

# Correlative Raman microscopy, SEM and EDX – fundamentals and applications

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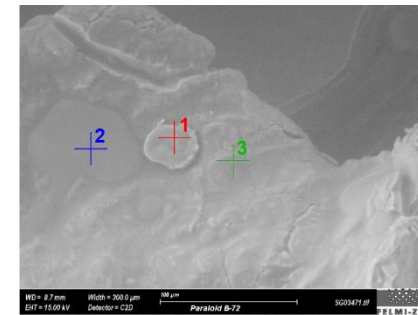
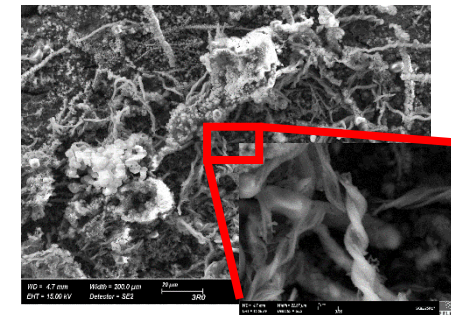
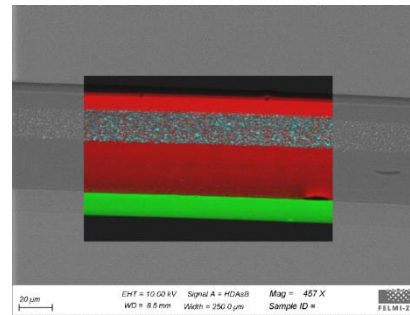
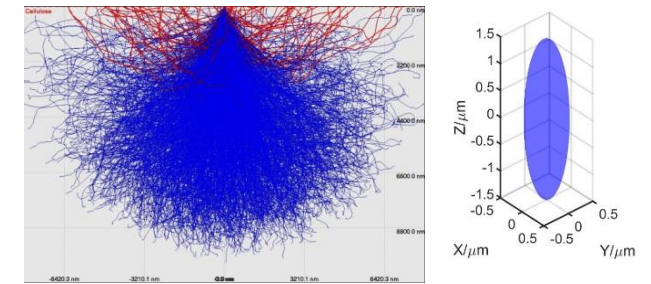
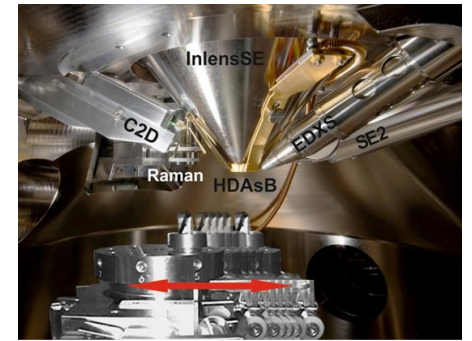
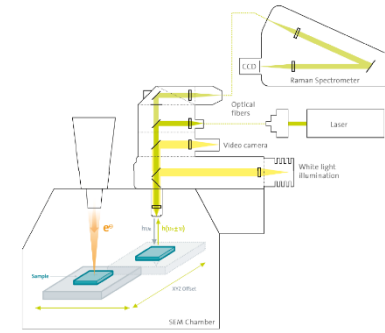
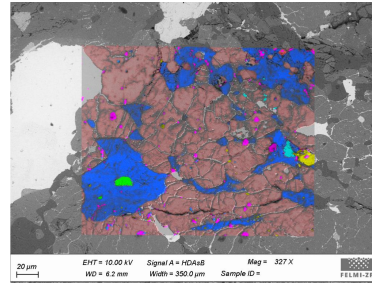
# FELMI-ZFE: Application meets Research



55 employees: from experienced scientists to fresh minds and reliable office staff

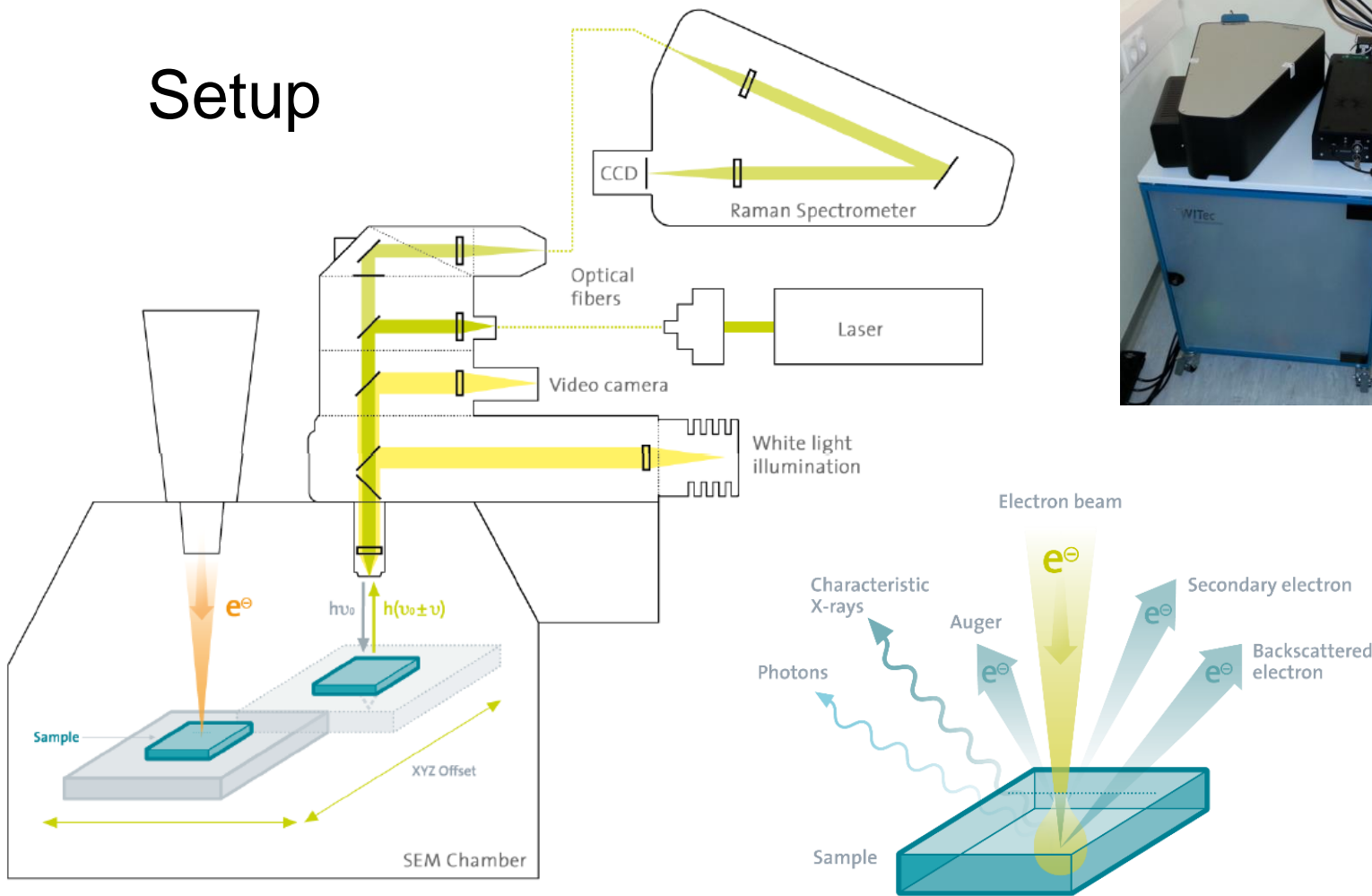
# Outline

- Introduction of the Sigma 300 & RISE
- Application Examples
  - Cement powder
  - Polymer layers
  - Meteorite
- Best practices
  - Sample preparation
  - Limitations
  - Correlation & Contrast
- Projects
  - Polymeric materials
  - Microbial corrosion of steel
  - Dust particles
  - Heat distribution on a TEM Lamellae
  - Conservation of Art
  - Laser-induced graphene

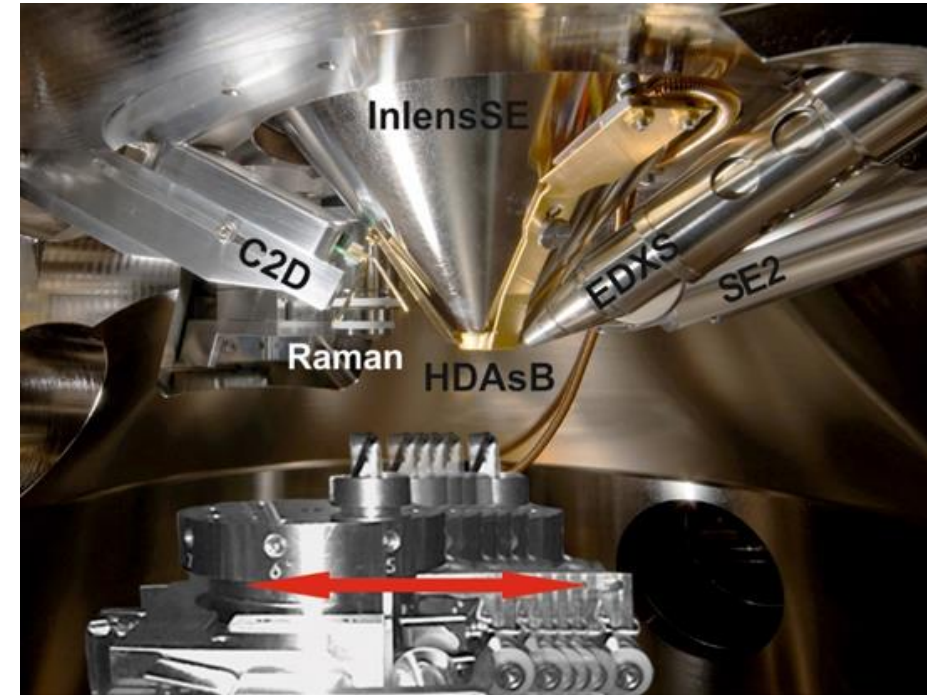
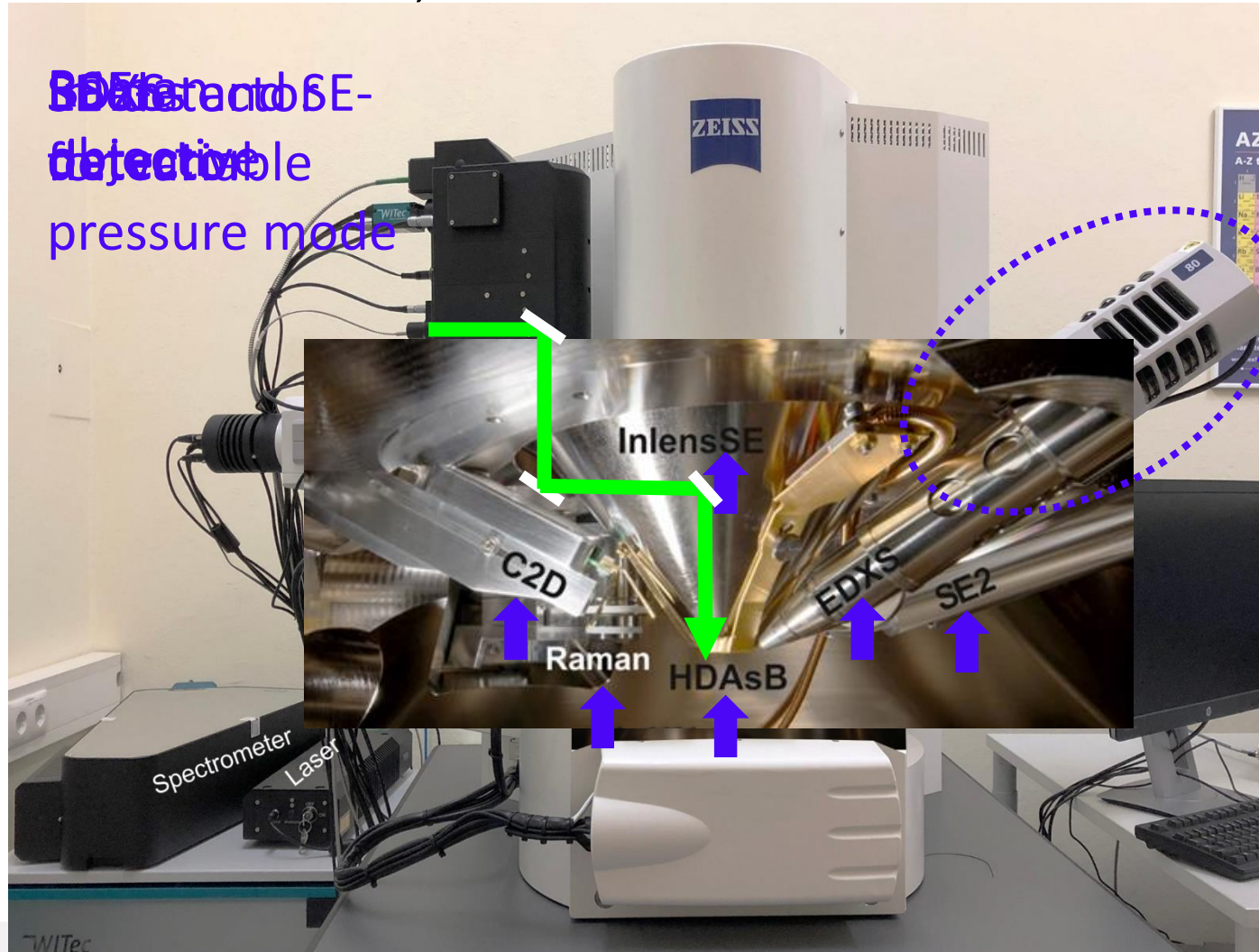


# RISE: Raman Imaging and Scanning Electron Microscopy

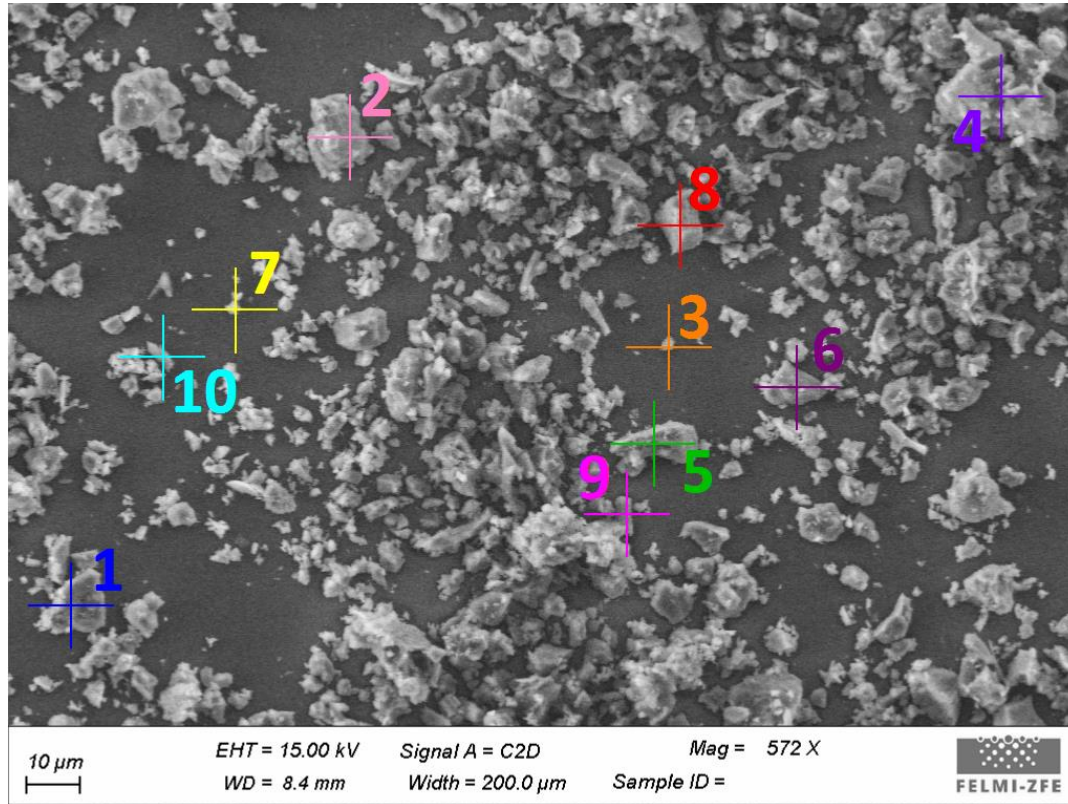
## Setup



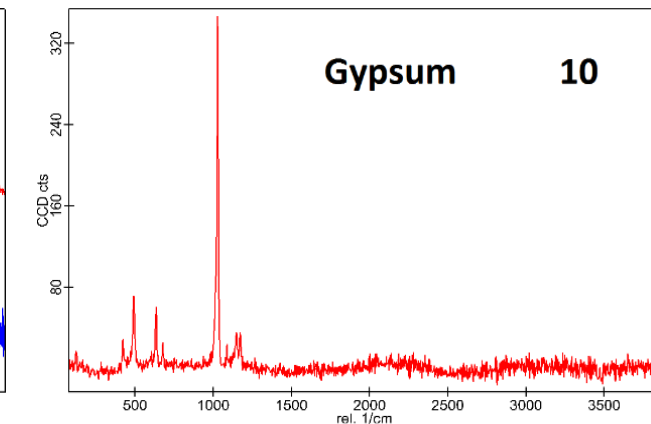
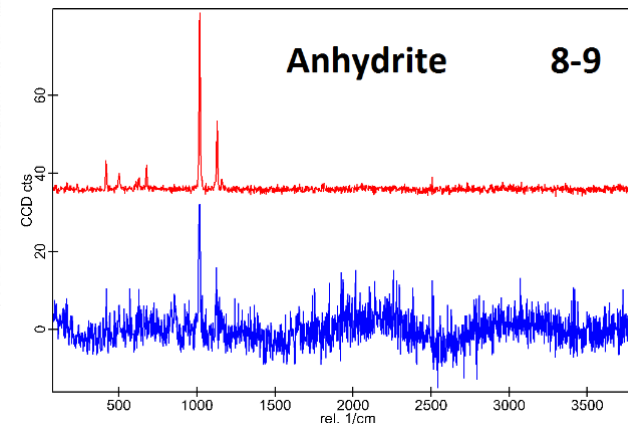
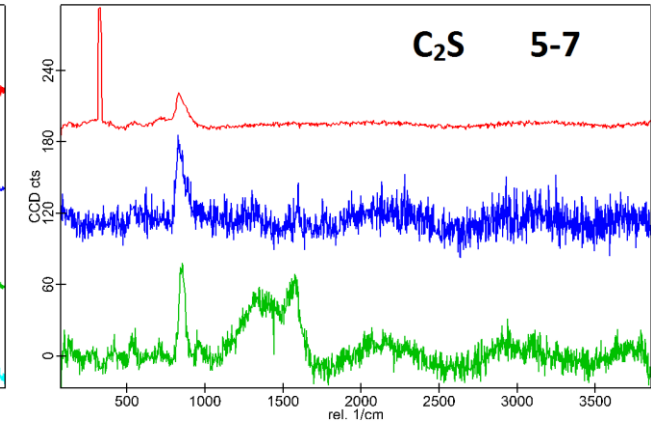
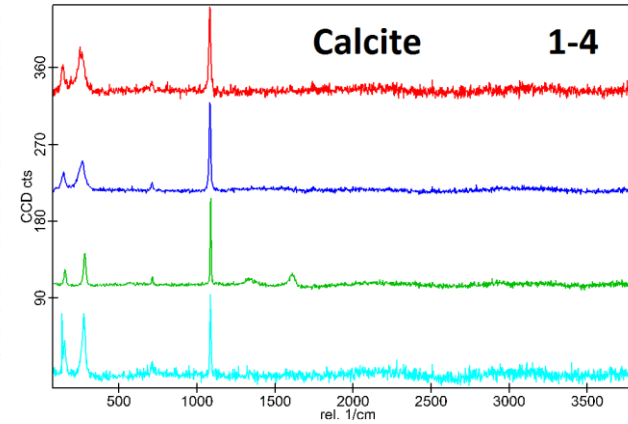
# All in One: SEM (Sigma 300) & Raman (RISE) & EDX (Oxford X-Max) = Six Detectors, Two Positions



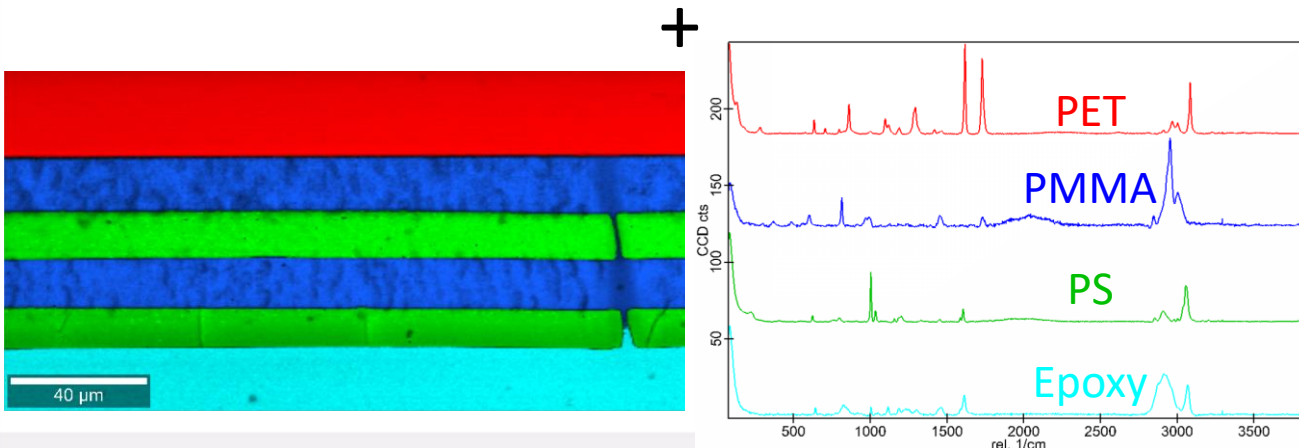
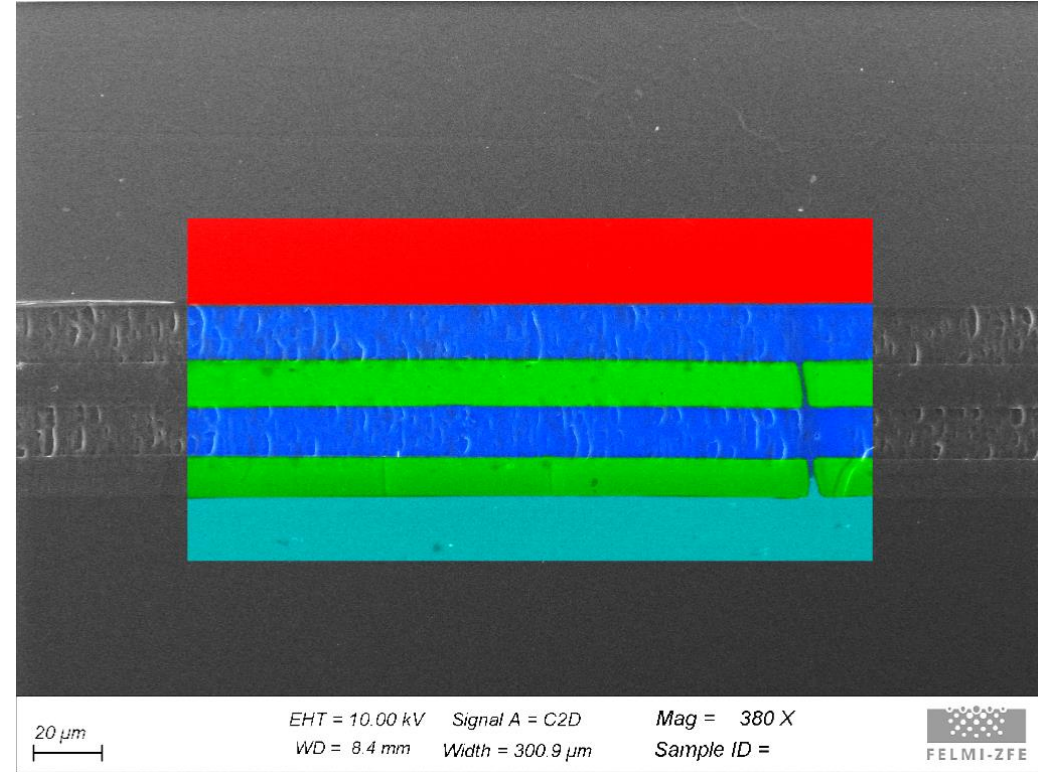
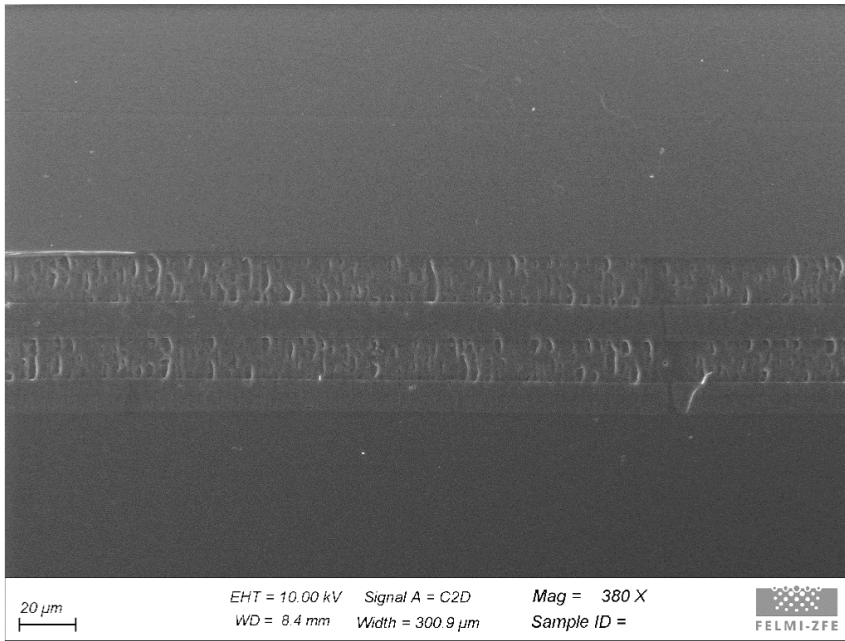
# Examples: Point Measurements on Cement Powder



Note that for the SEM imaging rough surfaces is not a problem, because of its large depth of focus.  
 => Point Measurements require no sample preparation.

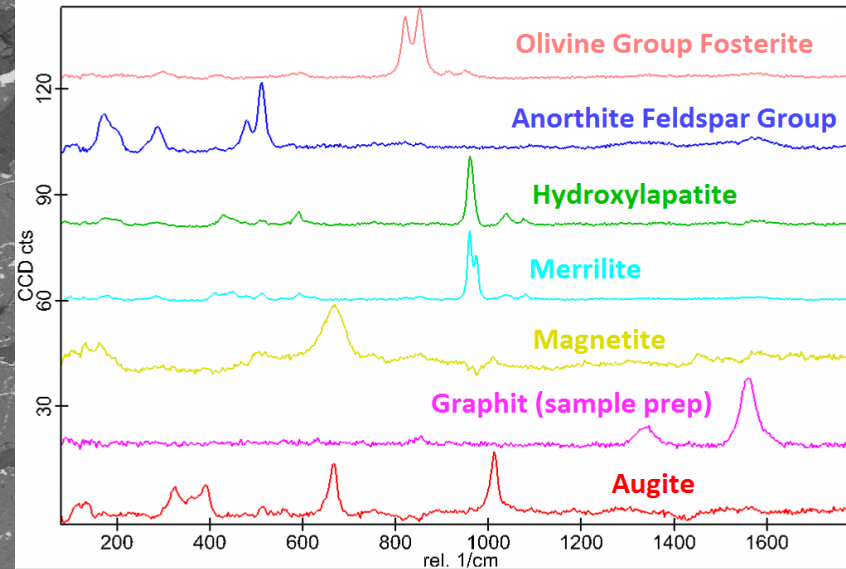
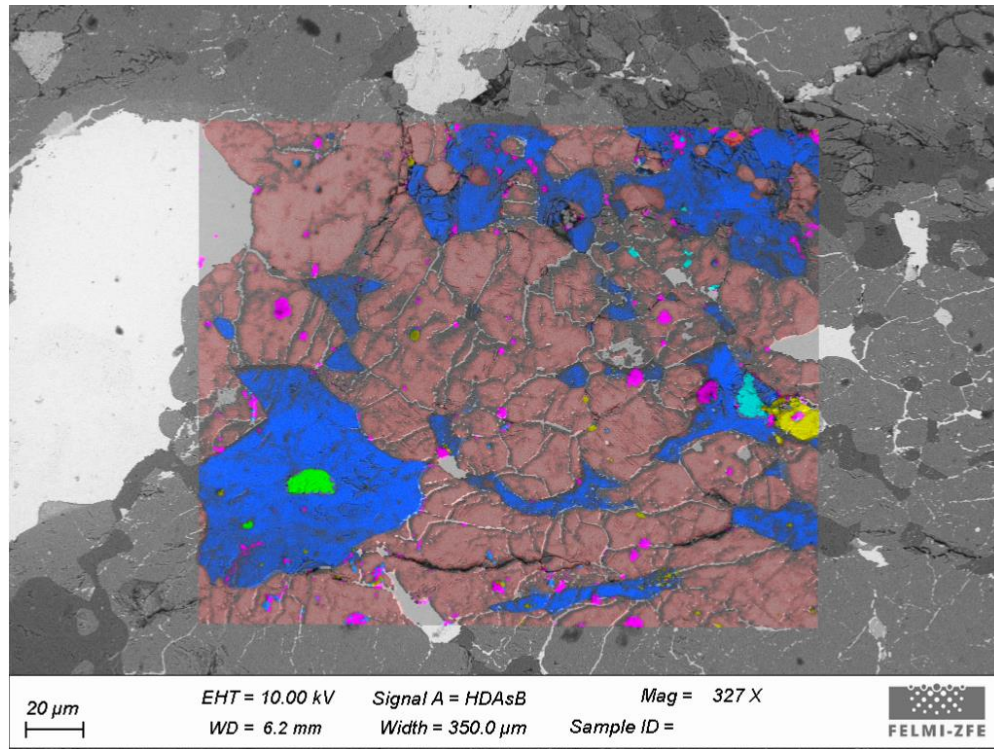


# Examples: Mapping of Polymer Layers (Cut)

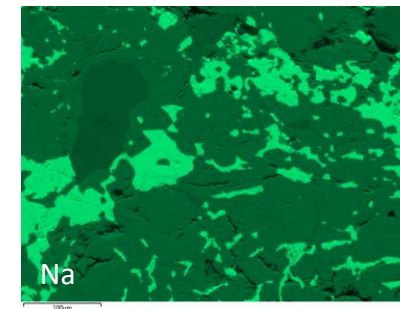
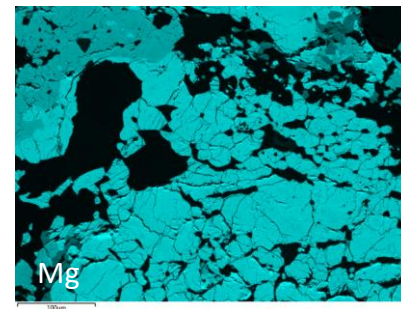
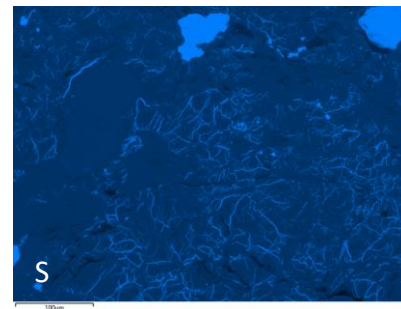
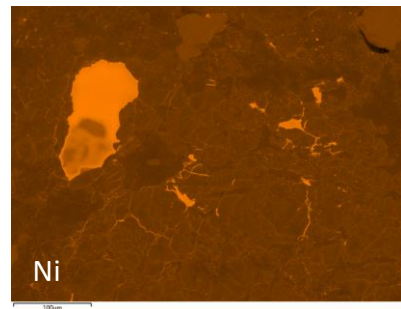
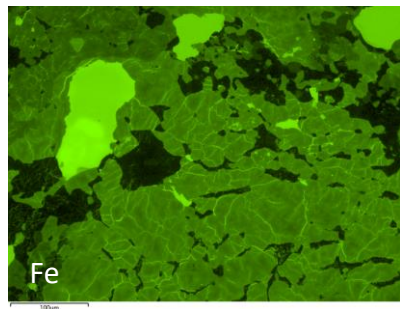


The information gained from Raman is especially helpful on organic samples, where there are limited analytic options and contrast in SEM.

# Examples: Meteorite (Polished)

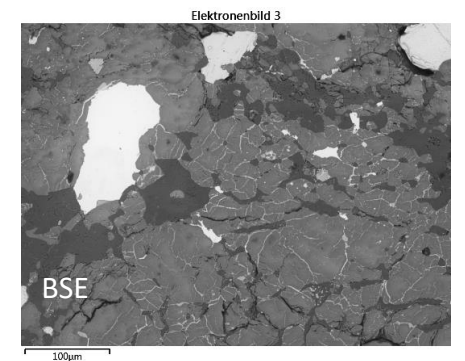
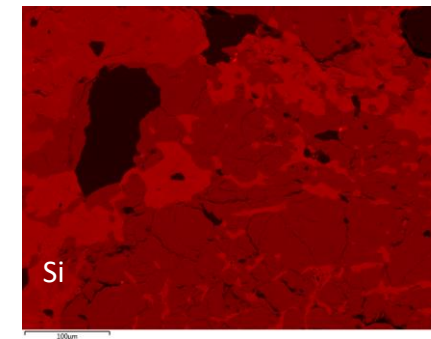
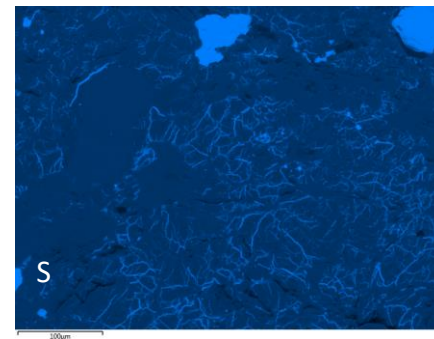
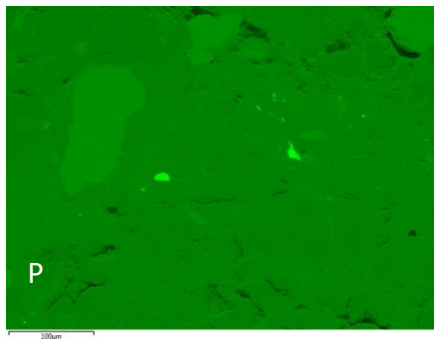
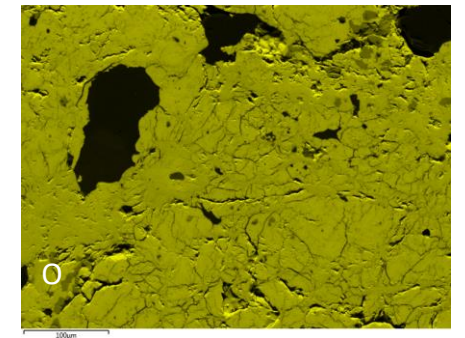
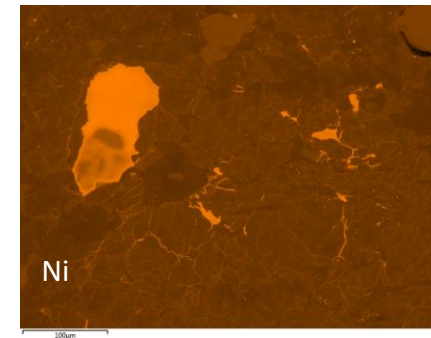
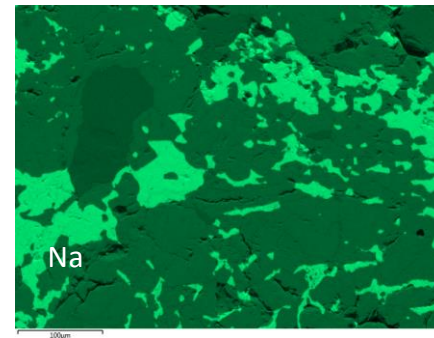
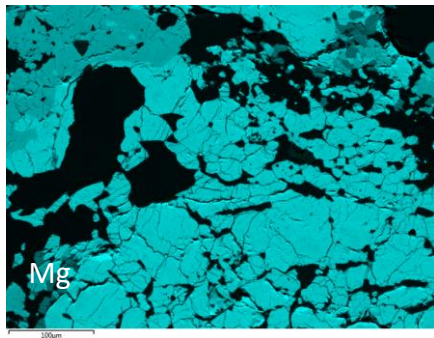
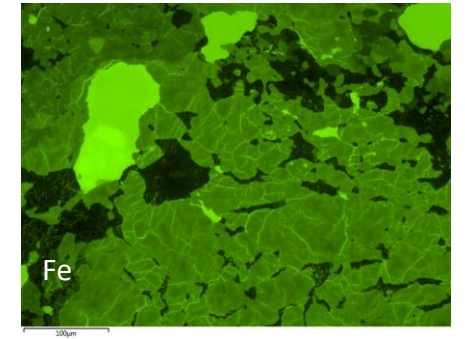
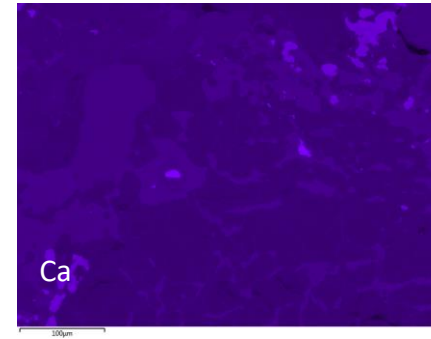
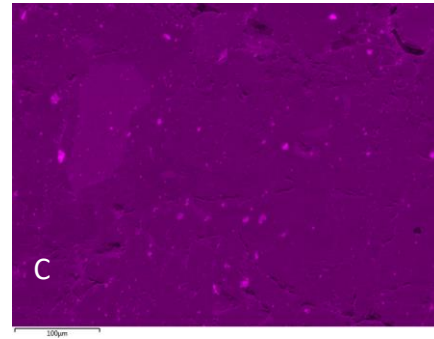


Complementary EDX gives crucial information about the metallic phase not detectable by Raman as well as about the composition of crystal groups with varying elemental composition.

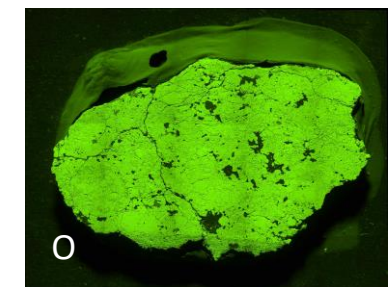
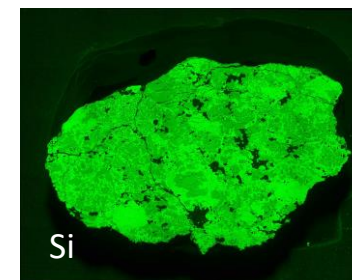
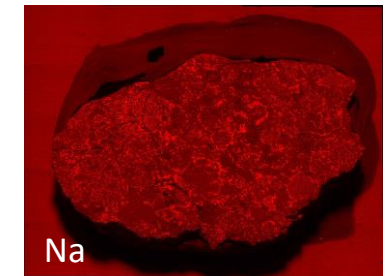
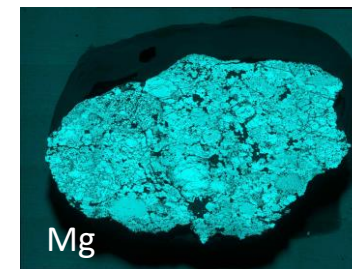
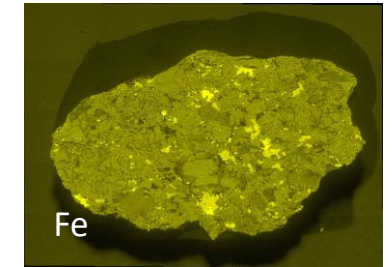
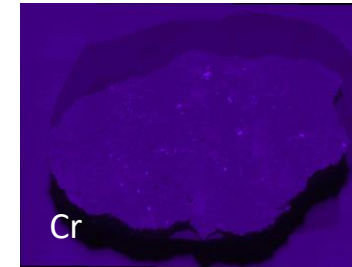
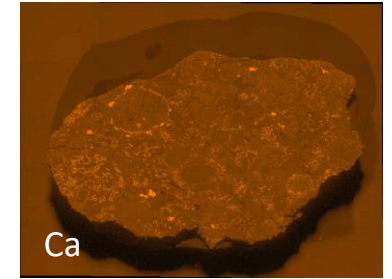
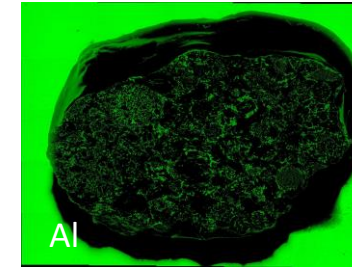
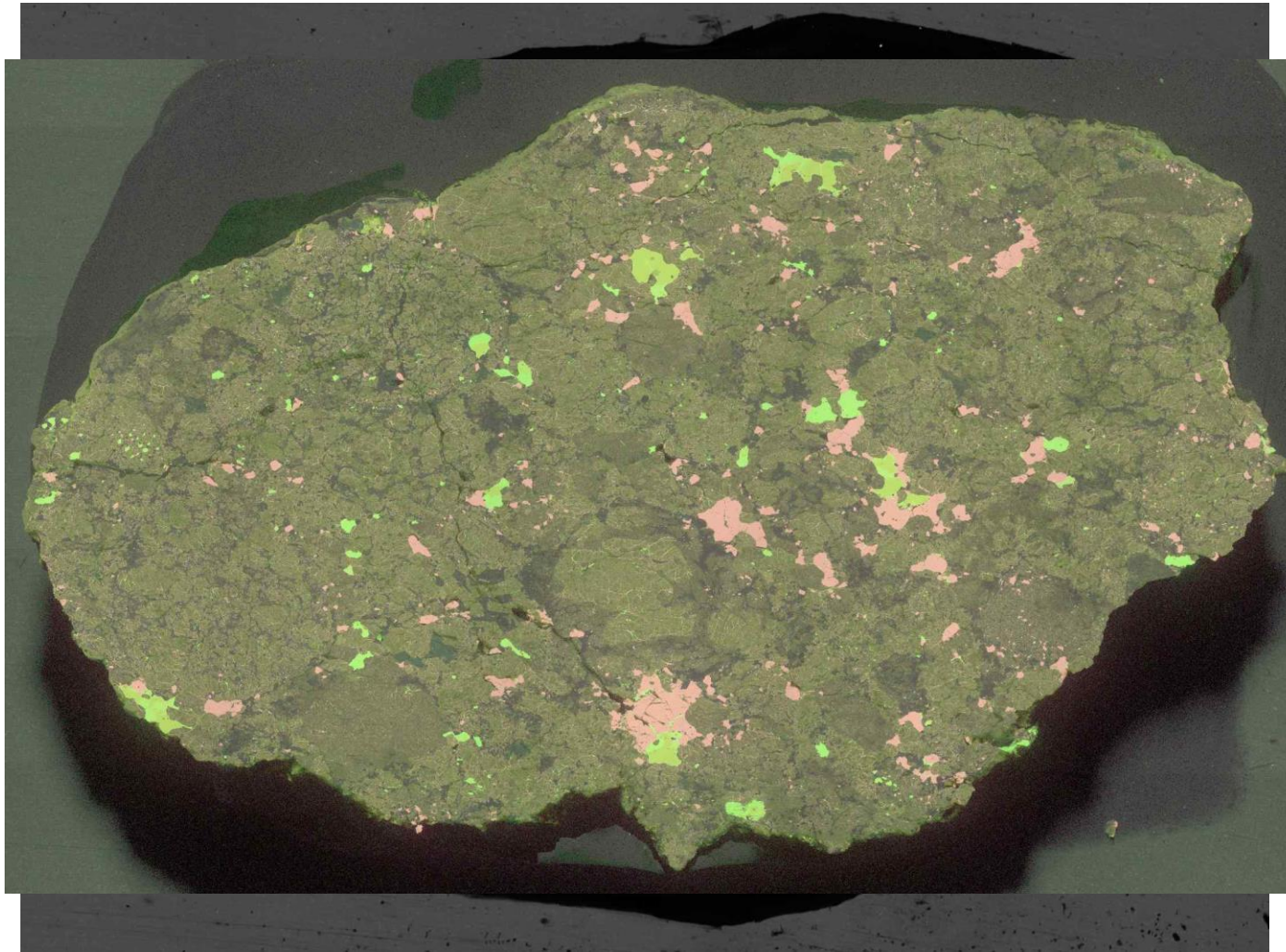




# Examples: Meteorite (all elements EDX)

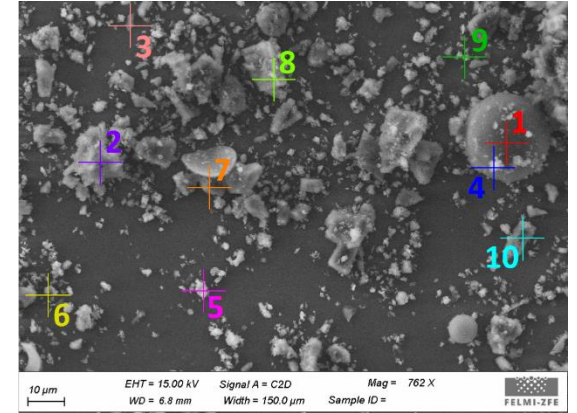


# Examples: Meteorite (Large Area EDX)

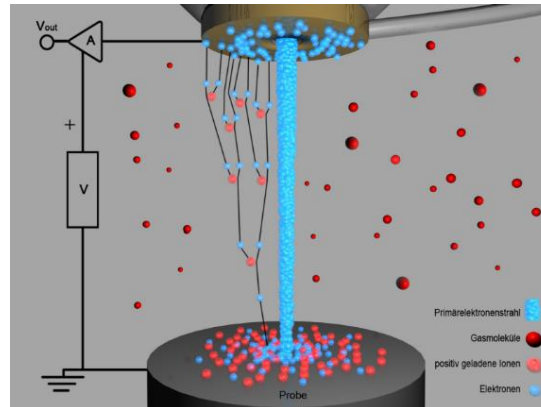


# Specimen Preparation

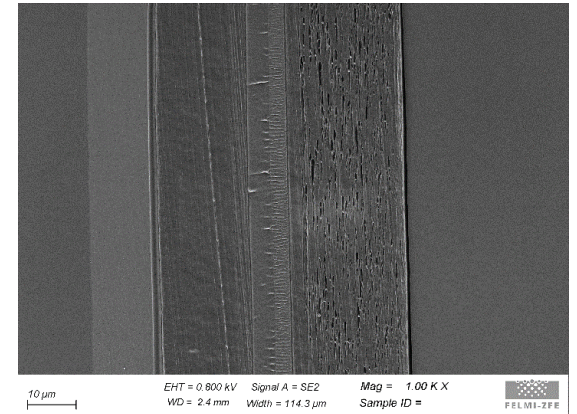
- The sample has to be flat (polished or microtome cut)



- The sample **can not be coated** (VP mode necessary in SEM)



Except if no mapping is necessary!



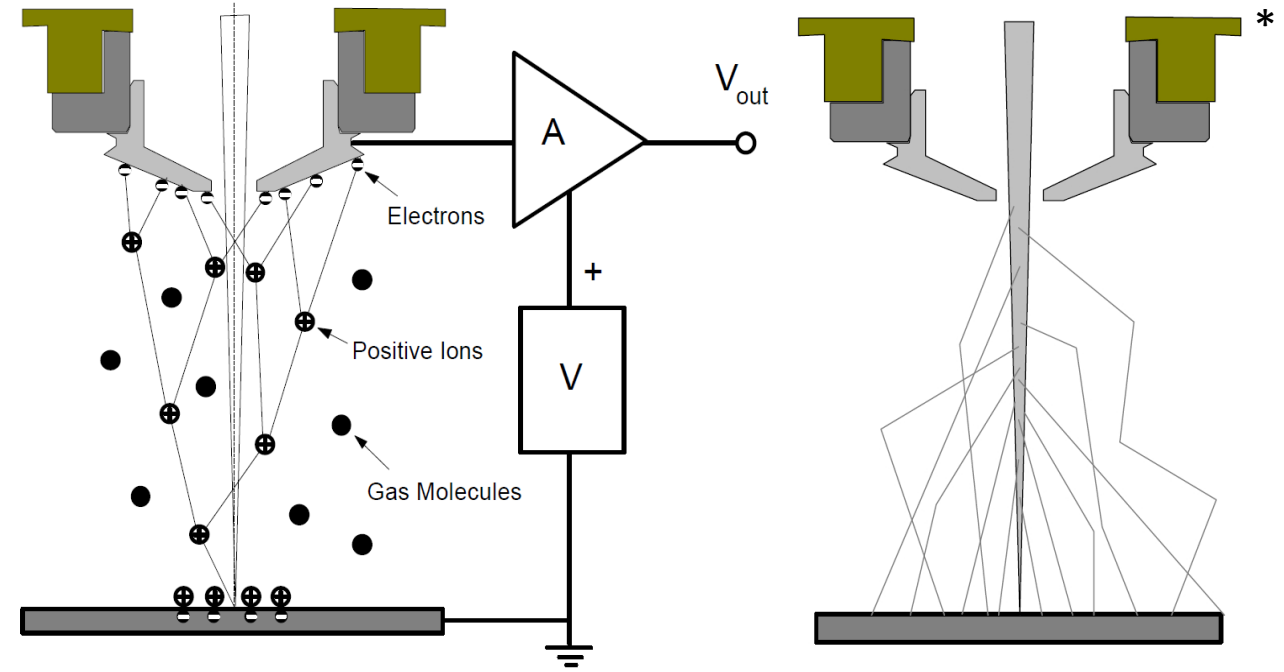
Except Low Voltage SEM (no BSE or EDX)!

- Vacuum stability

# Limitations: Variable pressure SEM & Charging



Mirror by charging (extreme chase of charging)



Schematics of a Variable pressure SEM: left SE detection; right scattering of the primary beam

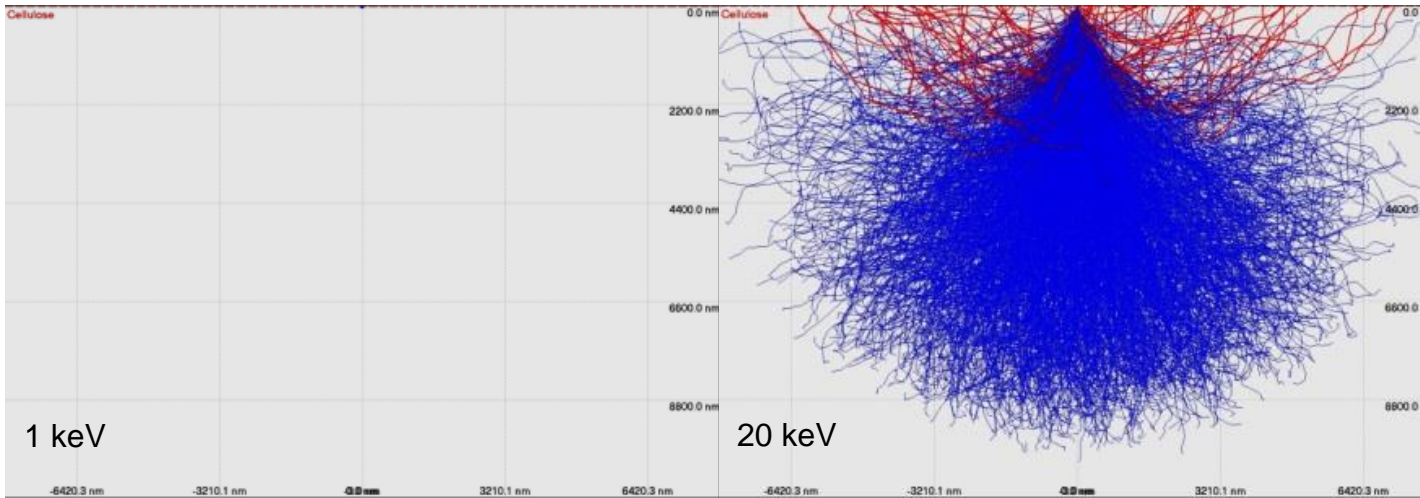
## Advantages:

- Simple to operate
- BSE imaging possible
- EDX possible

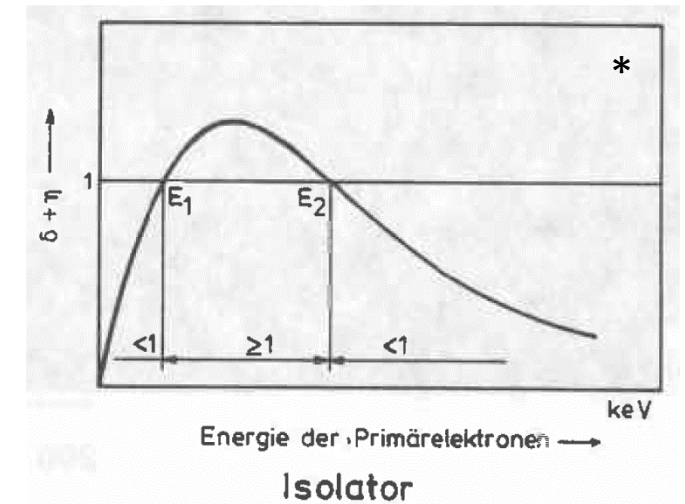
## Disadvantages:

- Reduced contrast
- Limited EDX mapping
- High acceleration voltages/currents advisable

# Limitations: Low voltage SEM



Simulation of the interaction volume of the electron beam with cellulose for different beam energies



At the right energy:  
Beam electrons = emitted electrons

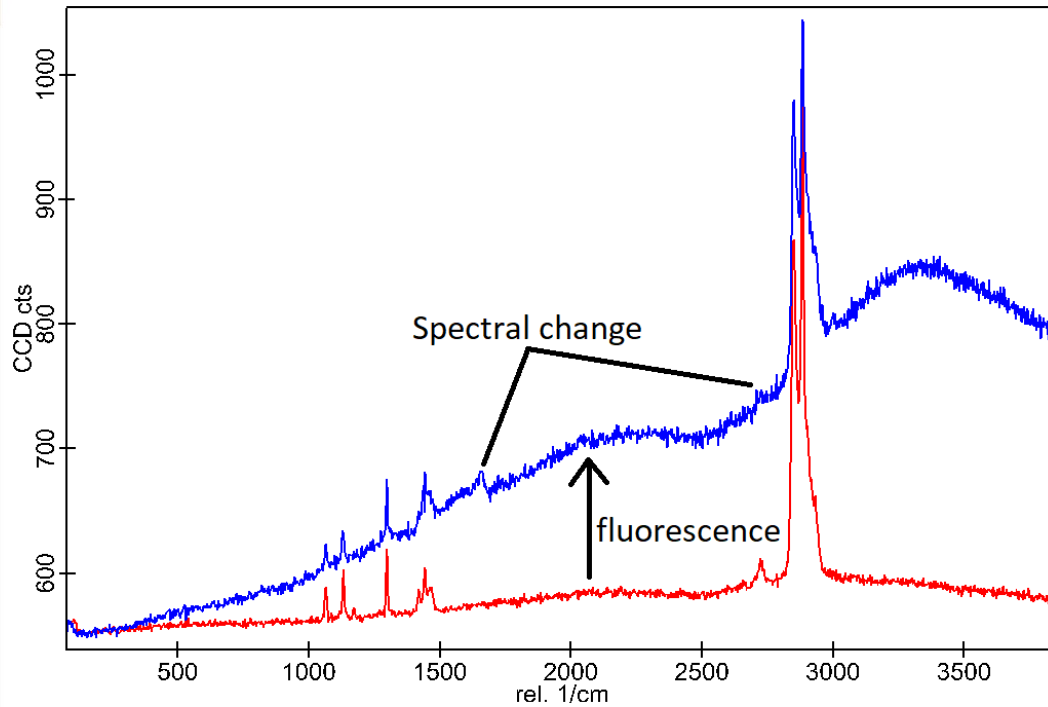
## Advantages:

- Low penetration depth
- Reduced specimen damage
- Better topographic contrast

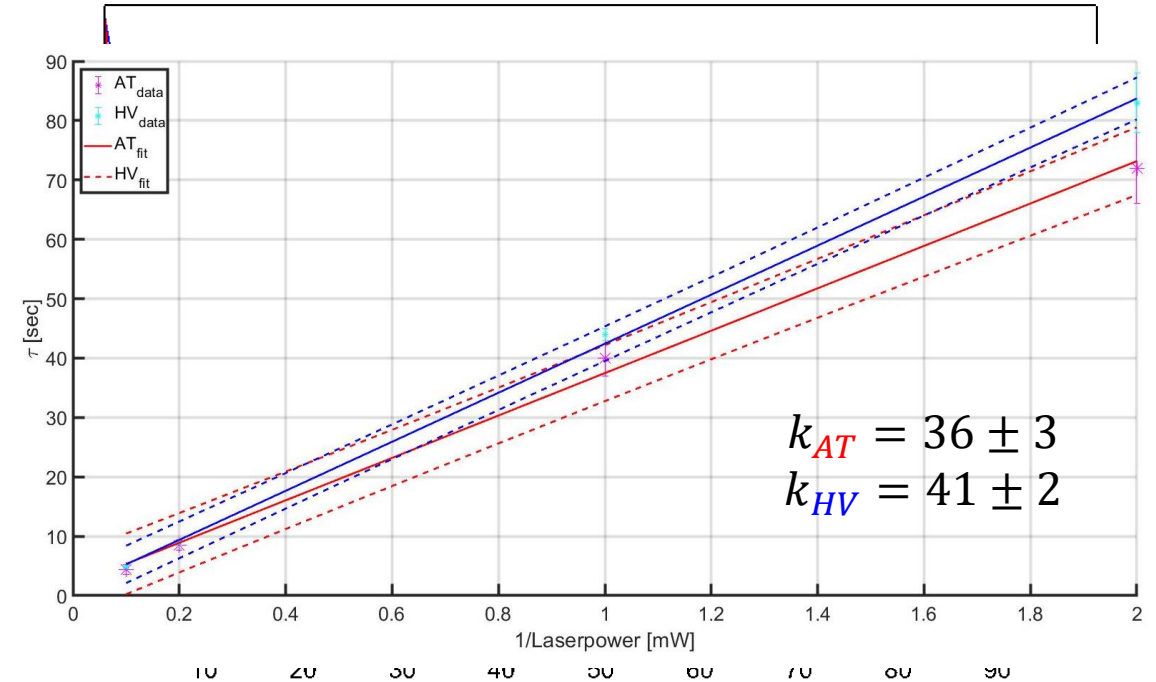
## Disadvantages:

- Difficult to operate
- Severely limited BSE & EDX
- High contamination rates

# Limitations: Beam damage & Raman in vacuum



Raman spectra of PE before (red) and after (blue) exposure to the electron beam



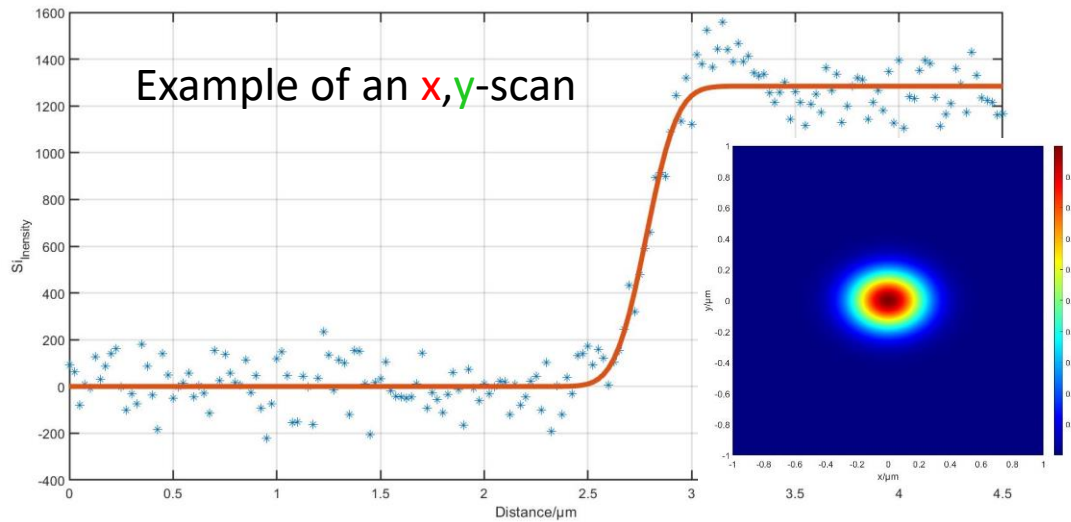
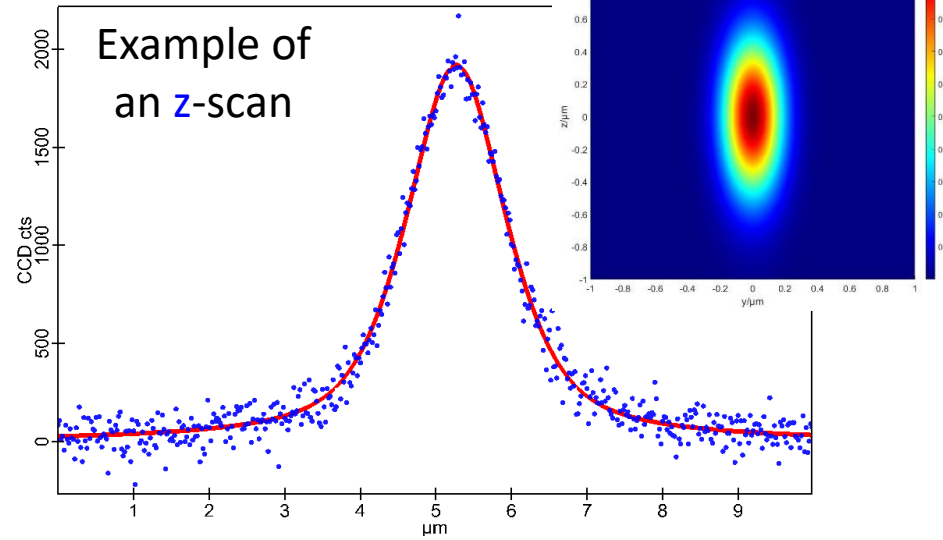
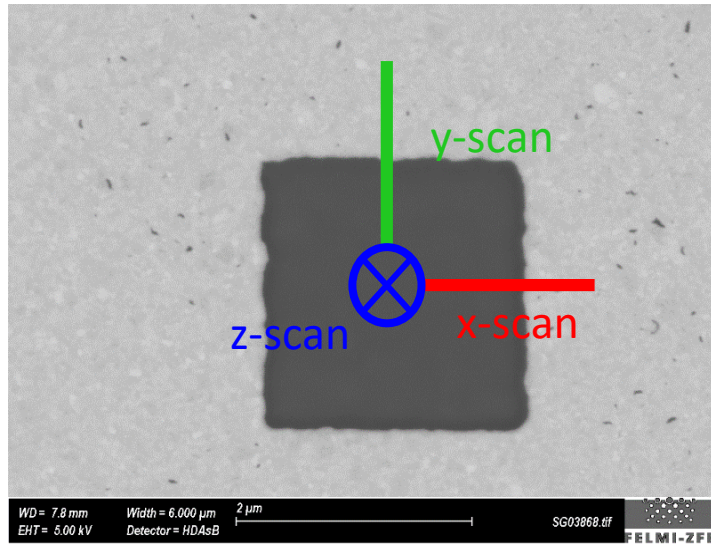
Fluorescence-decay constants for a yellow epoxy resin as a function of  $1/\text{Laserpower}$  in high vacuum (blue) and at ambient air (red)

Signal decline of the 1225  $\text{cm}^{-1}$  band of Trypan blue on Si over time in high vacuum (blue) and at ambient air (red)

*Measurement strategy:* Quick low quality SEM (find position)  $\Rightarrow$  all Raman measurements  $\Rightarrow$  high quality SEM  $\Rightarrow$  EDX-Mapping

This can vary depended on the circumstances but **EDX last** is always a good idea.

# Limitations: Resolution Raman



$$\omega_x = 340 \text{ nm}$$

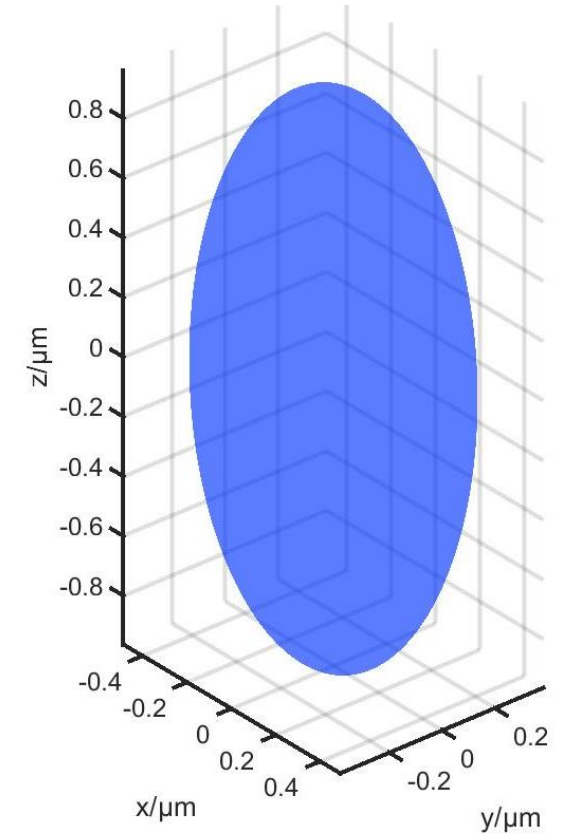
$$\Rightarrow \text{Res}_x \approx 480 \text{ nm}$$

$$\omega_y = 260 \text{ nm}$$

$$\Rightarrow \text{Res}_y \approx 370 \text{ nm}$$

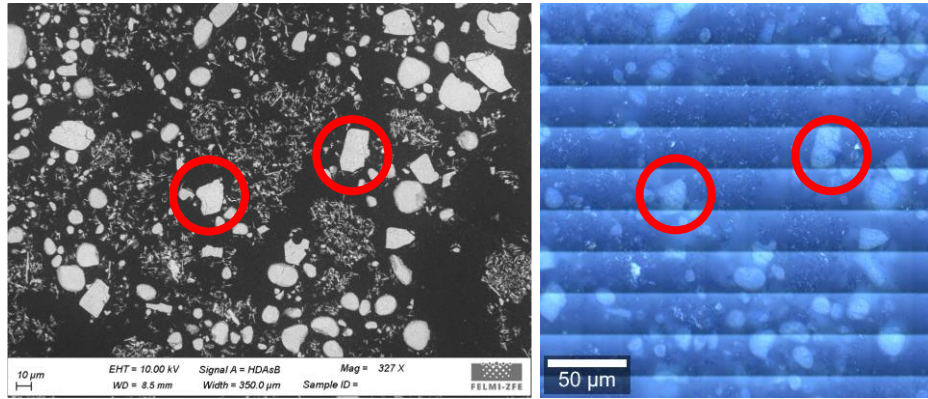
$$\omega_z = 680 \text{ nm}$$

$$\Rightarrow \text{Res}_z \approx 950 \text{ nm}$$

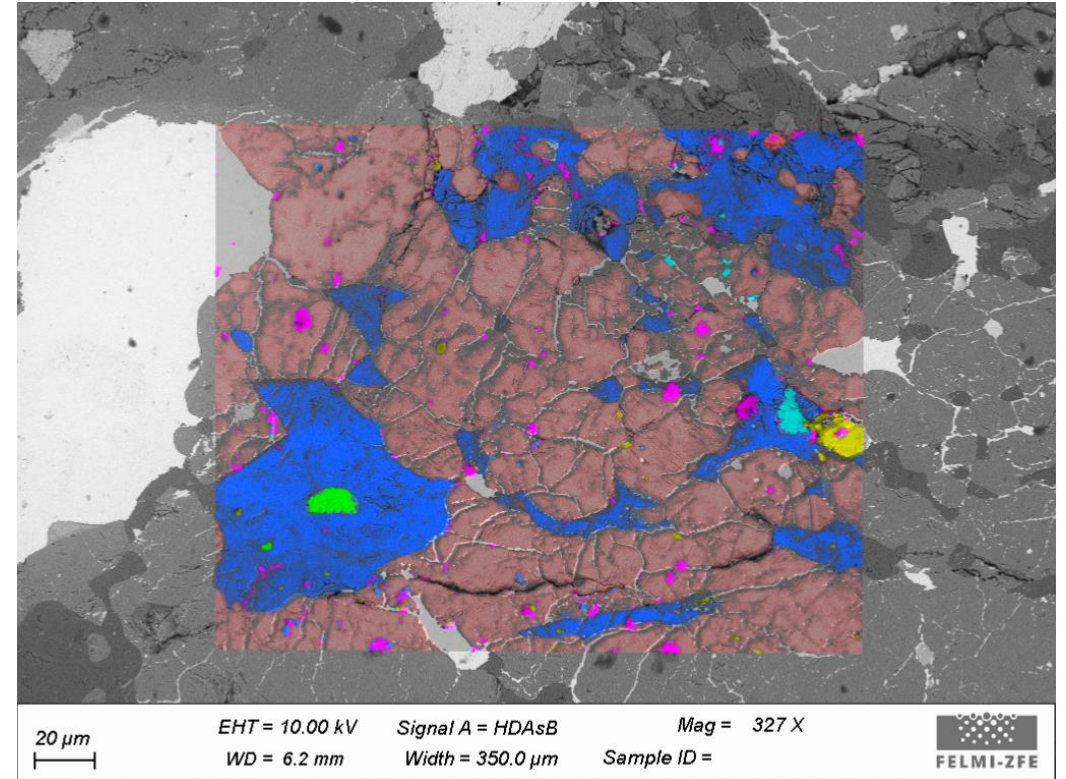
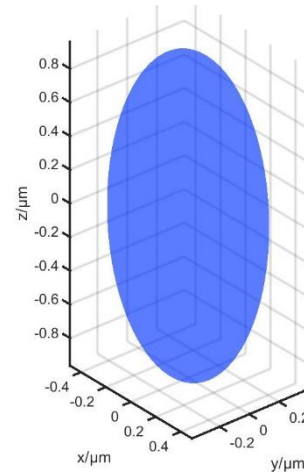
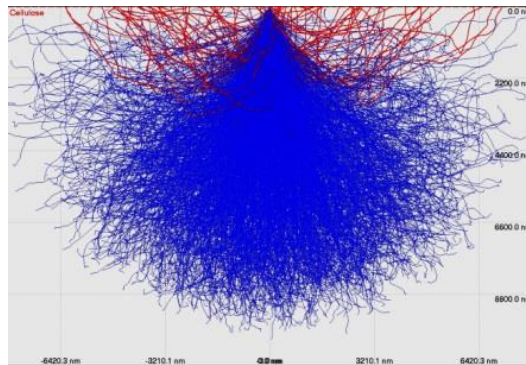


$1/e^4$ -isosurface of the point spread function

# Correlation & Contrast



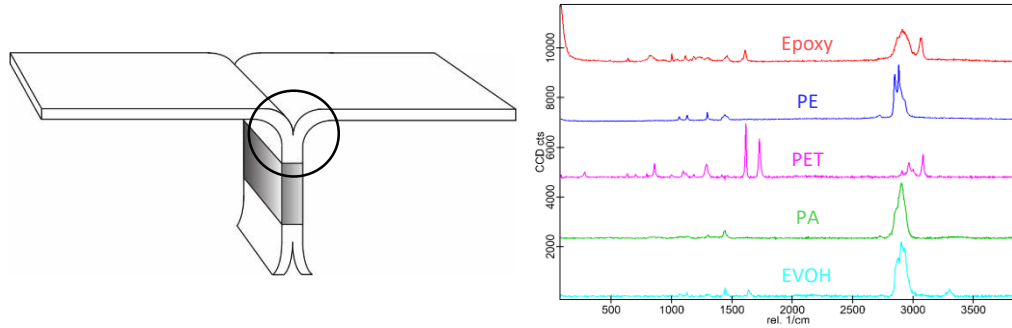
Correlation is done using features visible with both the SEM and light microscope.  
=> Good correlation is always enforced!



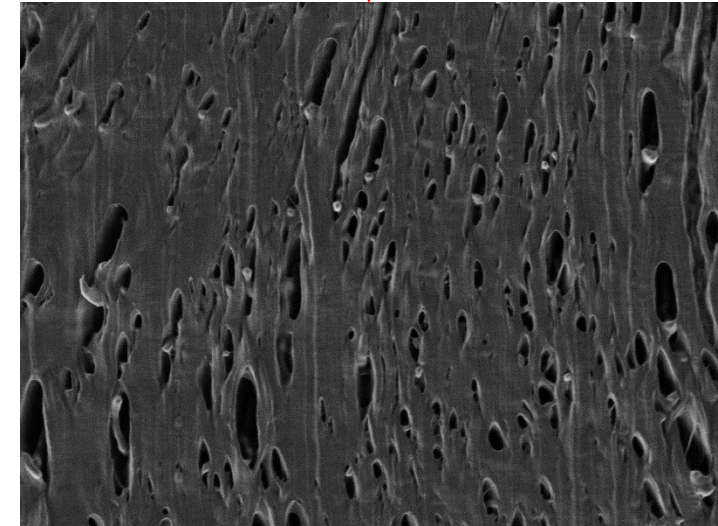
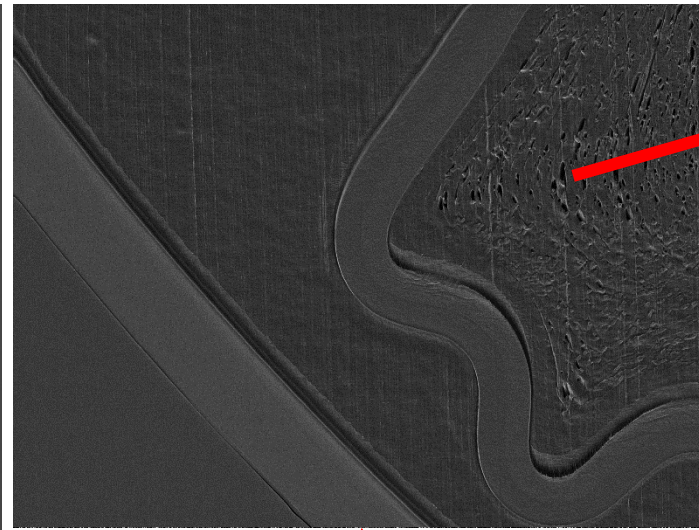
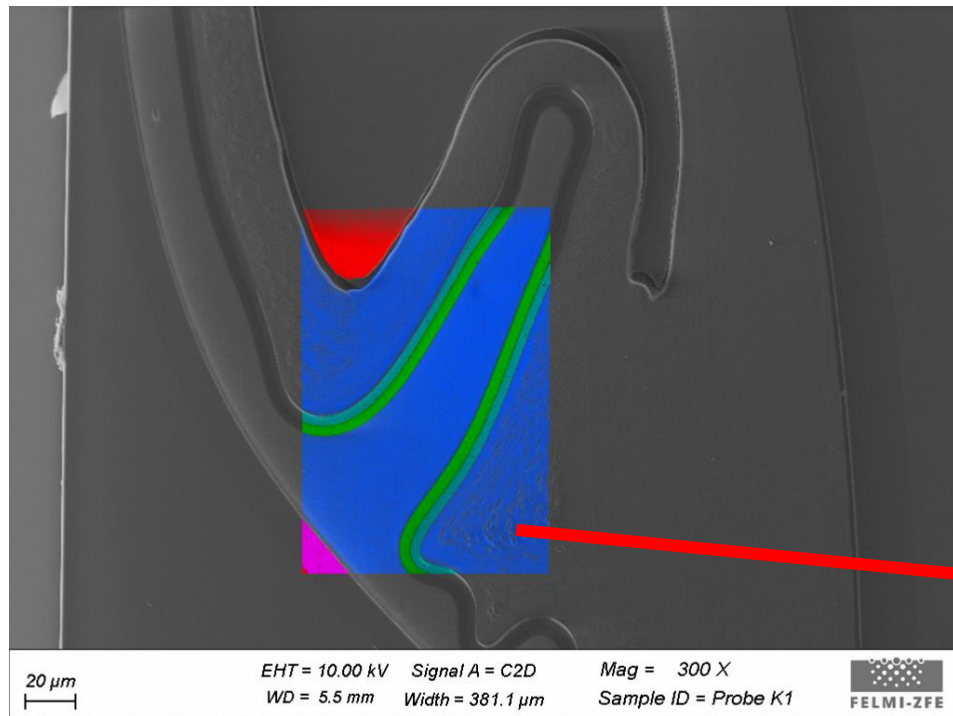
The recorded  $W_D$  &  $W_S$  spectra were then fitted to the Raman map visible in SEM & BSE-TOPO contrast stage split (60x) visible from the pore wall particle.



# Analysis of polymeric materials: peel film\*

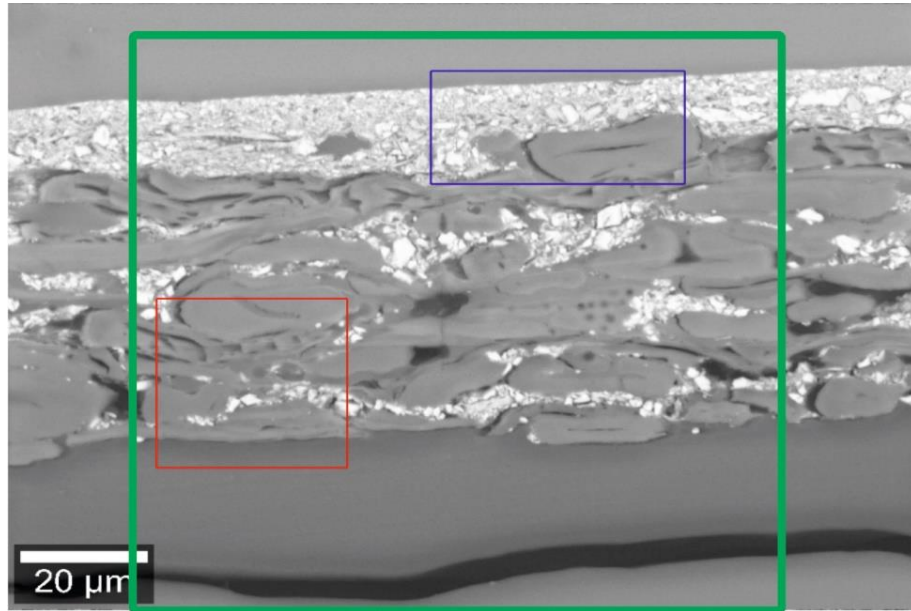


Investigation of details using the Low voltage mode (0.8 keV); SE images



\*Schmidt, Ruth, et al. "The Combination of Electron Microscopy, Raman Microscopy and Energy Dispersive X-Ray Spectroscopy for the Investigation of Polymeric Materials." *Macromolecular symposia*. Vol. 384. No. 1. 2019.

# Analysis of polymeric materials: paper\*

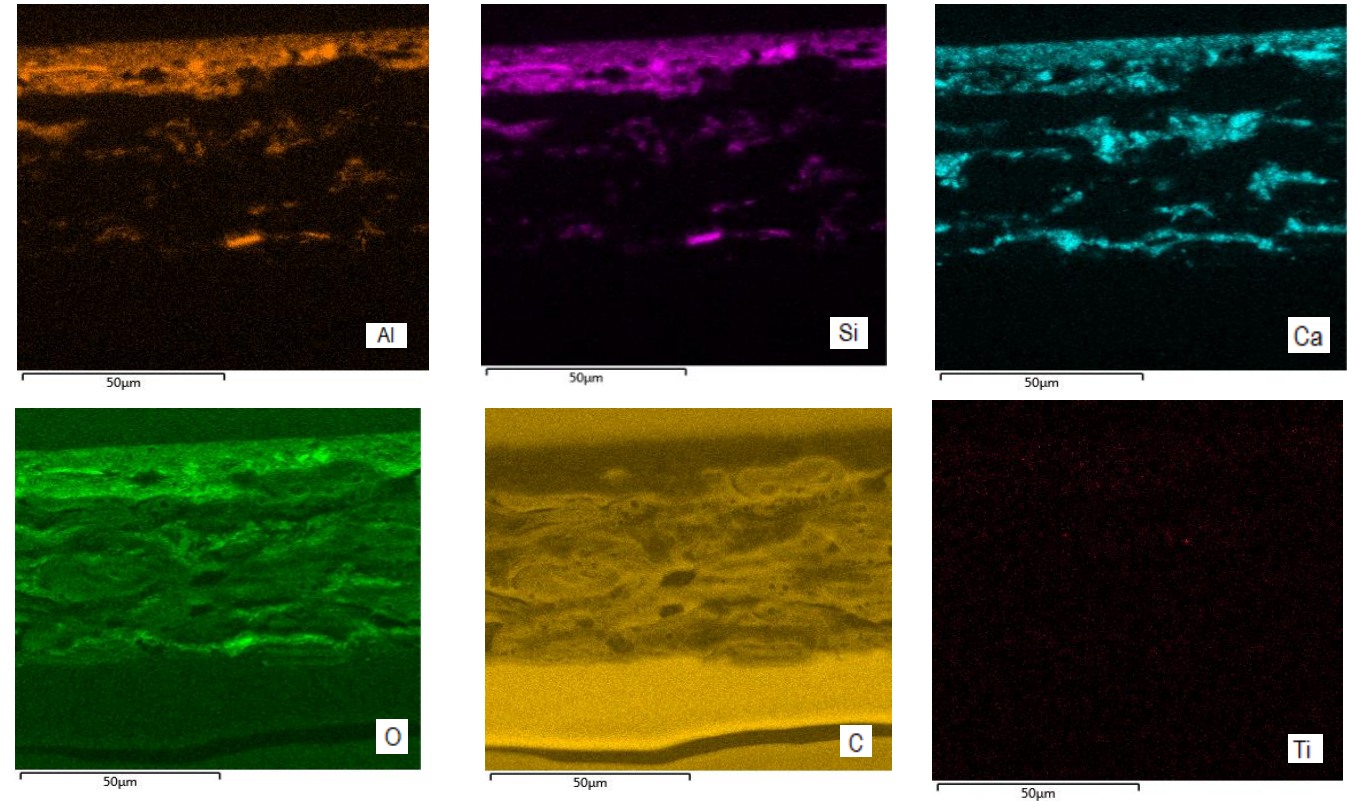


BSE of a PE-coated paper cross-section

Green area: EDX-Mapping

Red & Blue area: Raman-Mapping

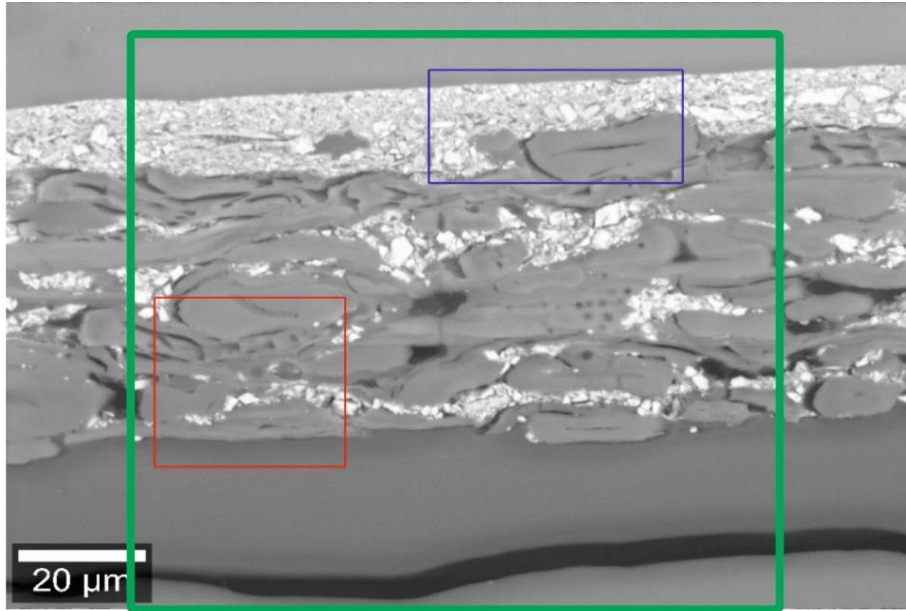
From EDX one might expect 4 compounds:  
Ca, Al-Si & C (x2 different)



Elemental distribution measured with EDX

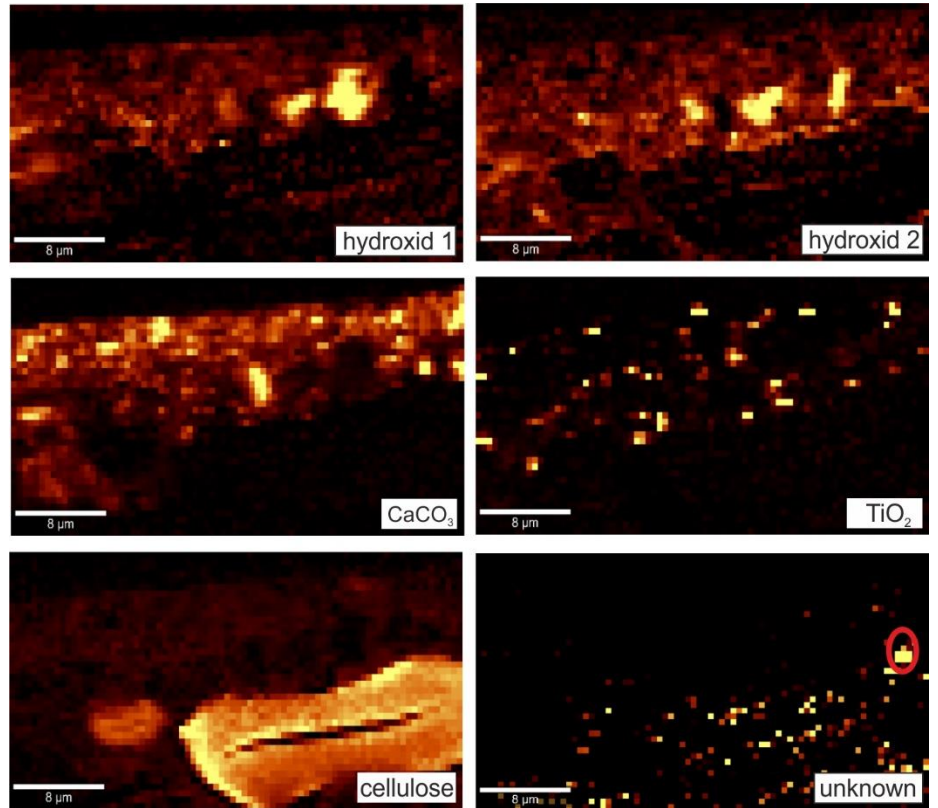
\*Schmidt, Ruth, et al. "The Combination of Electron Microscopy, Raman Microscopy and Energy Dispersive X-Ray Spectroscopy for the Investigation of Polymeric Materials." *Macromolecular symposia*. Vol. 384. No. 1. 2019.

# Analysis of polymeric materials: paper\*

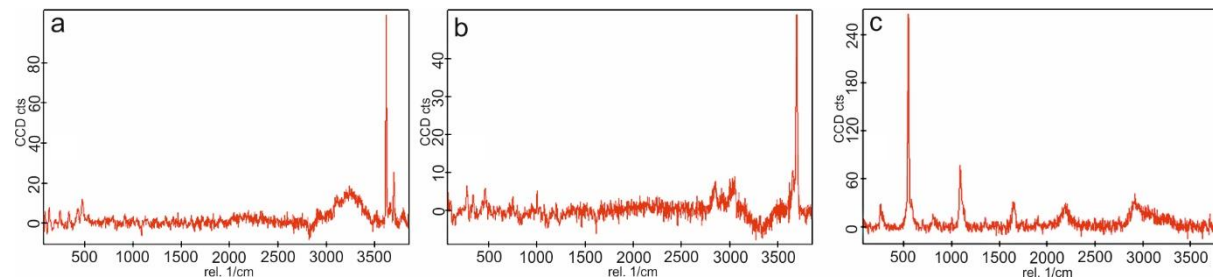


There are 6 compounds found in the blue area by Raman.

Note that Ti (in  $\text{TiO}_2$ ) was missed by EDX!



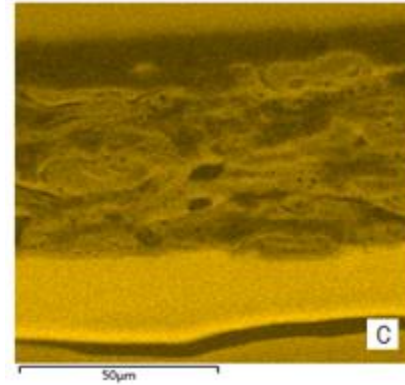
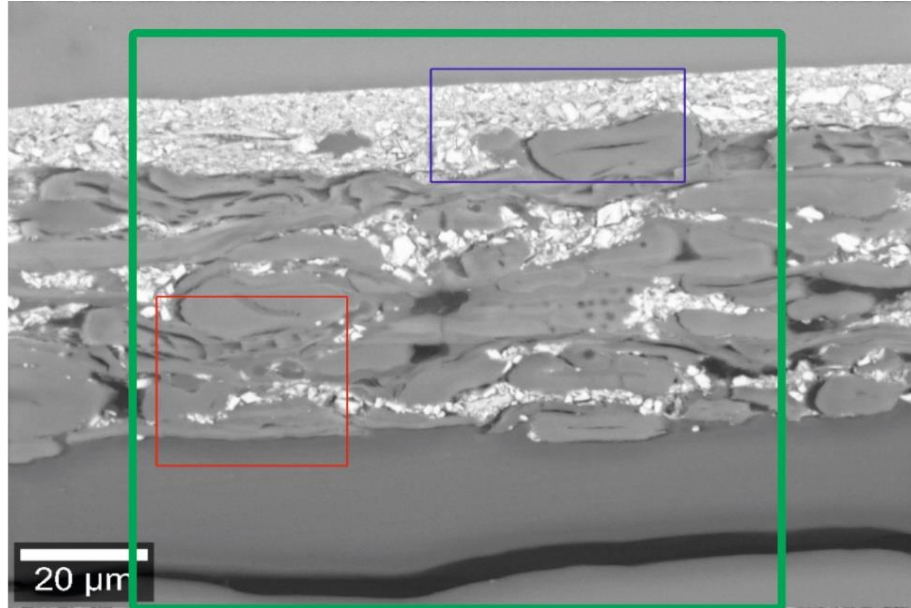
Raman mapping of the blue area



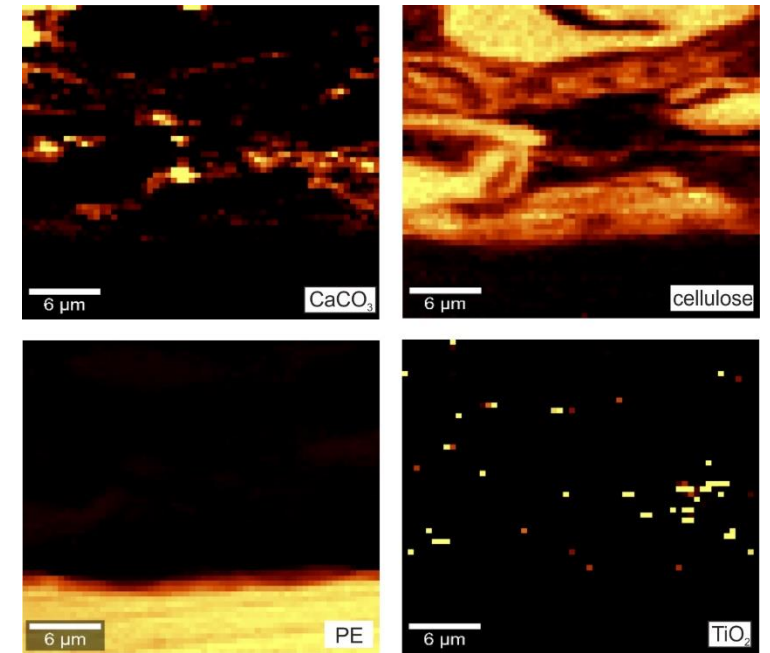
Raman spectra of the not fully identified compounds

\*Schmidt, Ruth, et al. "The Combination of Electron Microscopy, Raman Microscopy and Energy Dispersive X-Ray Spectroscopy for the Investigation of Polymeric Materials." *Macromolecular symposia*. Vol. 384. No. 1. 2019.

# Analysis of polymeric materials: paper\*



An identification of the two organic compounds suspected by EDX is possible with Raman.

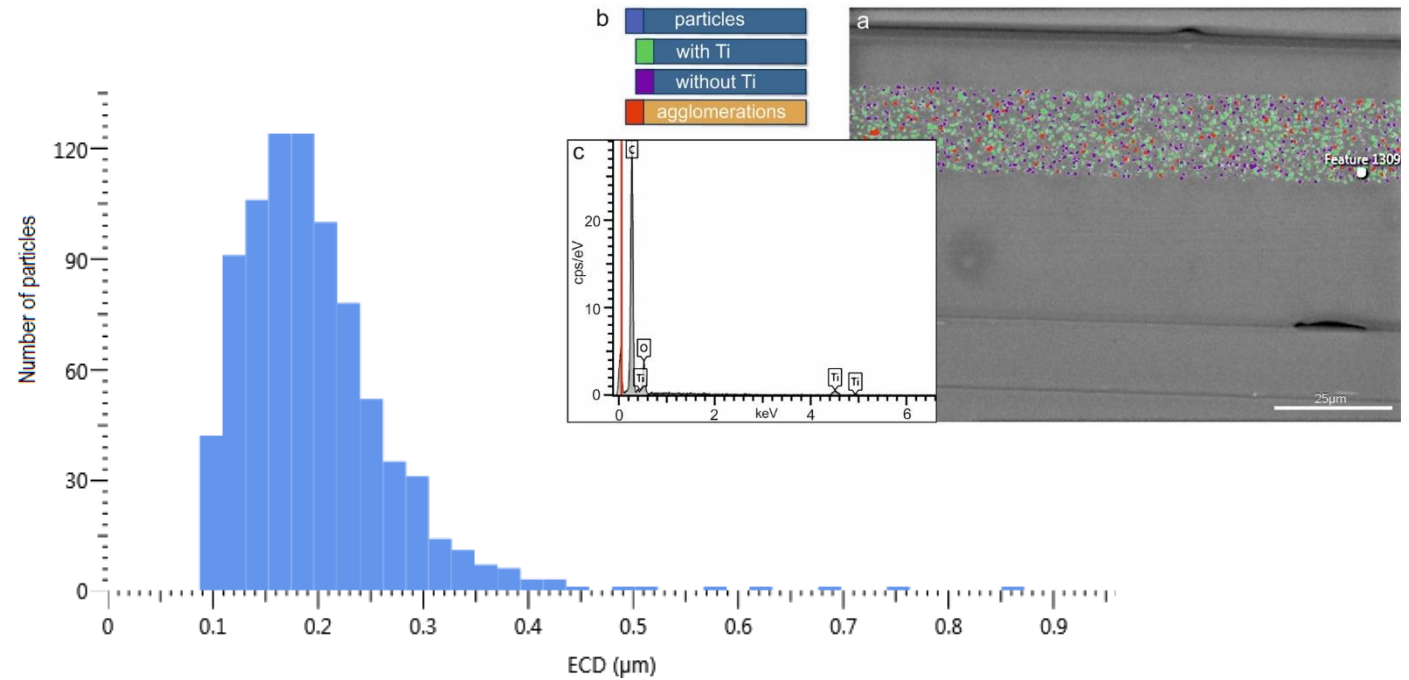
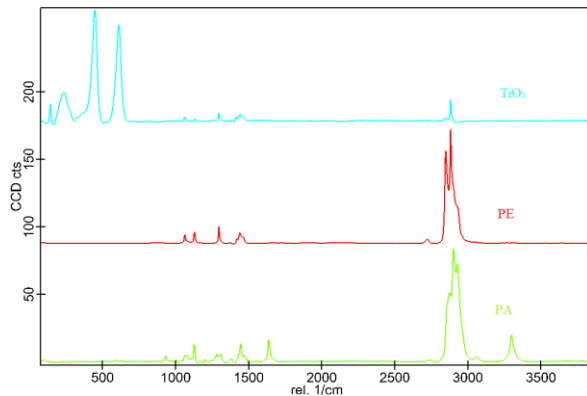
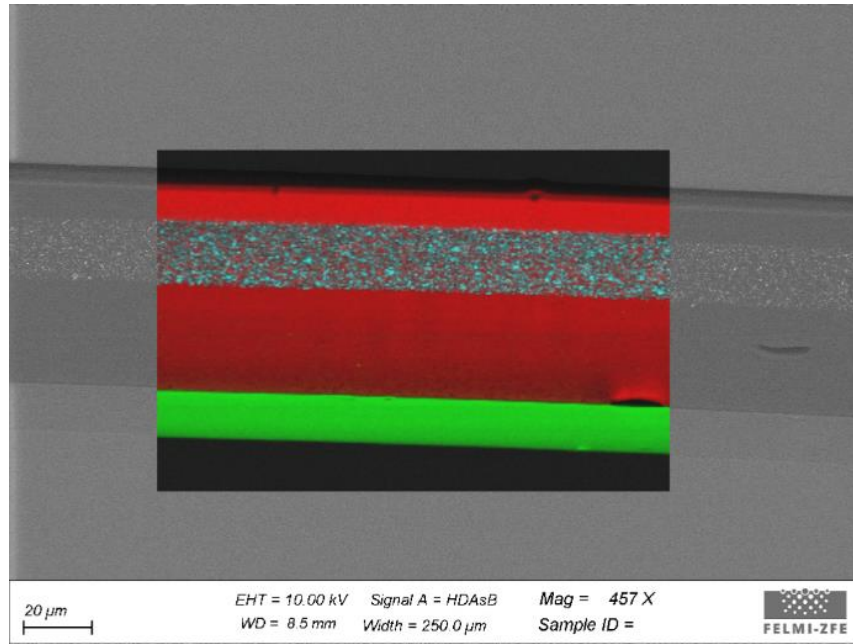


Raman mapping of the red area

Further improvements would be possible by directly correlating the EDX and Raman, especially with regard to identifying small unknown compounds.

\*Schmidt, Ruth, et al. "The Combination of Electron Microscopy, Raman Microscopy and Energy Dispersive X-Ray Spectroscopy for the Investigation of Polymeric Materials." *Macromolecular symposia*. Vol. 384. No. 1. 2019.

# Analysis of polymeric materials: packaging foil\*

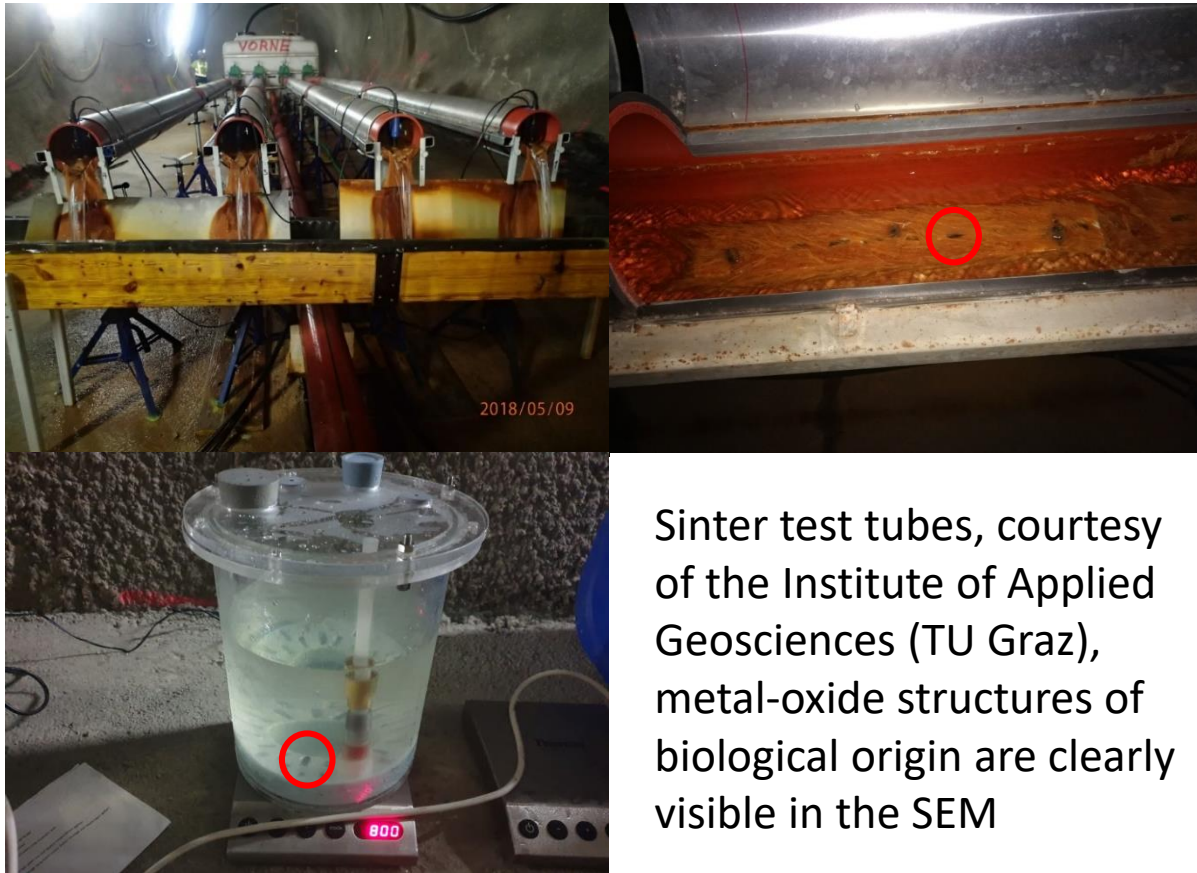


SEM: a fine layer of particles is visible -> Raman: Polymers and particles identified -> EDX: Size distribution of the particles is measured.

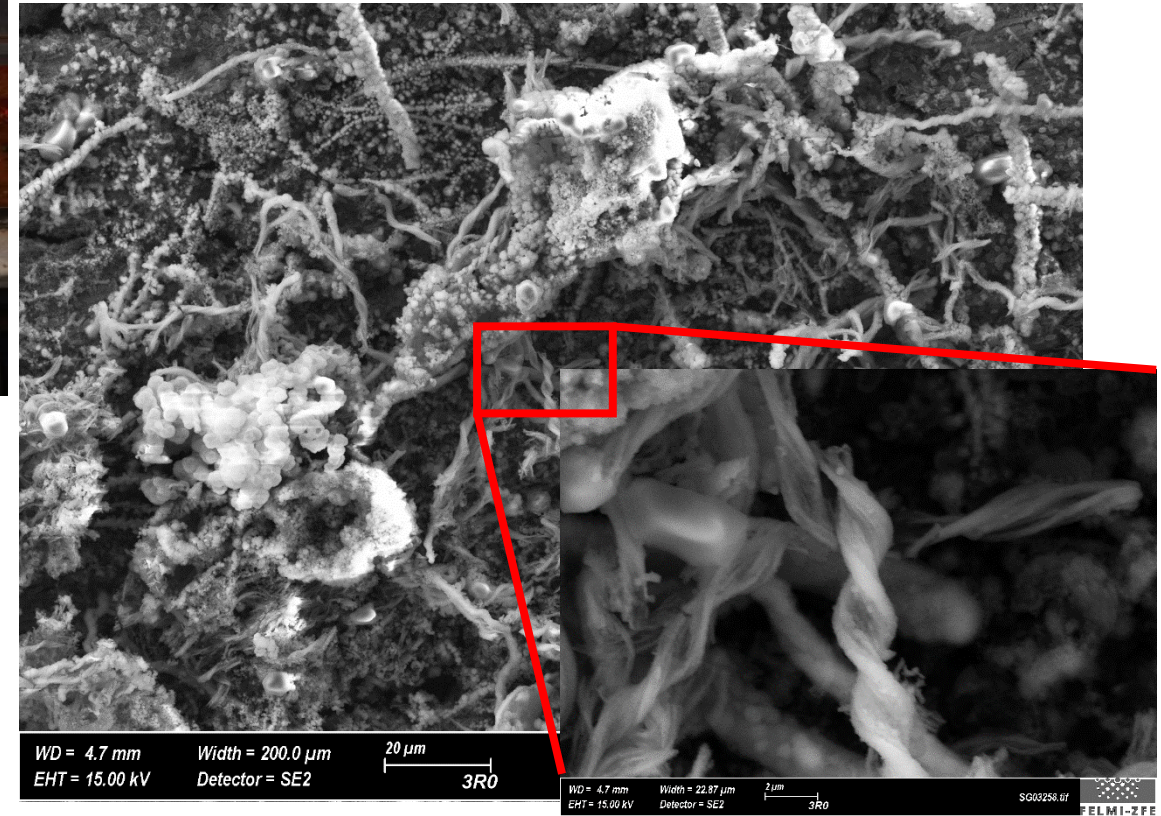
Note that the particles are too small for a size distribution by Raman **and** too small for EDX quantification!

\*Schmidt, Ruth, et al. "The Combination of Electron Microscopy, Raman Microscopy and Energy Dispersive X-Ray Spectroscopy for the Investigation of Polymeric Materials." *Macromolecular symposia*. Vol. 384. No. 1. 2019.

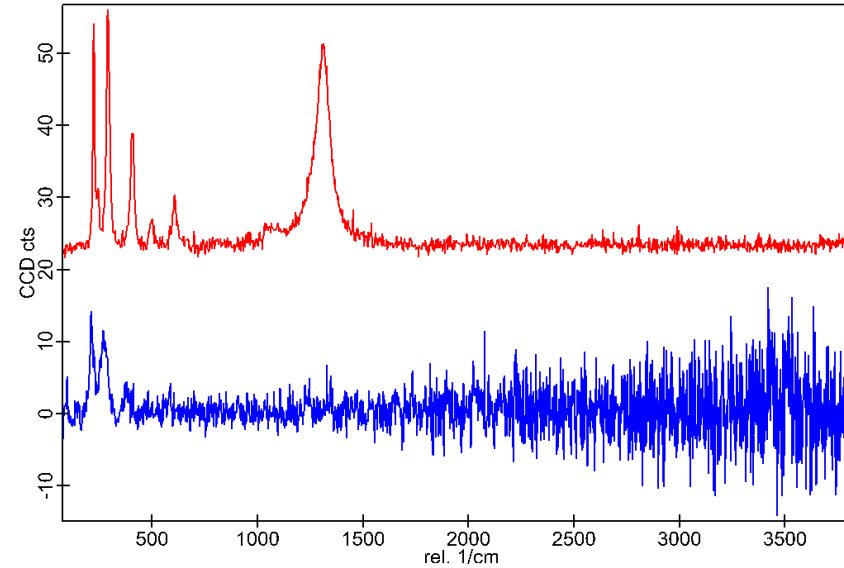
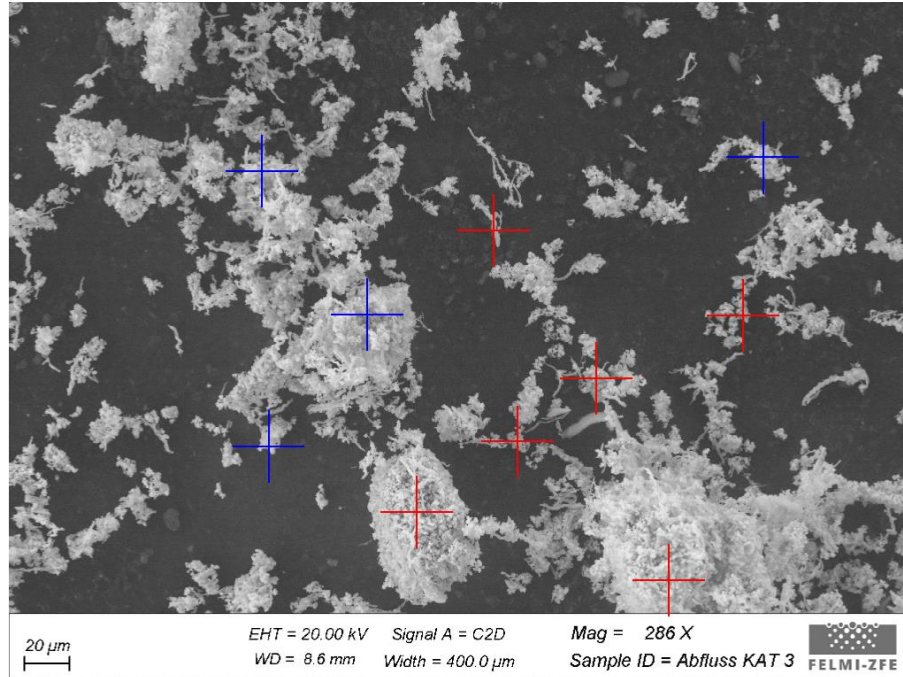
# Microbial corrosion of steel



Sinter test tubes, courtesy of the Institute of Applied Geosciences (TU Graz), metal-oxide structures of biological origin are clearly visible in the SEM



# Microbial corrosion of steel



The Fe-oxide were identified as hematite (red) & biotic maghemite (blue); High Si and Ca content is also visible in the EDX.

A Raman mapping was impossible, because the samples are too sensitive to the laser.

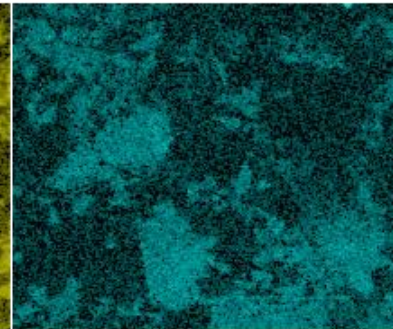
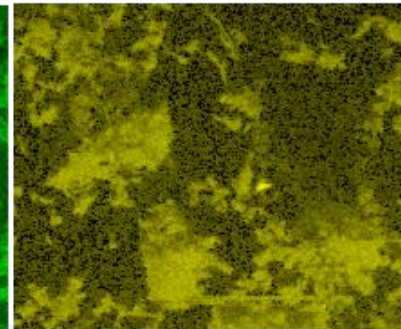
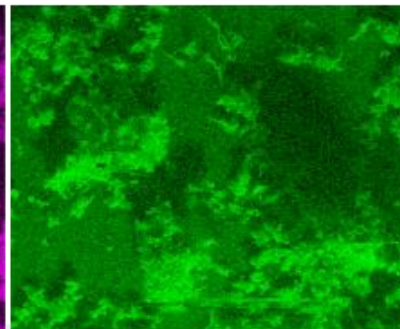
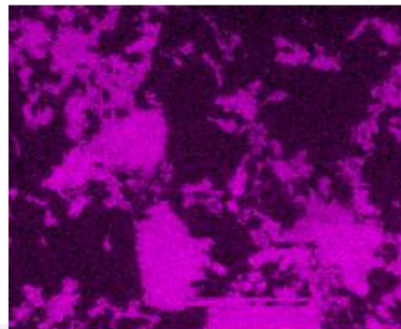
A systematic analysis of different steel types is currently ongoing.

Fe K-Serie

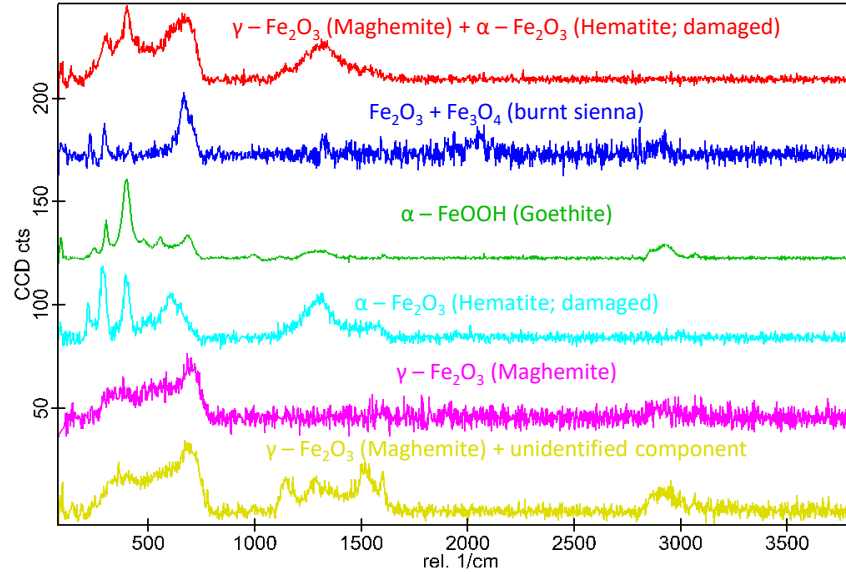
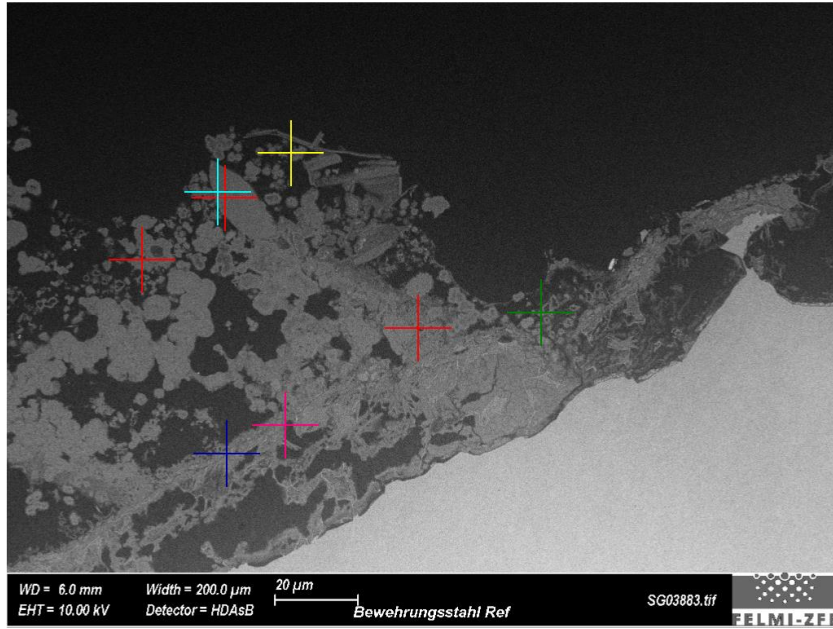
O K-Serie

Si K-Serie

Ca K-Serie



# Rebar (reference)



On the reference sample some typical iron-oxides are found.

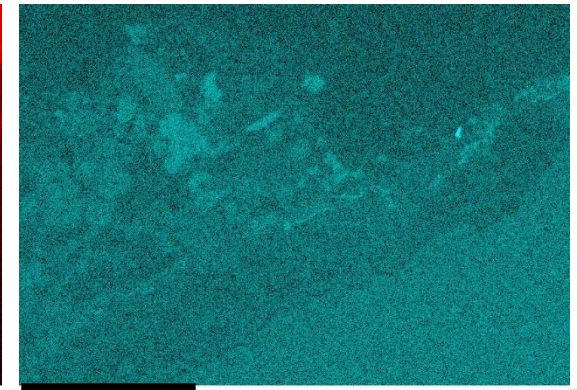
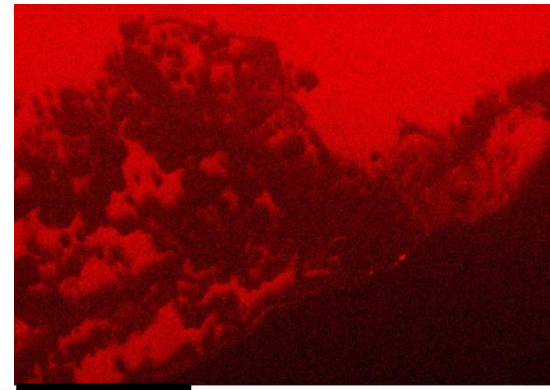
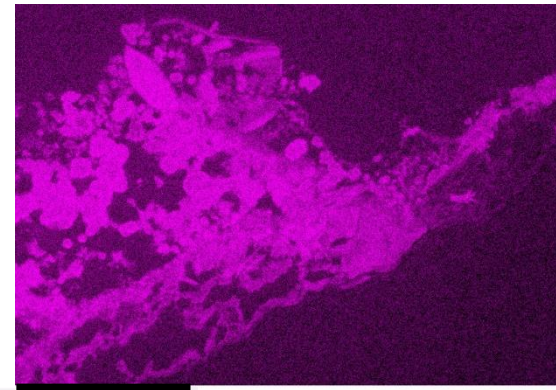
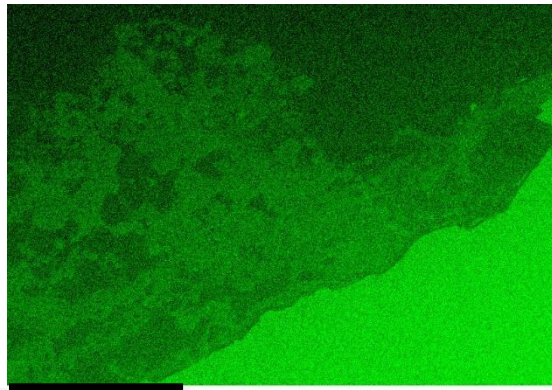
The most important elements found in the EDX-mapping are shown below.

Fe Kα

O Kα

C Kα

Si Kα



50μm

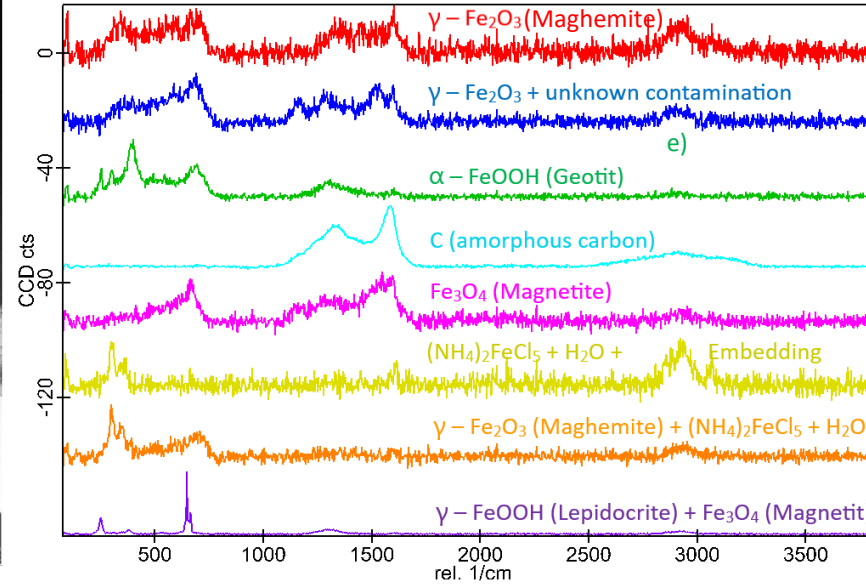
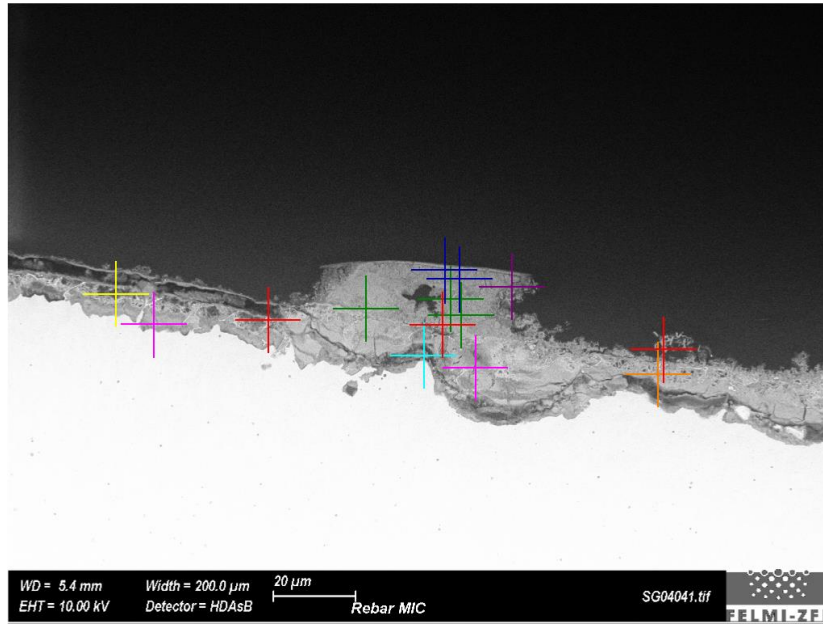
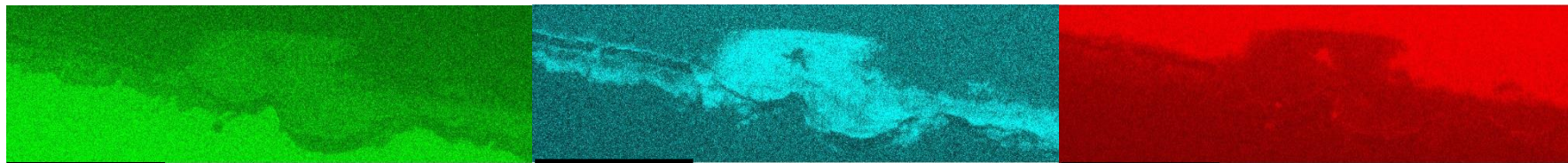
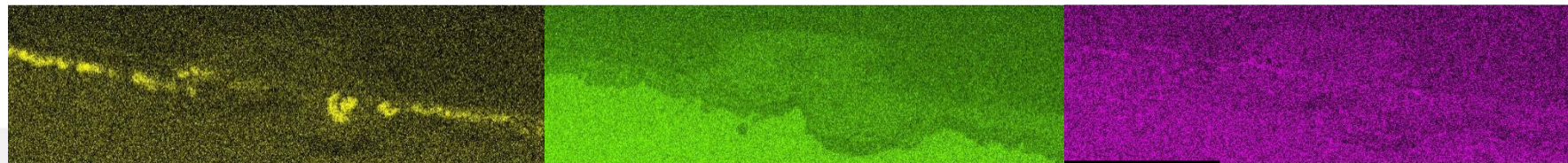
50μm

50μm

50μm



# Rebar (MIC)

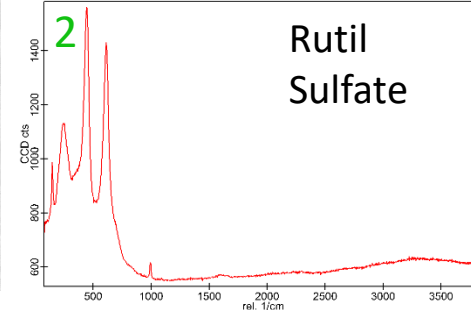
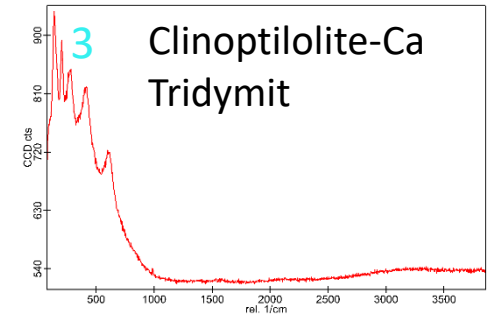
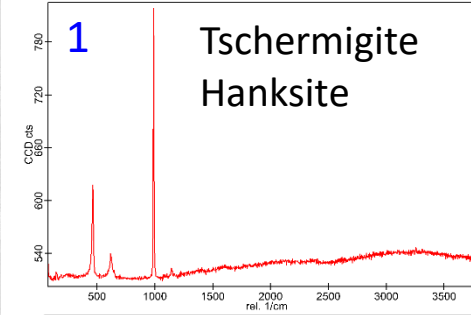
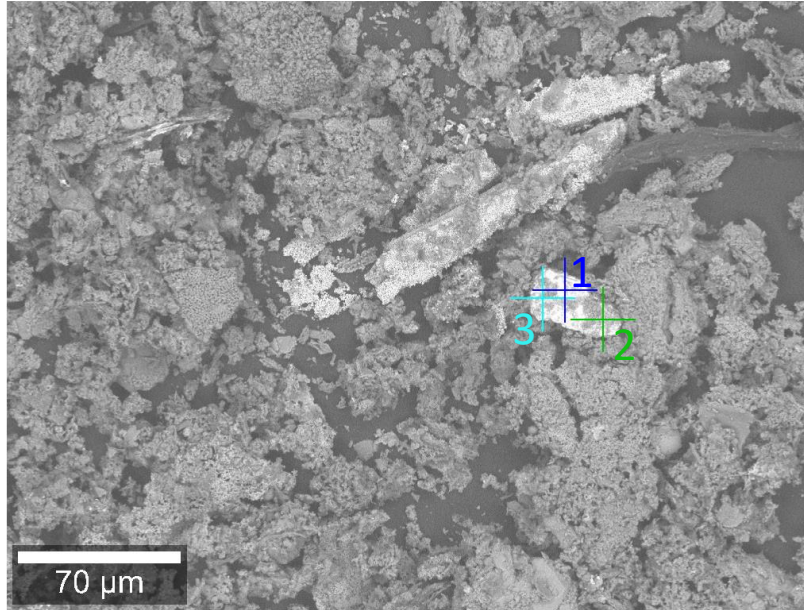
Fe K $\alpha$ O K $\alpha$ C K $\alpha$ 50 $\mu$ mS K $\alpha$ 50 $\mu$ mMn K $\alpha$ 50 $\mu$ mSi K $\alpha$ 50 $\mu$ m50 $\mu$ m50 $\mu$ m

In addition to the iron-oxides found on the reference *Magnetite*, *Lepidocrocite* and *a-C* could be detected.

In the EDX mapping a S layer is clearly visible that presumably formed due to MIC.

**Open question:**  
A Raman spectrum was measured that can best be identified as  $(\text{NH}_4)_2\text{FeCl}_5$  but no N was found by EDX.

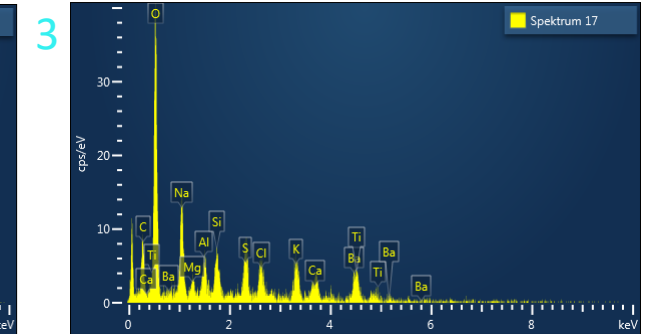
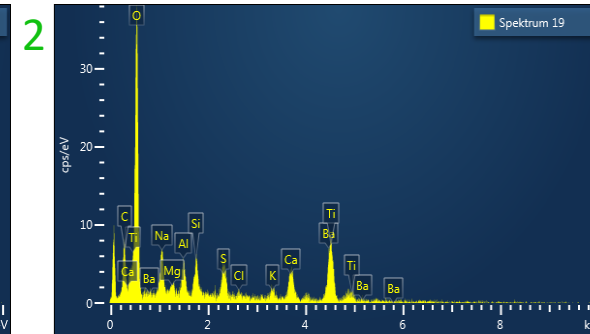
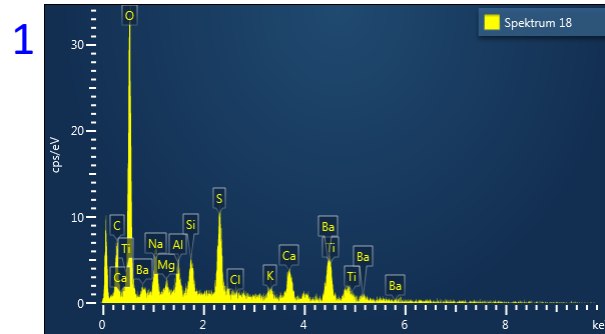
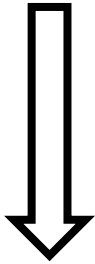
# Dust particles



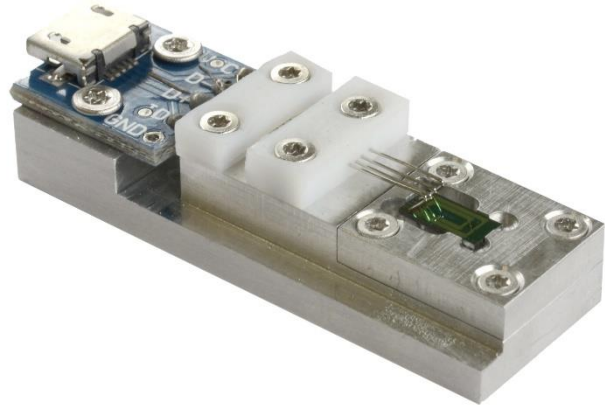
Marginal differences in the EDX Spectra, but completely different Raman identification!



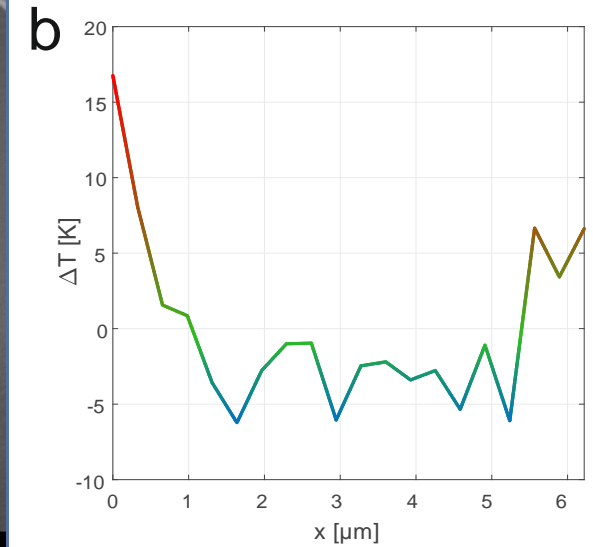
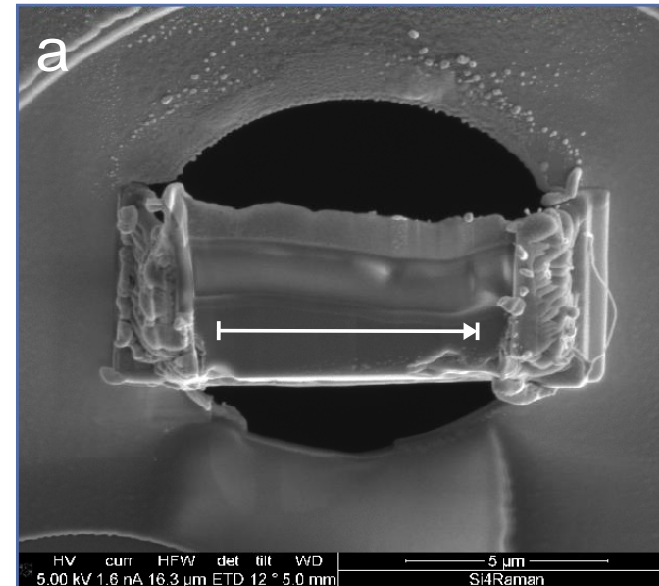
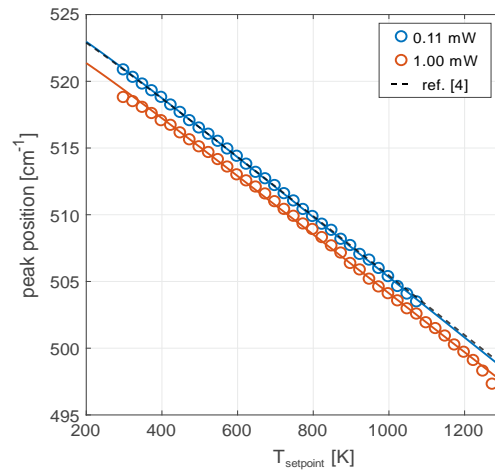
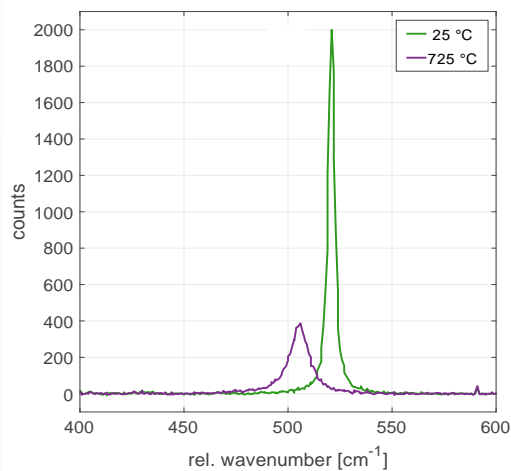
Transfer for further analysis



# Heat distribution on a TEM Lamellae



Custom built SEM-holder for a MEMS heater chip (DENSsolutions; insitu TEM)



The heat distribution across a FIB lamella (Si) was measured using Raman spectroscopy. This is a crucial contribution for the characterization of the actual temperature gradients during heating experiments.

***Or is this due to stress?***

# Conservation of Bread (& Art)



“Nailed Bread” by  
**Dragoljub Raša Todosijević,**  
*Museum of Contemporary Art,*  
*Zagreb. Inv. No 1810\**



Original Sample

Simple cut



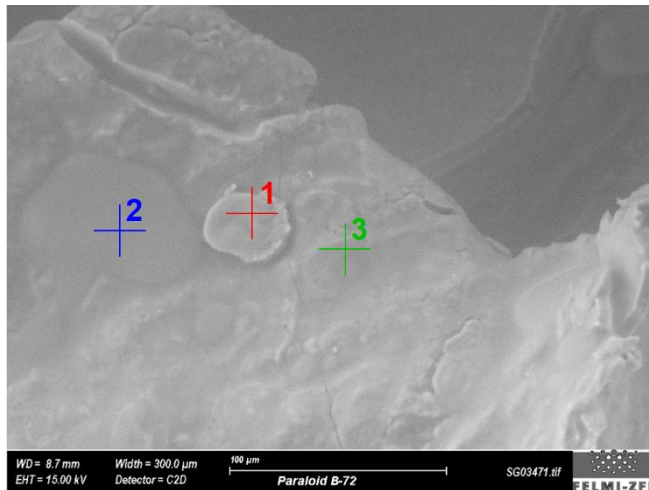
Sample used for  
Raman-SEM



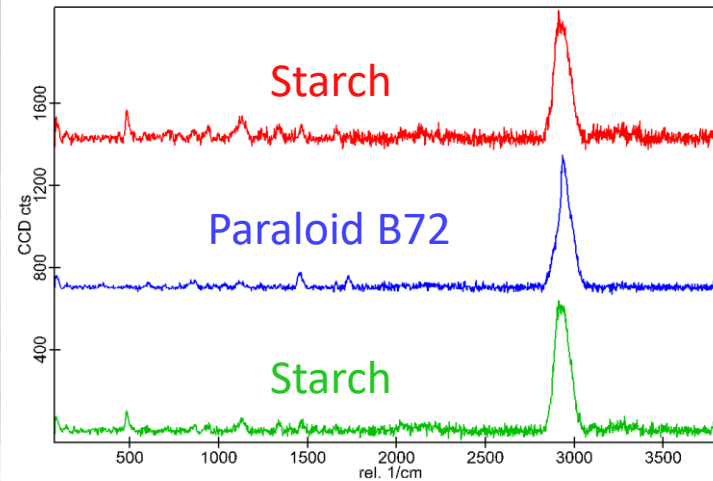
epoxy resin &  
ultramicrotome-cut



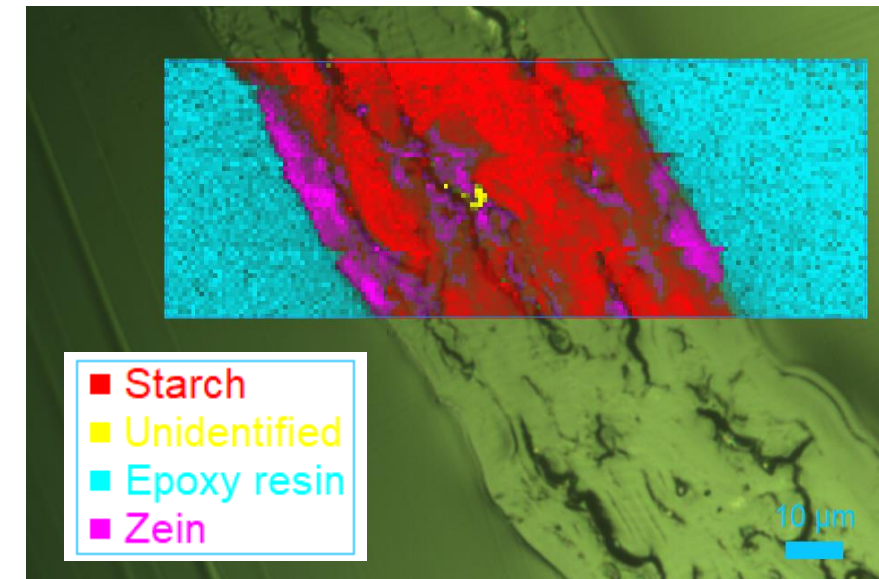
Sample used in the  
Raman mapping



Top: Point measurements on the rough bread surface (SEM-Raman)

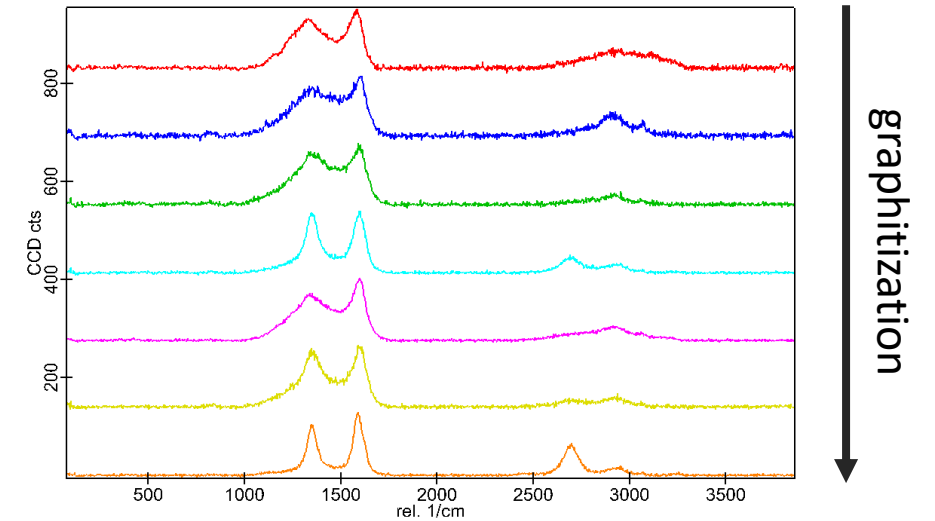
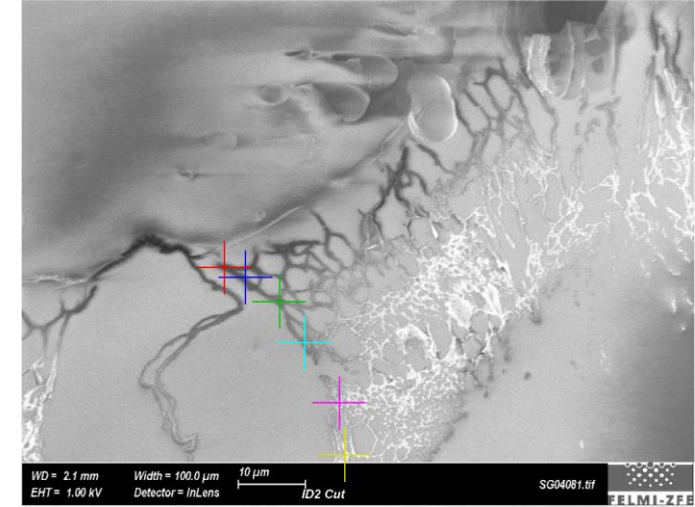
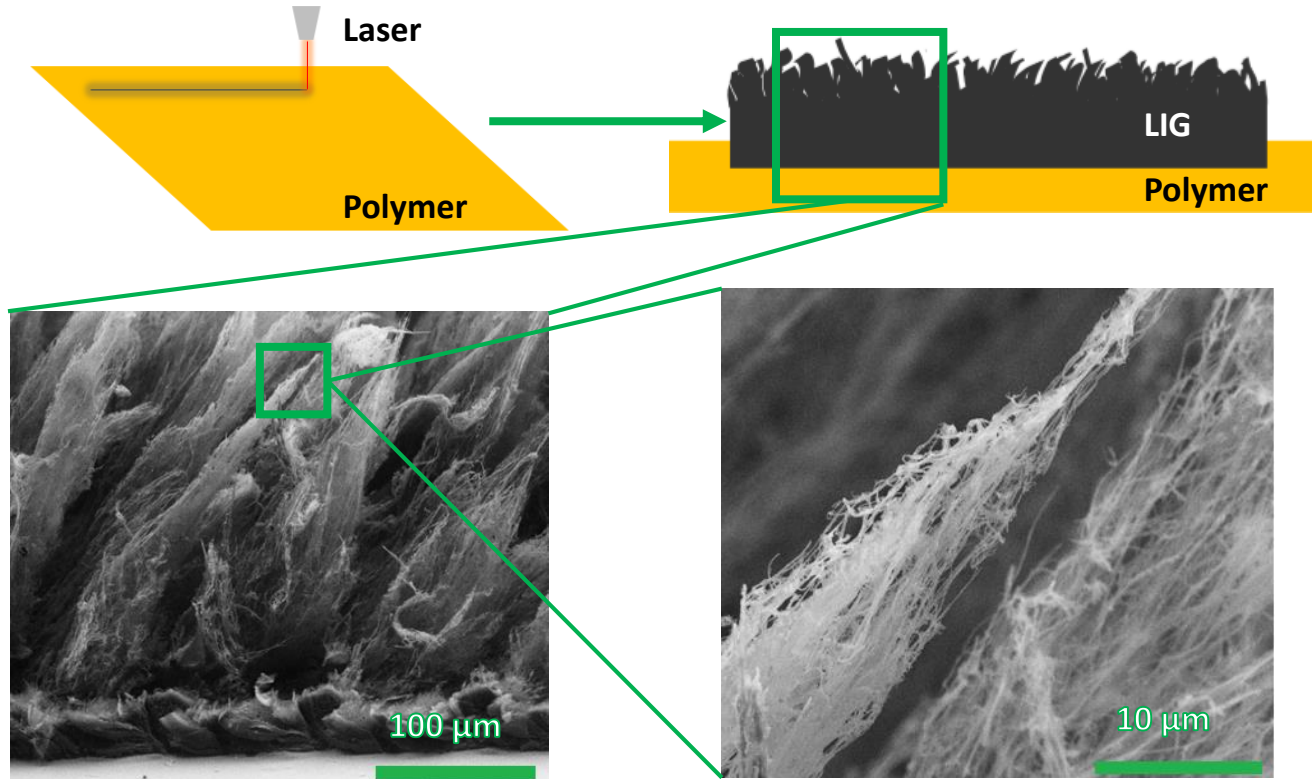


Right: Raman Mapping in a standalone Raman microscope on the prepared sample



\*Photograph by Jovan Kliska

# Laser-induced graphene (LIG)



Both the morphology and the chemical evolution of Laser-induced graphene (LIG) along the growth of the fiber are analyzed.

# Acknowledgements

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## Thank You for Your Attention!