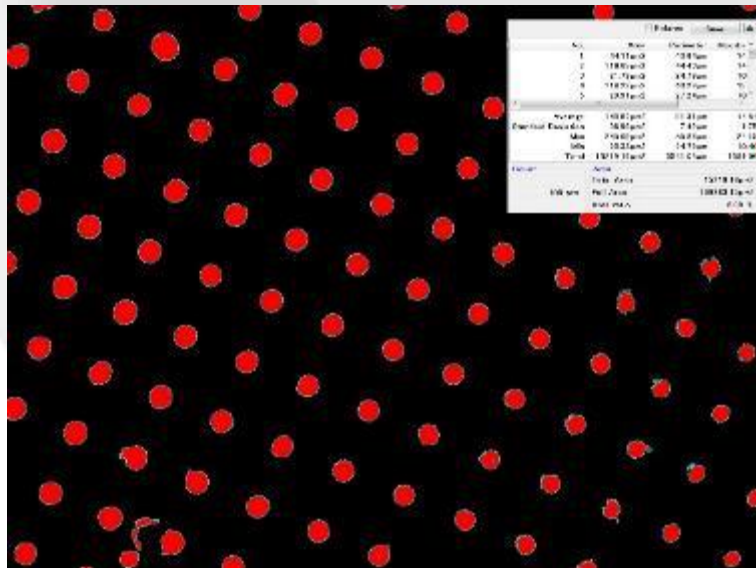
**Originates from computer chip  
Quality control  
No objectives but cameras  
Magnification up to x5000  
3D  
Most sophisticated image  
Analysis technique**



# Digital Microscopy: Examples

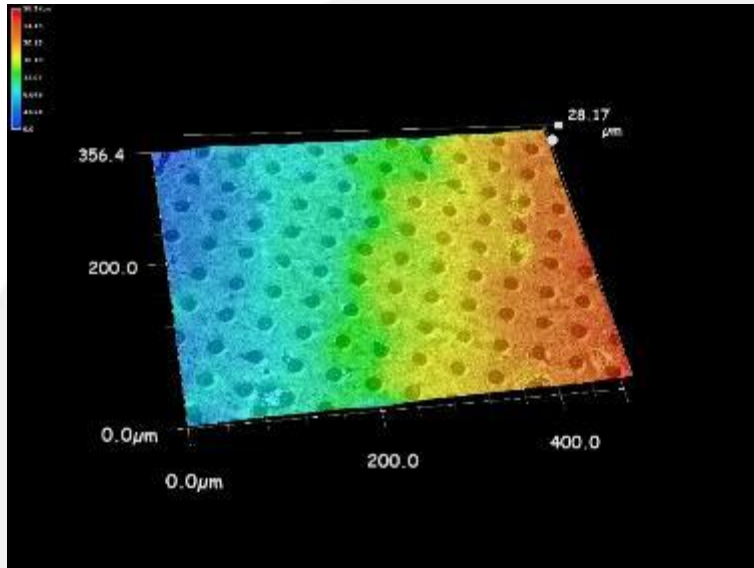


**Presentation of distinct structures  
By digital subtraction  
(e.g. newly formed bone only)**

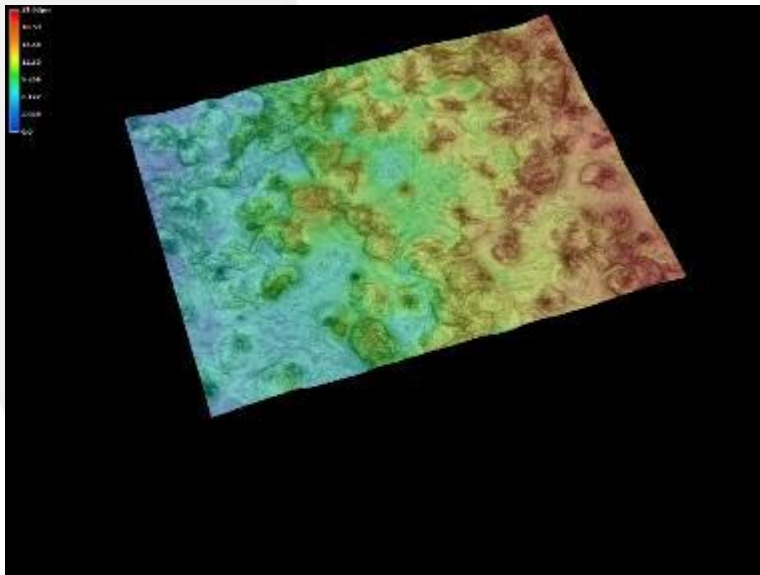


**Organ-on-chip  
Surface image analysis**

# Digital Microscopy: Examples



**Device texture and surface analysis**



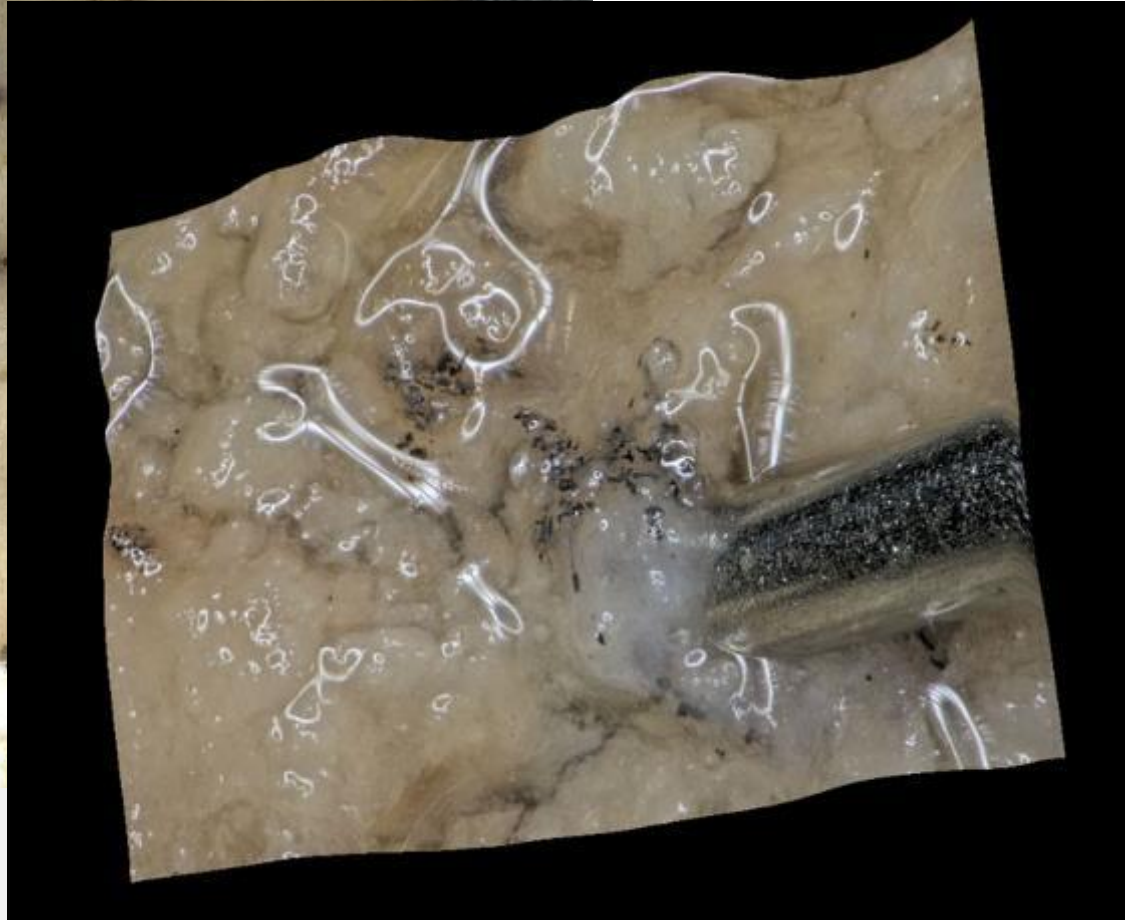
**Device surface analysis on Cell covering**

# Preparation of unknown crystals from liver tissue by Digital Microscopy

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X 200. Note 27 G Syringe



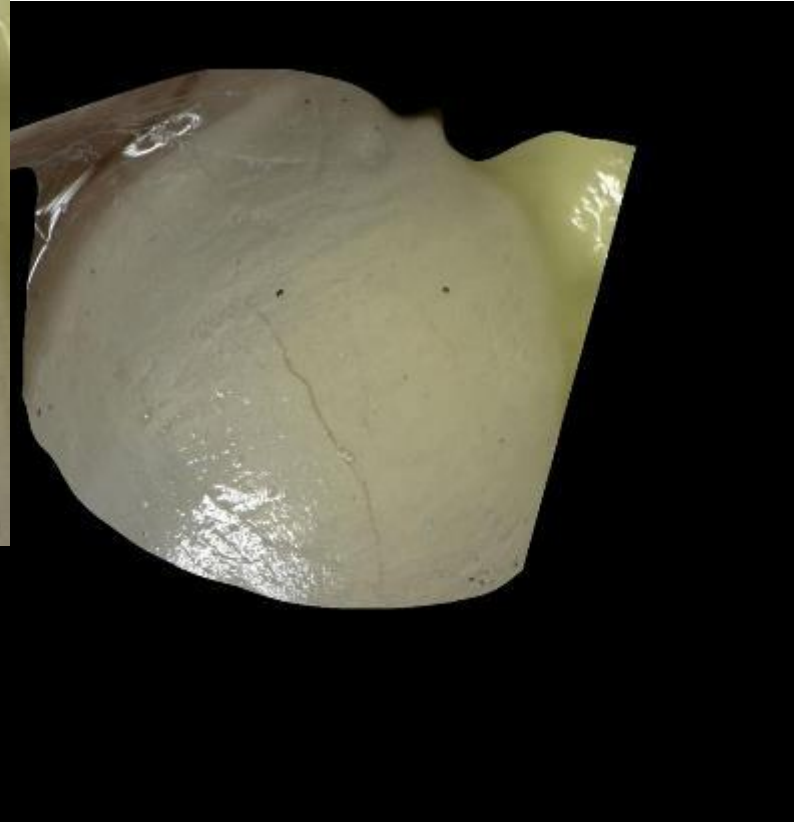


# Example: Evaluation of Rat Pups by Digital Microscopy (Special Problems – Special Requests)

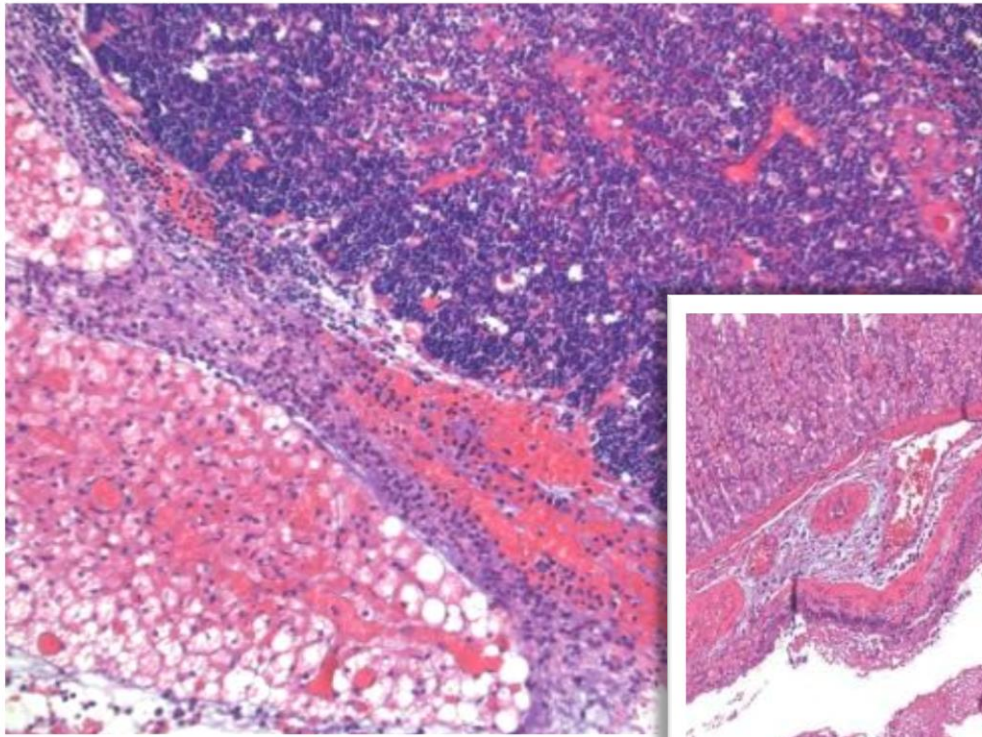
Urinary bladder x 300



Urinary bladder x 300, 3D

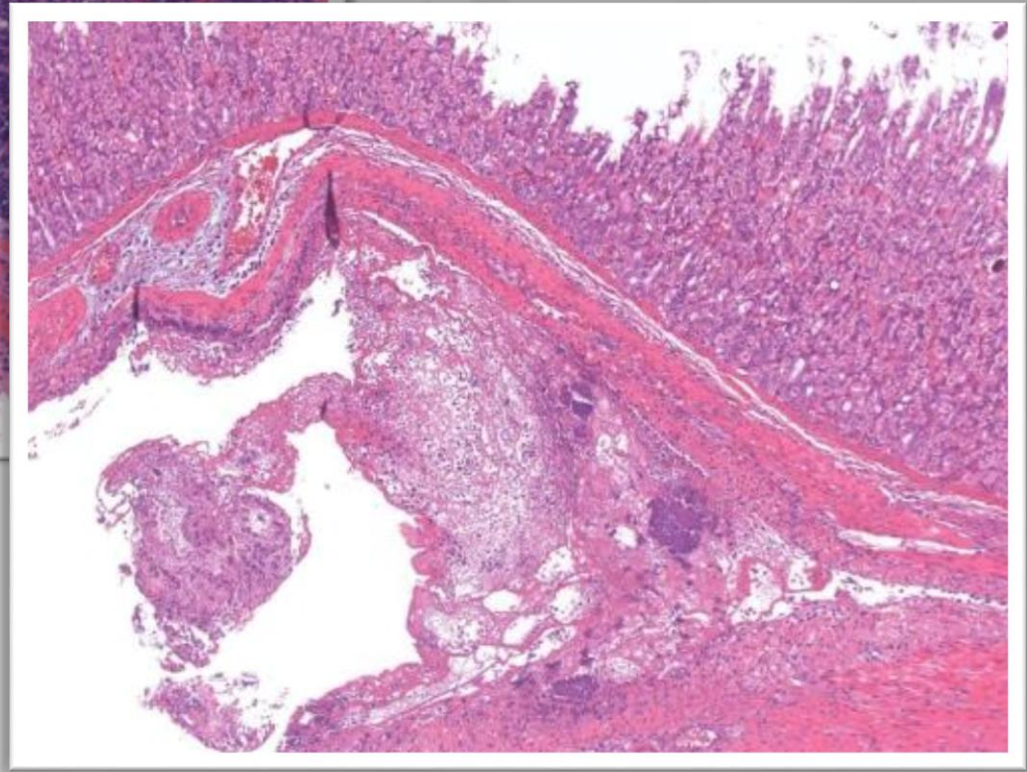


# Example: Extreme high mortality rate by gavage (perforation)



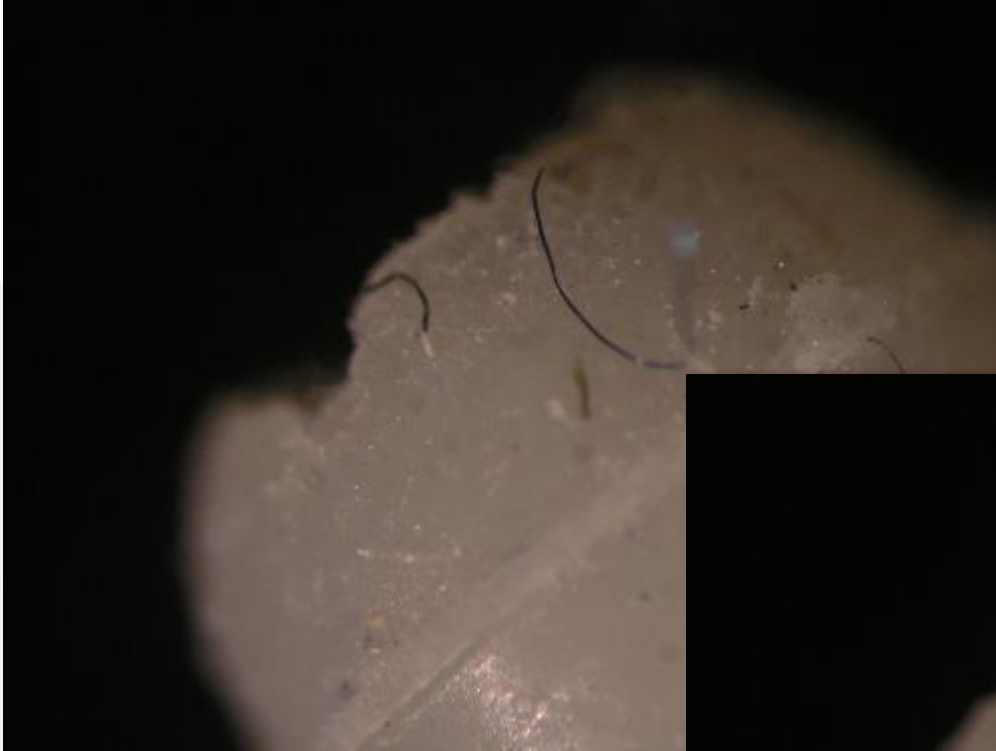
.... mediastinal inflammation (thymus)

....and occasionally perforation and ascites



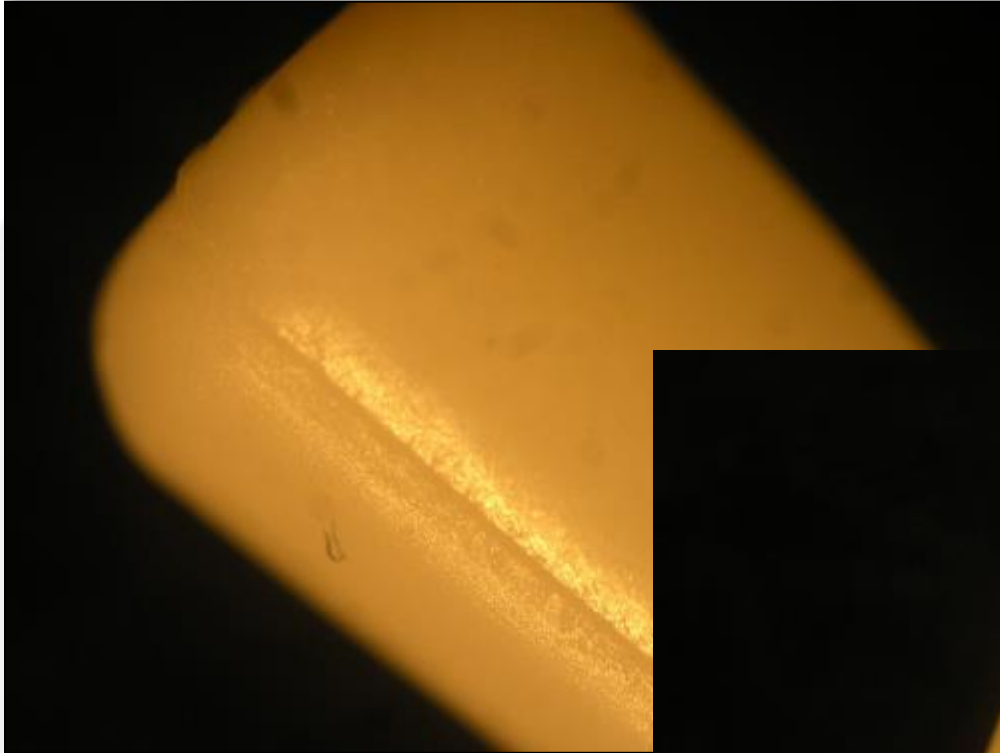
# The gavage probe killed (Digital Microscopy)

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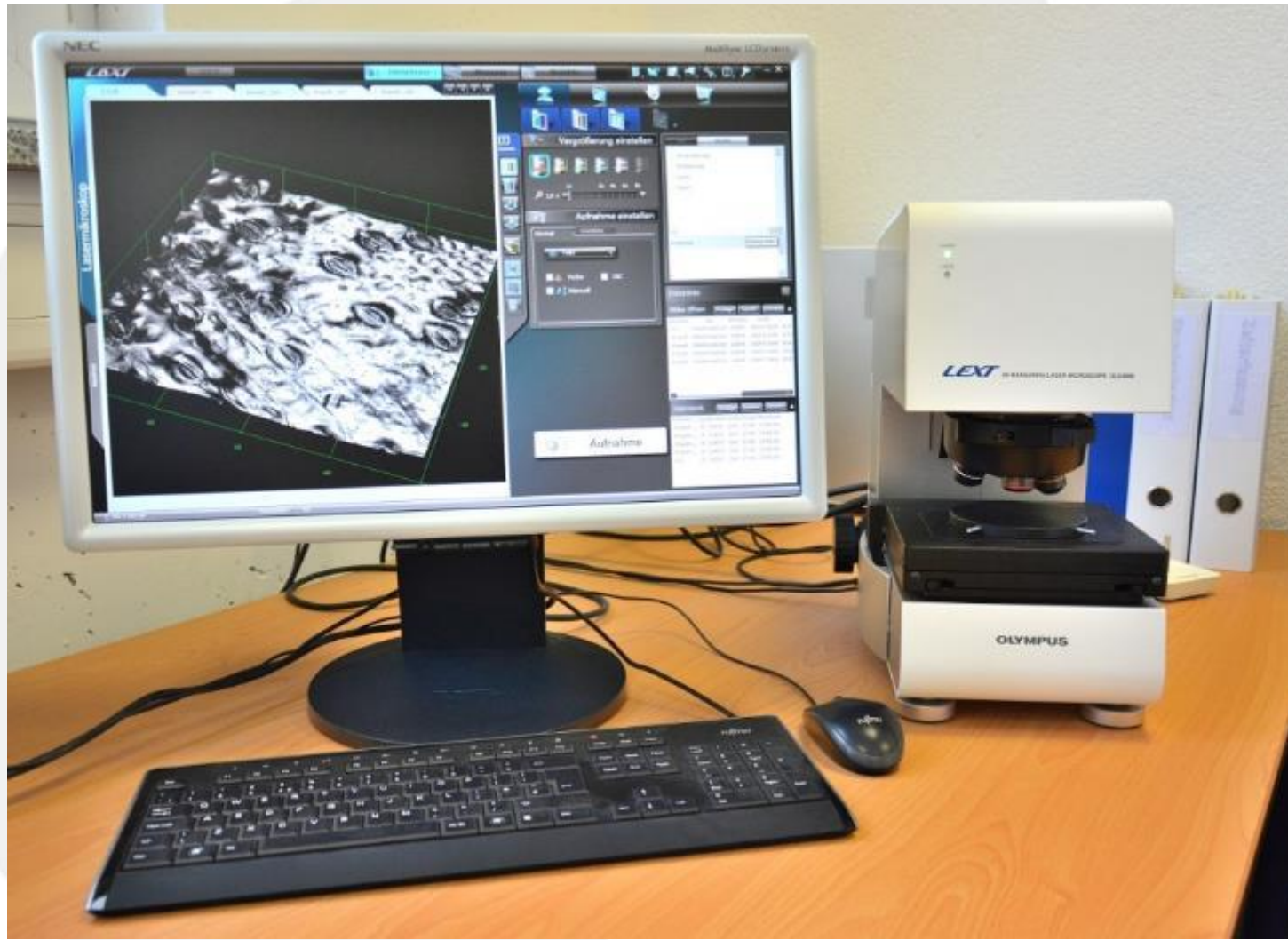


**This gavage probe (Silicone Head) looks normal!**

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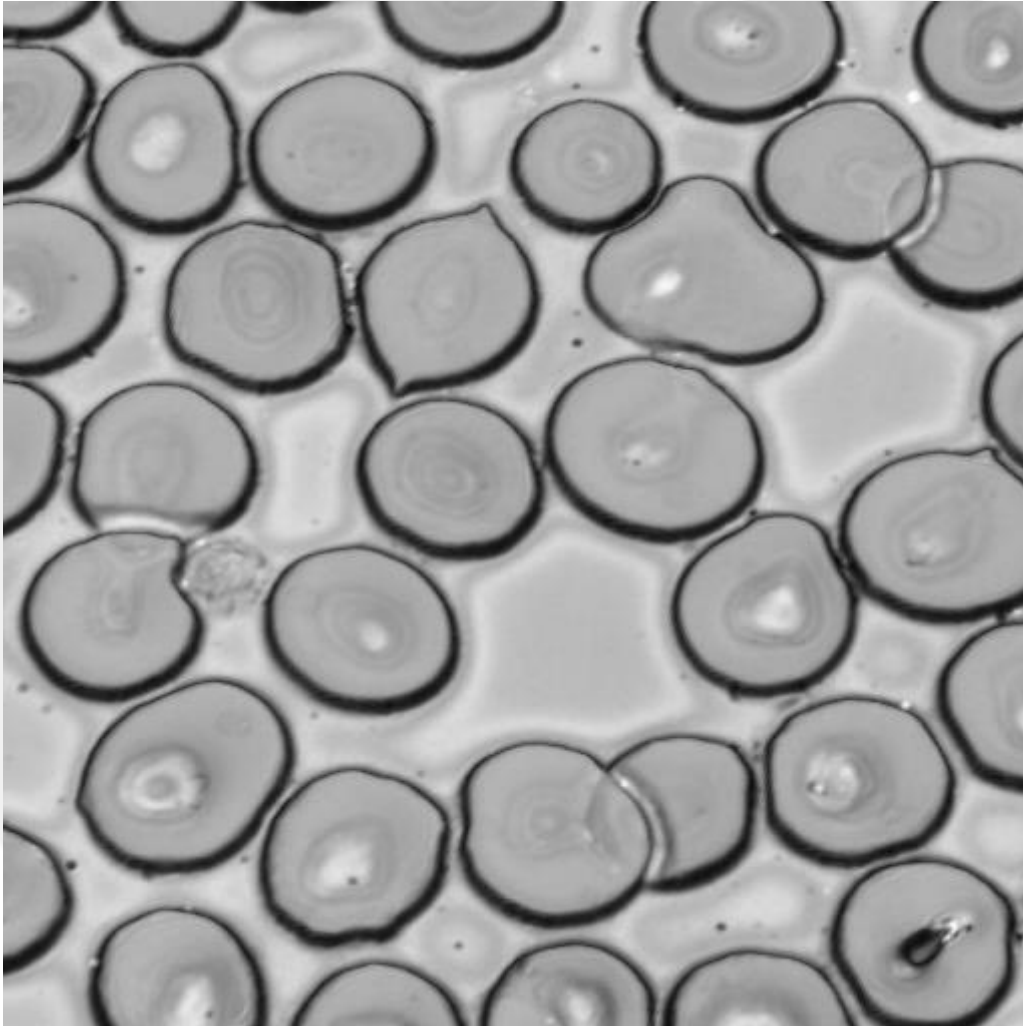
# Laser Scanning Microscopy (LEXT, Olympus)



# Erythrocyte Shaping: Smear Evaluation by 3D Digital Microscopy

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## Evaluation of Isocytosis/Anisocytosis



**Fresh Smear  
No staining  
Human  
x3000**

# Other Issues: Hemocompatibility and Morphology

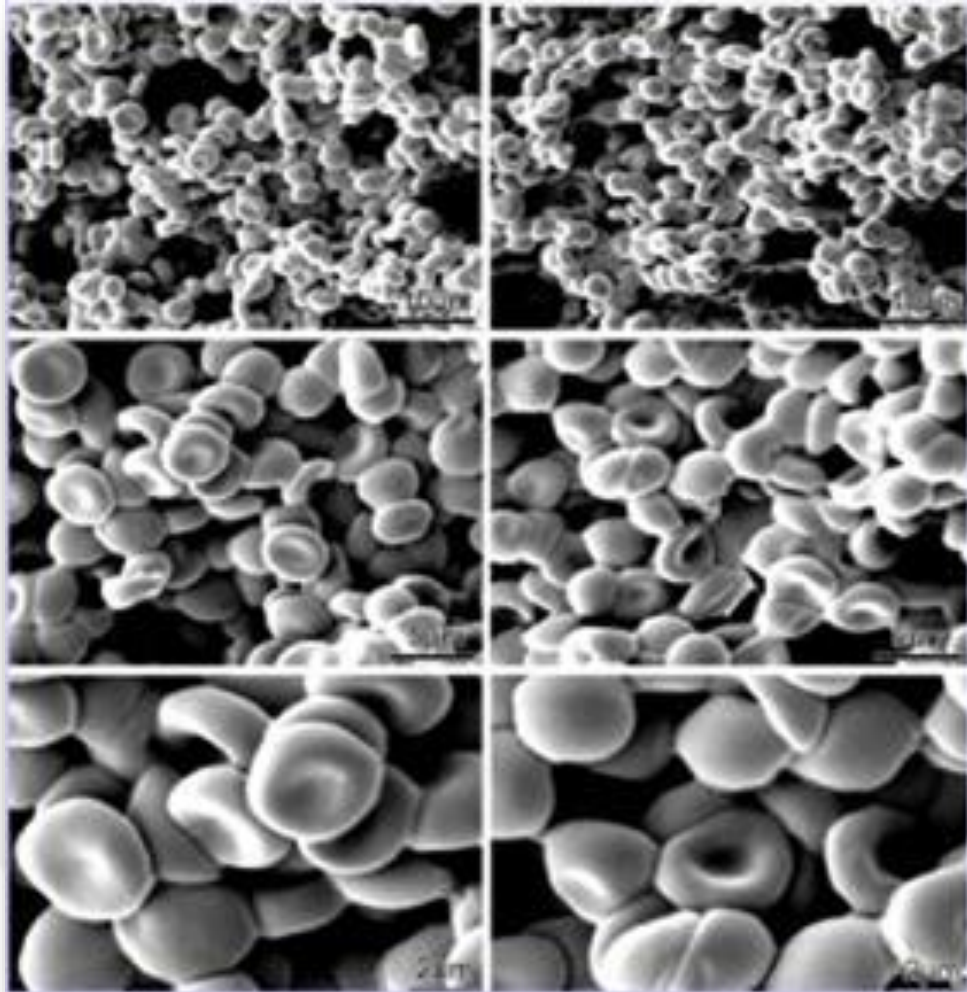
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In case of suspected membrane instability, e.g. anemia of unknown reason or with indication in smear:

- Testing by Parpart (not always reliable)
- Testing by Scanning Electron Microscopy, i.e. sampling and fixation of erythrocytes with subsequent measurements of concavity depths
-

# Hemocompatibility and/or Membrane Instability: SEM

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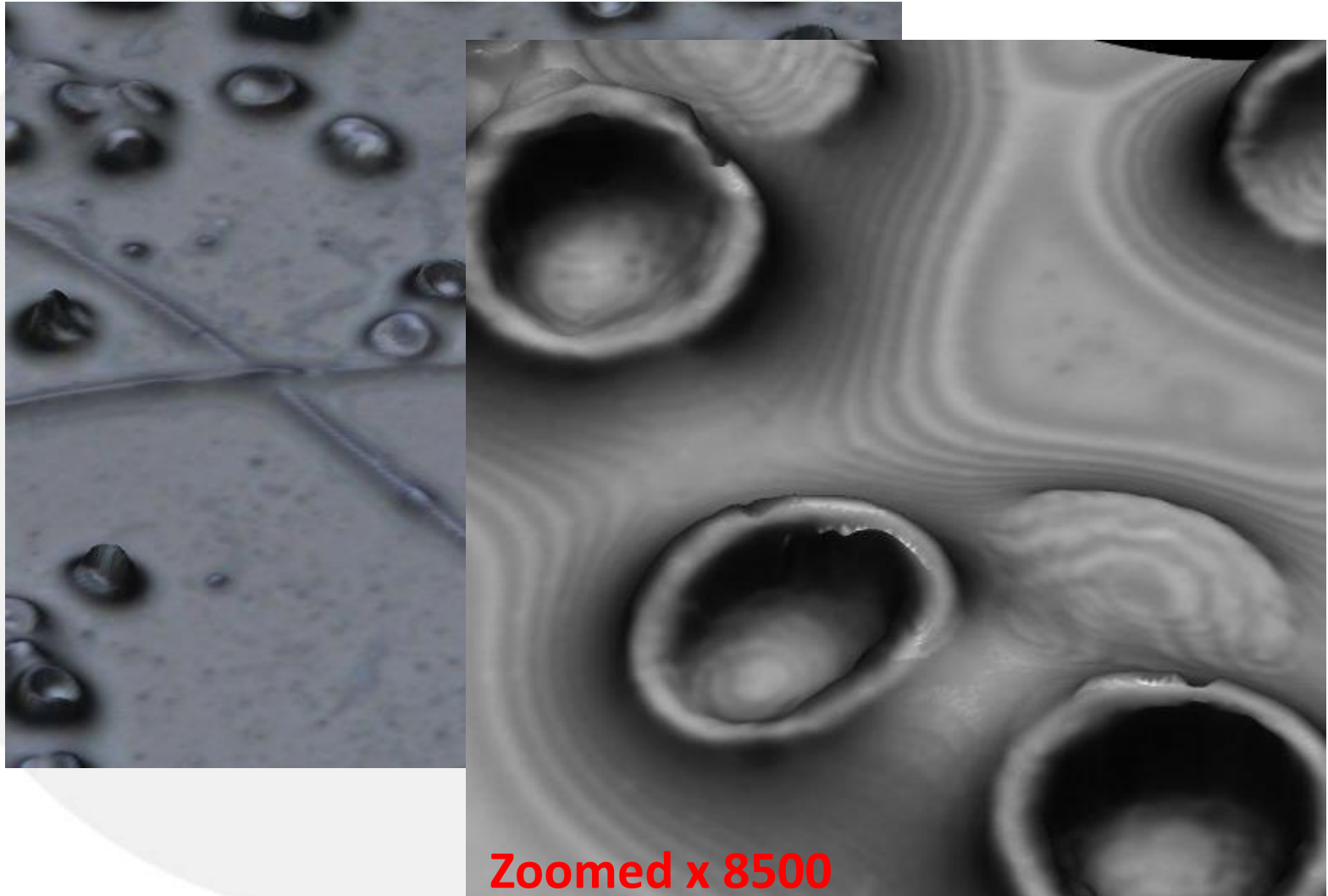
**Concavity measurement:  
Groups with differences  
in concavity in a case  
of anemia**



# Hemocompatibility and/or Membrane Instability: Laser Scanning Digital

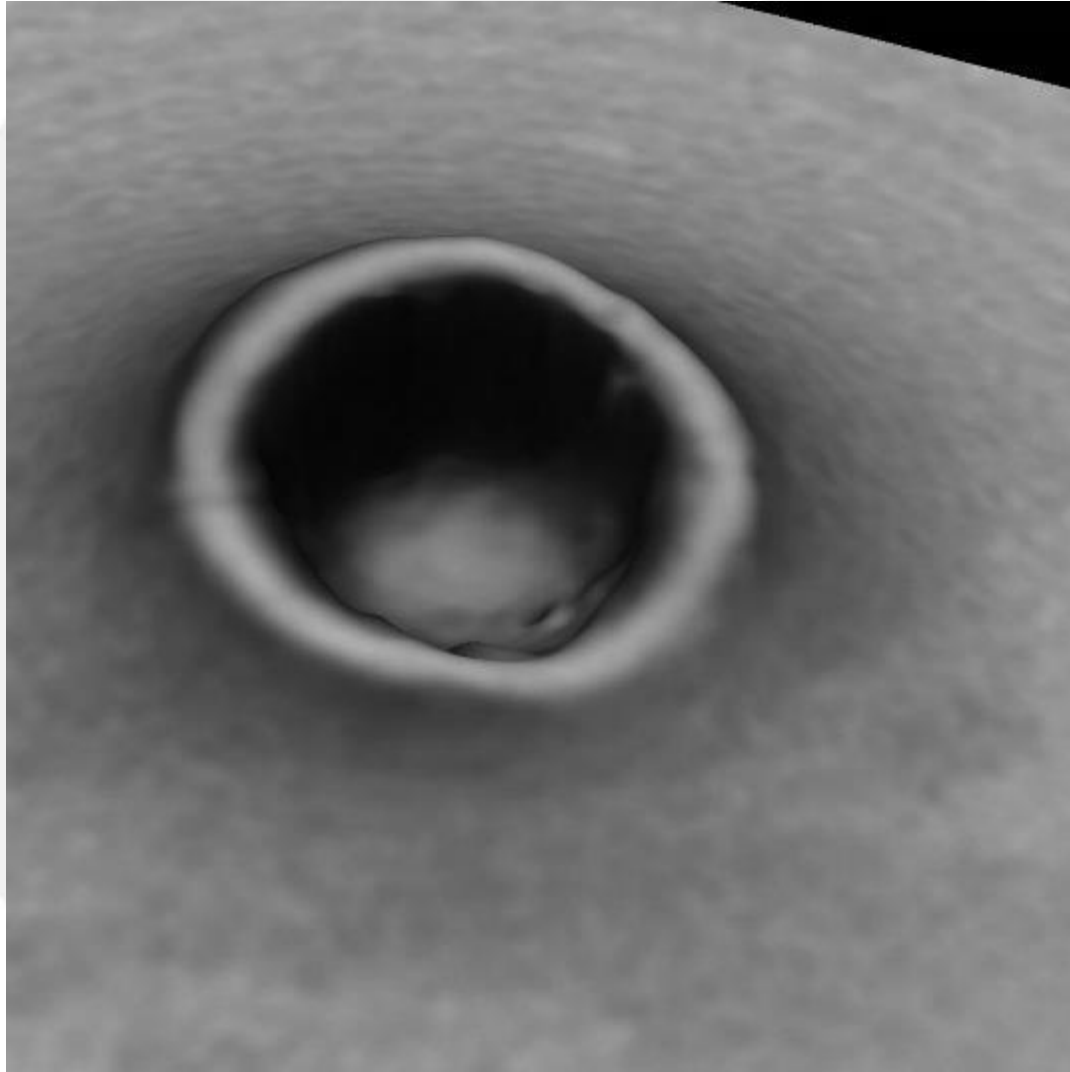
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Example from smear with pre-fixed erythrocytes



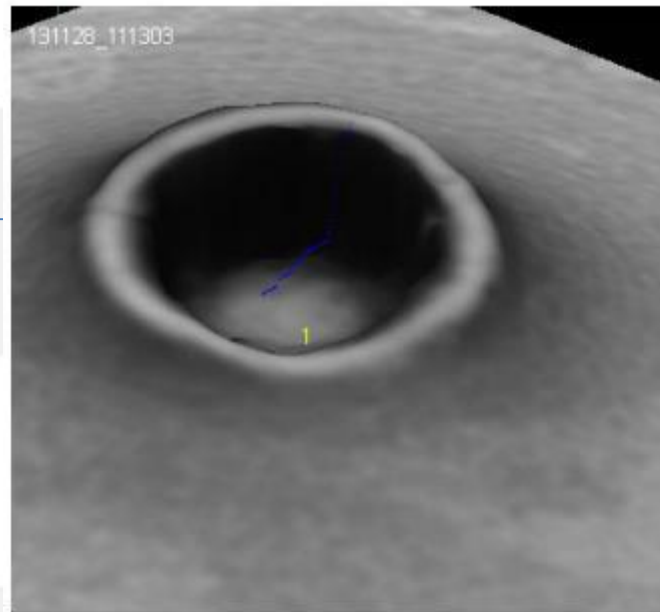
# Hemocompatibility and/or Membrane Instability: Laser Scanning Digital

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**Erythrocyte**  
**Zoomed x 12500**

# Reporting

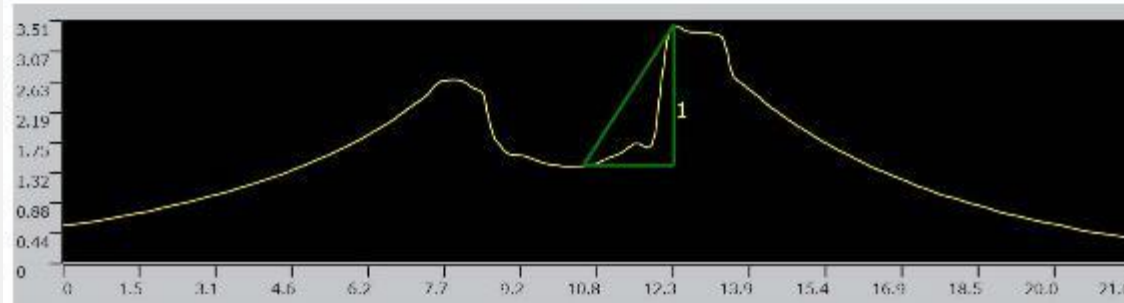


BenutzerID: GUEST  
 Benutzername: GUEST  
 Beschreibung: GUEST USER

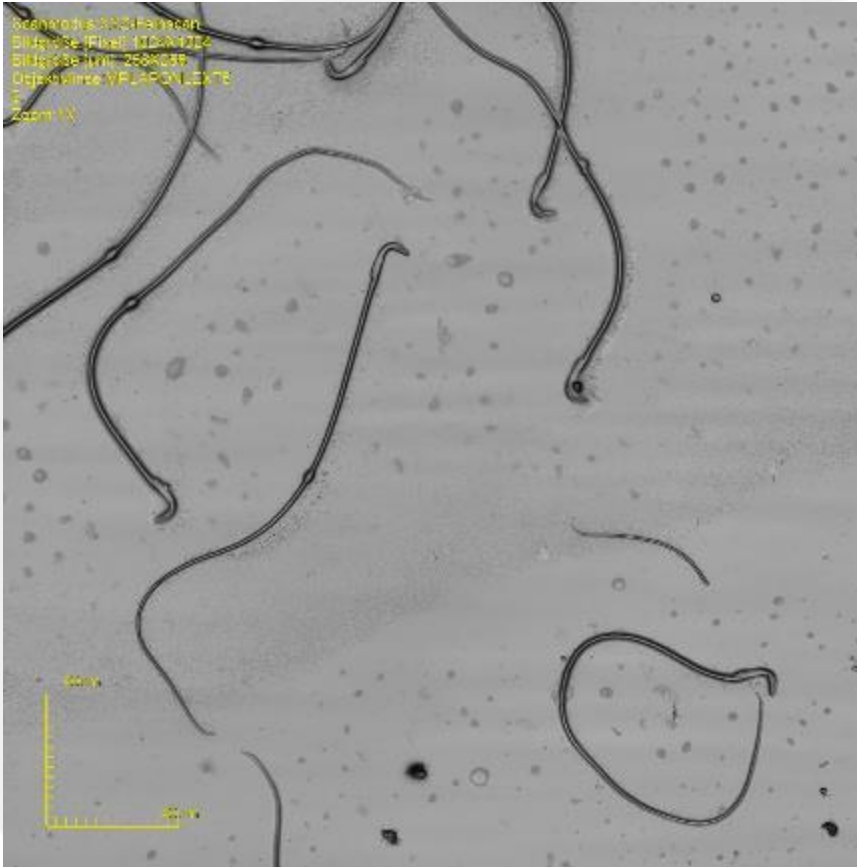
131128\_111303  
 [Aufnahmeparameter]  
 Scanmodus: XYZ-Feinscan + Farbe  
 Bildgröße [Pixel]: 1024X1024  
 Bildgröße [µm]: 22X22  
 Objektivlinse: MPLAPONLEXT100x  
 Zoom: 6X  
 DIC: Aus

Comment

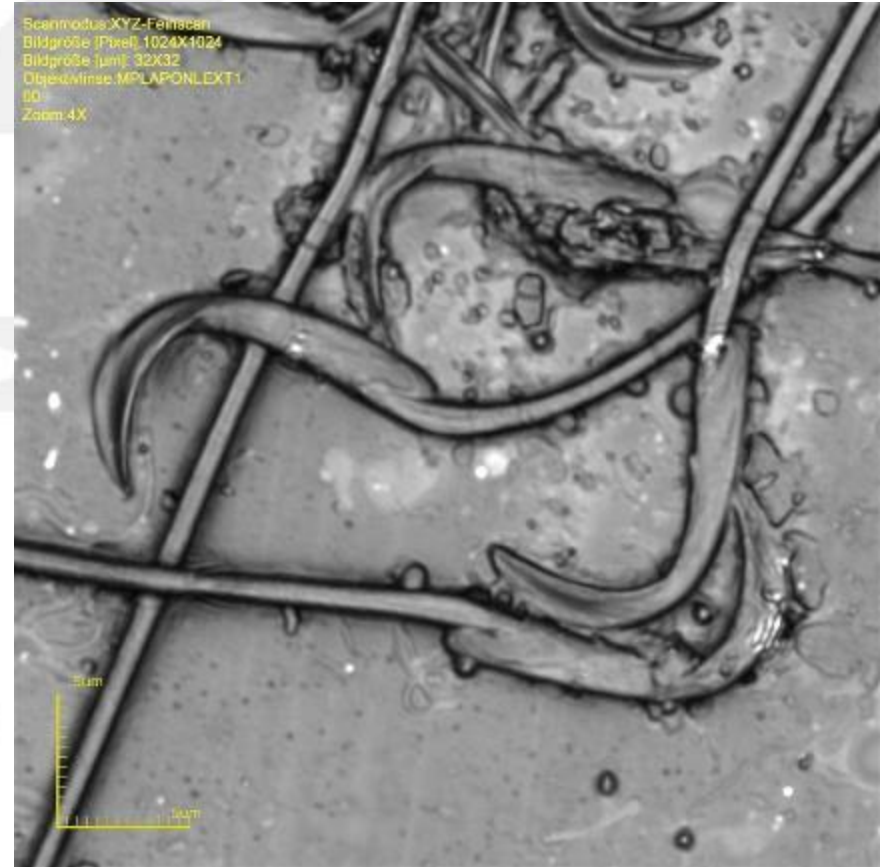
| Nr.                                 | Ergebn | Breite[µm] | Höhe[µm] | Länge[µm] | Winkel[°] | Dateiname   |
|-------------------------------------|--------|------------|----------|-----------|-----------|-------------|
| <input checked="" type="checkbox"/> | 1      | 1.812      | 2.035    | 2.724     | 48.321    | 131128_1113 |
| Zählung                             |        | 1          | 1        | 1         | 1         | 1           |
| Durchschnitt                        |        | 1.812      | 2.035    | 2.724     | 48.321    |             |
| Min.                                |        | 1.812      | 2.035    | 2.724     | 48.321    |             |
| Max.                                |        | 1.812      | 2.035    | 2.724     | 48.321    |             |
| Bereich                             |        | 0.000      | 0.000    | 0.000     | 0.000     |             |
| σ                                   |        | 0.000      | 0.000    | 0.000     | 0.000     |             |
| 3σ                                  |        | 0.000      | 0.000    | 0.000     | 0.000     |             |
| Toleranz                            |        | Aus        | Aus      | Aus       | Aus       | Aus         |
| Toleranz oben                       |        | 0          | 0        | 0         | 0         |             |
| Standard                            |        | 0          | 0        | 0         | 0         |             |
| Toleranz unten                      |        | 0          | 0        | 0         | 0         |             |



# Sperm Analysis by Digital Microscopy



**Zoomed x2000**  
**Fixed sperm, Smear, Rat**



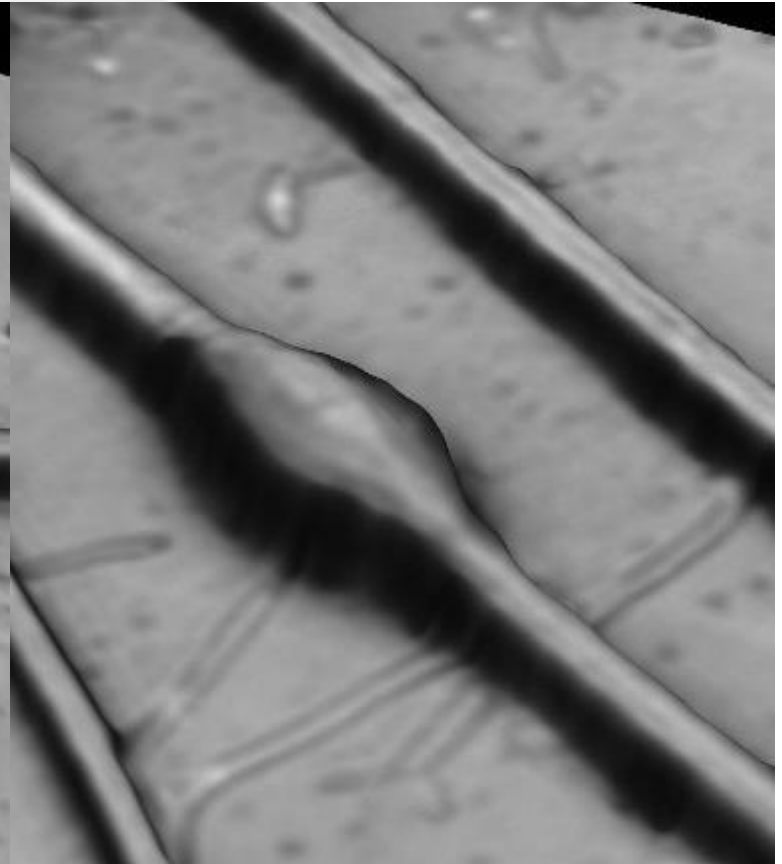
**Zoomed x8500**

# Sperm Analysis by Laser Digital Microscopy

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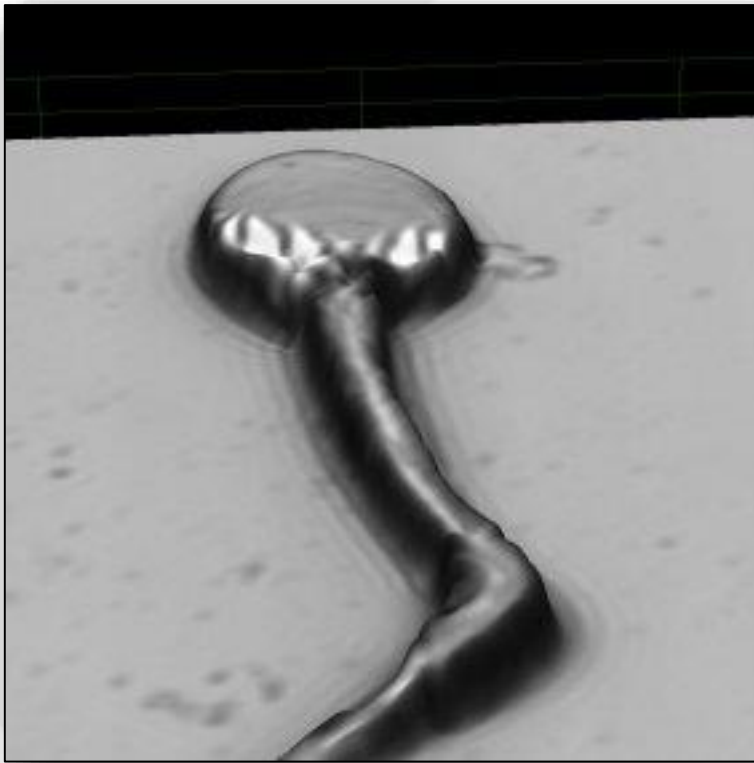
**Zoomed x 8500**



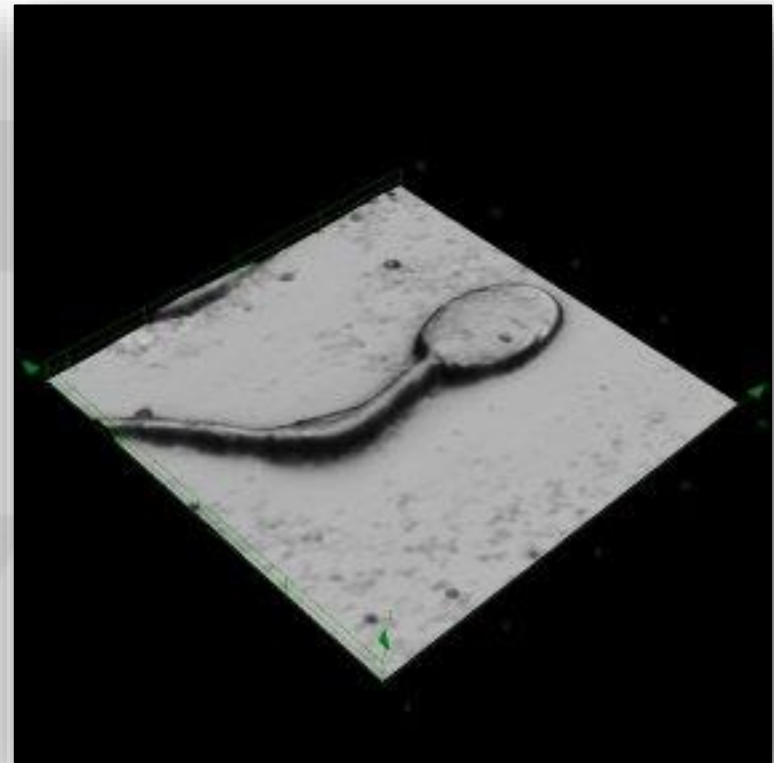
**Zoomed x 15000**

# Lesions

- In contrast, in primate and rabbit, >90% of sperm without CD
- If CD present, sperm is abnormal



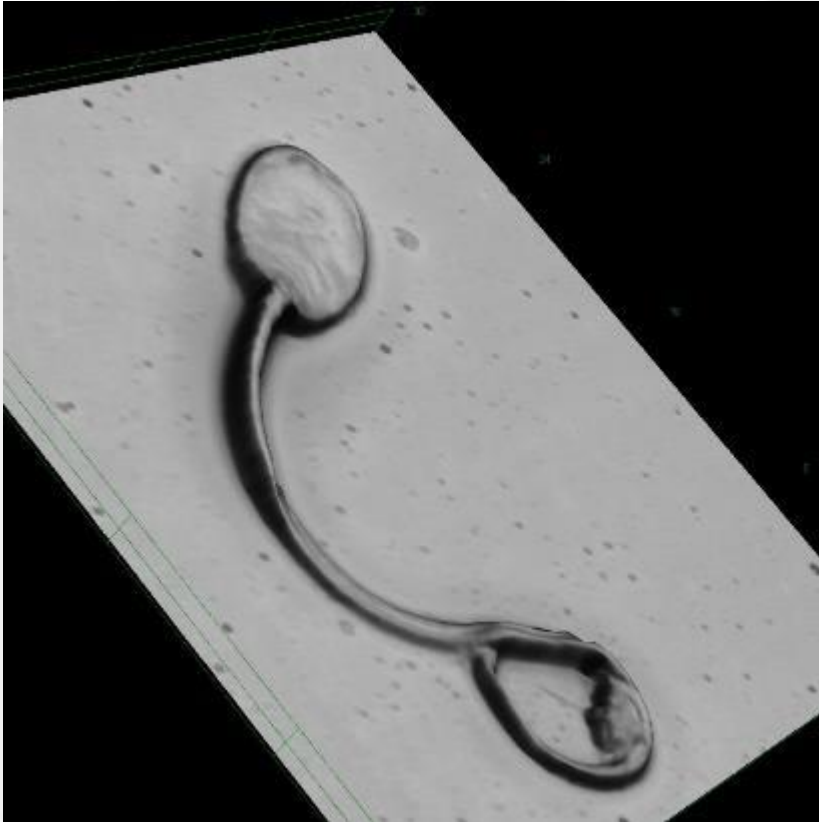
**CD in cynomolgus sperm**



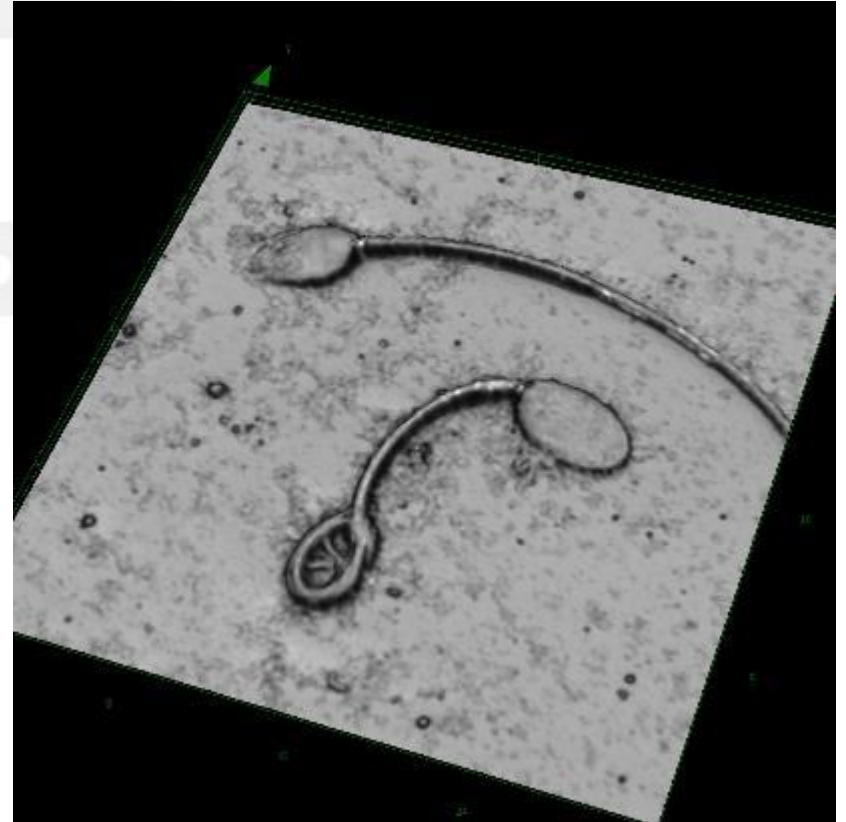
**Cytoplasmic remnant in rabbit sperm**

# Lesions

- Coiled tails at reasons for infertility

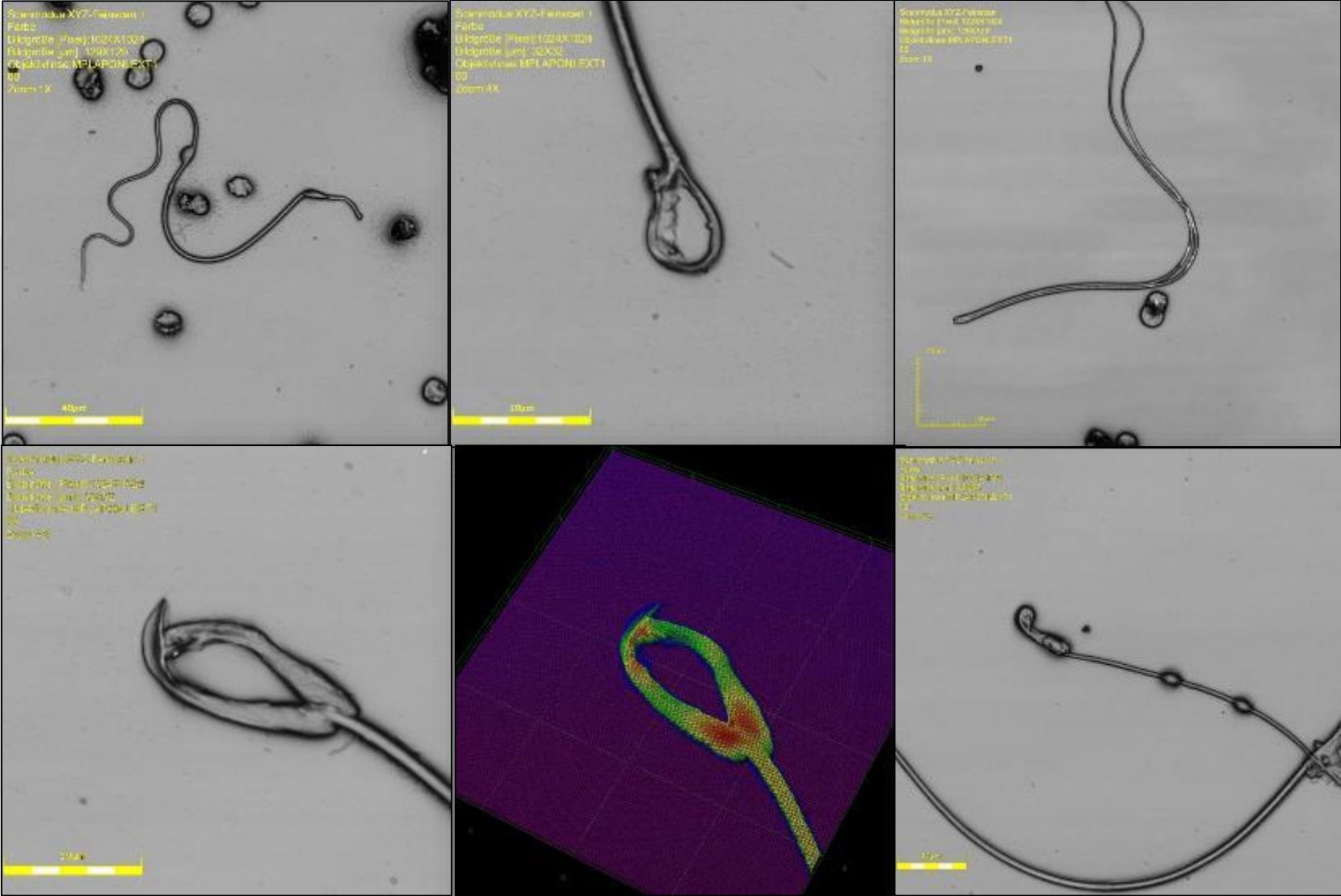


**Coiled tail in rabbit sperm**



**Fake coiled tail in cynomolgus**

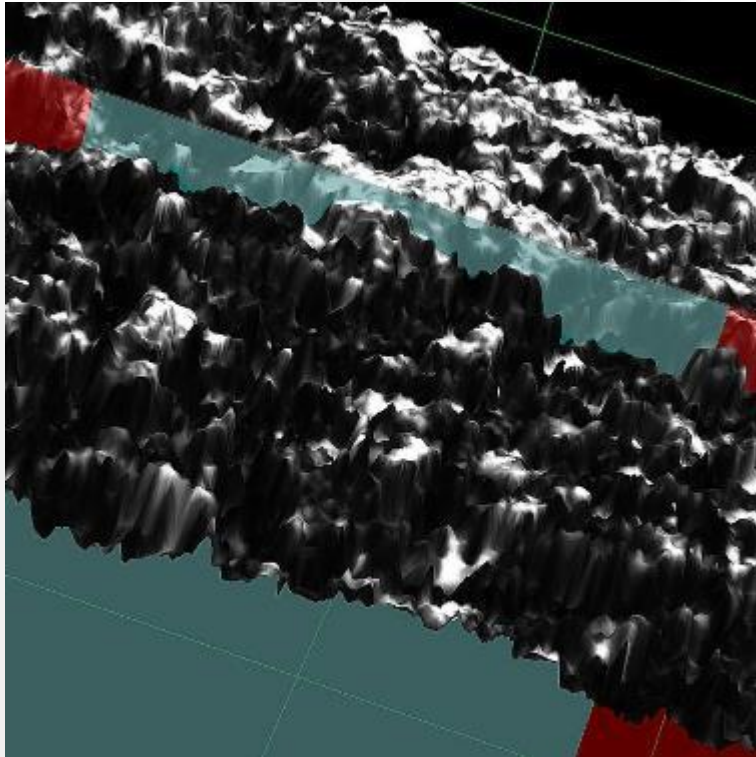
# Induced Lesions



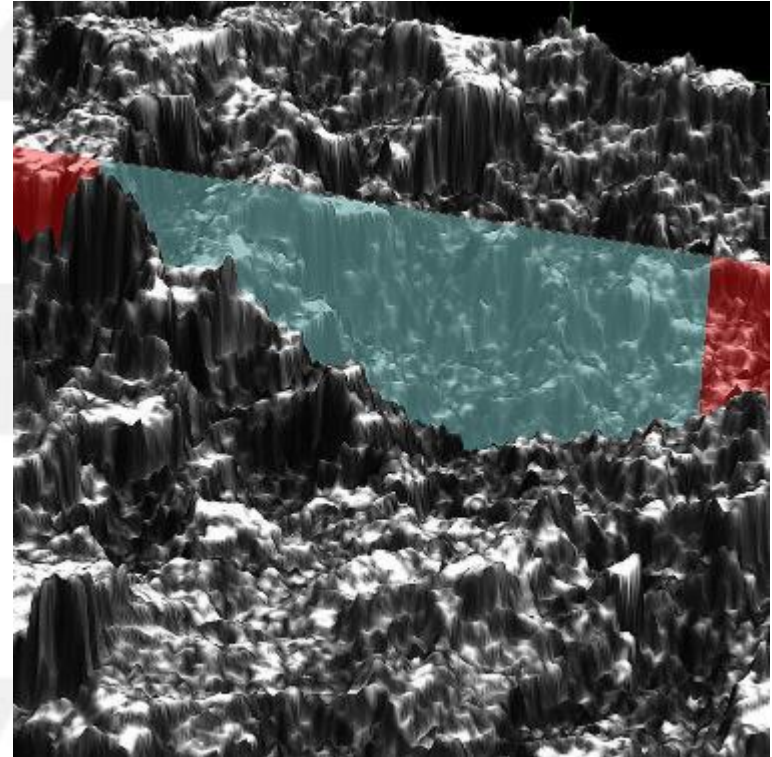


# Surface evaluation by LEXT

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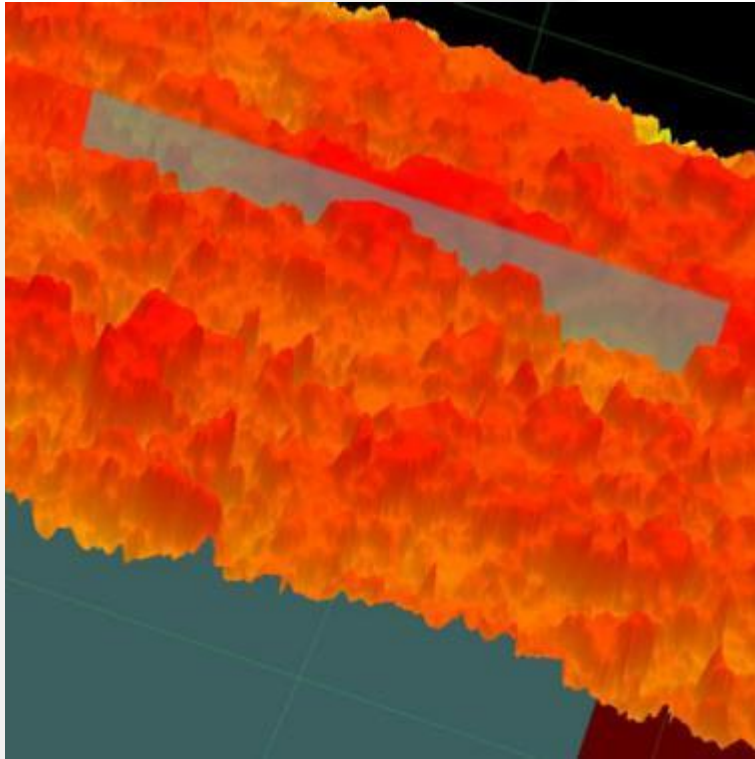
**Implant 1**



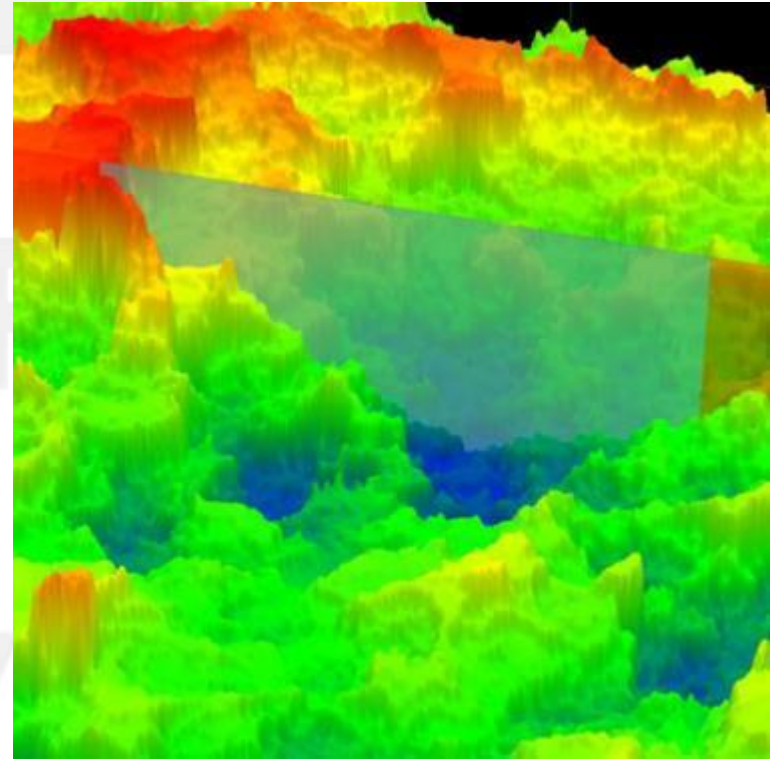
**Implant 2**

# Surface evaluation by LEXT

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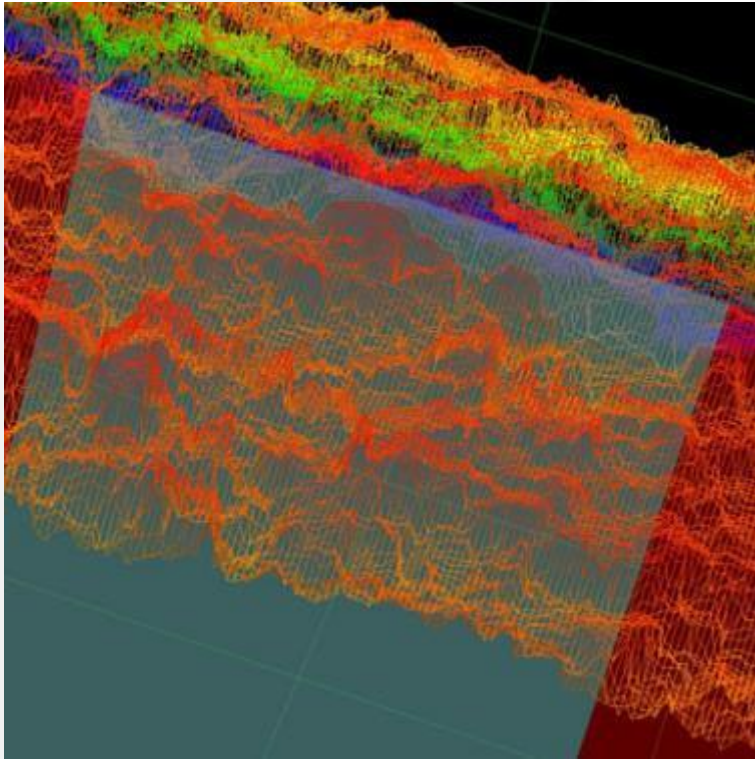
**Implant 1**  
**Reverse reflectance**



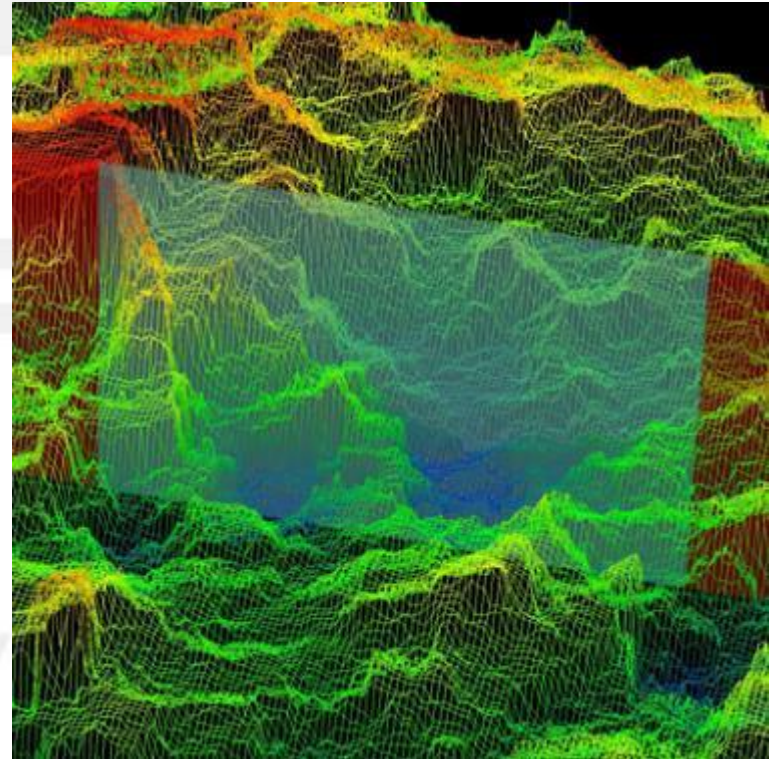
**Implant 2**  
**Reverse reflectance**

# Surface evaluation by LEXT

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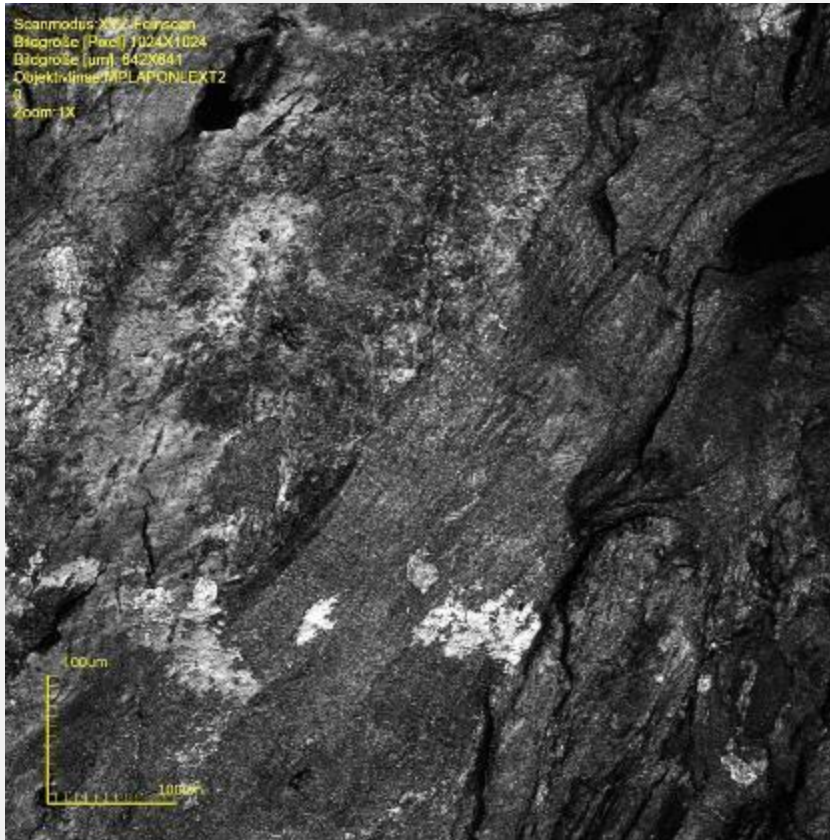


**Implant 1**  
**Image Slope**

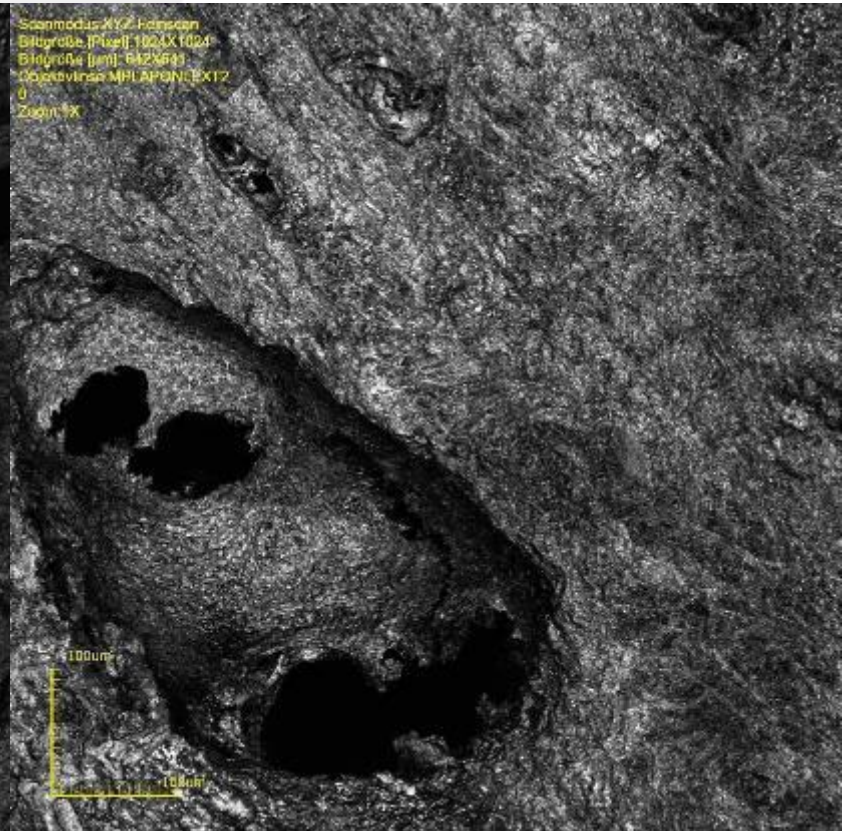


**Implant 2**  
**Image Slope**

# Differences in bone surfaces: different surgically used bone saw techniques

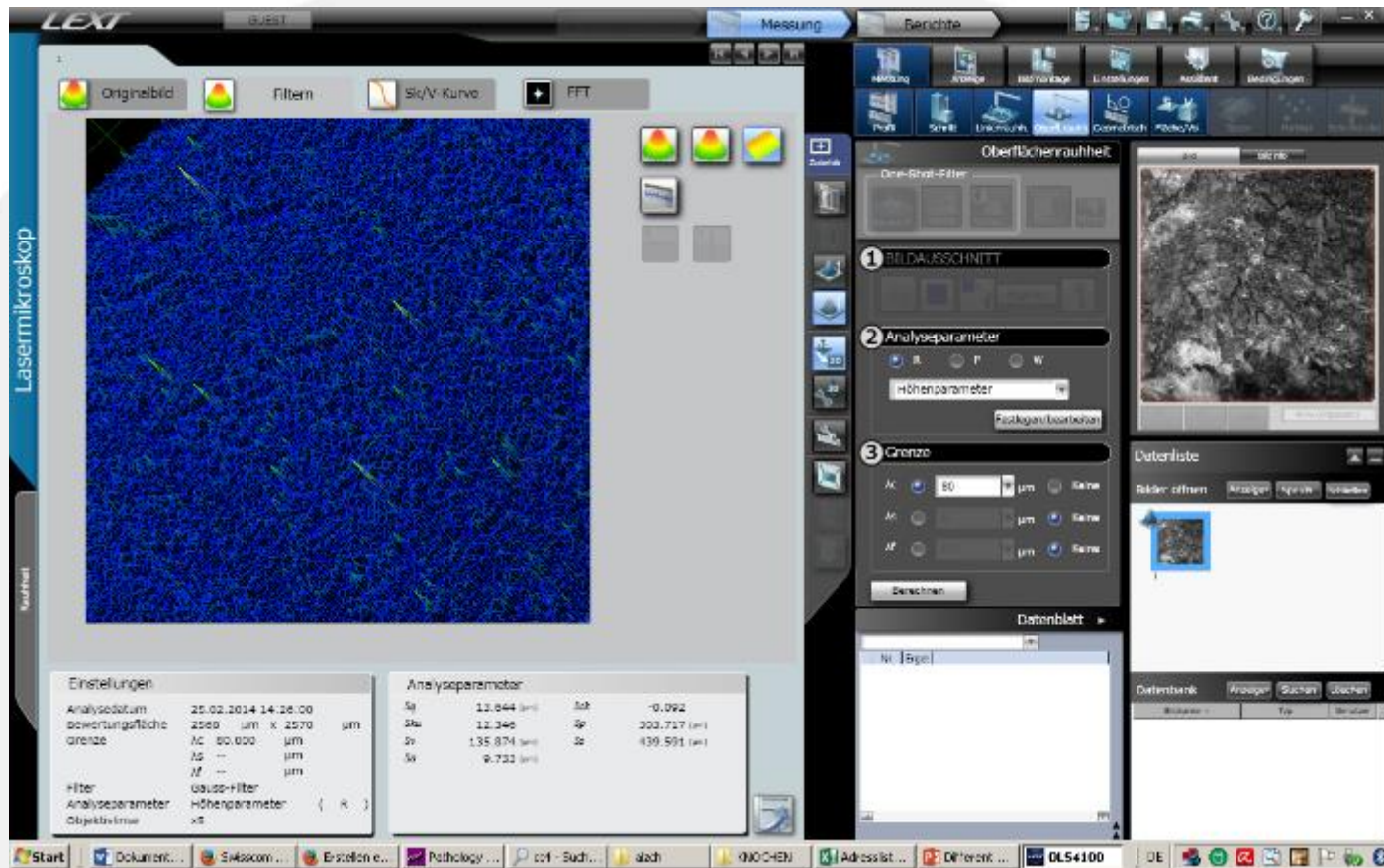


**Conventional technique.  
Note detritus.**



**New technology.  
Note free pores.**

# Surface evaluation by LEXT



Sq: root mean square wavelength

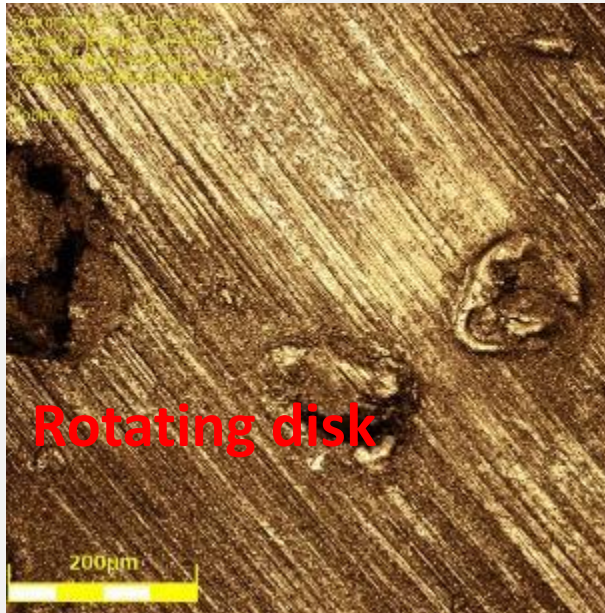
Ssk: skewness - measure of the asymmetry of the probability distribution

Sku: kurtosis - measure of the "peakedness" of the probability distribution of a real-valued random variable

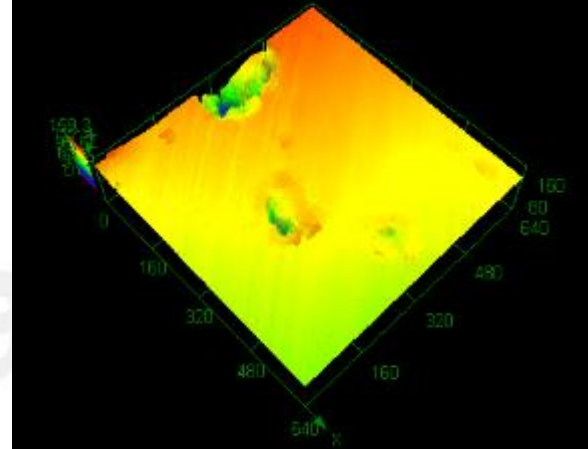
Sp: maximum peak height; Sv: maximum pit depth (Sz: maximum (Sp + Sv))

Sa: arithmetic mean height

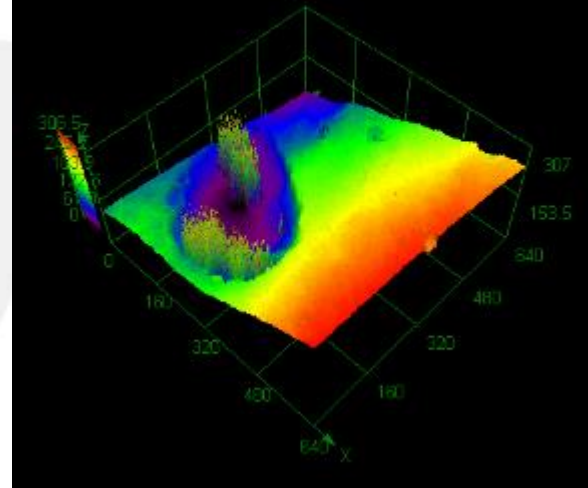
# Examples: Bone Surface Roughness



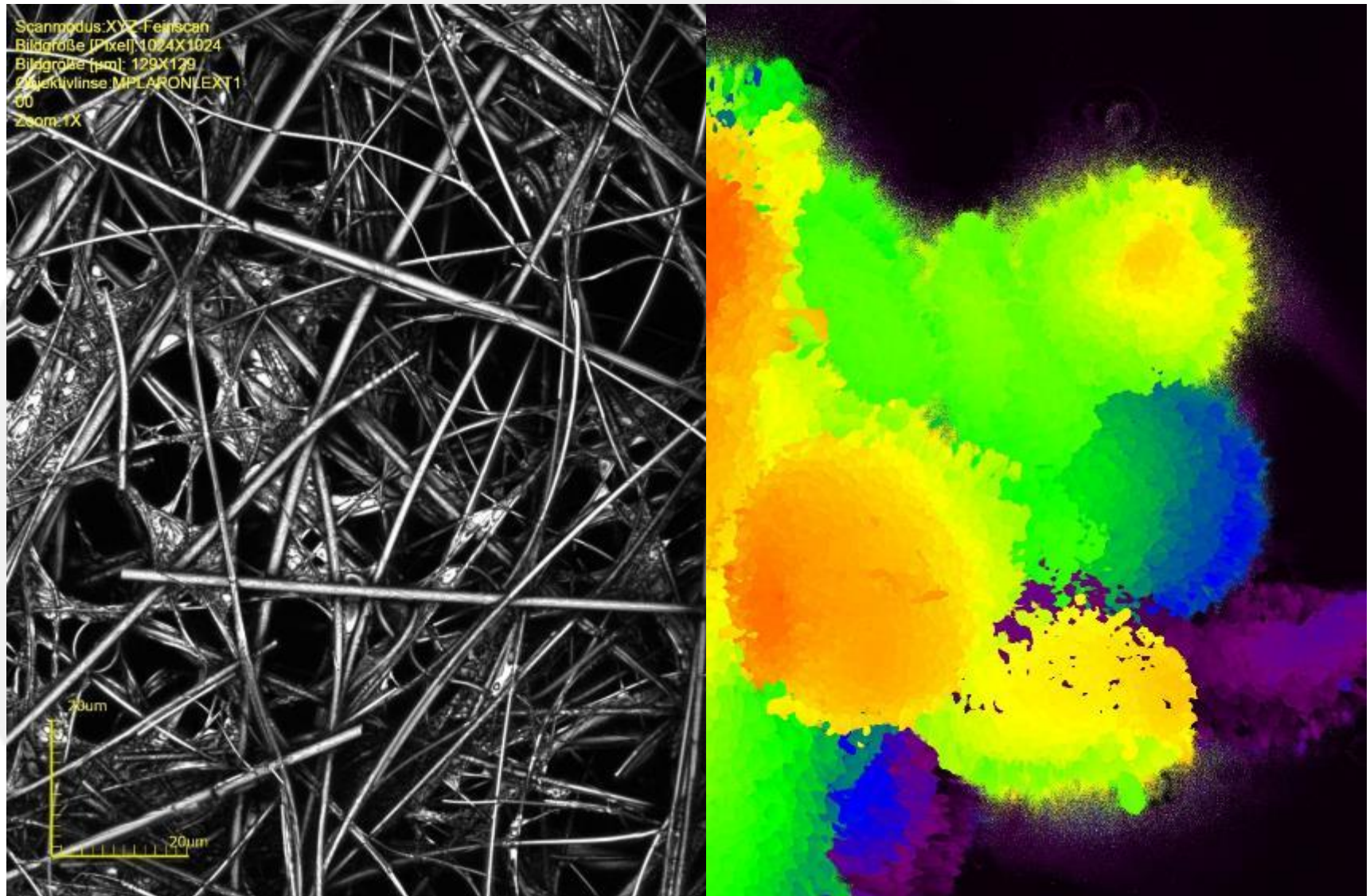
Rotating disk



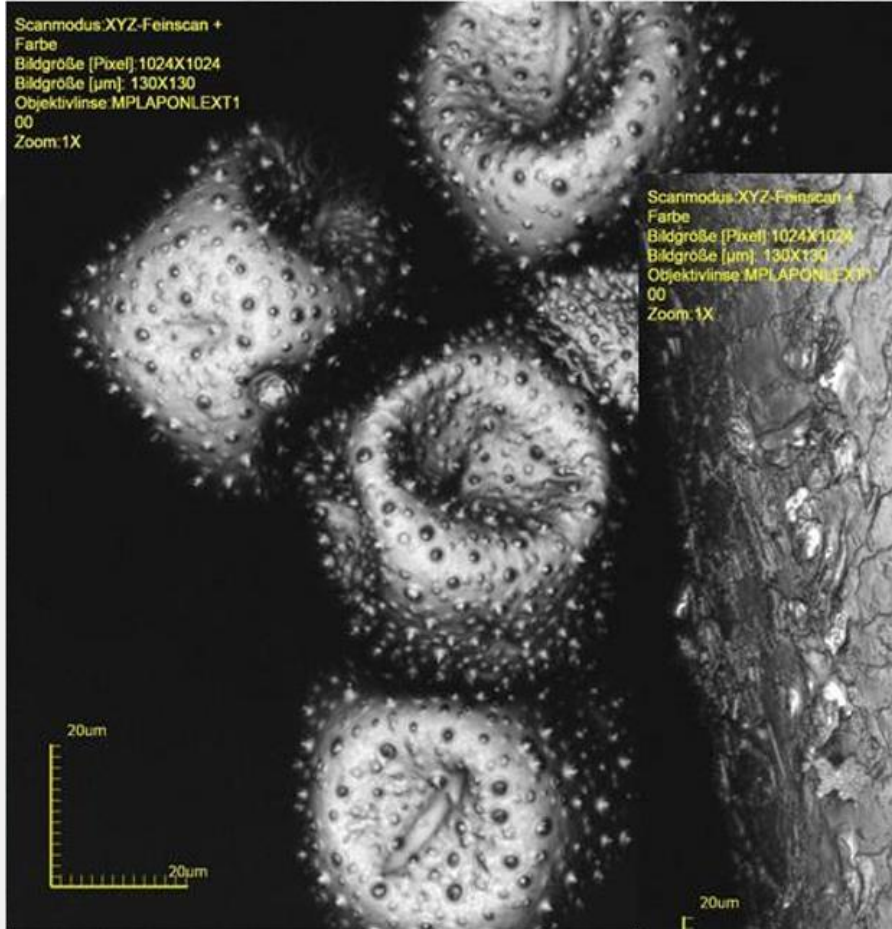
Laser ablation



# Application on Nanoparticles

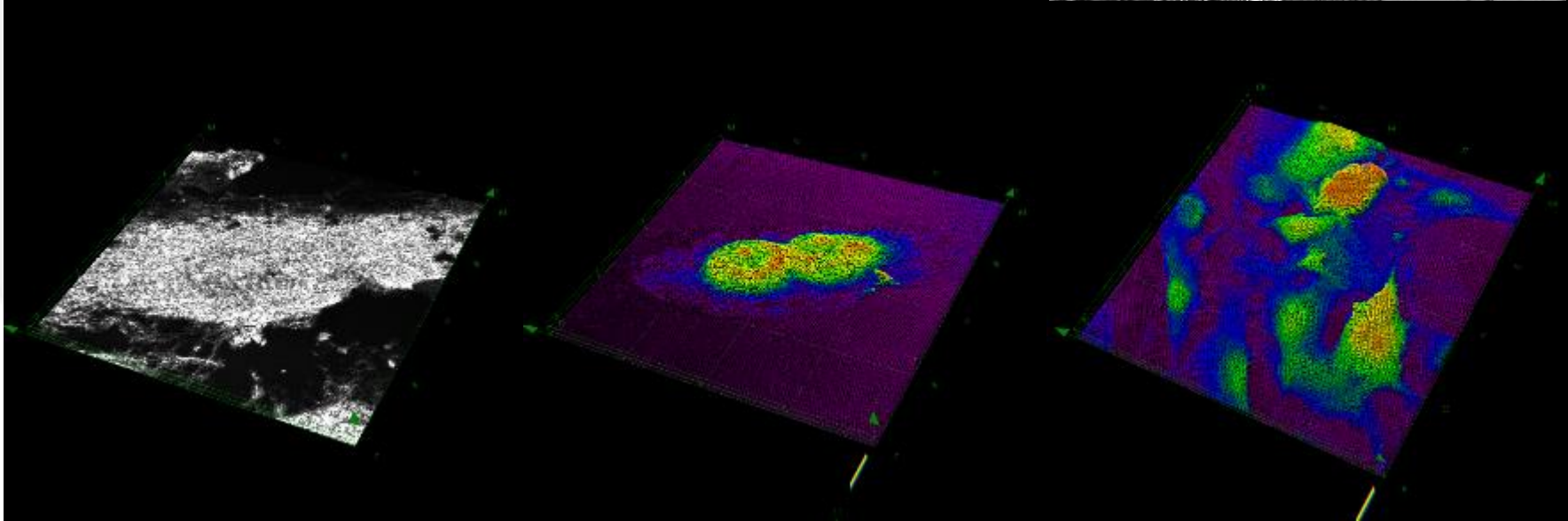
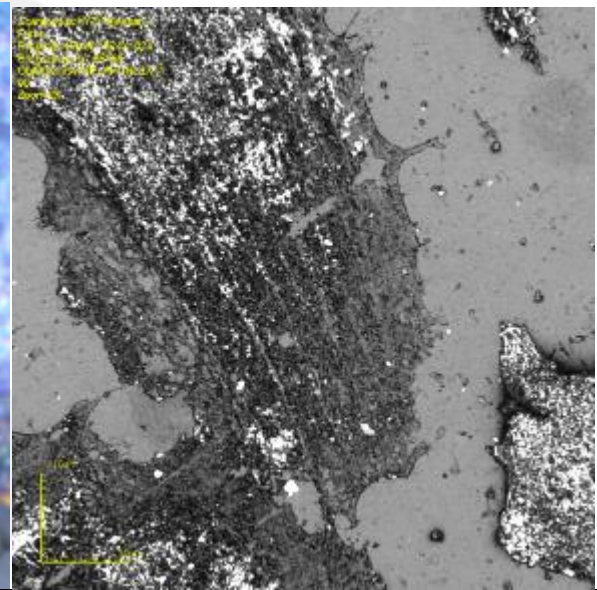
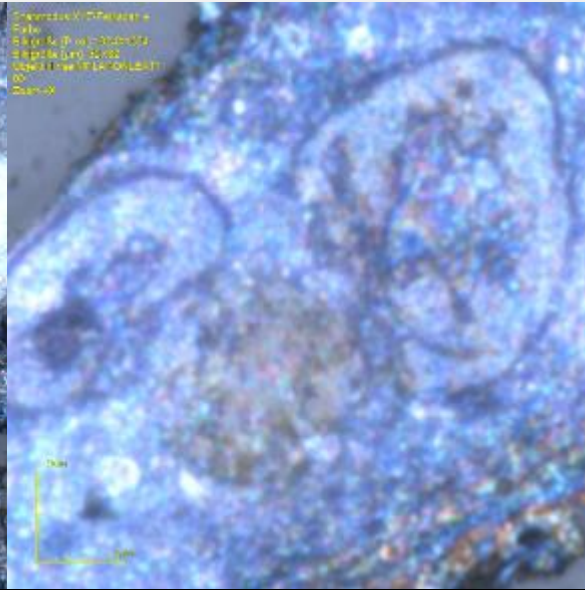
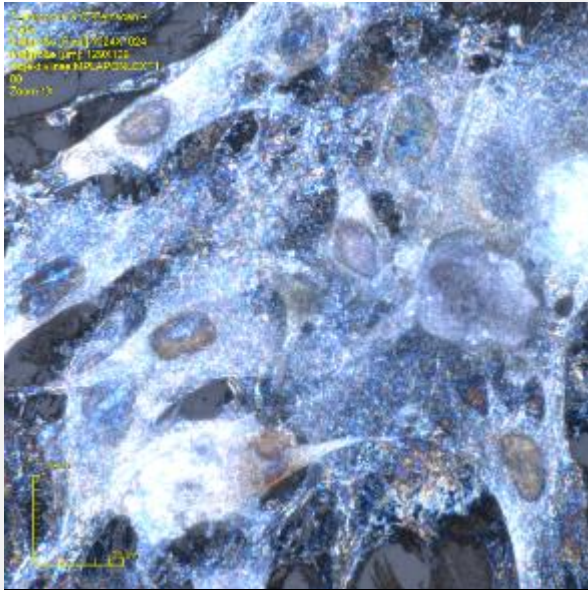


# Other examples by laser digital microscopy



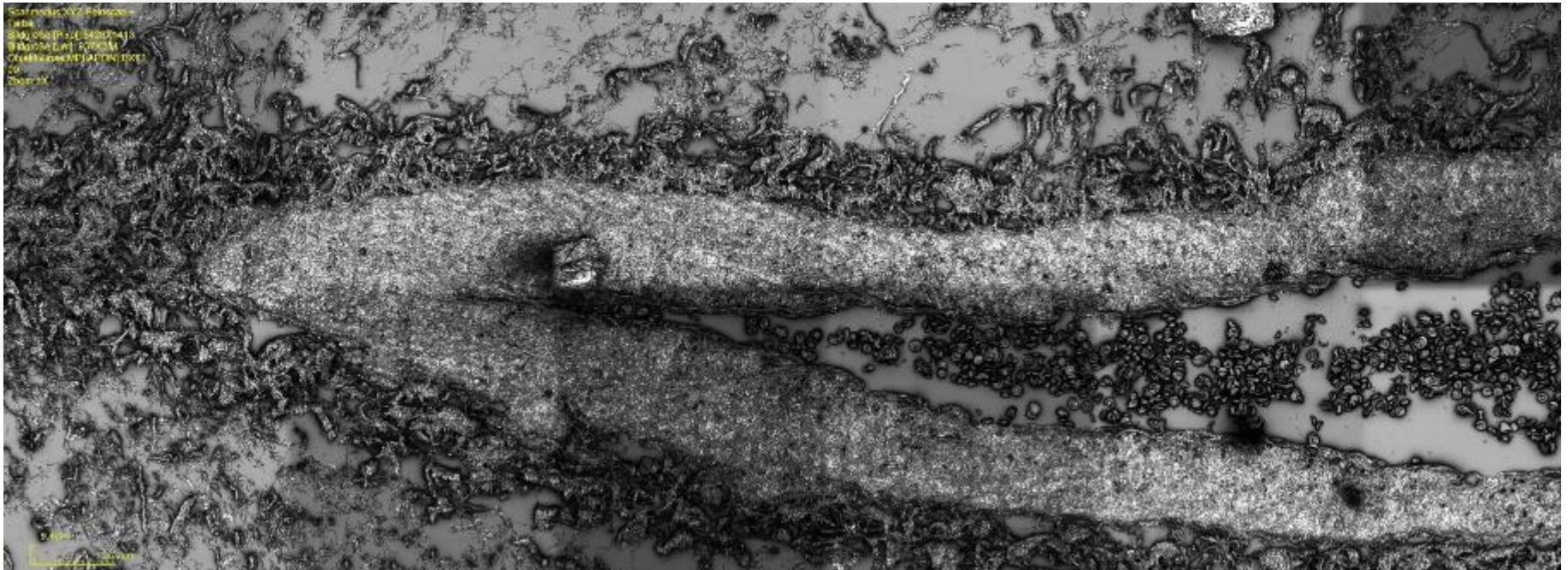


# New possibilities: cell culture



# New possibilities: tissue (Example: lung)

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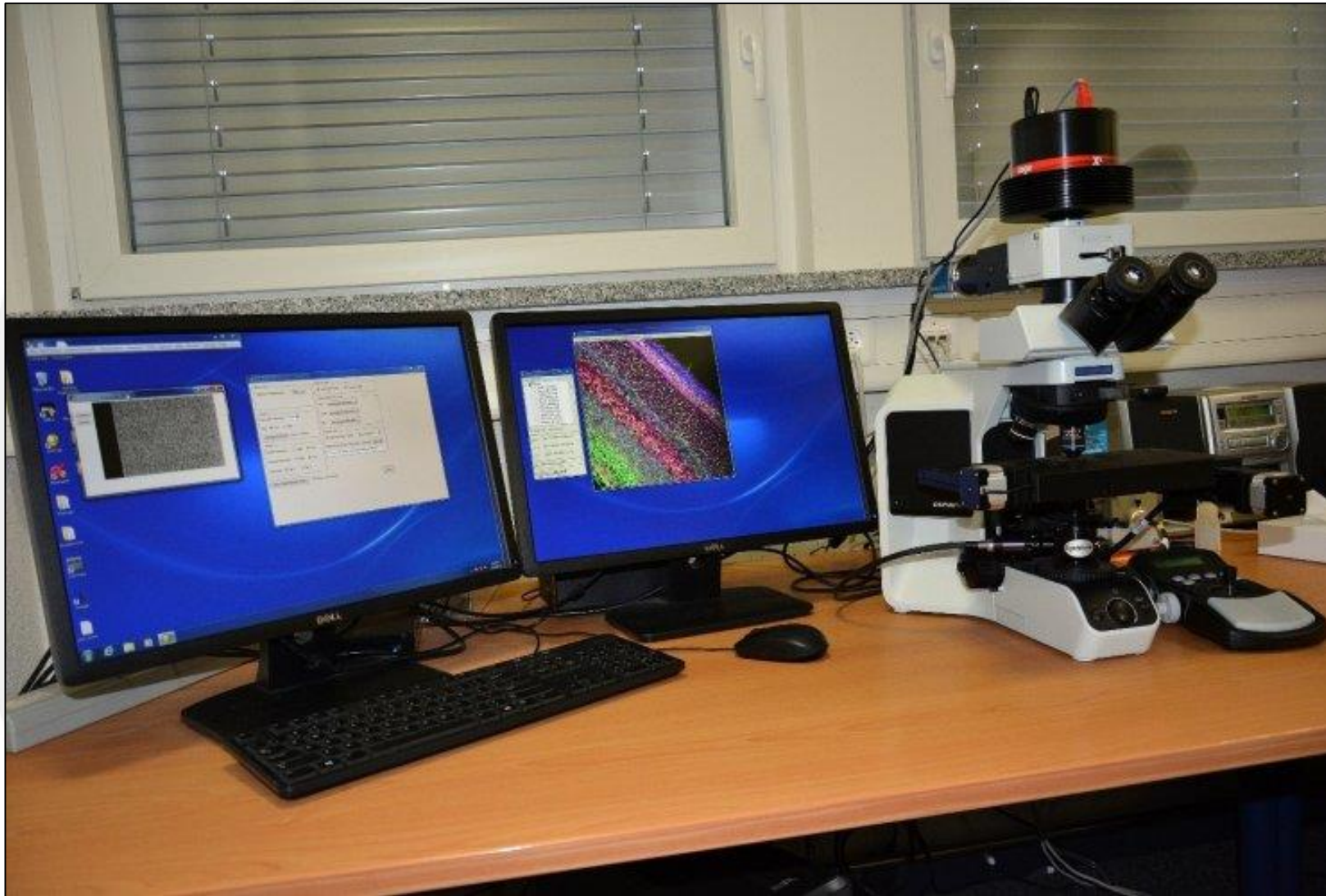
# Another Technique:

## Cytoviva Nanoscale Hyperspectral Analysis

- **image at with an Olympus BX-51 microscope equipped with the patented CytoViva illumination system and a 100W Quartz-Halogen light source**
- **spectral data capture with CytoViva spectro-photometer and integrated CCD camera**
- **spectral analysis by CytoViva Hyperspectral analysis software program**

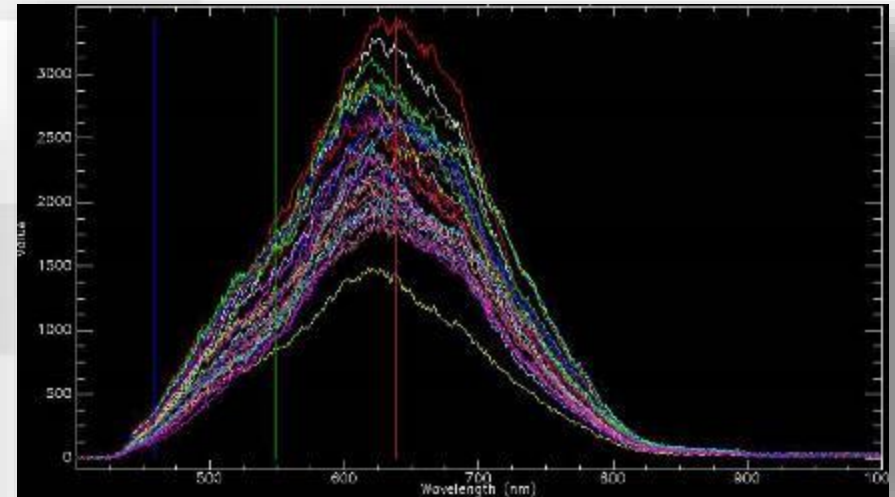
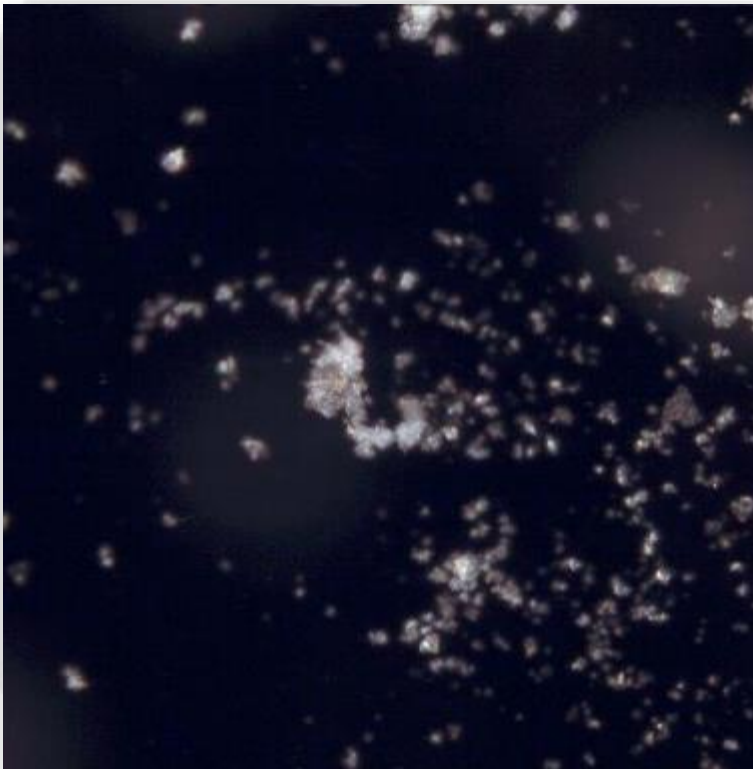
# Hyperspectral Analysis

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# How to make it...

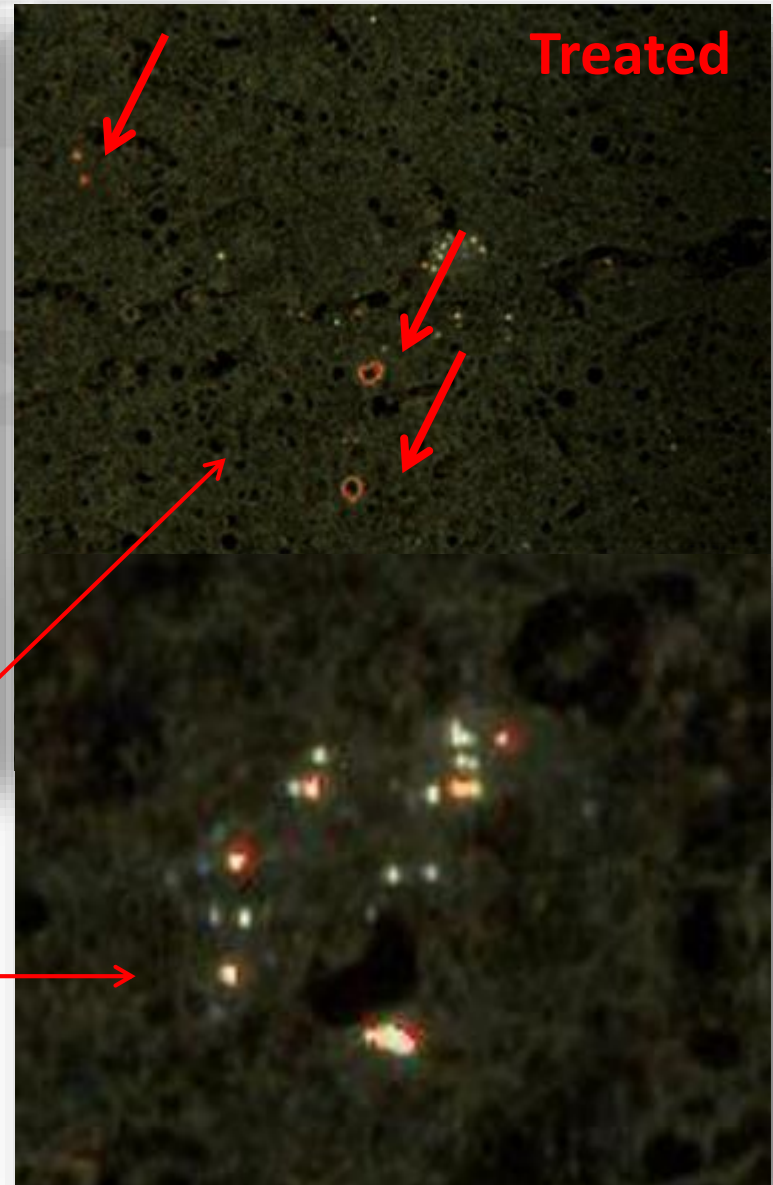
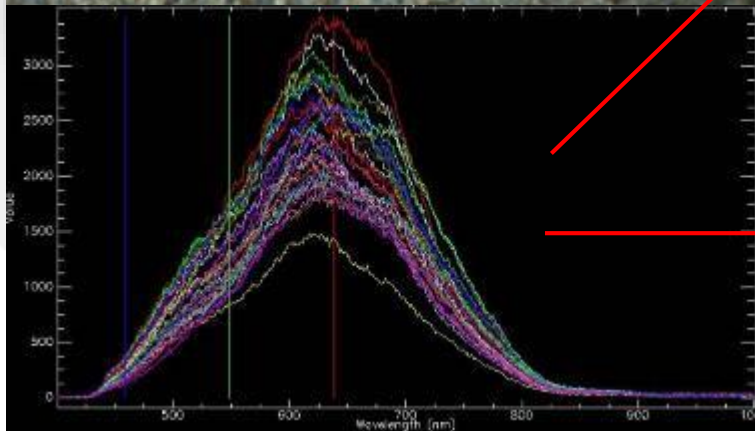
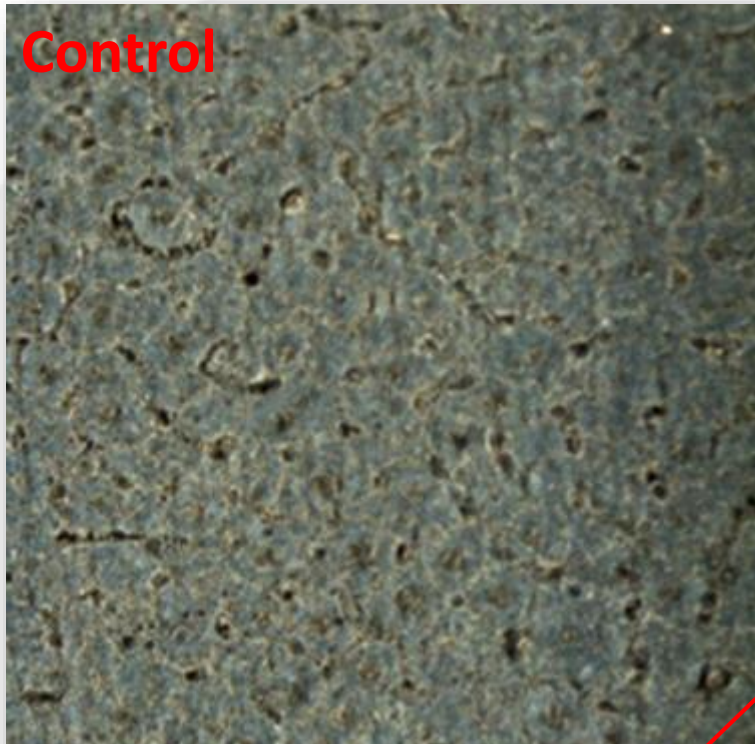
## Dissolved Compound Y (dark field)



## Nanoscale Hyperspectrum of test item

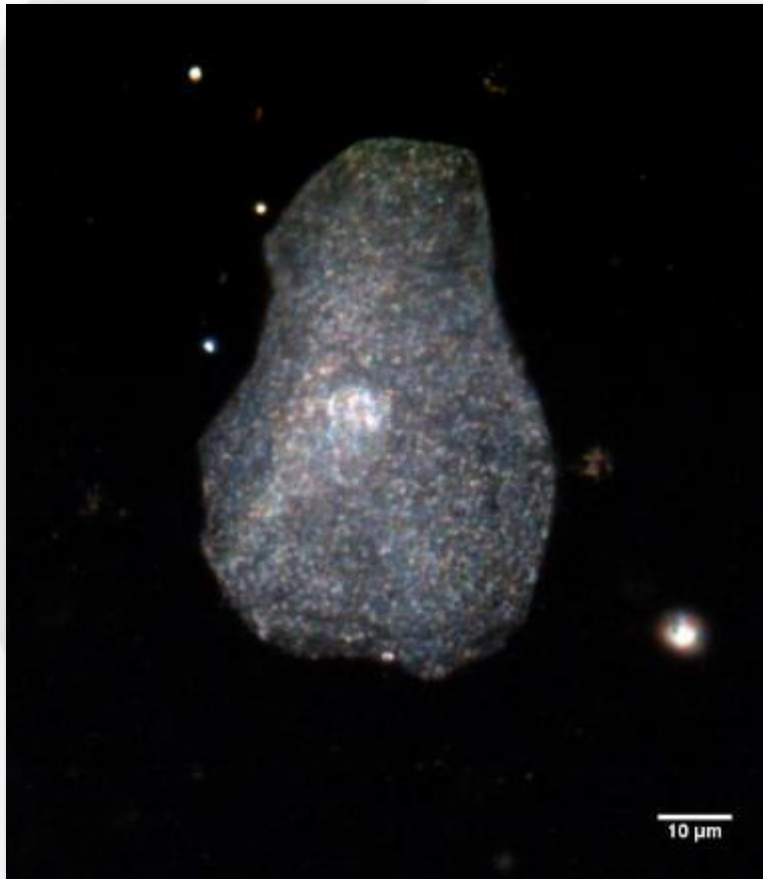
Weber K, Canut L, Xanxo S, Sander J, Maraschiello C, Djonov V, Yamate J, Marino K: Hepatotoxic compounds. Classic Examples in Toxicologic Pathology (4th Edition) Eds: Drommer W, Karbe E, Germann PG, 4th Edition, ISBN 978-3-9814653-0-3, 2011

# How does it look like...

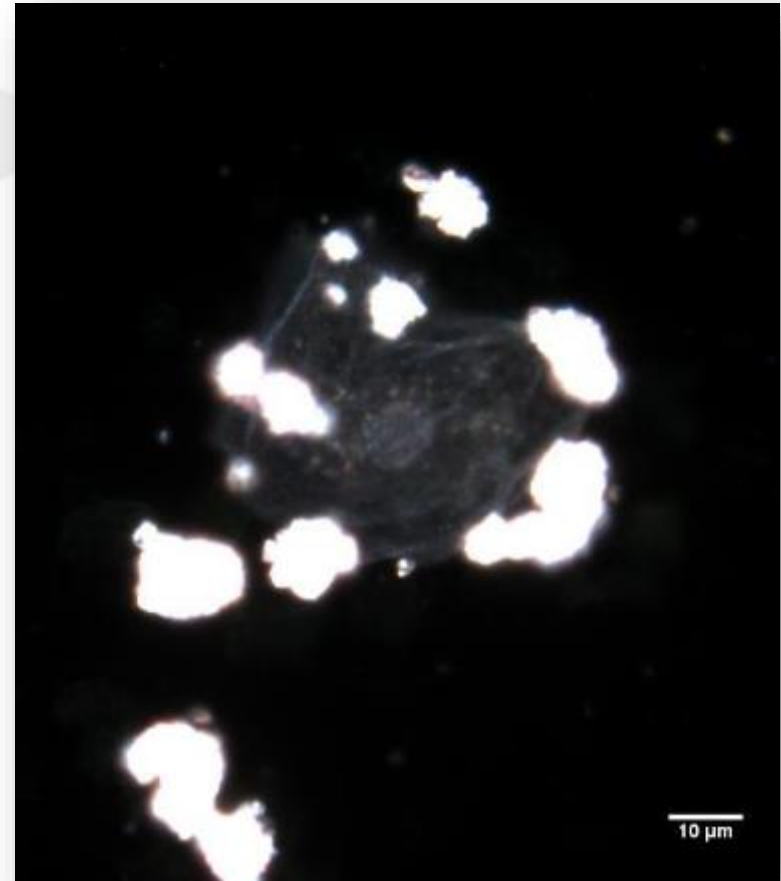


# Particle mapping (by courtesy of Cytoviva)

- CytoViva optical image of live epithelial control cell:  
No particles present



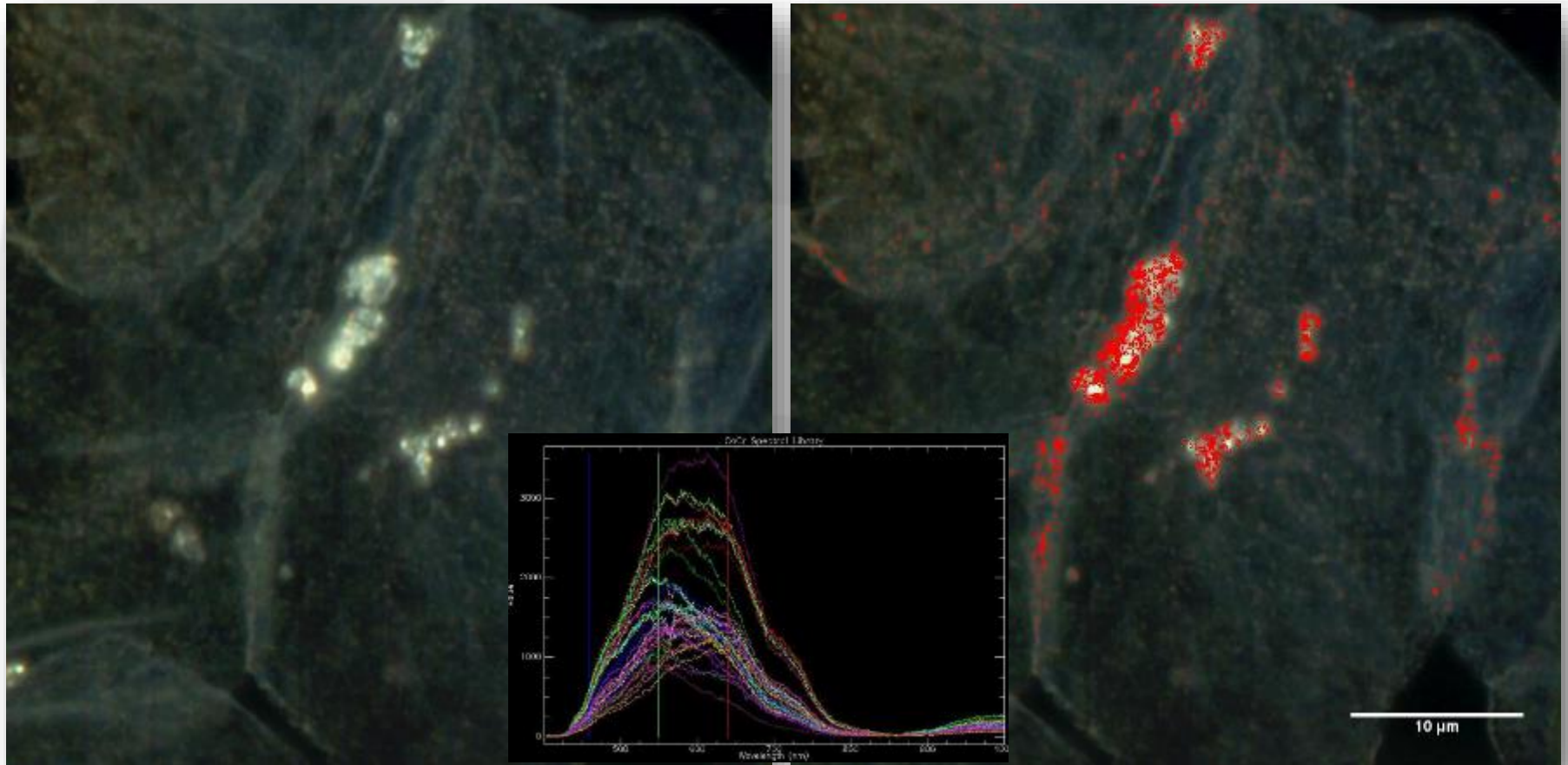
- CytoViva optical image of live epithelial cell incubated with CoCr particles #1



# Particle detection in cells

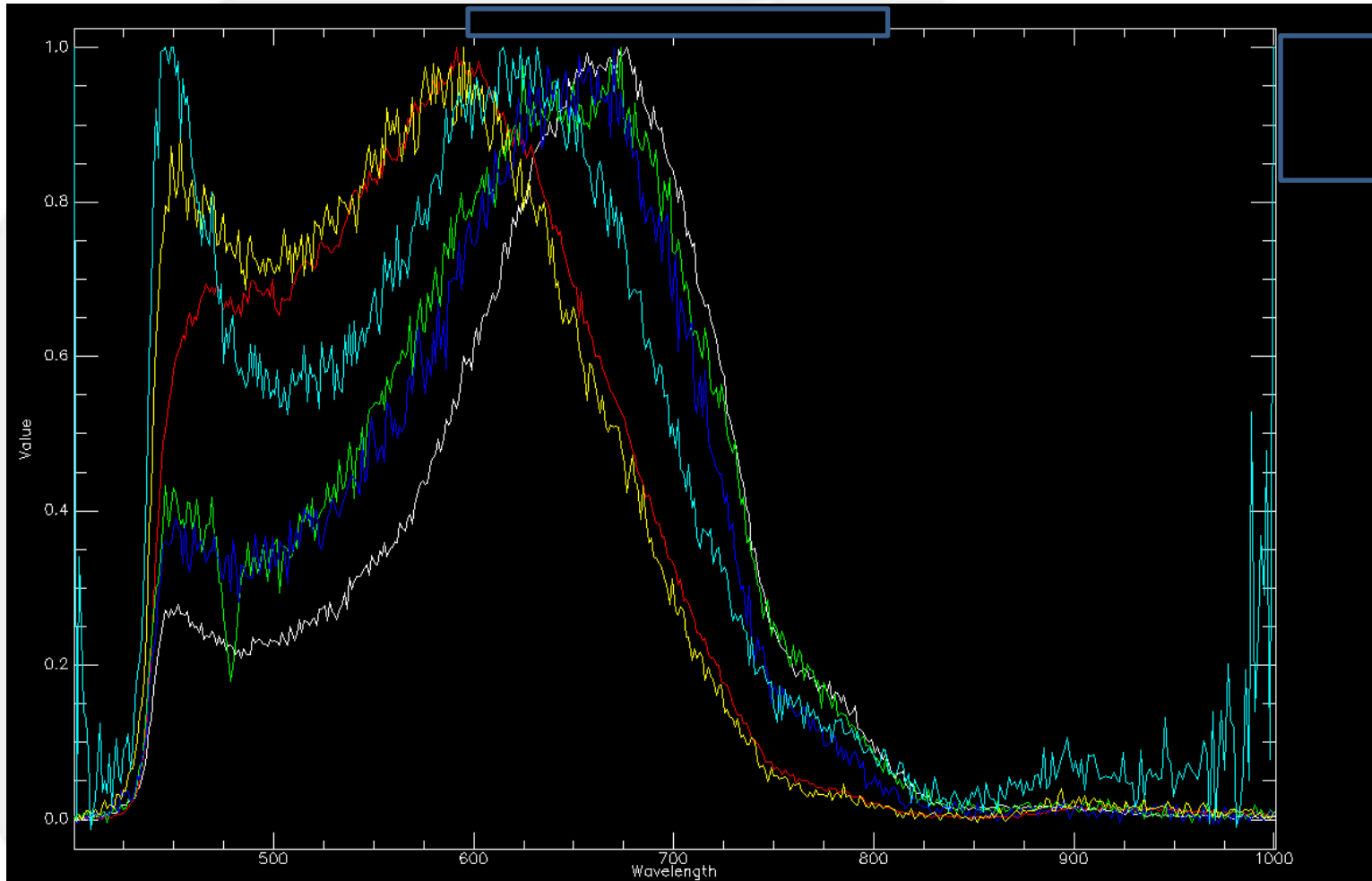
**CytoViva Hyperspectral Imaging scan of live epithelial cells incubated with CoCr particles**

**Pixels mapped in red match the exact spectral signature of CoCr particles**





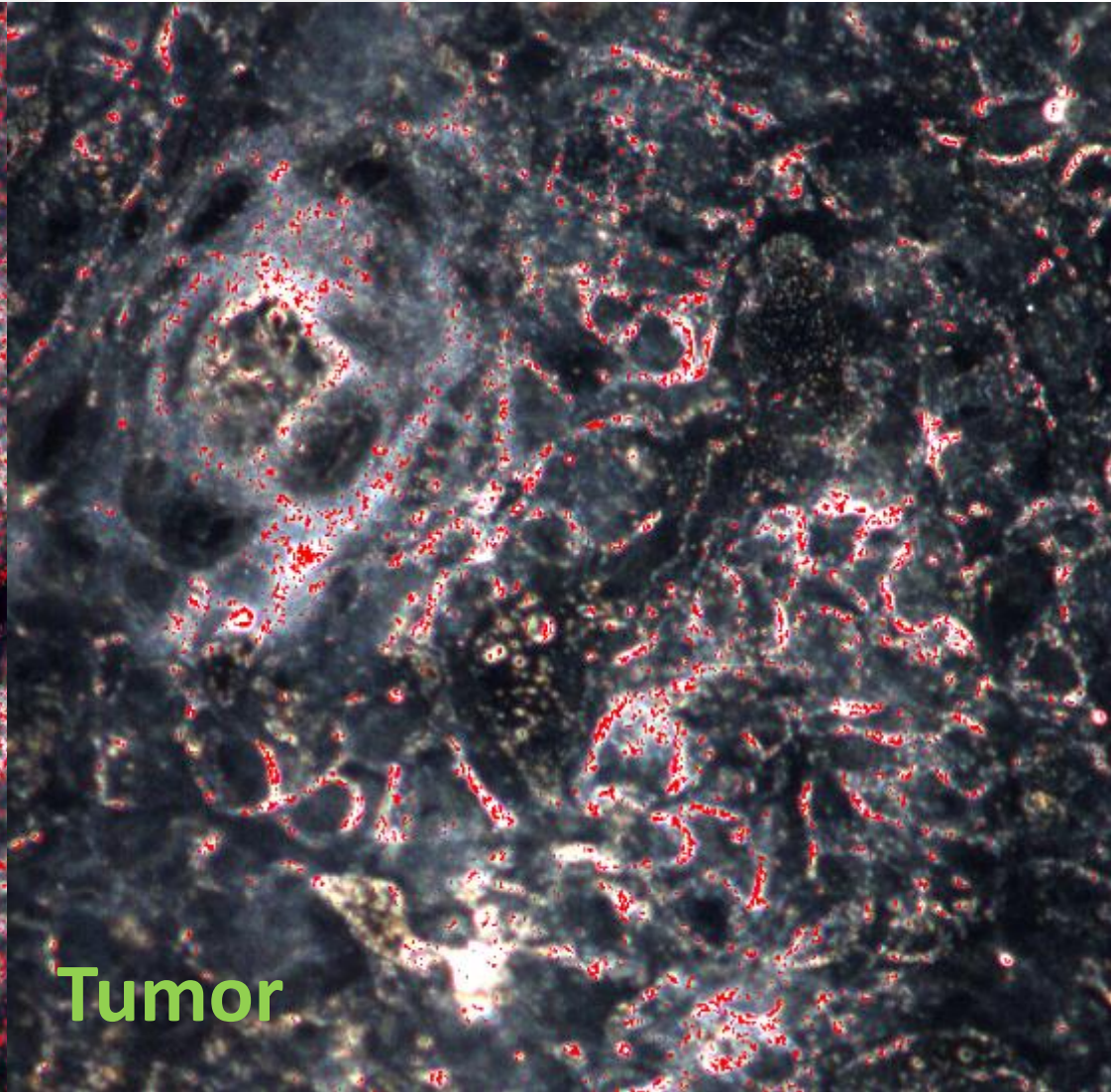
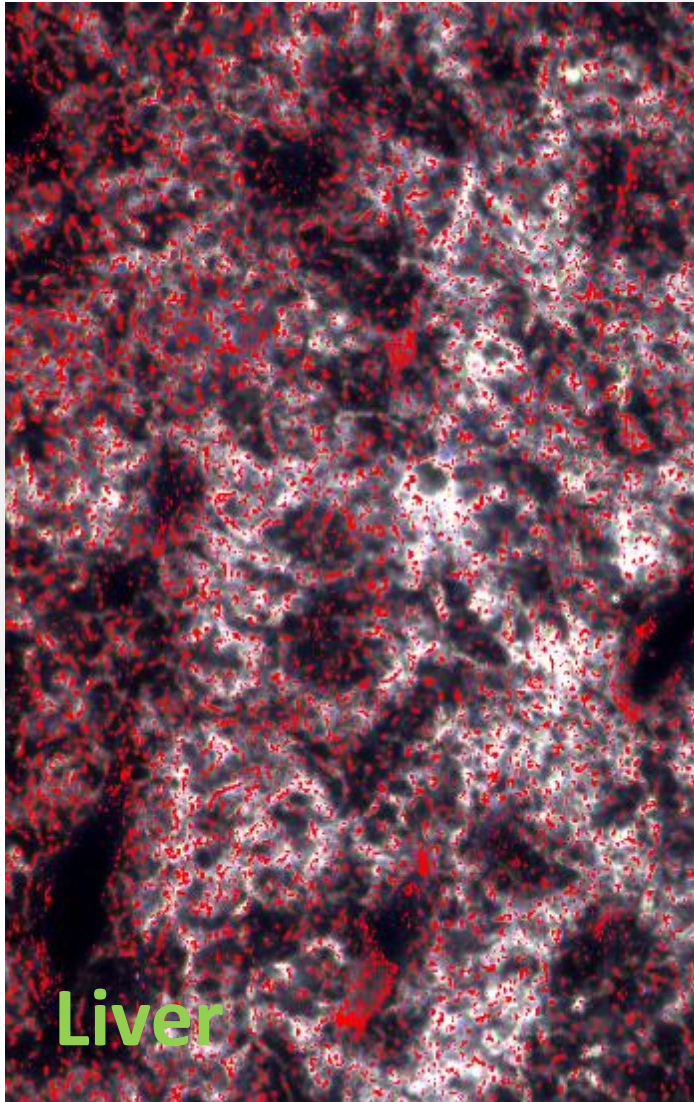
# Detection of different carbohydrates



**Collected Spectra from different sugars**

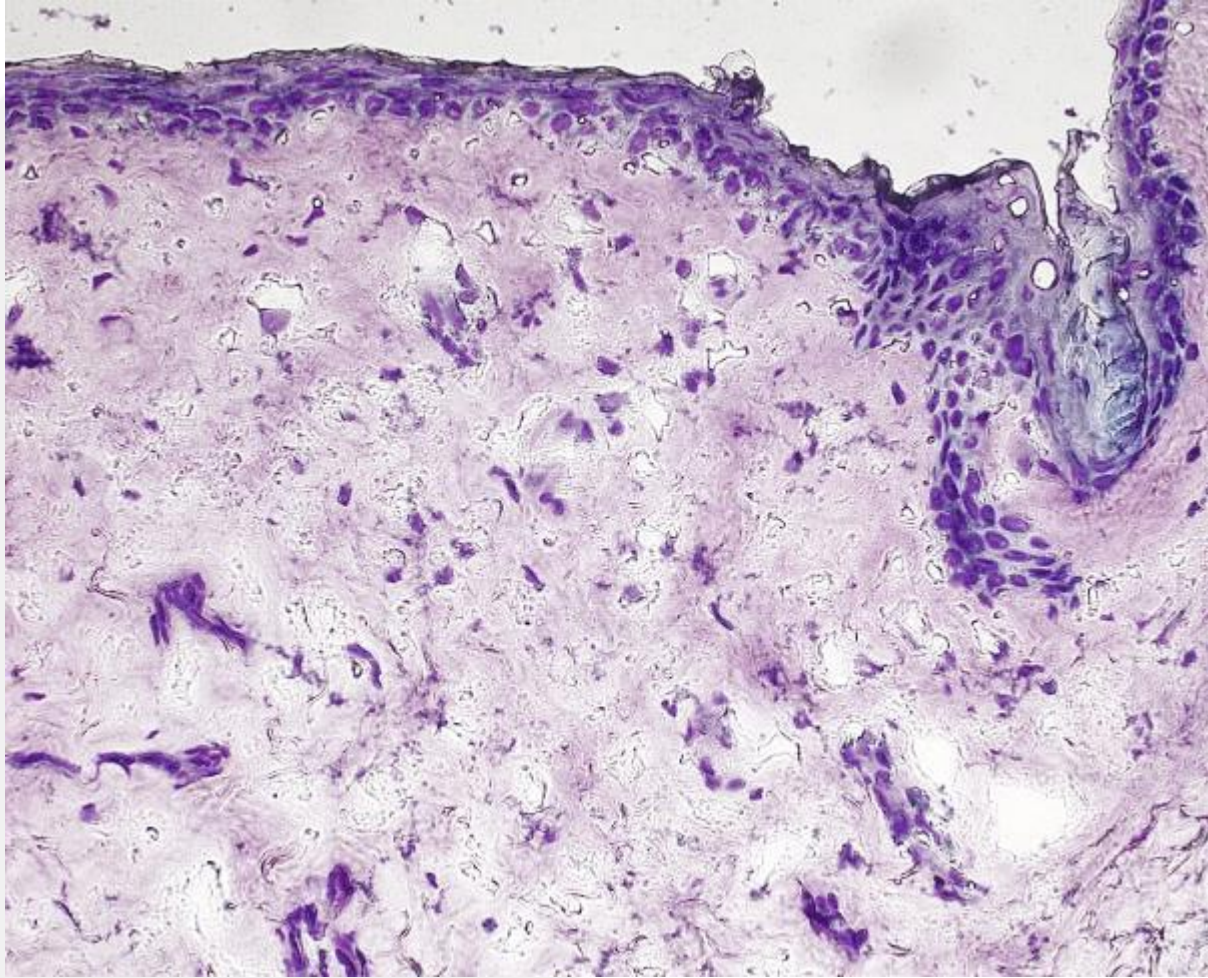
# Detection of different carbohydrates in cryosections

---



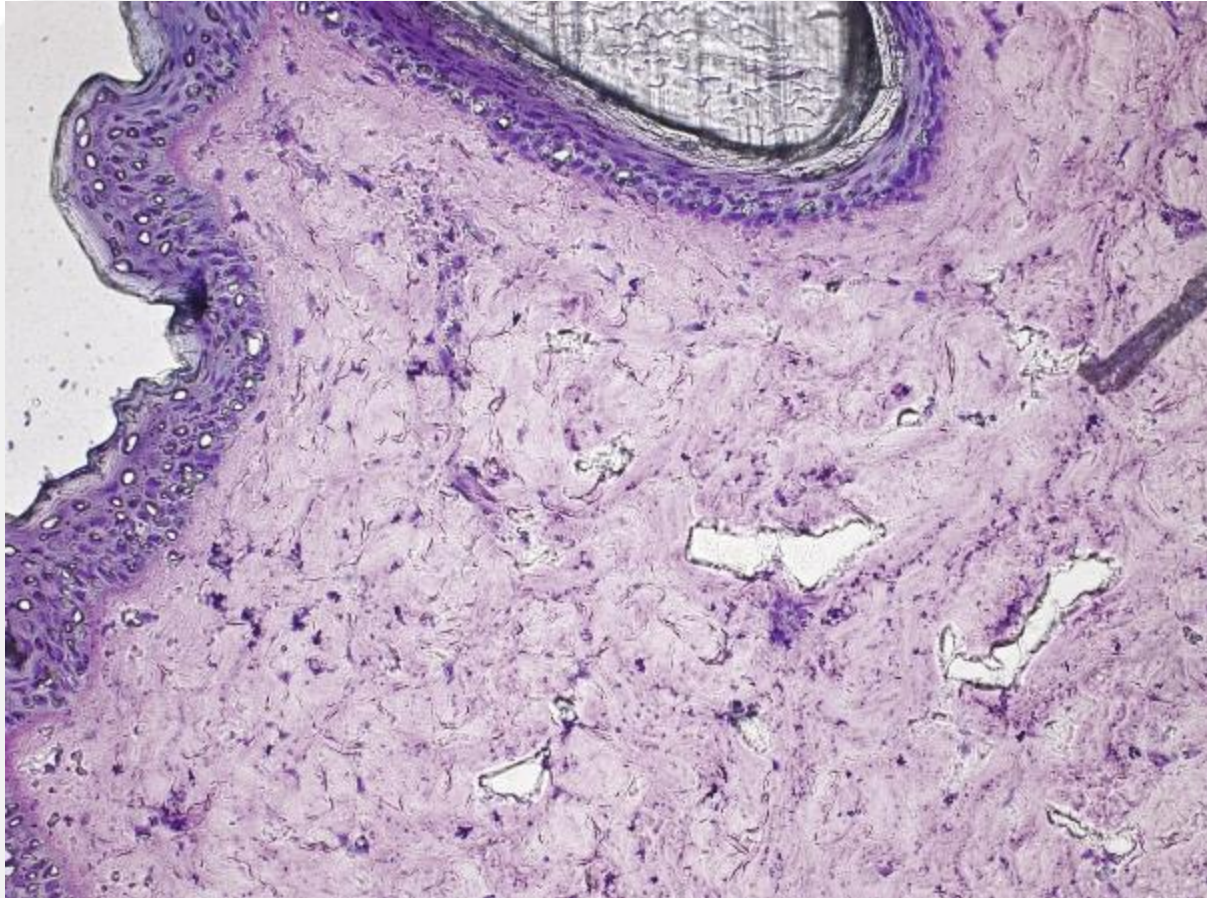
# Example for Compound Tracing: Skin Samples: Human, Giemsa, 1 min

---



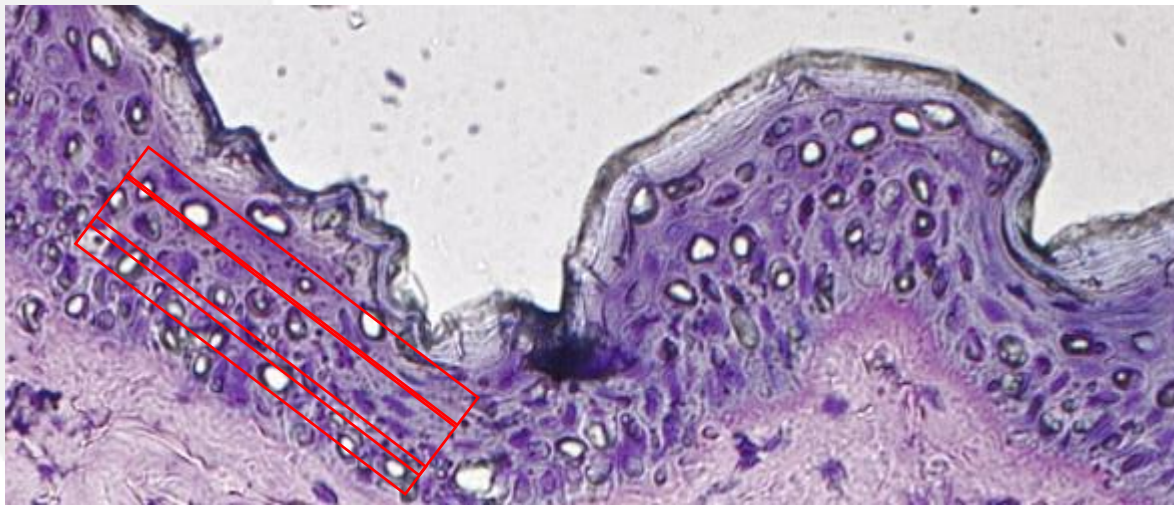
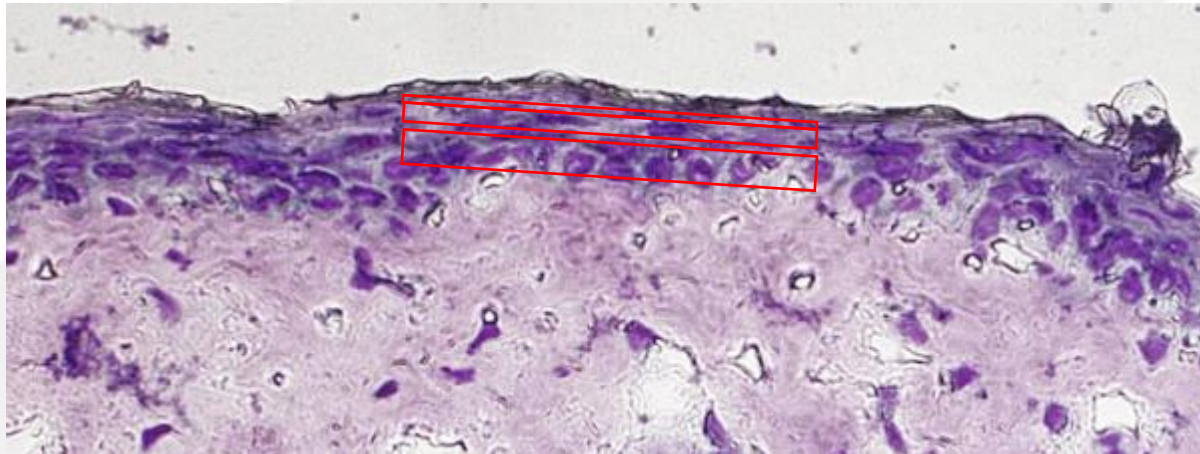
# Skin Samples: Pig, Giemsa, 1 min

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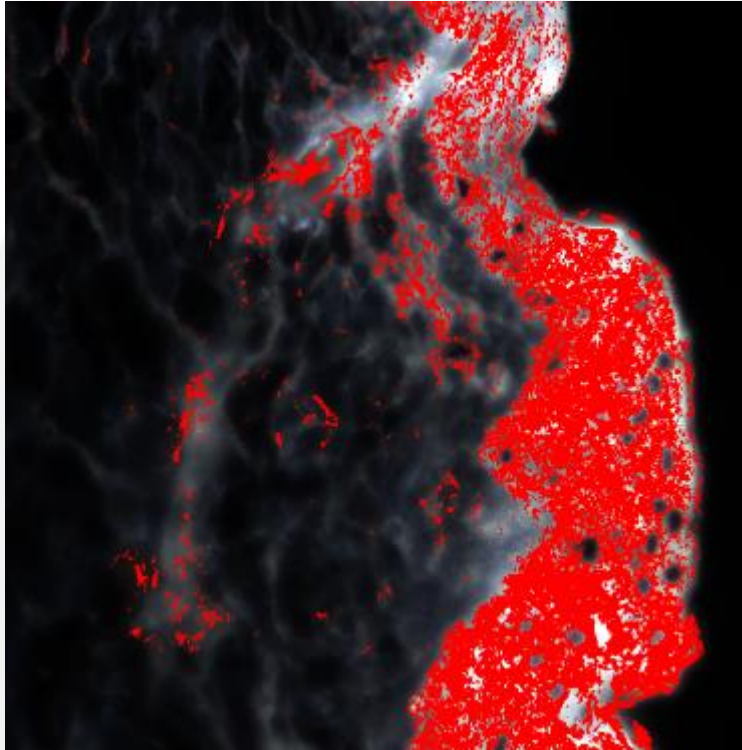
# Skin: Comparison

---

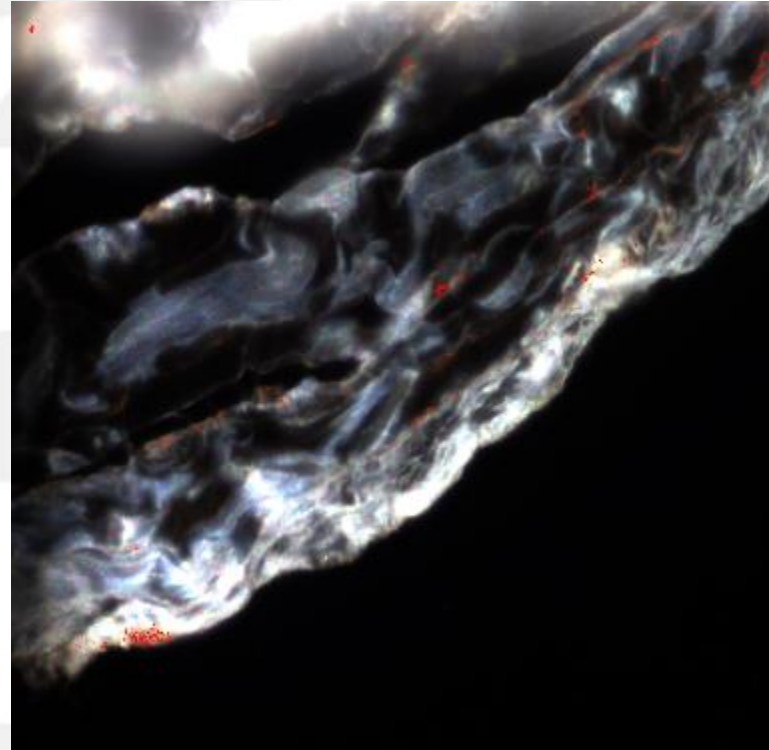


# Measurements

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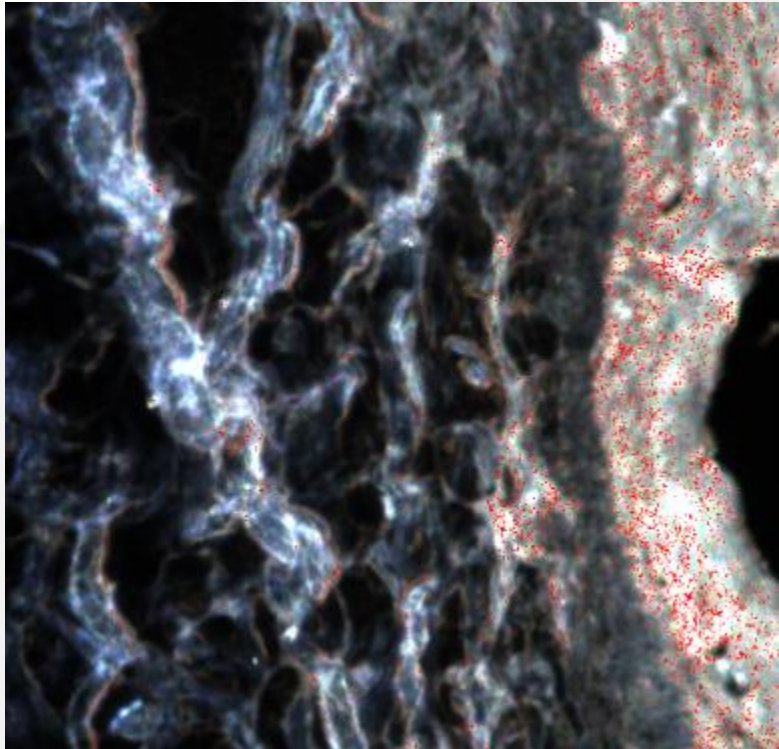
**Sample No 1.: Cell 7, Minipig, 4mm,  
Formulation A**



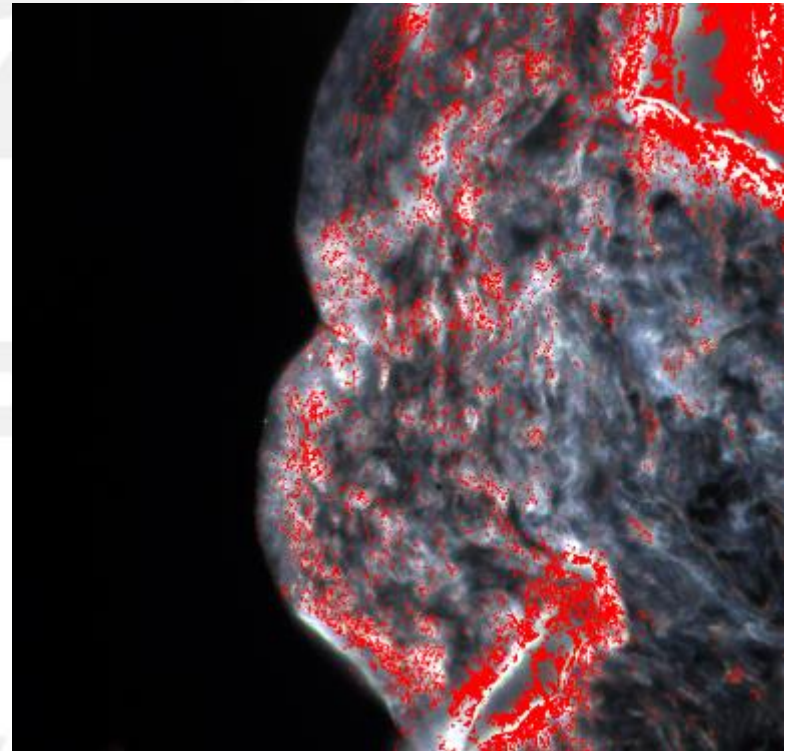
**Sample No. 7.: Cell13, Human, 4mm,  
Formulation A**

# Measurements

---

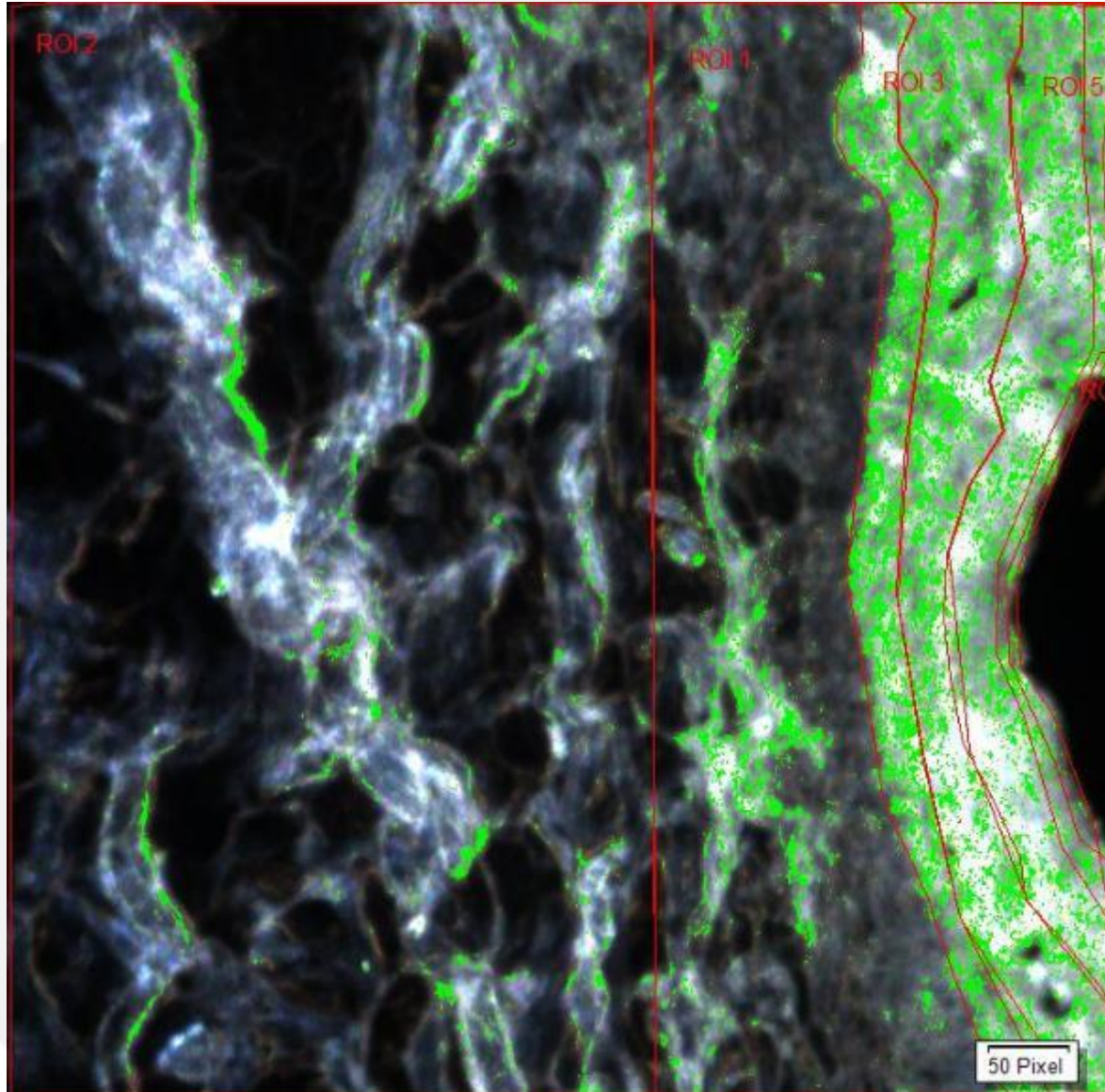


**Sample No. 4.: Cell 11, Minipig, 4mm,  
Formulation B**



**Sample No. 10.: Cell17, Human, 4mm,  
Formulation B**

# ROI's





# ROI Print

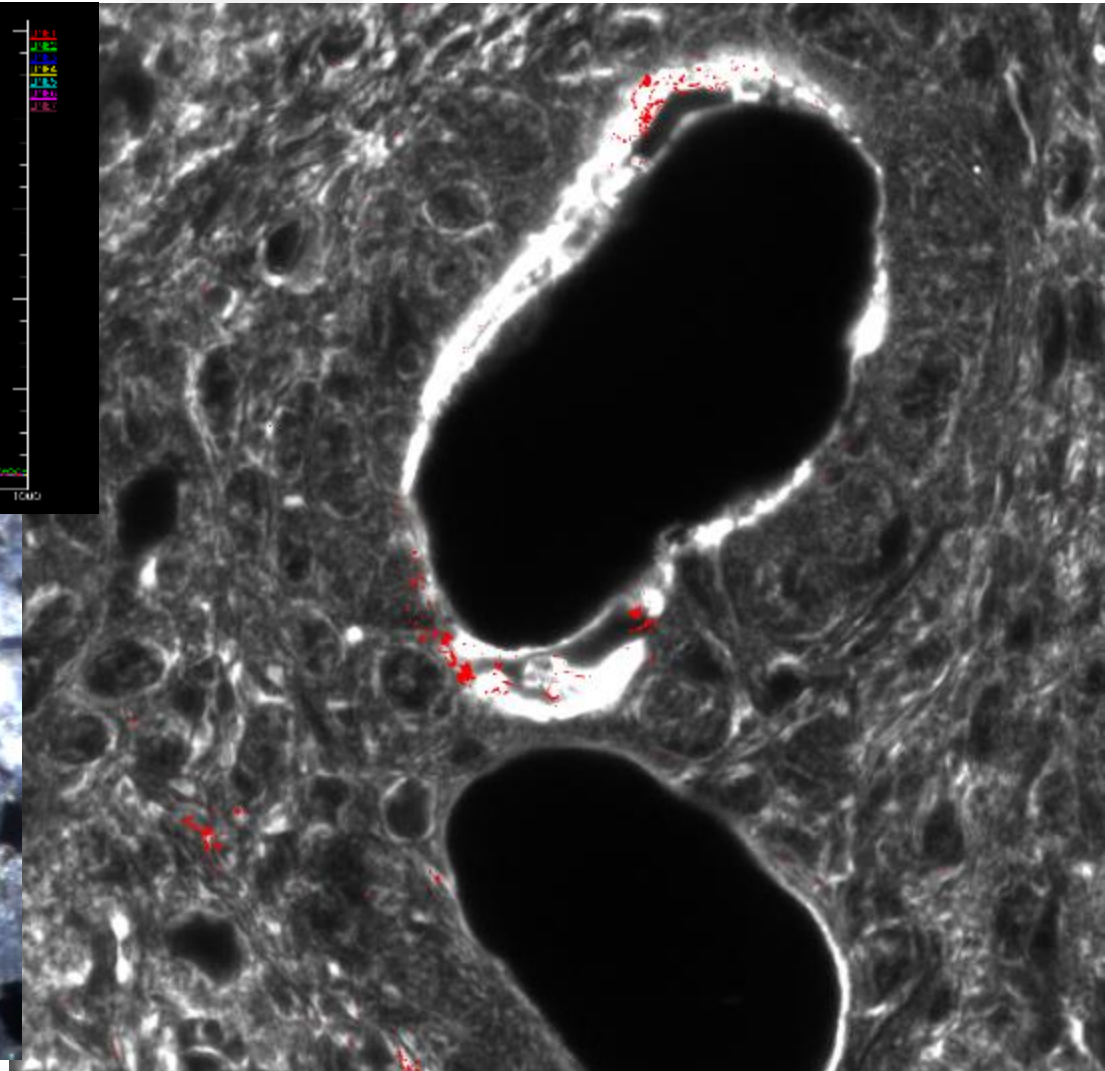
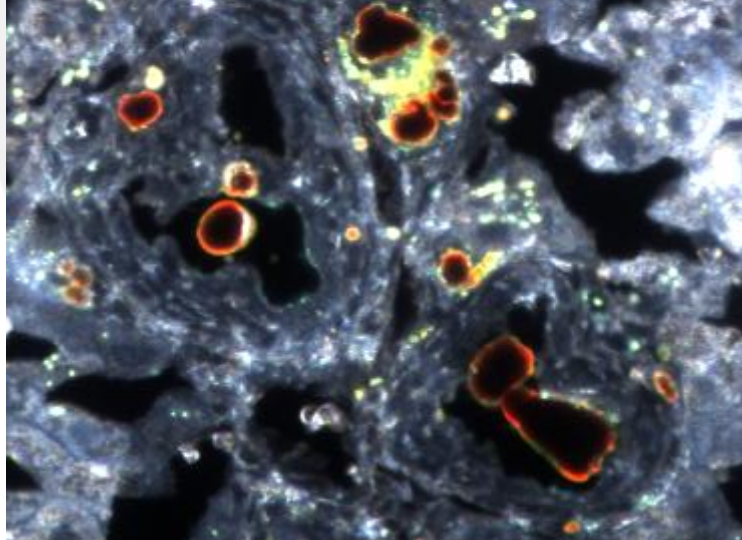
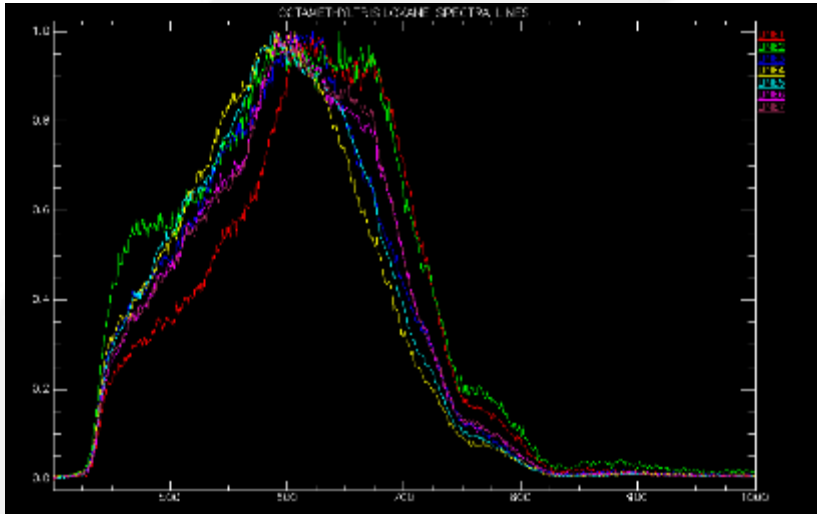
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| <b>Statistik (%)</b> | <b>Objektflächenanteil ROI</b> | <b>ROI-Fläche</b> | <b>ROI</b> |
|----------------------|--------------------------------|-------------------|------------|
|                      | 1.77                           | 276429            | ROI 2      |
|                      | 14.78                          | 199645            | ROI 1      |
|                      | 48.56                          | 19990             | ROI 3      |
|                      | 41.93                          | 32743             | ROI 5      |
|                      | 29.37                          | 31260             | ROI 4      |
|                      | 35.25                          | 8877              | ROI 6      |
| <b>Anzahl</b>        | <b>6</b>                       | <b>6</b>          |            |

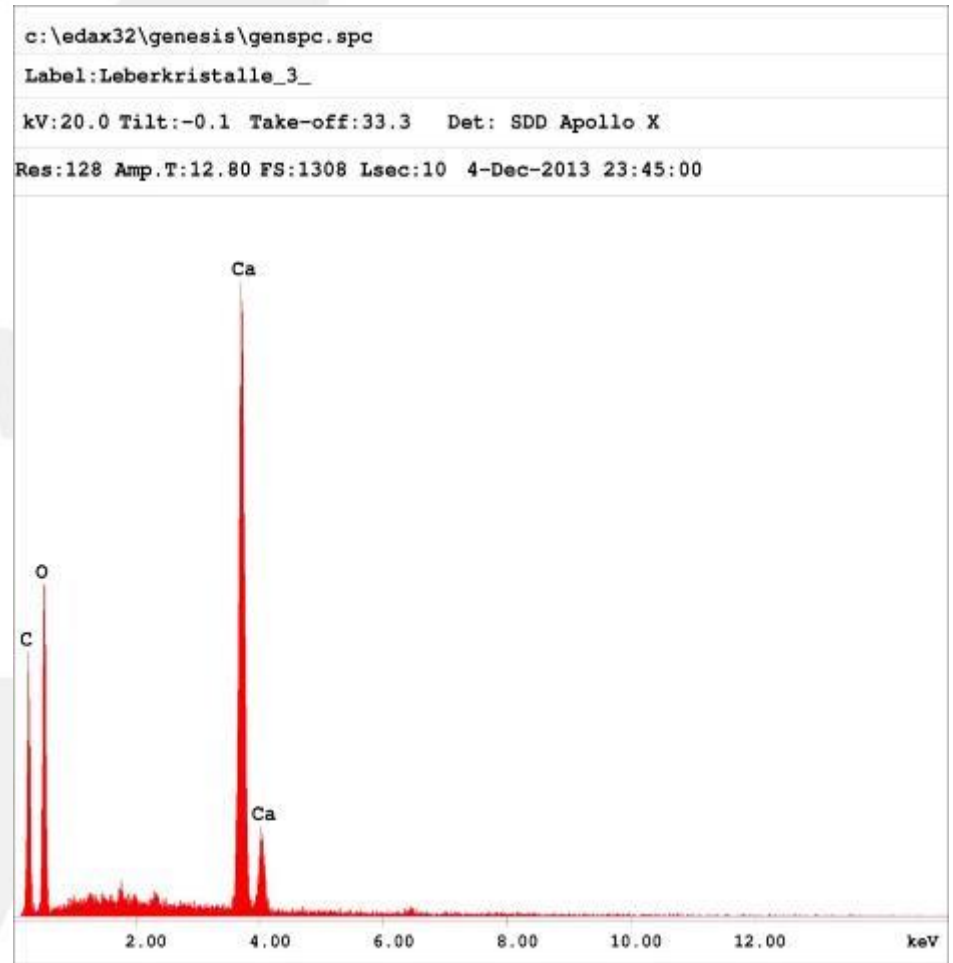
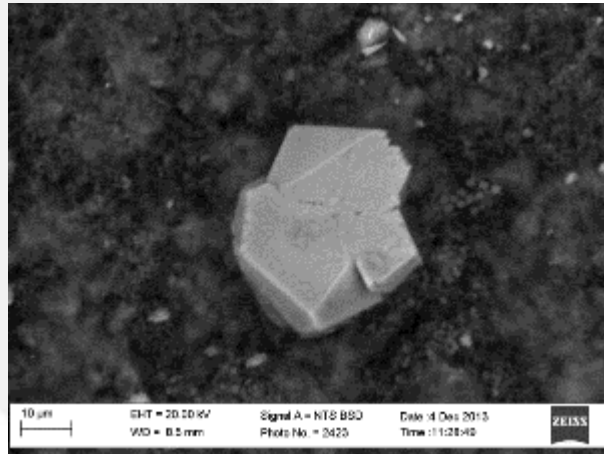
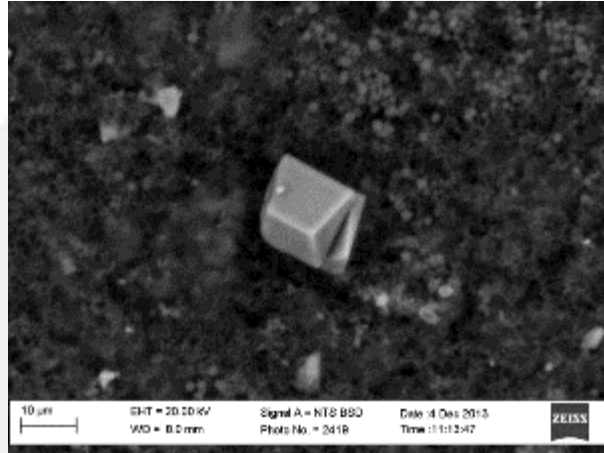
|                           |   |               |   |           |
|---------------------------|---|---------------|---|-----------|
| ROI 1: Stratum papillare  | } | Corium        | } | Epidermis |
| ROI 2: Stratum reticulare |   |               |   |           |
| ROI 3: Stratum basale     | } | germinativum  |   |           |
| ROI 4: Stratum spinosum   |   |               |   |           |
| ROI 5: Stratum granulosum |   |               |   |           |
| ROI 6: Stratum corneum    | } | mortificatium |   |           |

# Particle detection in bile ducts

Crystal formation by test item. See red pixels indicating test item within bile ducts.



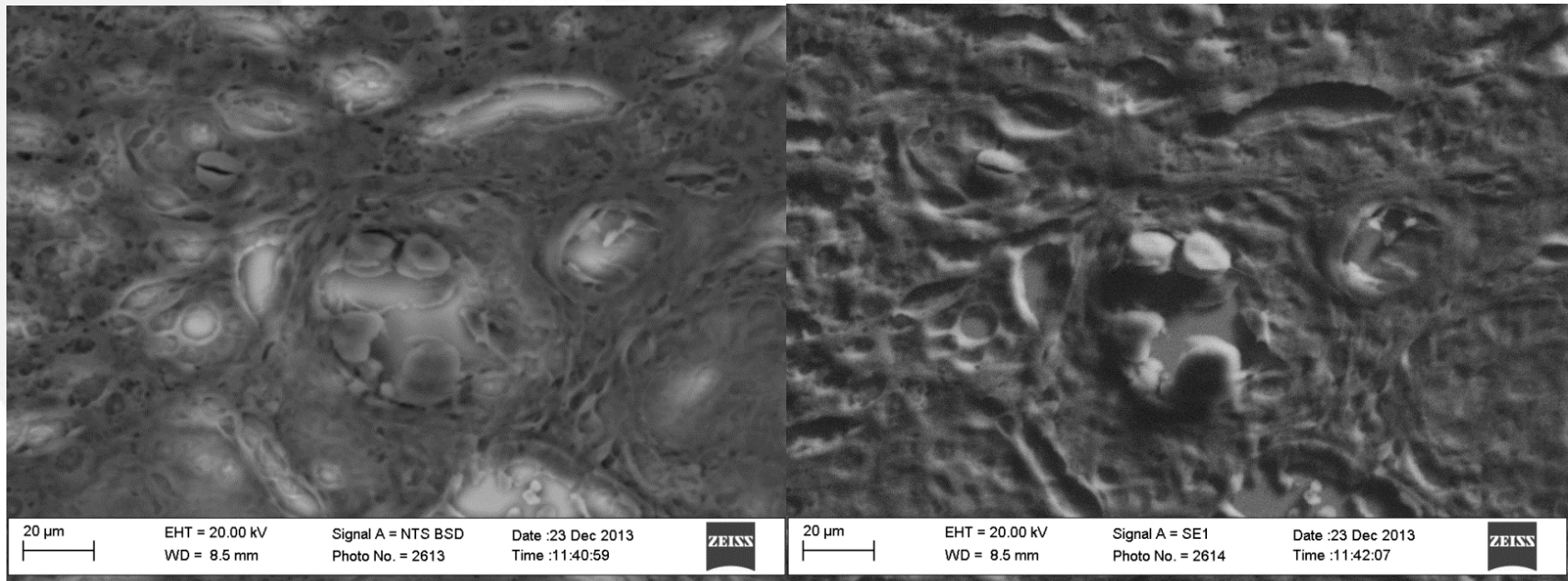
# Evaluation of crystals by EDX (SEM)



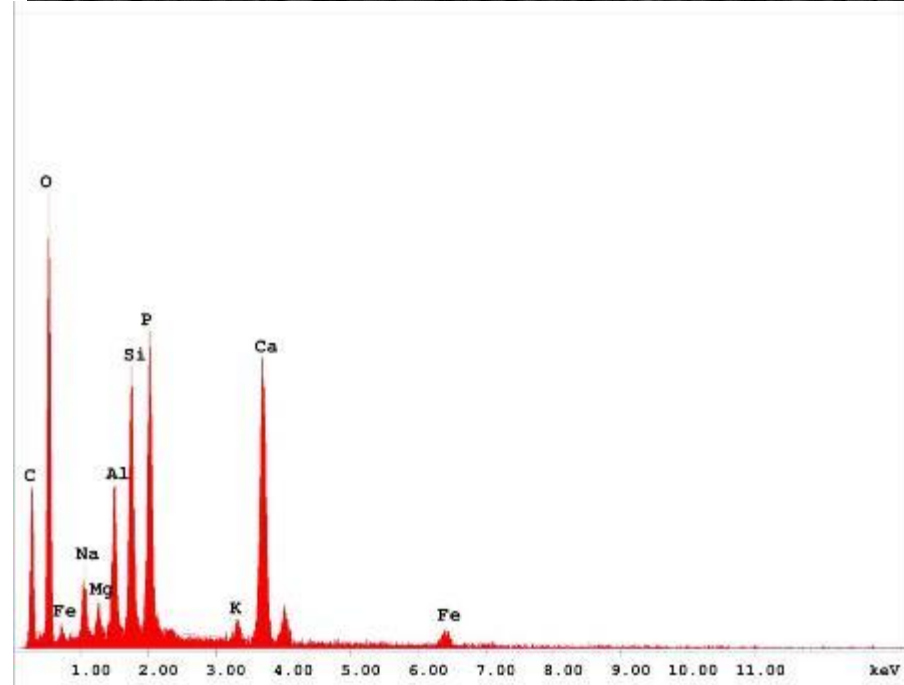
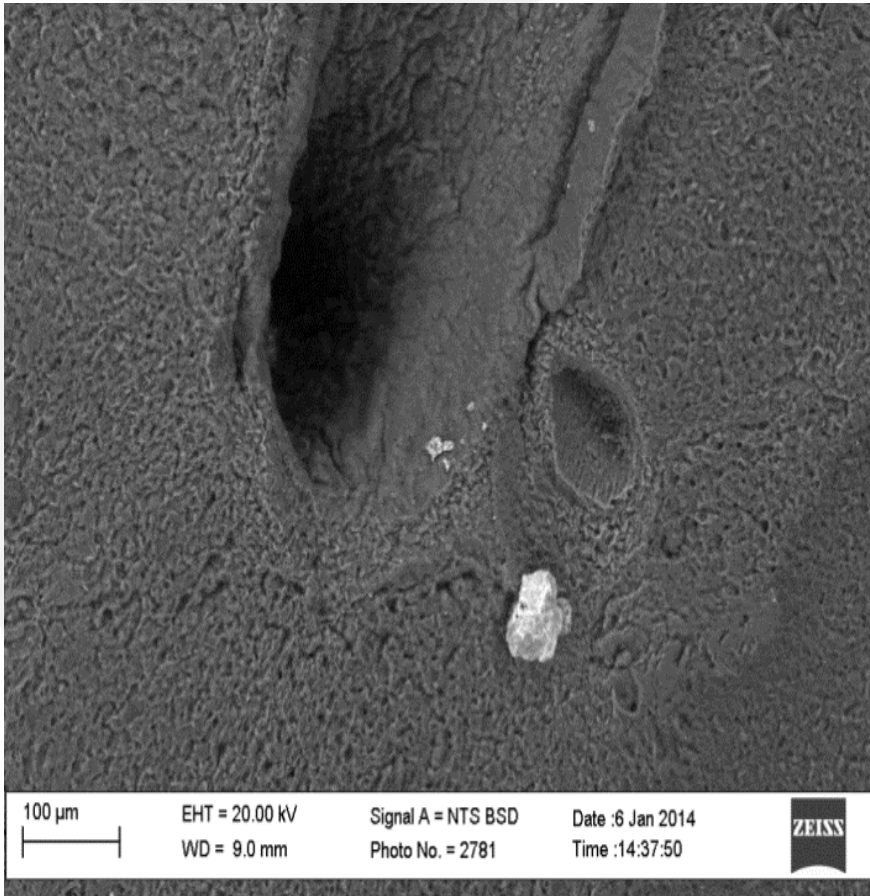
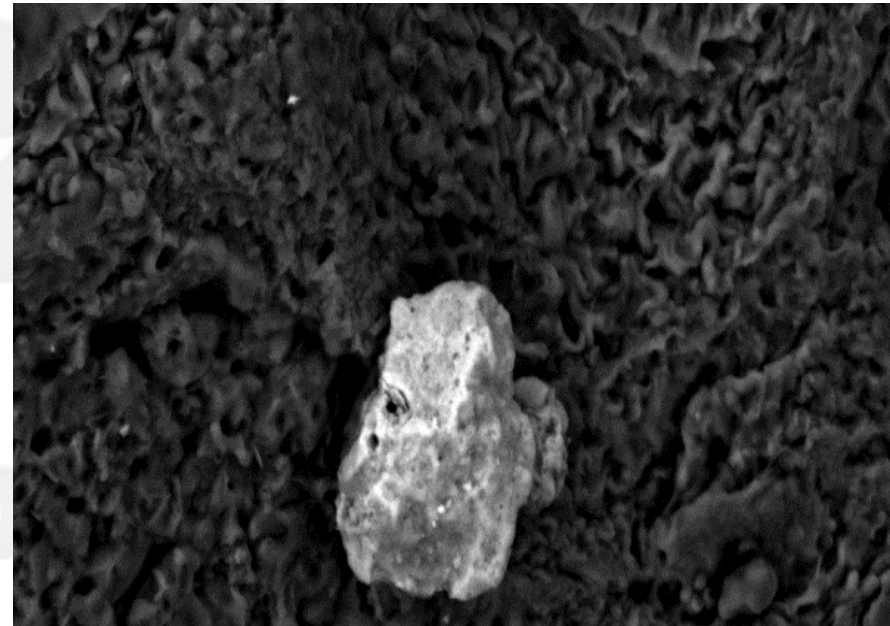
# EDX: HE-stained sections

- Backscattered electron picture HE-stained liver section
- The bright gray indicates the Si-glass-slide below the sample, which is denser than the tissue.
- Also possible by TEM

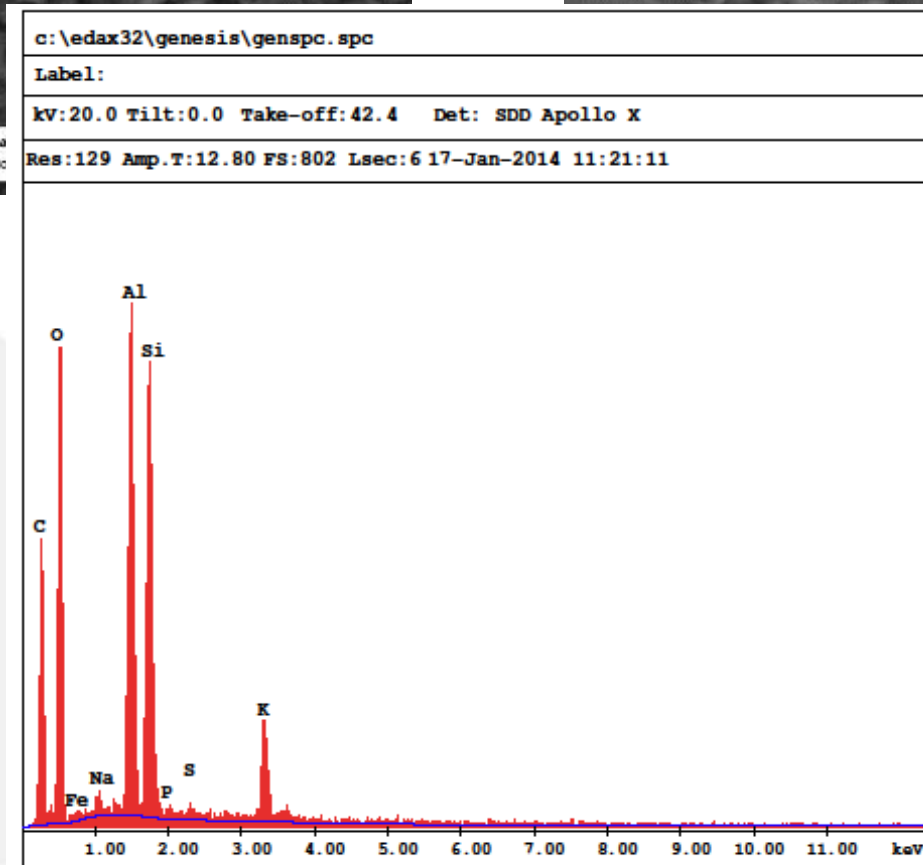
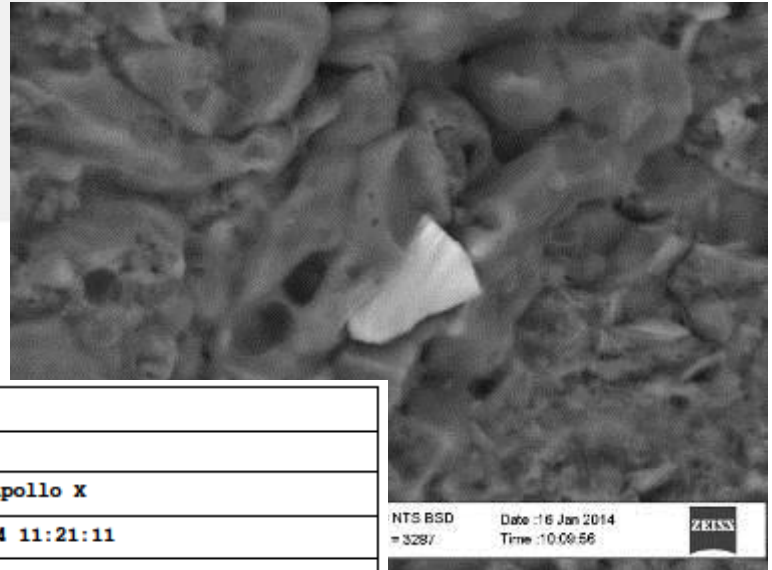
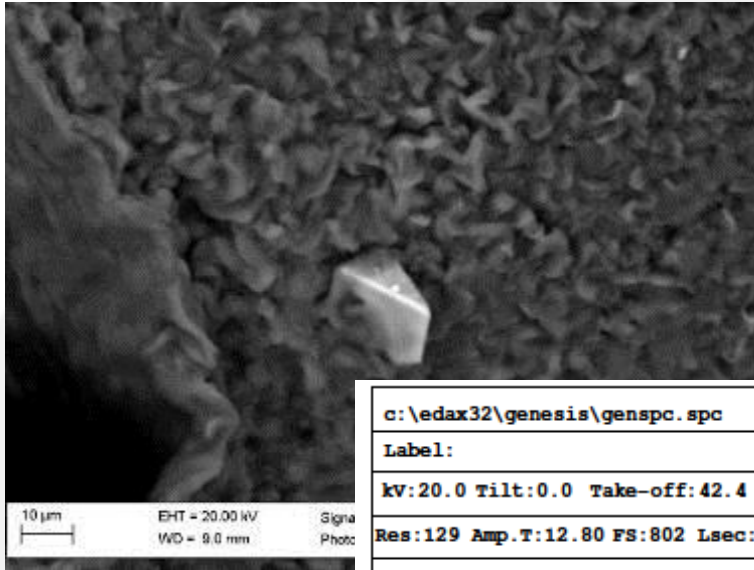
AnaPath



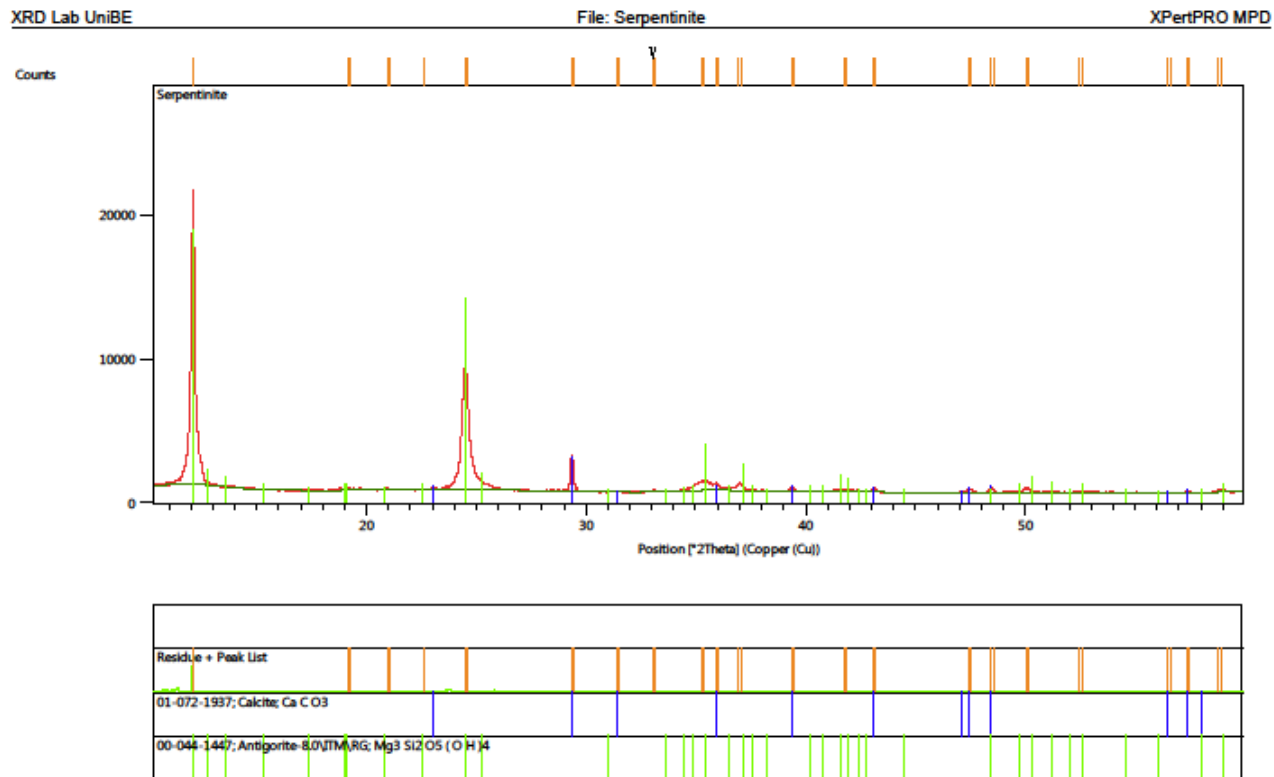
# Evaluation of Unknown Precipitations in Dried Liver Sections precisely Formalin- fixed by EDX (SEM)



# More examples



# Use of further mineralogical/crystallographical technologies incl. X-ray powdered diffraction, RAMAN etc. (Example Mineral deposition)



# Use of 3D Cell Cultures

Scanmodus:XYZ-Feinscan +  
Farbe  
Bildgröße [Pixel]:1024X1024  
Bildgröße [µm]: 258X258  
Objektivlinse:MPLAPONLEXT5  
0  
Zoom:1X

