Elemental Analysis of Biological Samples using SEM EDS

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Presenter

- Max Patzschke
- Working for 10 years as an application scientist for SEM/EDS and µXRF for Bruker Nano Analytics, Berlin, Germany
- In his previous employment as a senior mineralogist in the technical development department of Vale S.A. in Brazil, he was responsible for various analytical techniques such as QEMSCAN, SEM-EDS and XRD analysis, as well as mineral phase interpretation and sample preparation.
- Before that he worked in Australia for Intellection as a SEM/QEMSCAN operator and as a core logger for Gnomics Exploration and Barrick Gold.
- In 2008 he completed his Master of Science in mineralogy at the Free University Berlin

Outline

01 SEM EDS analysis challenges

02 Technical details - FlatQUAD

03 FlatQUAD vs. conventional detector

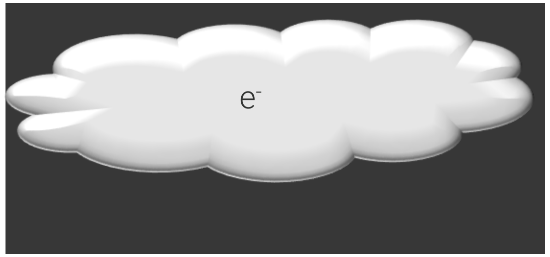
04 Application examples – Biological topographic complex samples

05 Large area mapping

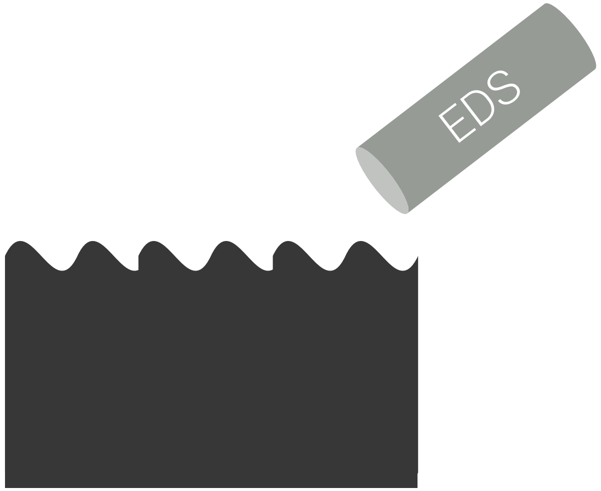
06 Summary

SEM-EDS analysis common challenges

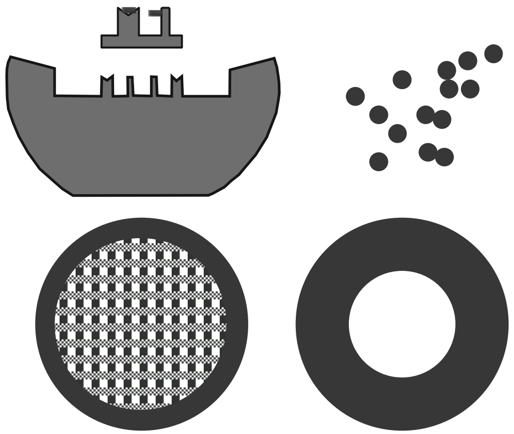
Charge accumulation



Topography

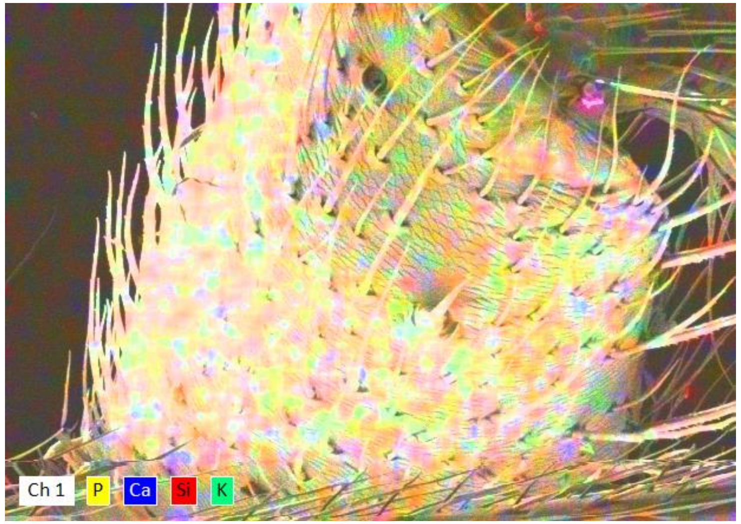


Low X-Ray yield



SEM-EDS analysis common challenges
Sample charging

Charge accumulation



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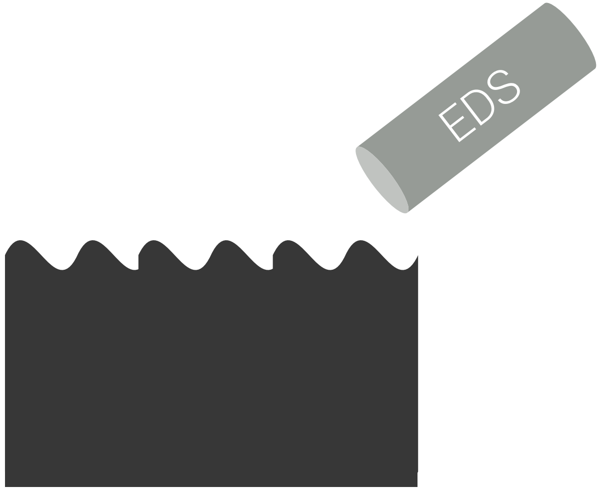

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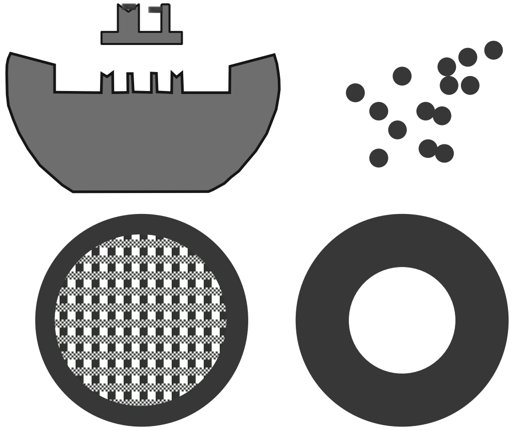

SEM-EDS analysis common challenges
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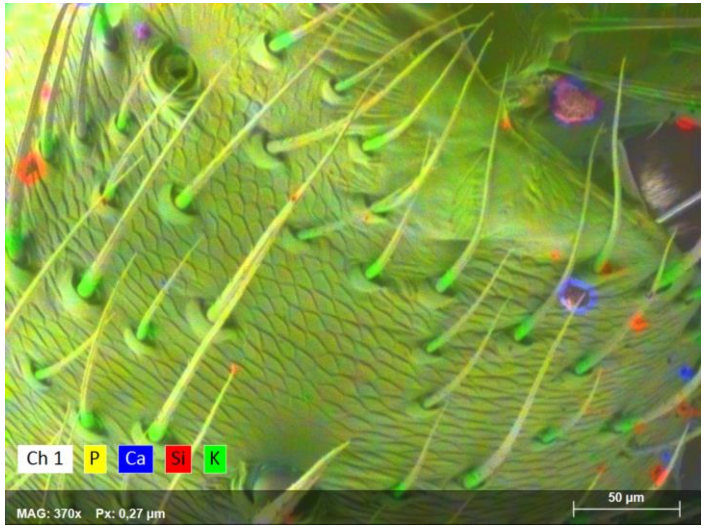
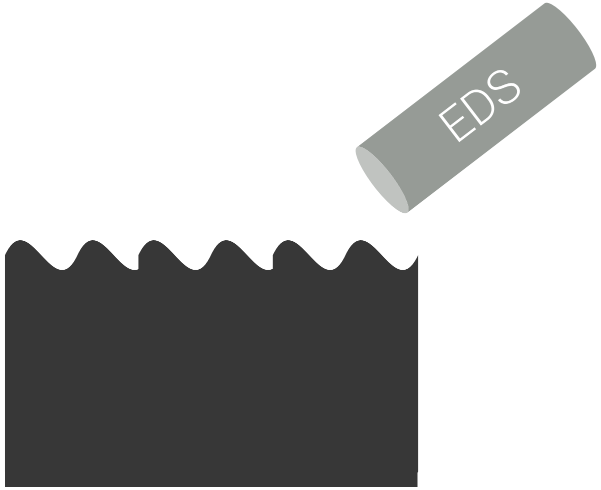
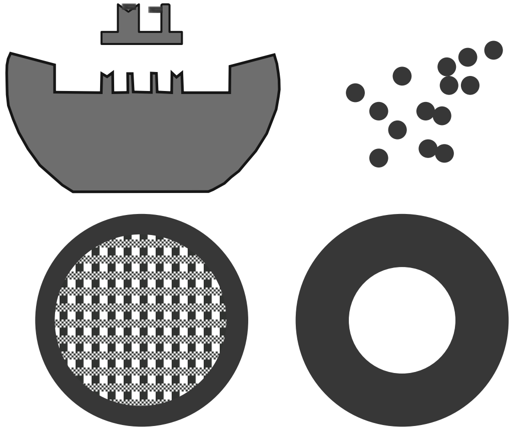
Low X-Ray yield

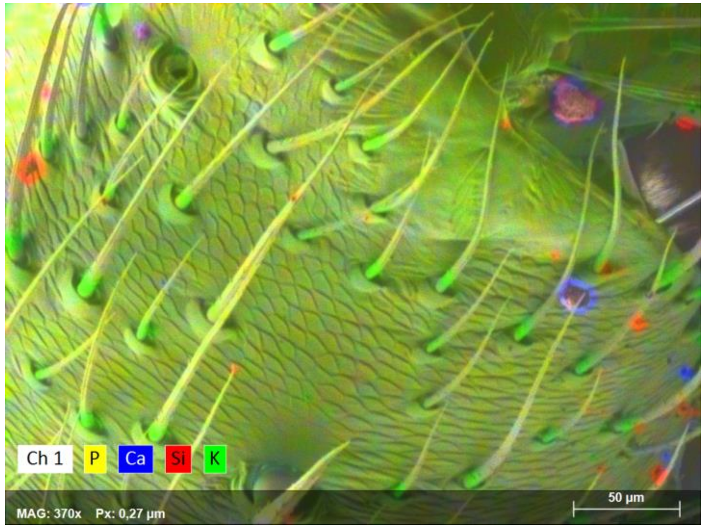






SEM-EDS analysis common challenges
Sample charging

Charge accumulation



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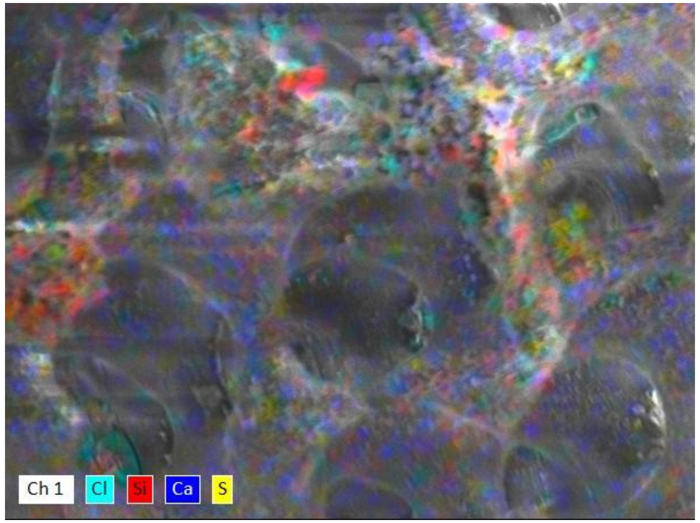

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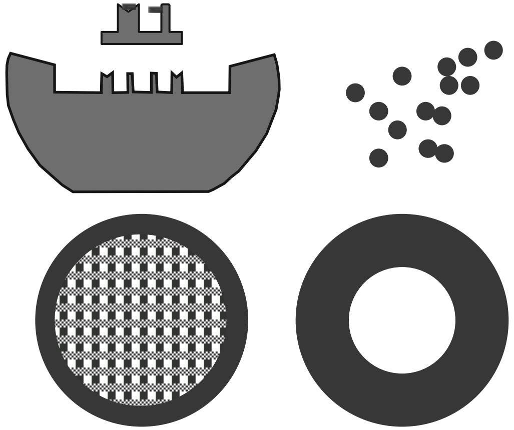
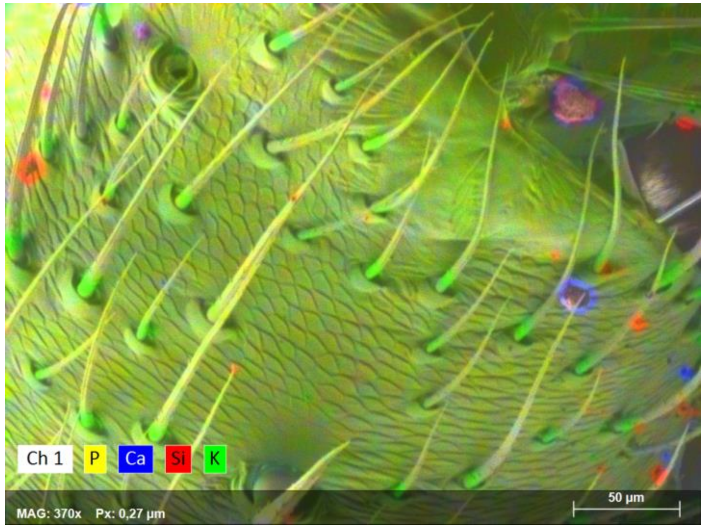


SEM-EDS analysis common challenges
Topography

Charge accumulation



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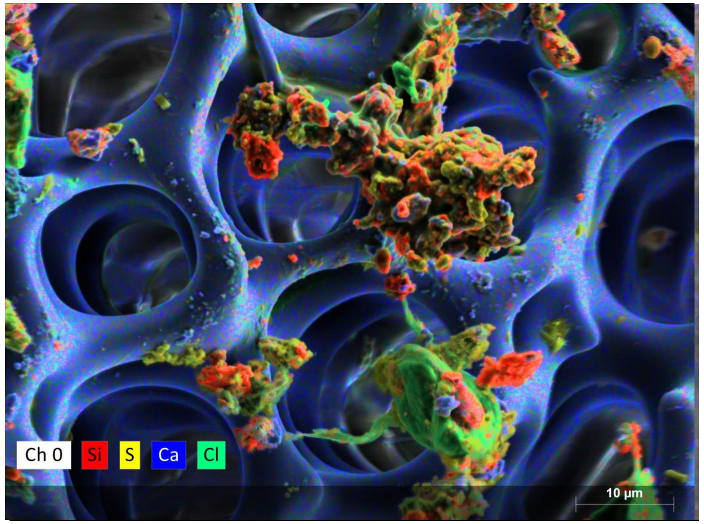
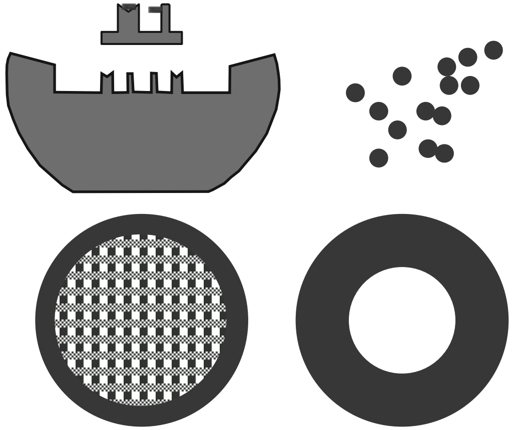
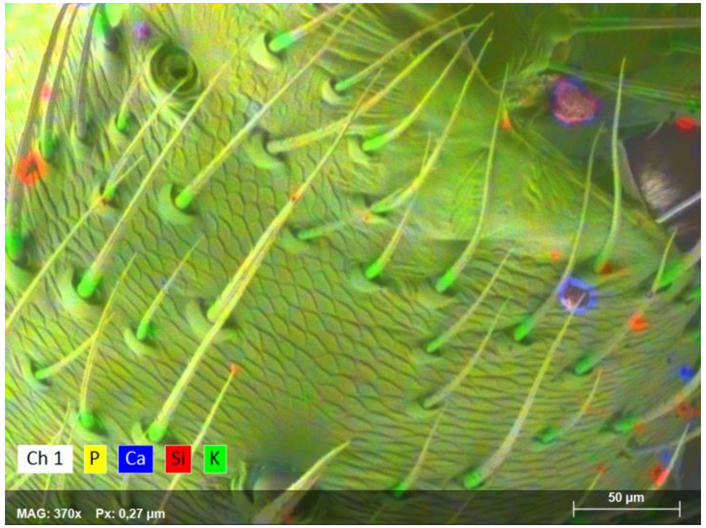


Low X-Ray yield



SEM-EDS analysis common challenges
Low X-ray yield

Charge accumulation



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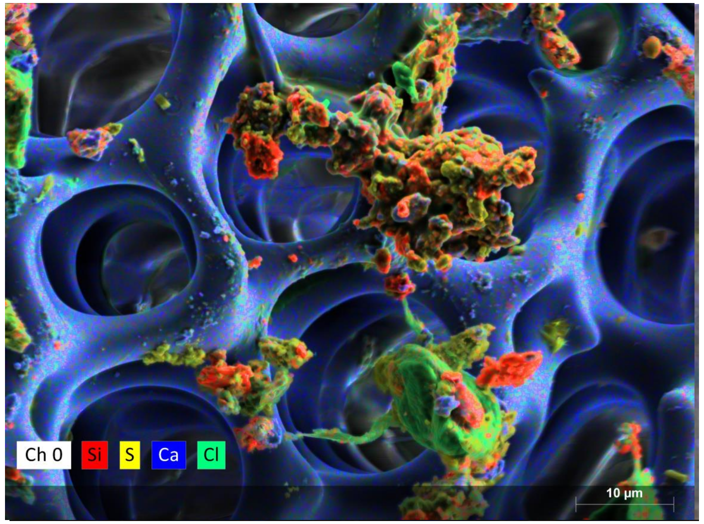

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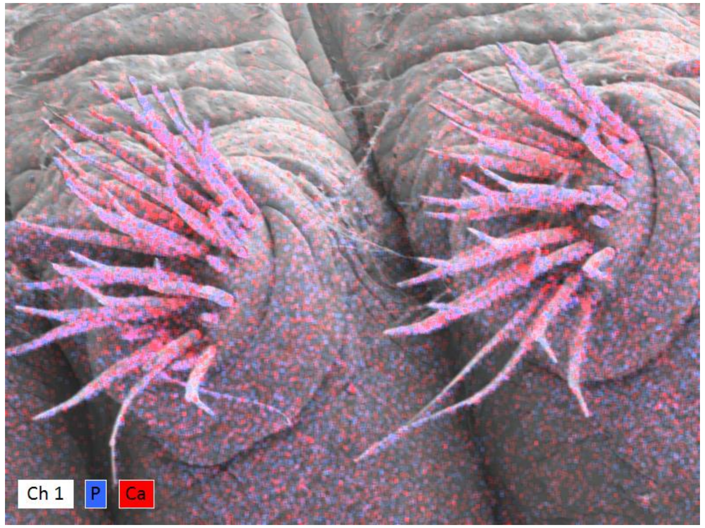
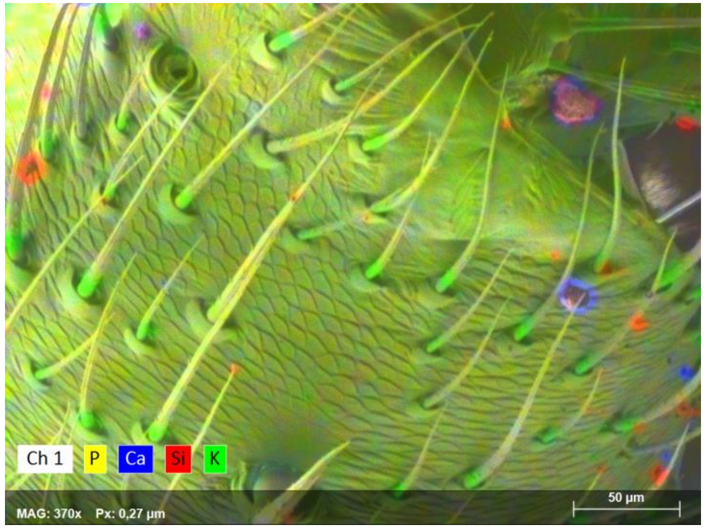


SEM-EDS analysis common challenges

Charge accumulation



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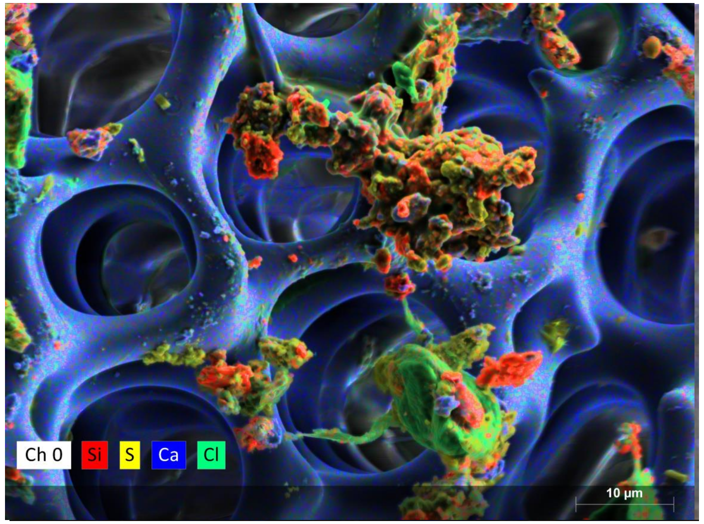
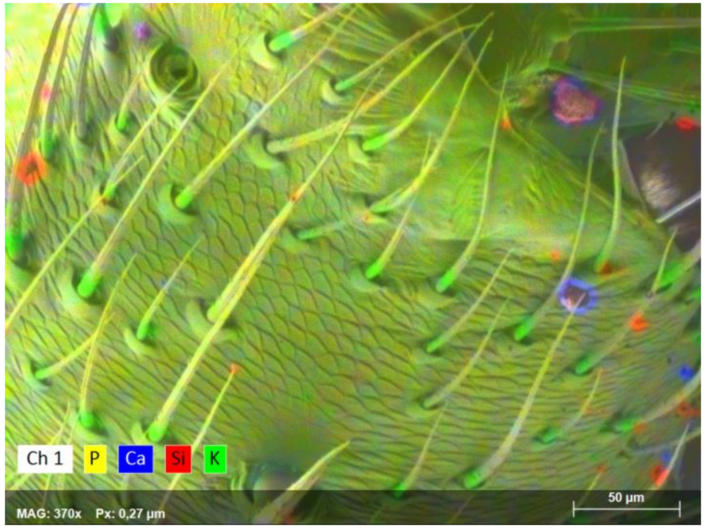


Low X-Ray yield

SEM-EDS analysis common challenges

How can we solve these challenges?

Charge accumulation



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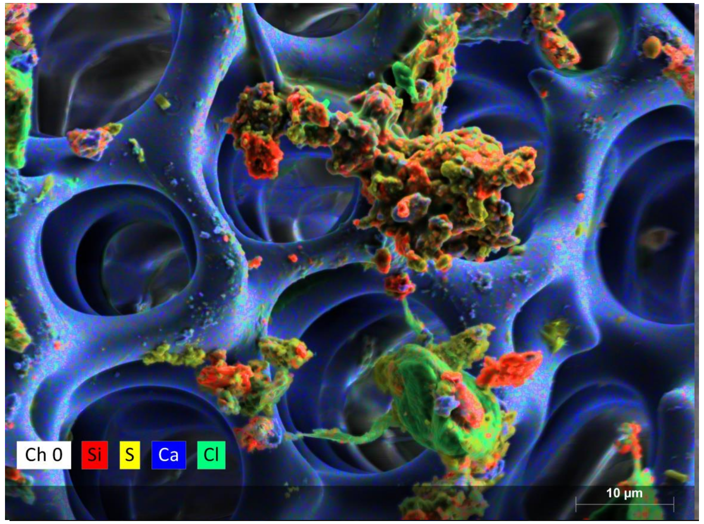
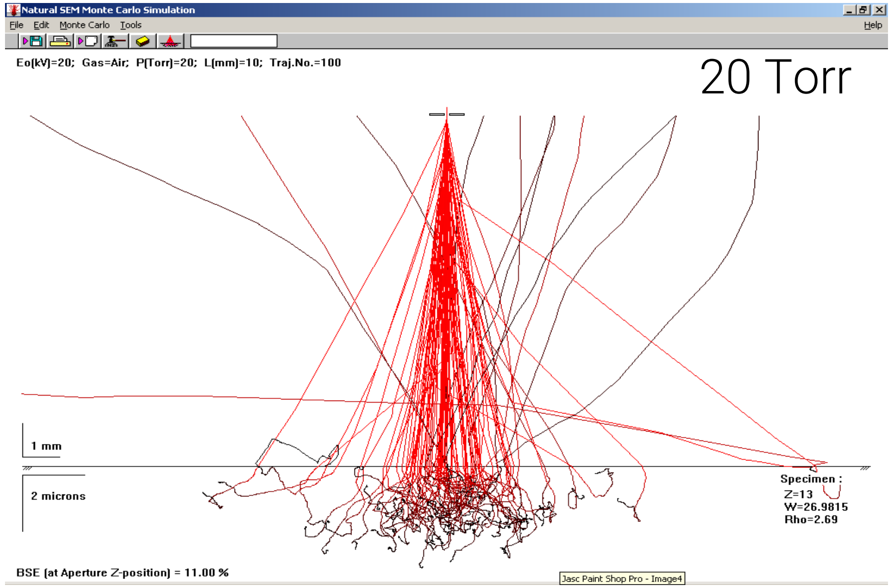
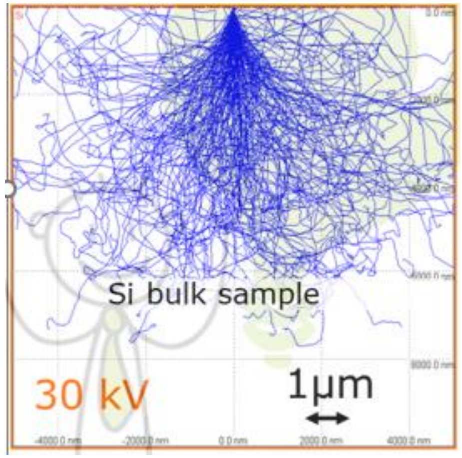
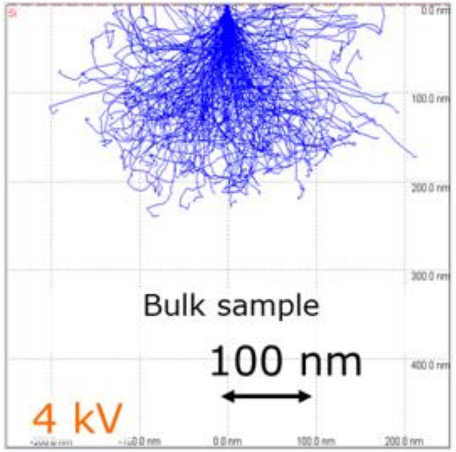


Low X-Ray yield

SEM-EDS analysis common challenges
SEM parameters we can change





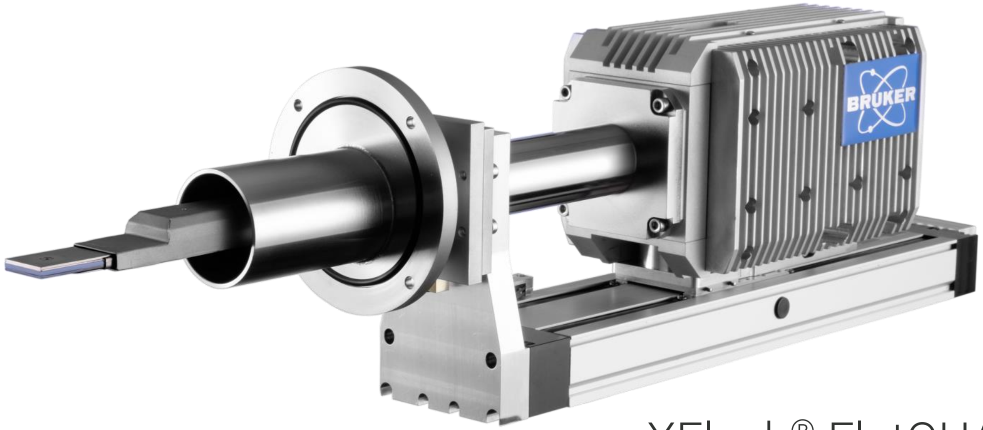


- **Low pressure**
  - Reduce charging
  - Beam skirting effects
  - Reduce the spatial resolution
  - Contributions from environmental gas

- **Low kV and low beam current:**
  - Reduce charging
  - Protects beam sensitive samples
  - Spatial resolution reduced
  - Leads to low X-ray count rates
  - Longer measurement time
  - More overlapping peaks
  - Background subtraction more difficult

SEM-EDS analysis common challenges
Possible solutions

Possible solution with existing Hardware on the SEM!



XFlash® FlatQUAD

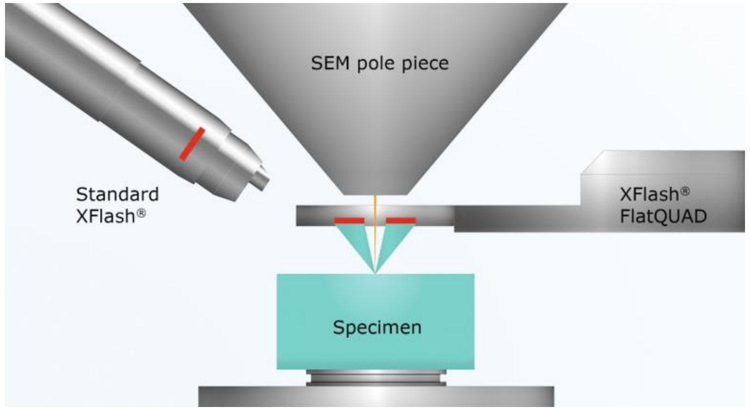
➔ Multiple EDS detectors (double detector system)

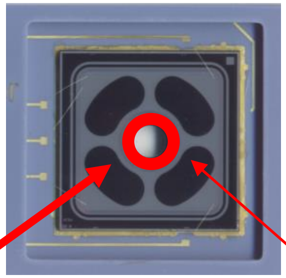
➔ XFlash® FlatQUAD detector (4 detectors in 1)

➔ XFlash® FlatQUAD inserted below pole piece -> high solid angle/collection efficiency

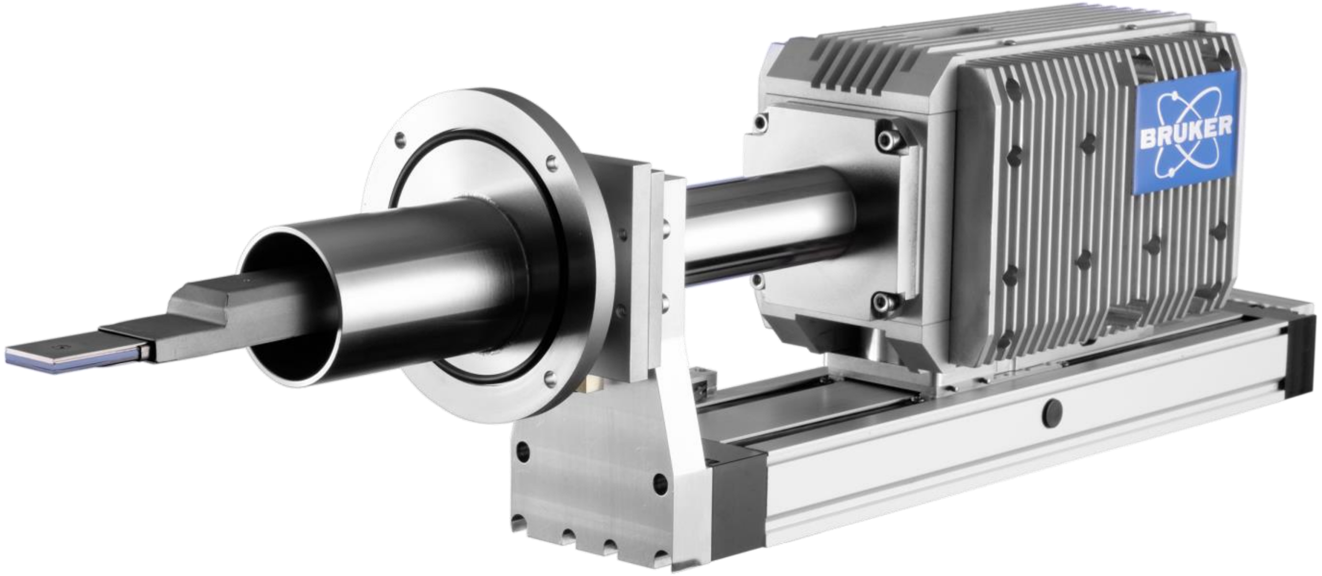
4-segment annular EDS detector - XFlash® FlatQUAD

Annular design with side entry





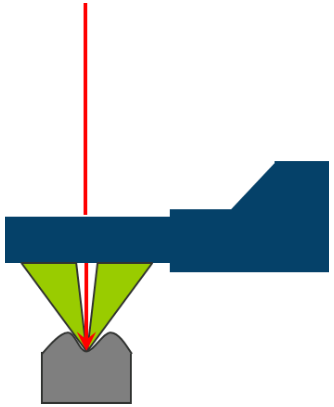
- Hole in the center for the e-beam surrounded by the 4 segments

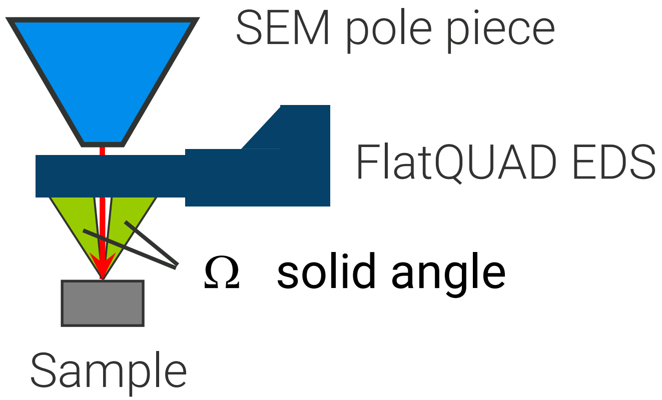


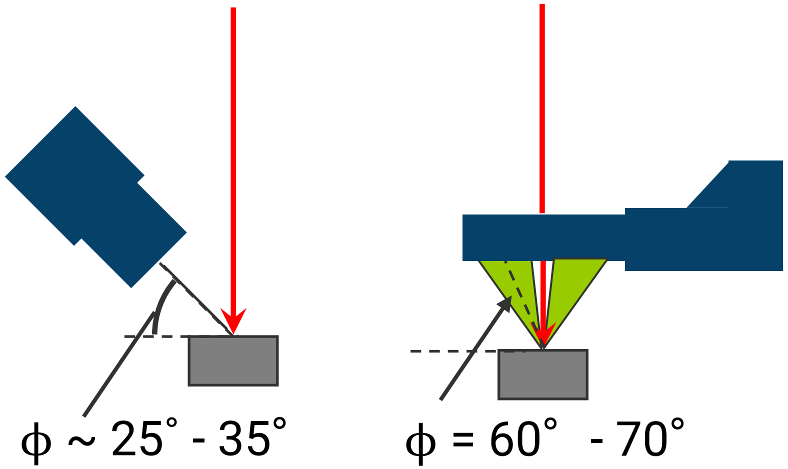
- Side entry – Similar to STEM or retractable BSE
- EDS detector under the polepiece above the sample
- Optimal geometry for signal collection
- Up to 2.400.000 cps throughput

XFlash® FlatQUAD

Geometrical features compared to conventional EDS detector







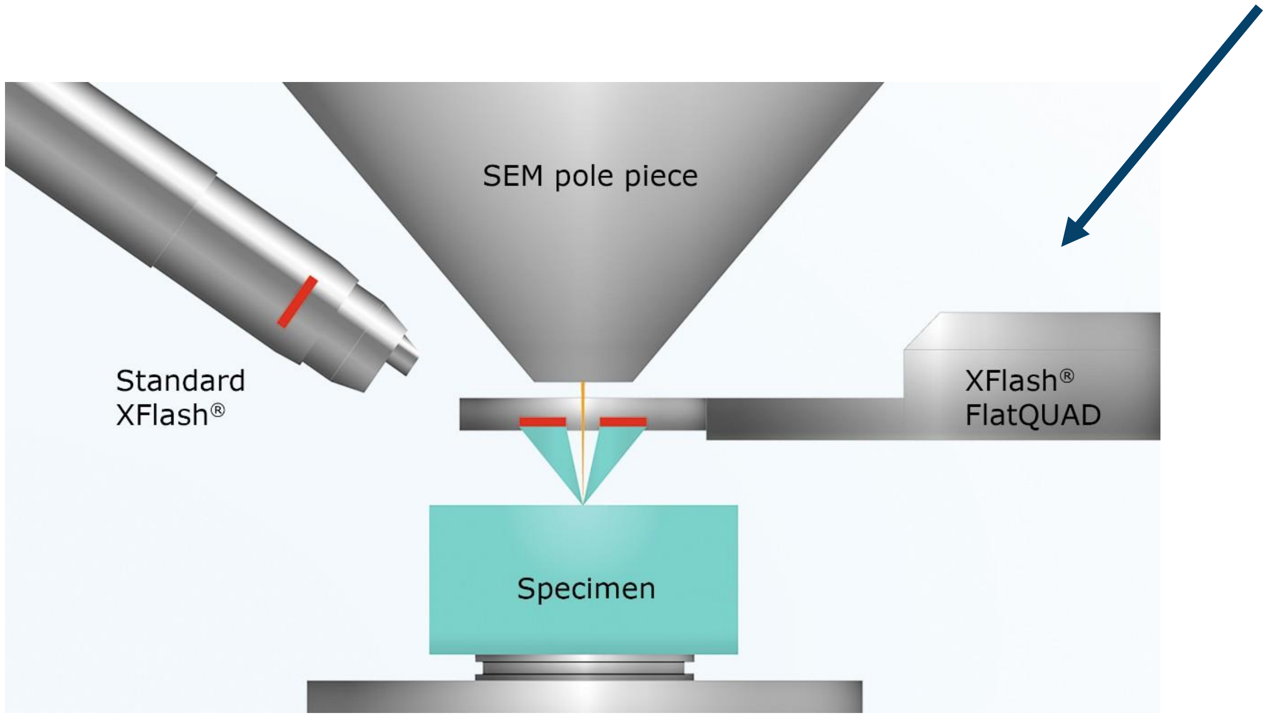
- Shadowing minimized for topographic samples
- Highest solid angle (up to 1.1 sr)
- High take-off angle (~60˚-70˚)

XFlash® FlatQUAD
Analytical advantages

- High sensitivity, high signal – low noise
- High count rate at low kV and low beam currents
- No/low shadowing for topographic samples
- Full quantitative mapping and quantification from each pixel in the map is possible

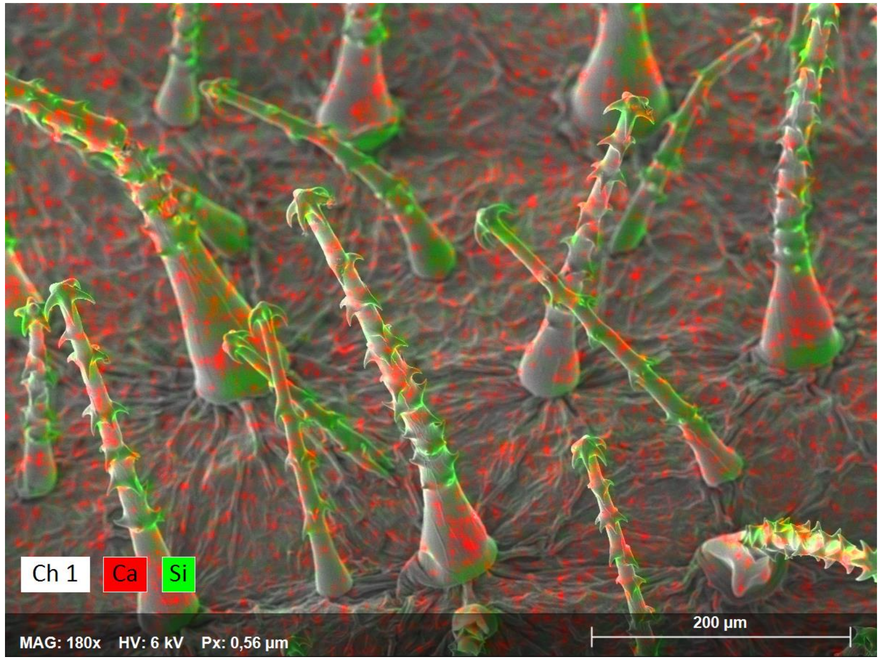
Conventional detector vs. XFlash® FlatQUAD

Conventional inclined detector vs. XFlash® FlatQUAD



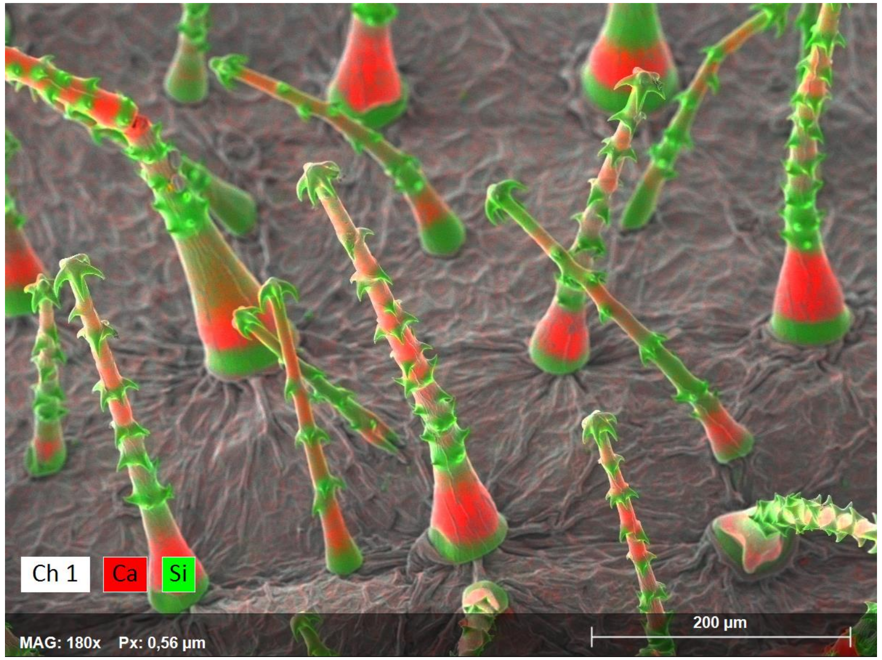
XFlash® FlatQUAD – vs. Conventional detector
Aosa rupestris (Asteraceae family)

Conventional EDS detector



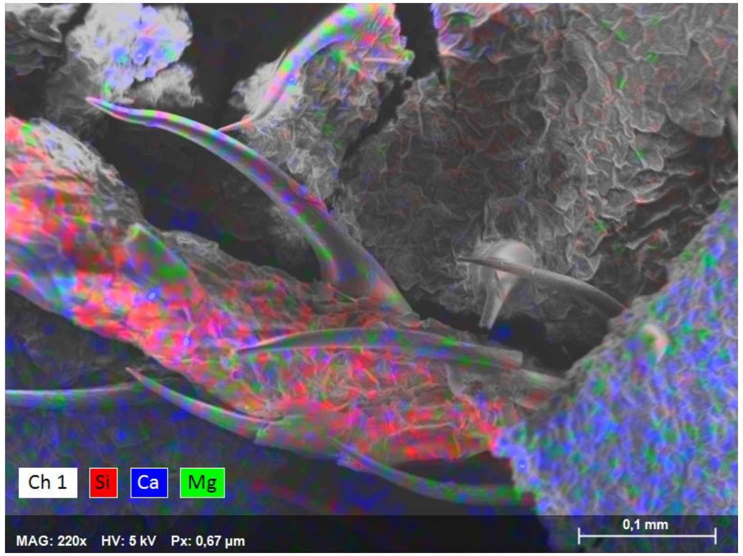
- 6 kV / Input count rate: 4,700 cps
- Pd coated
- Shadow effects

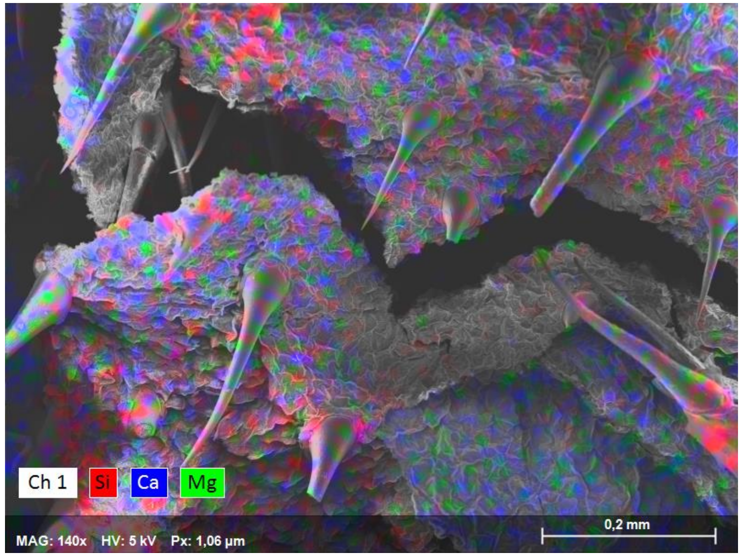
XFlash® FlatQUAD

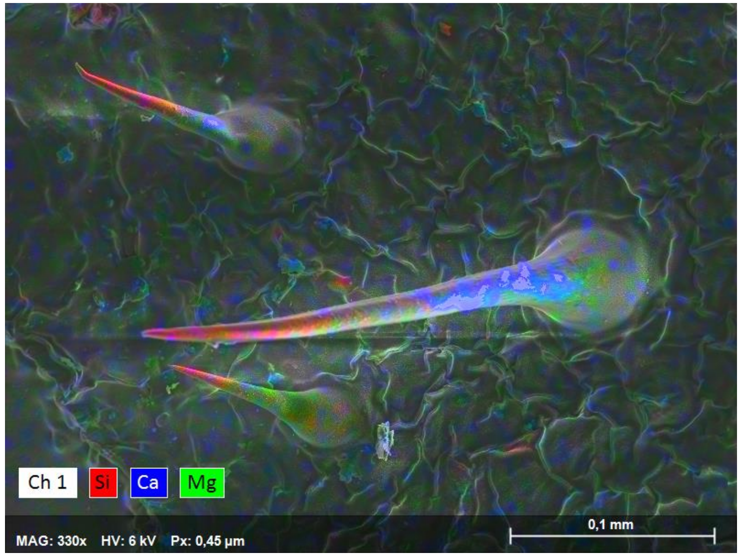


- 6 kV / Input count rate: 58,700 cps
- Pd coated
- No shadow effects

Conventional EDS detector
Sting nettle plant (Urtica sp.)





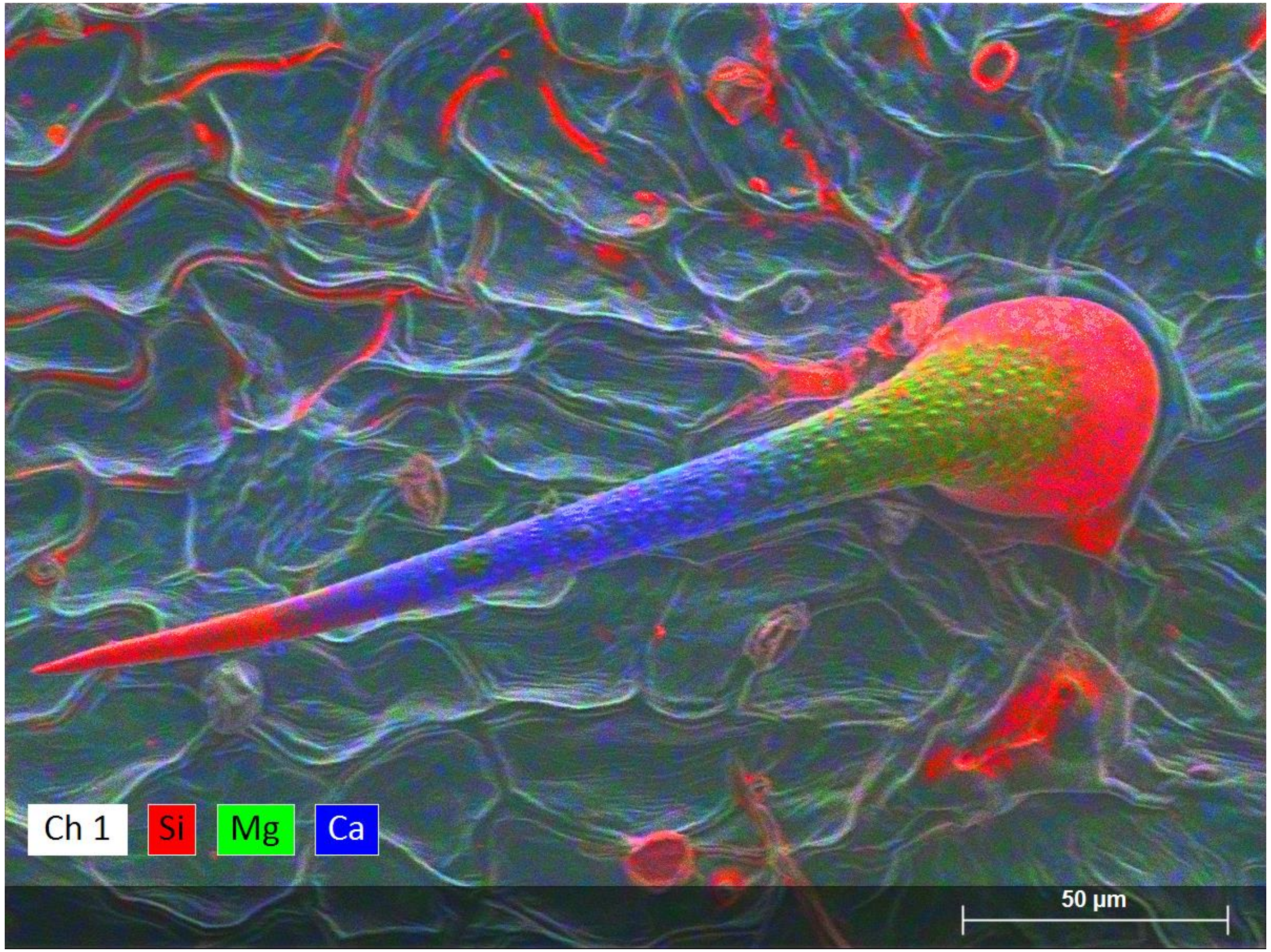


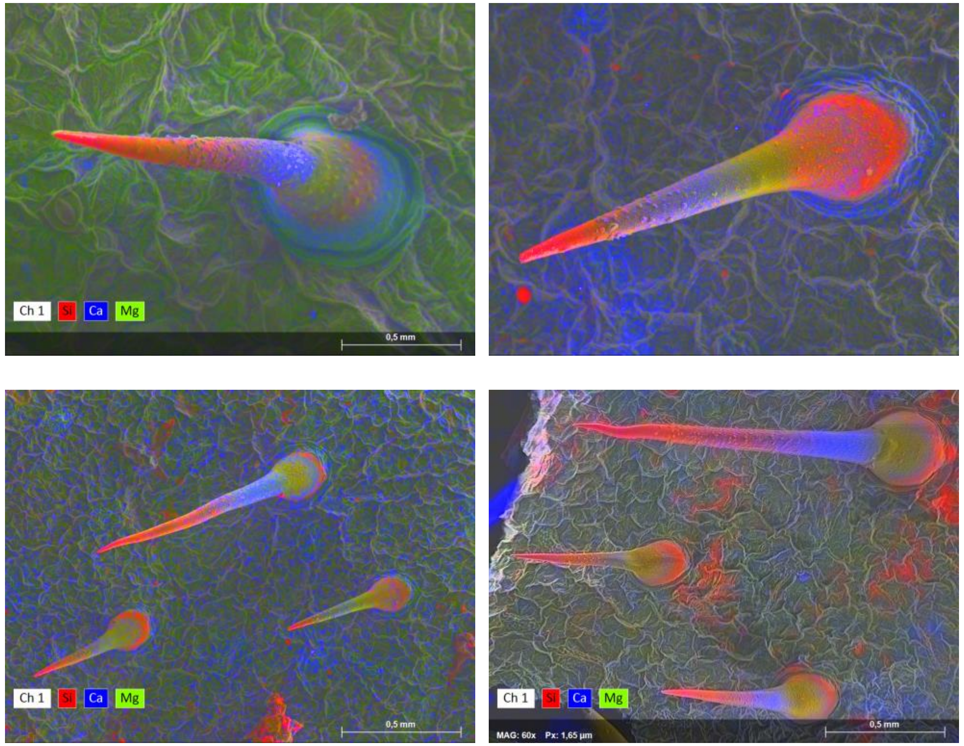
- Mapping parameters
  - 5-6kV
  - Measurement time: ~10min

- Conventional EDS detector shows shadowing, charging and low input count rate

XFlash® FlatQUAD

Sting nettle plant (Urtica sp.)



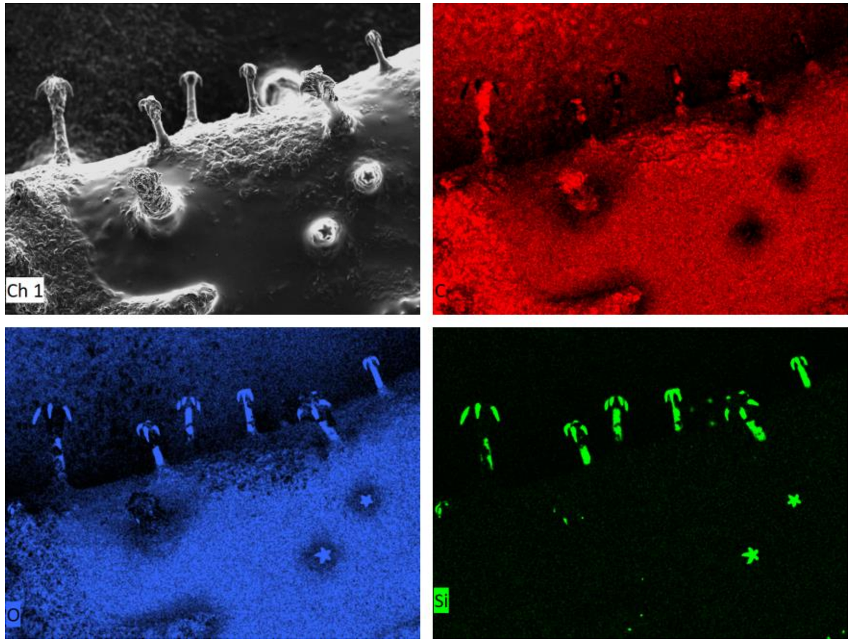


- Mapping parameters
  - 5-6 kV
  - Measurement time: 2-20 min

Topographic, beam sensitive samples measured with XFlash® FlatQUAD

Biological samples measured with XFlash® FlatQUAD

Aosa rupestris (Asteraceae family)
Flowering nettle family



- Mapping parameters:
  - 6 kV
  - Beam current ~180 pA
  - Magnification: 168
  - Pixel spacing: 0,97µm

Sample curtesy of University Bonn

UNIVERSITÄT BONN

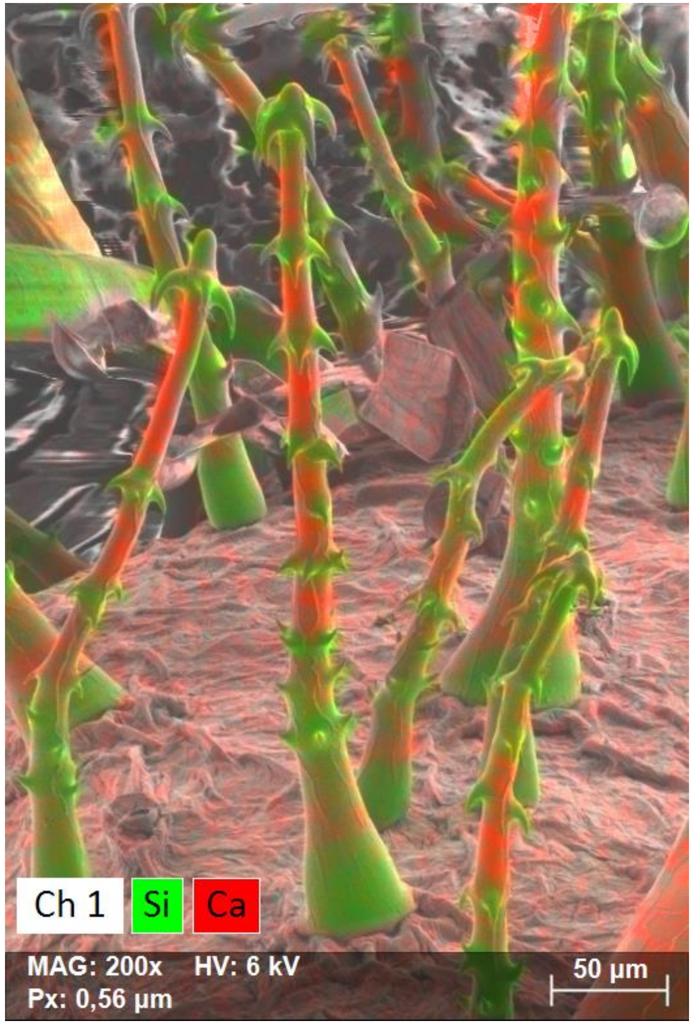
Aosa rupestris (Asteraceae family)
Flowering head of Aosa rupestris

- Mapping parameters:
  - 6 kV
  - Beam current ~80 pA
  - Image 1200x900 pixel
  - Pd coated
  - Magnification: 180
  - Pixel spacing: 1,5µm

Sample curtesy of University Bonn

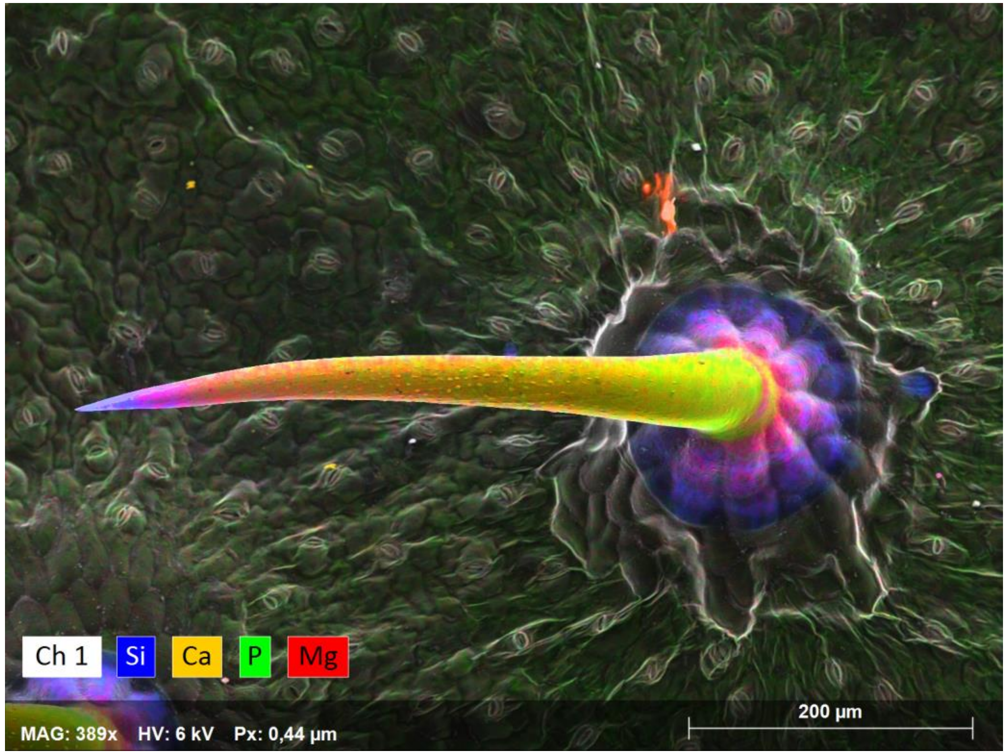
UNIVERSITÄT BONN

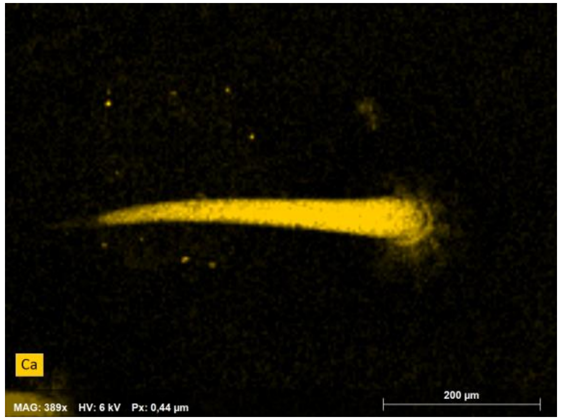
Aosa rupestris (Asteraceae family)
Mineralization of silicon, calcium and phosphorus

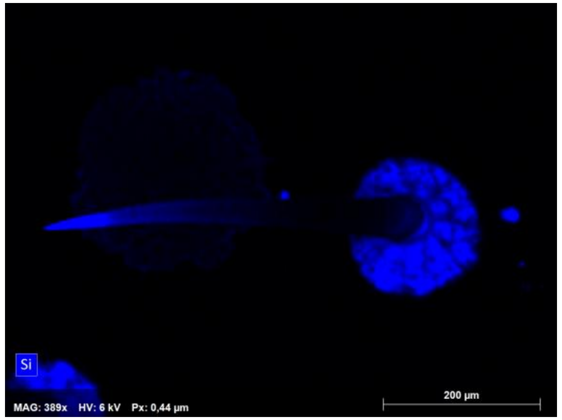


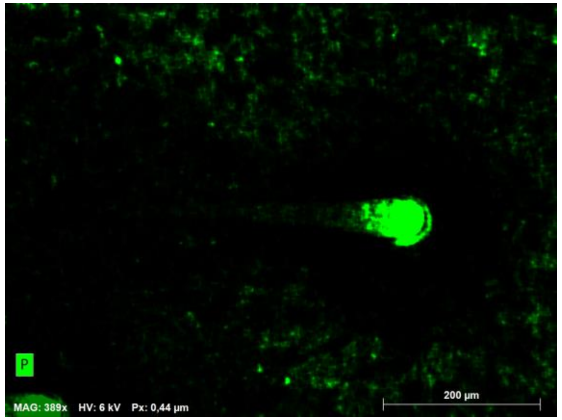
- Mineralization of silicon, calcium and phosphorus

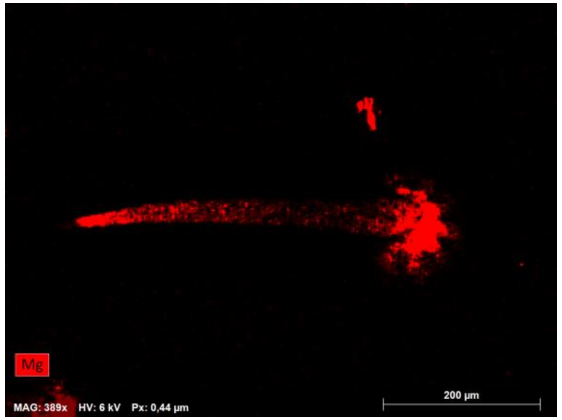
Echium hypertropicum
Exhibits mineralized hairs fresh on cooling stage









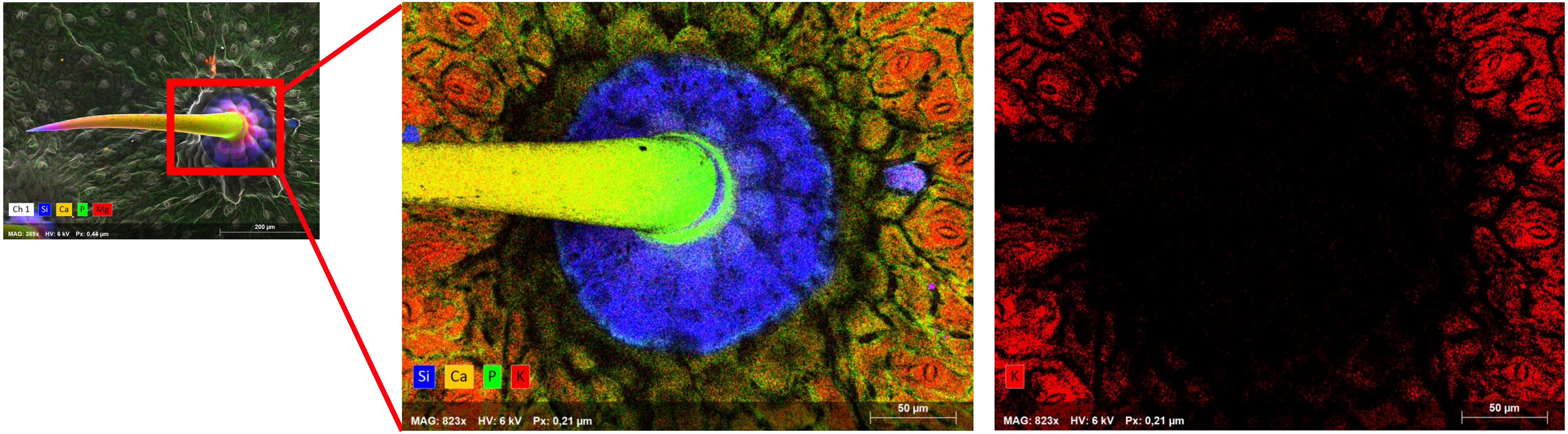


- Mapping parameters:
  - 6 kV
  - Image resolution 1600x1200 pixel
  - Magnification: 389
  - Pixel spacing: 0,4 µm
  - Time: 155sec

Sample curtesy of University Bonn

UNIVERSITÄT BONN

Echium hypertropicum
Potassium Regulation in Echium hypertropicum

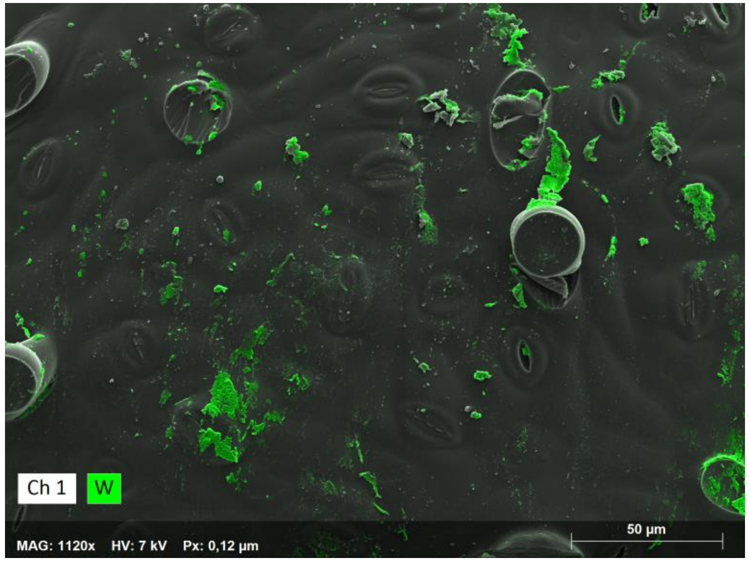


- Mapping parameters:
  - 6 kV
  - Image 1600x1200 pixel
  - Magnification: 823
  - Pixel spacing: 0,2 µm
  - Time: 126 sec.

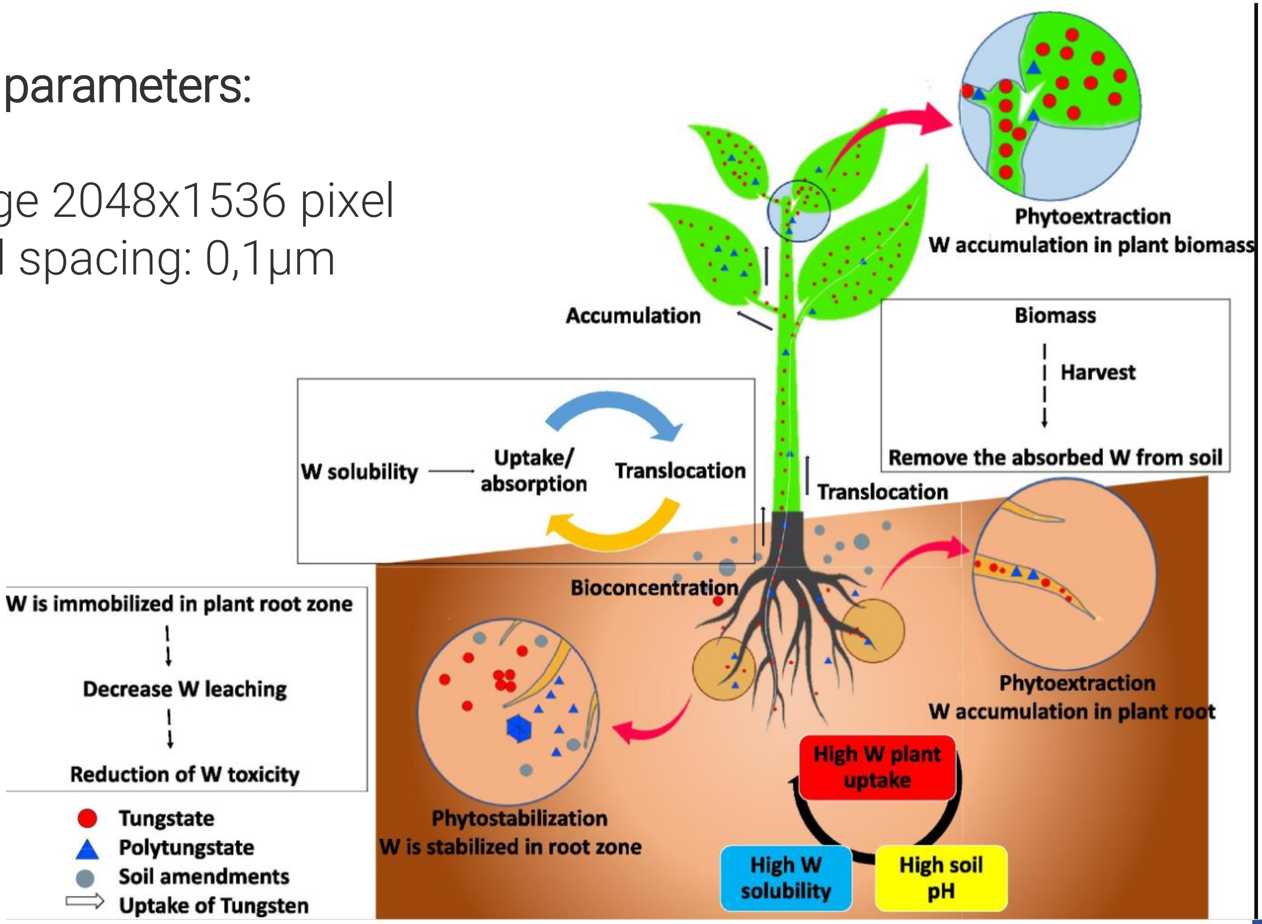
- The concentration of potassium observed around the stomata surrounding the hair, is essential for regulating their opening and closing

Sample curtesy of University Bonn

Leaf replicas made of synthetic resin
Pollution of Tungsten



- Mapping parameters:
  - 7 kV
  - Image 2048x1536 pixel
  - Pixel spacing: 0,1µm



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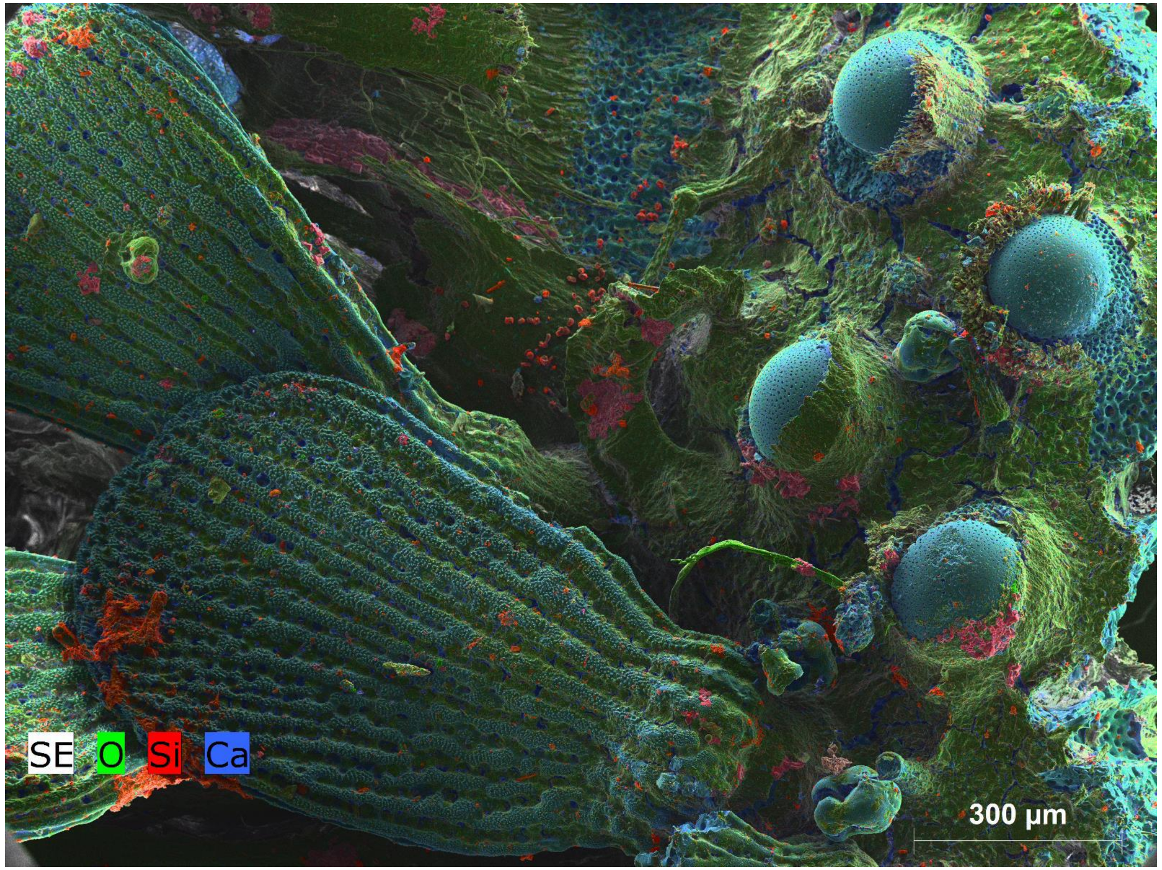
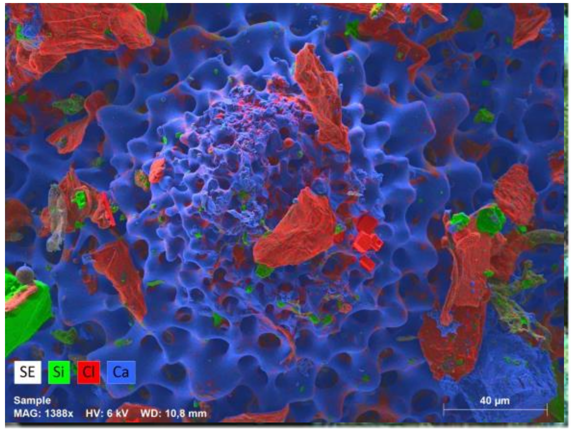
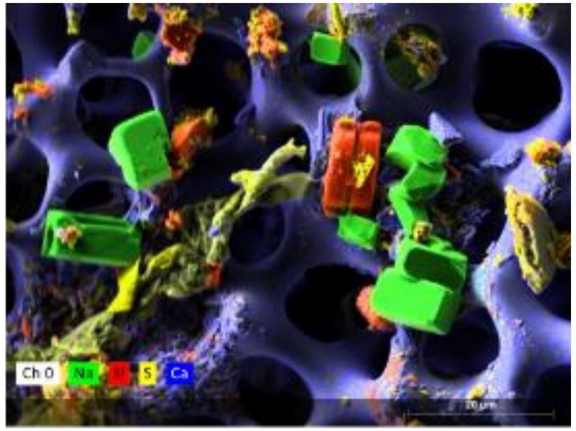
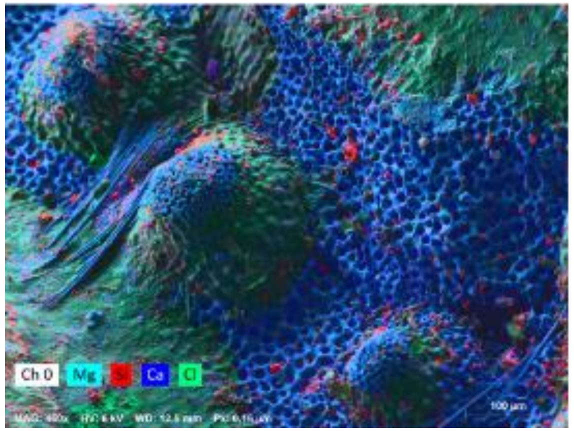
Sample curtesy of University Bonn

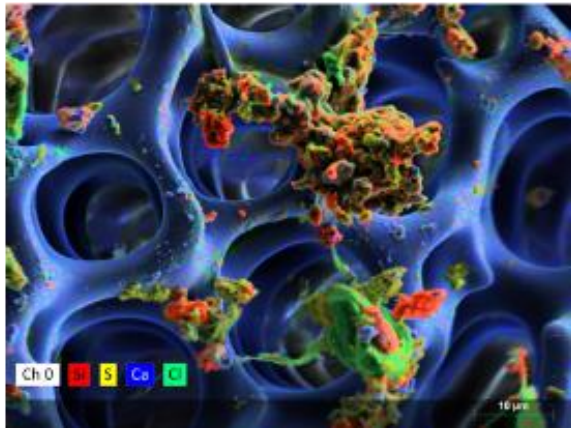
Sea Urchin Skeletons
Biomineralization and Hierarchical Structures





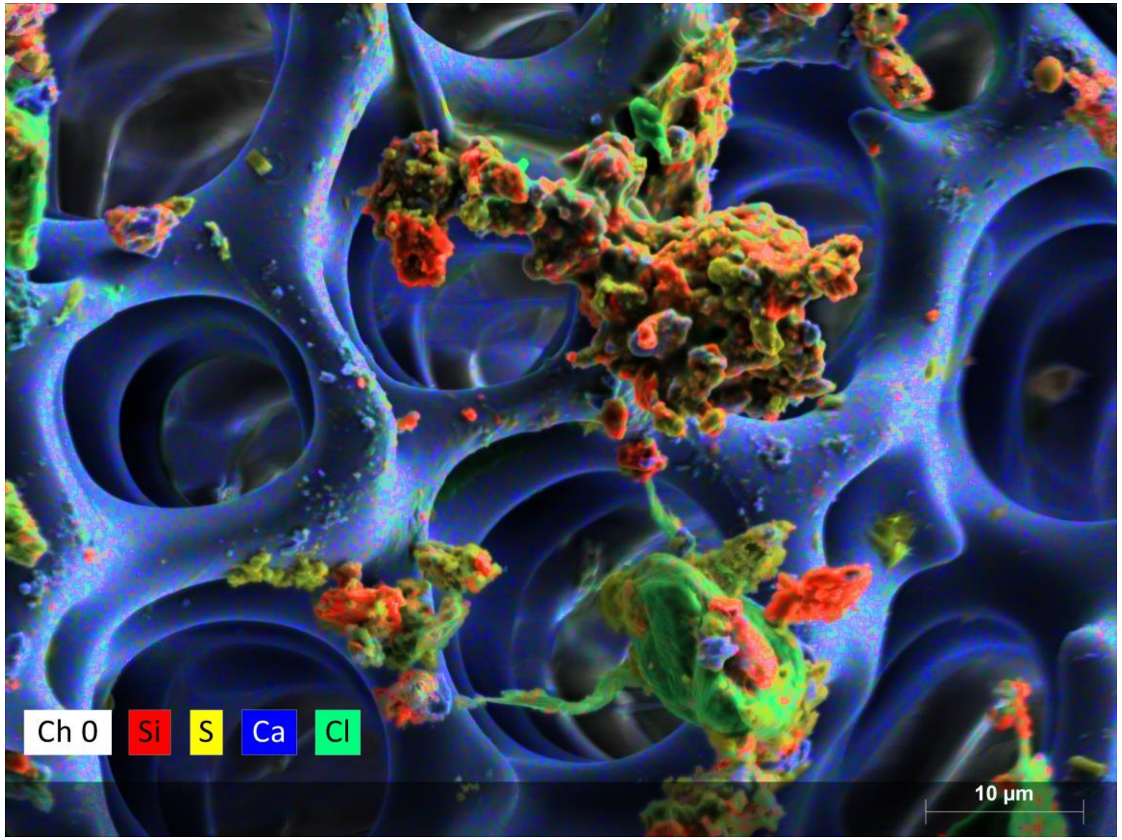


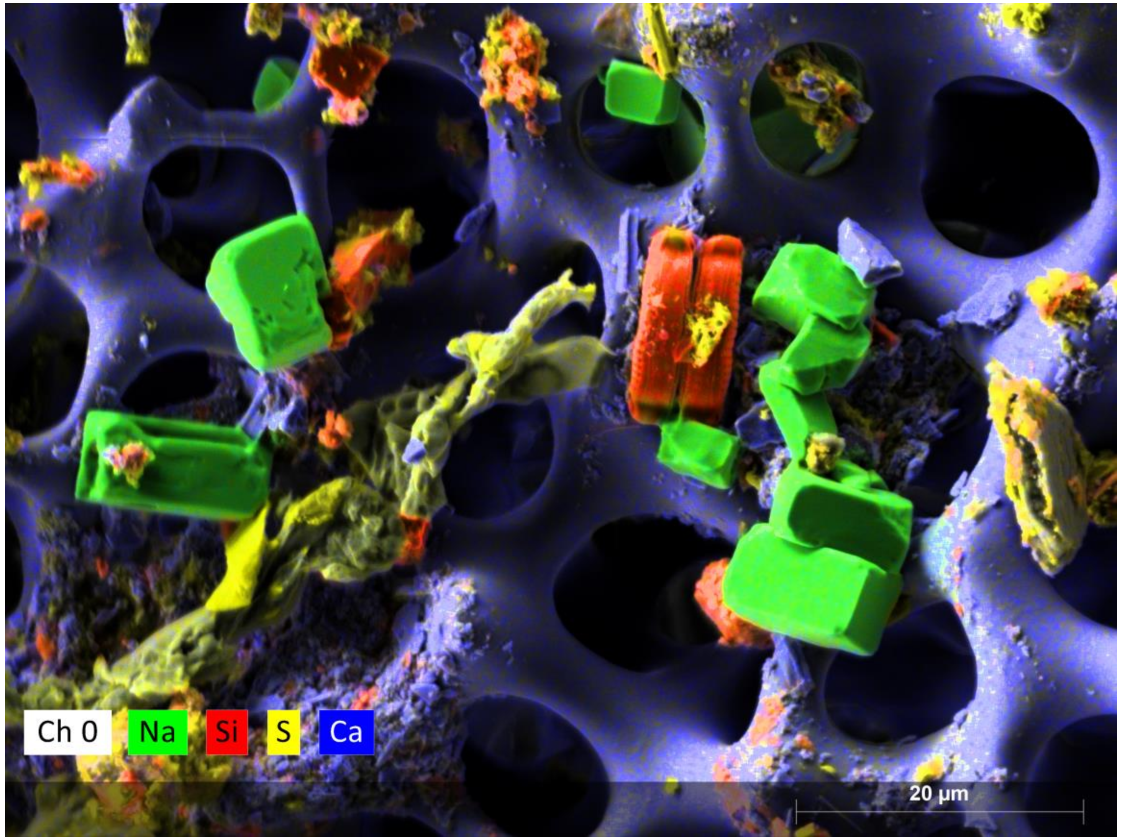




- 6 kV / ~55,000 cps / 51sec measurement time

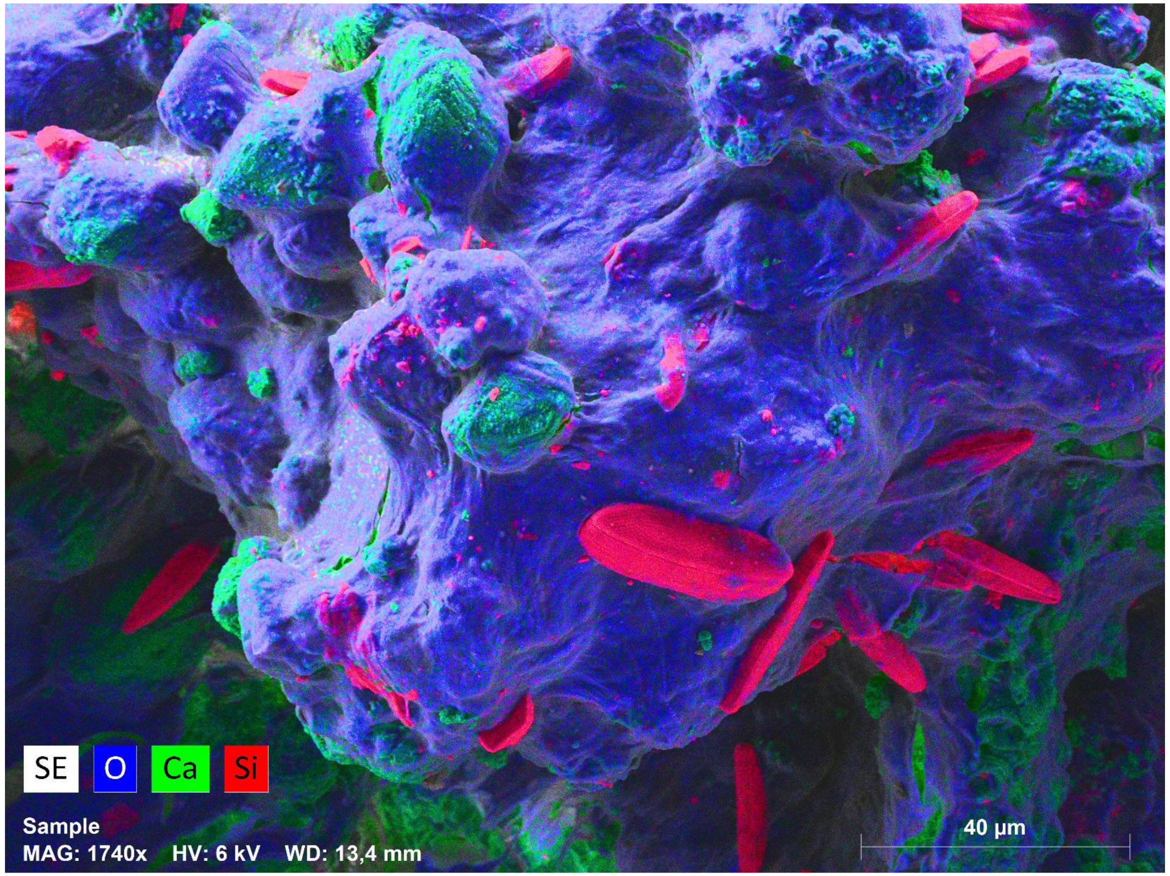
Sea urchin skeleton structure
No Shadowing

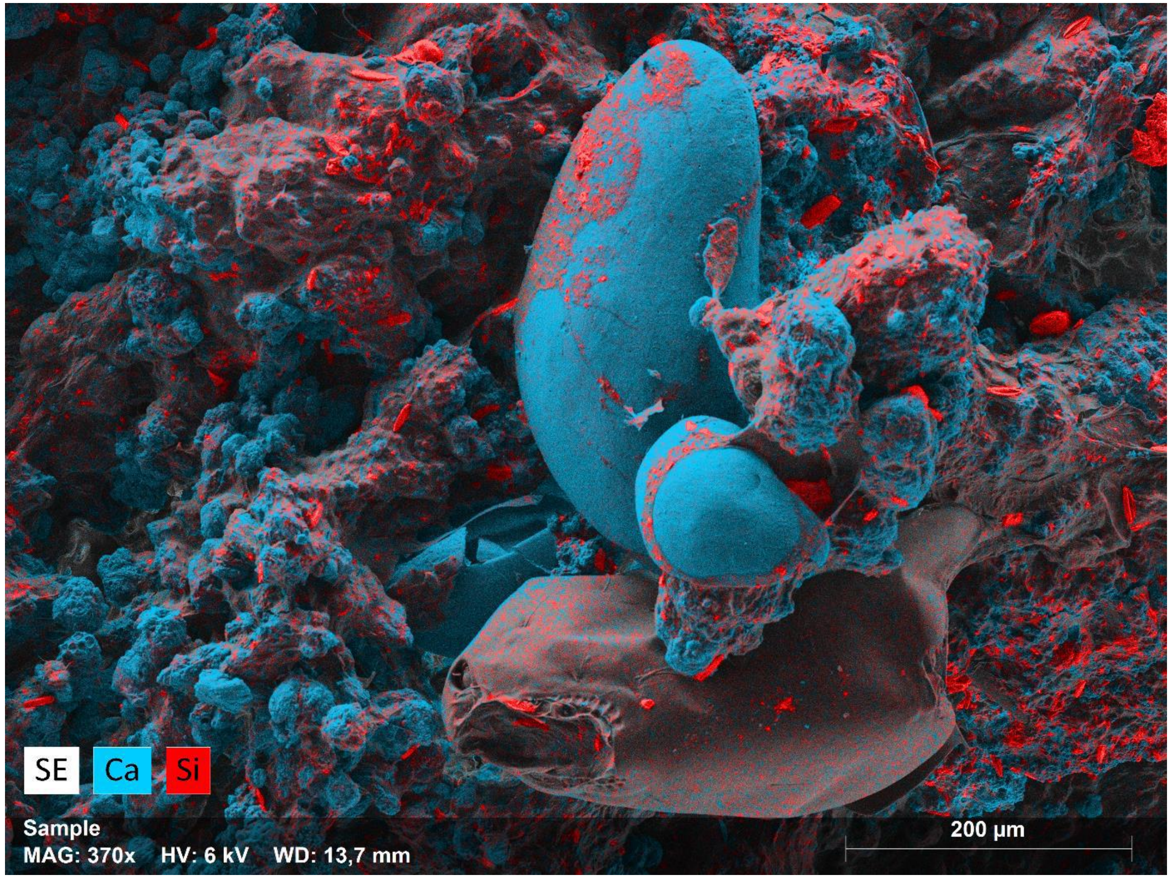




- 6 kV / ~58,000 cps / 51 sec measurement time

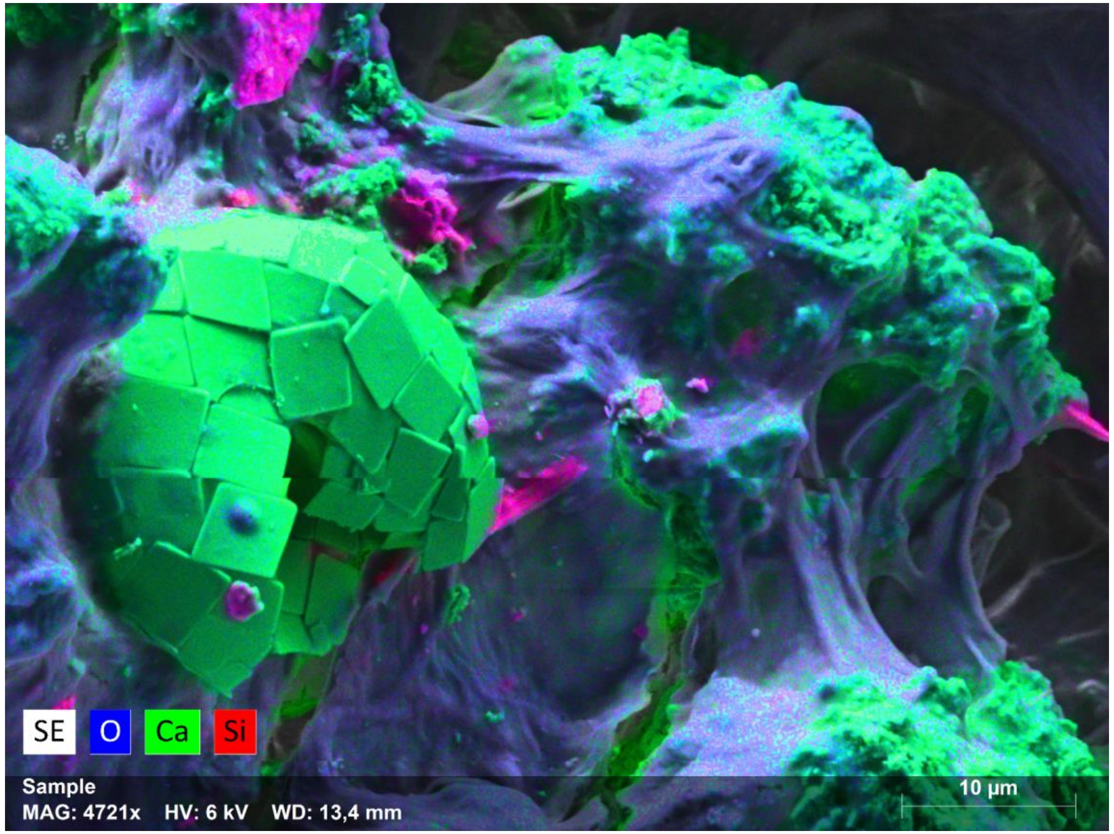
Unveiling Microbial Mat Diversity in Yellowstone's Hot Springs
No coating, no shadowing

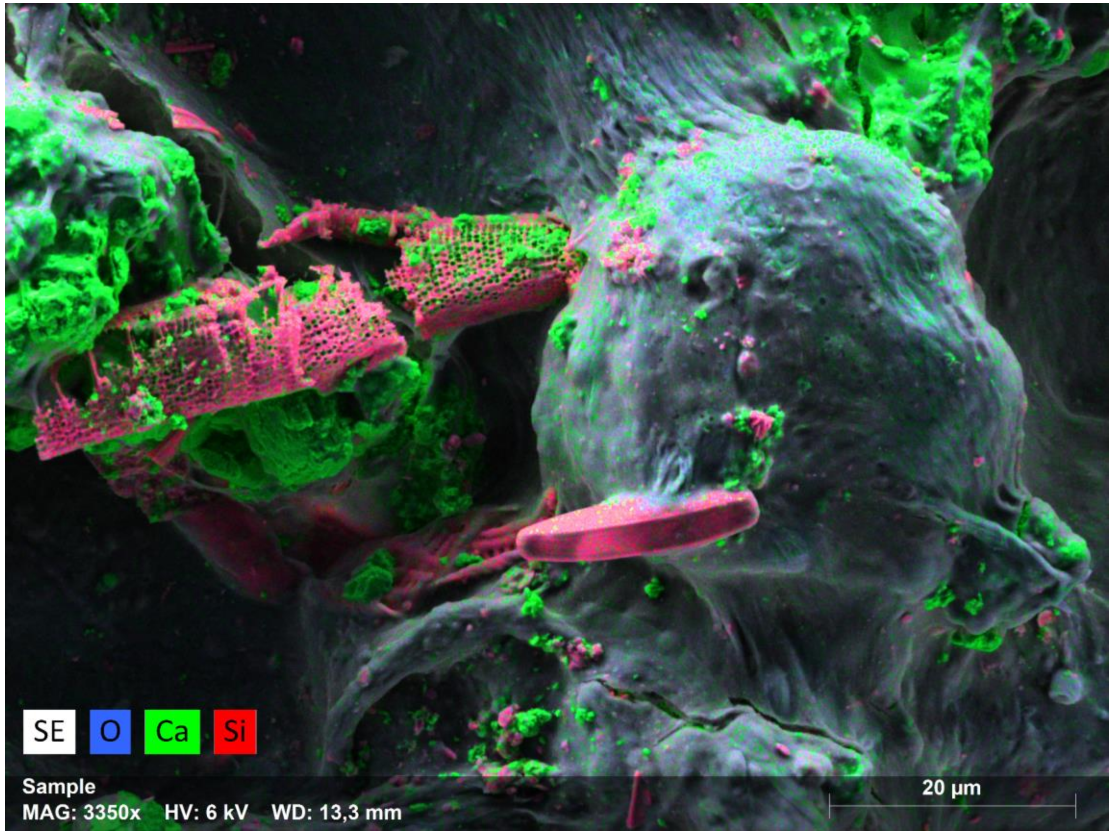




- 6 kV / ~60,000 cps / 51 sec measurement time
- Diatoms (red) sticking to the bluish biofilm matrix

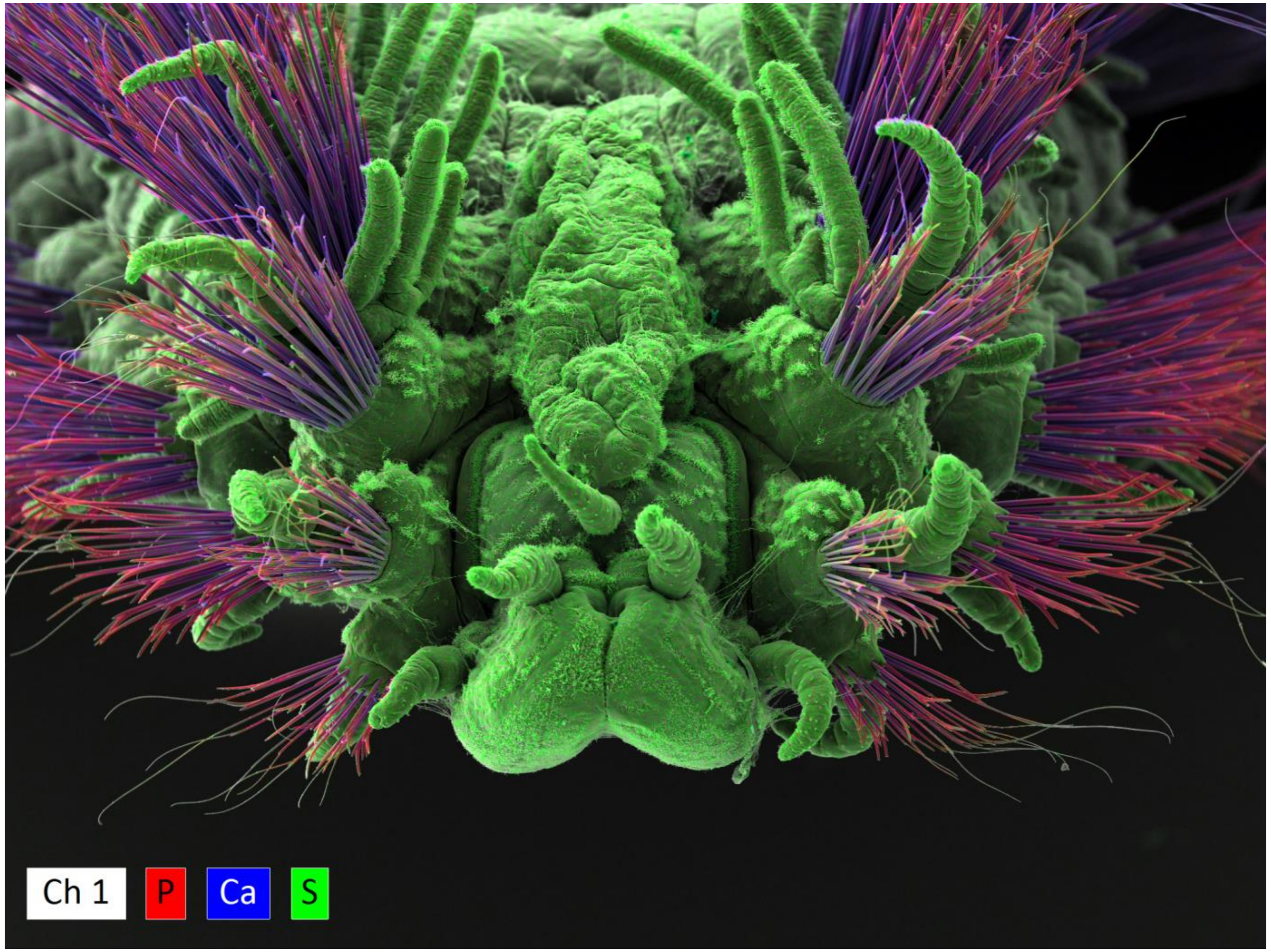
Calcium Carbonate Crystallization in Yellowstone's Microbial Mats
No coating, no shadowing





- 6 kV / ~50,000 cps / 51 sec measurement time,
- No sample preparation like carbon coating

Unlock the secrets of marine life with cutting-edge technology
Head of the polychaeta



- The head of Polychaeta, adorned with its mineralized bristles composed of calcium phosphate,
- These bristles, crafted from calcium phosphate, not only provide structural support to the organism but also serve a vital function in its defense mechanism

- Mapping parameters:
  - 10 kV
  - Image 2048x1536 pixel
  - Magnification: 160
  - Pixel spacing: 0,8 µm
  - Pd coated
  - Time: 176 sec.
  - WD 13 mm

Sample curtesy of University Bonn

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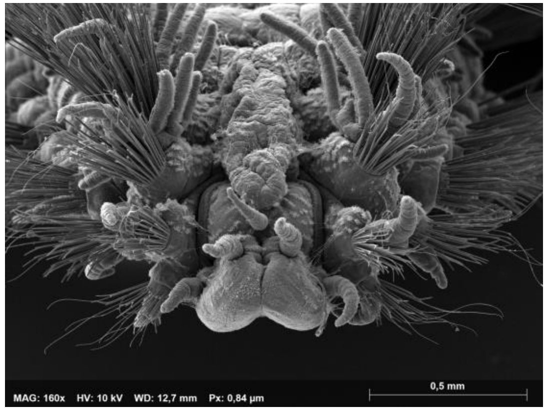
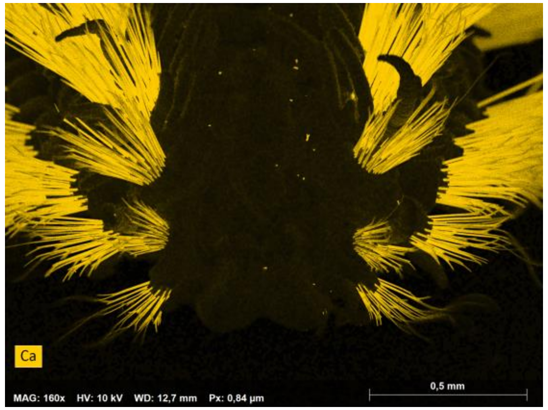
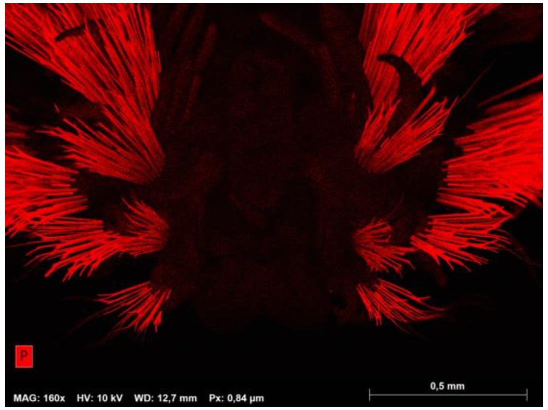
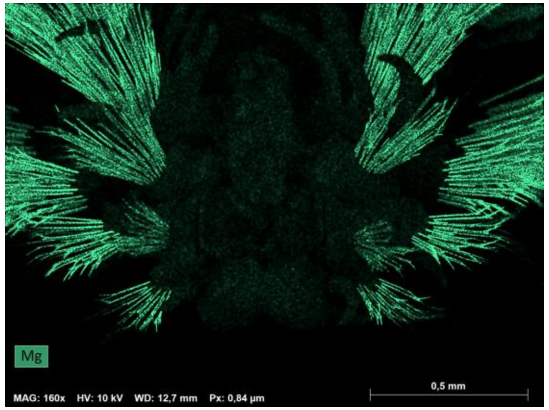
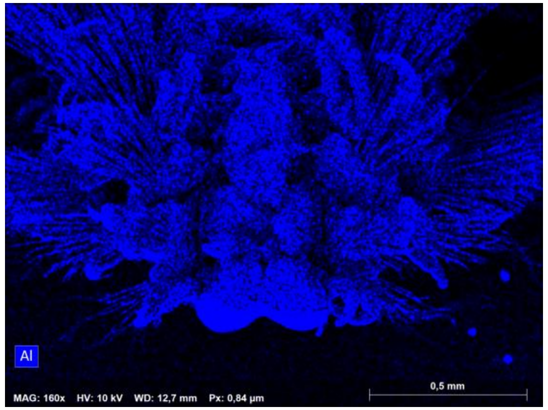
Head of the polychaeta
Single element maps



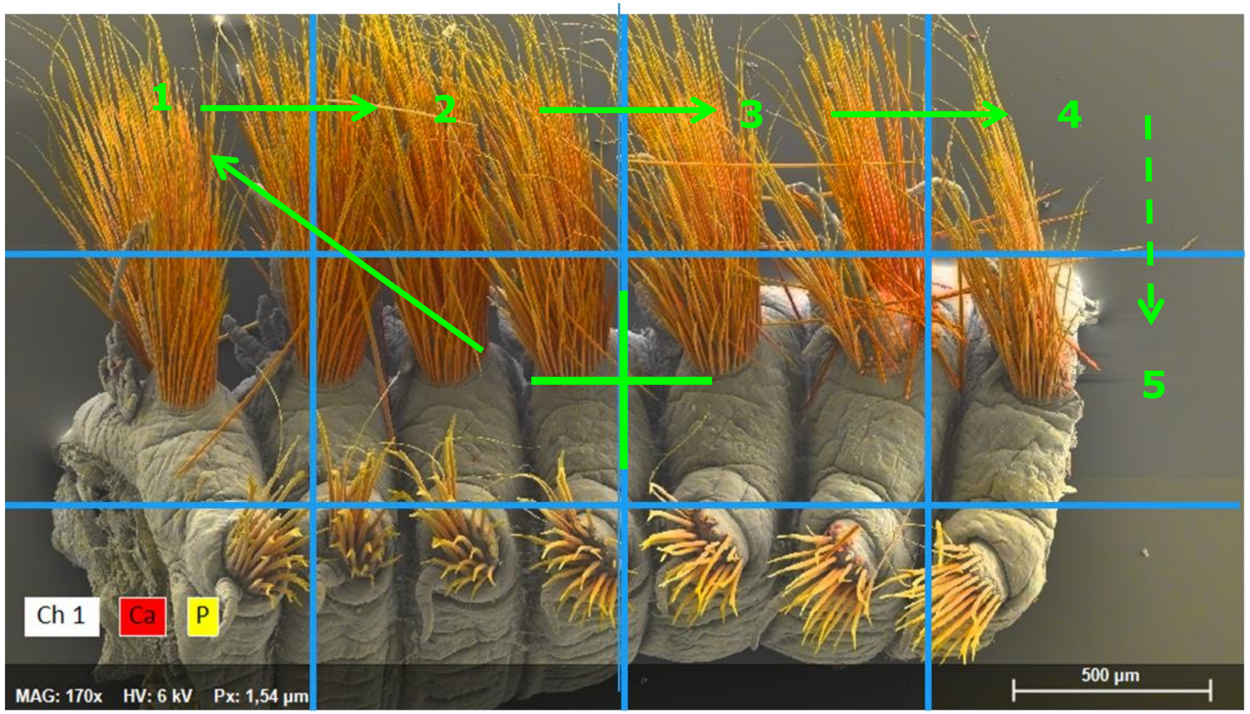


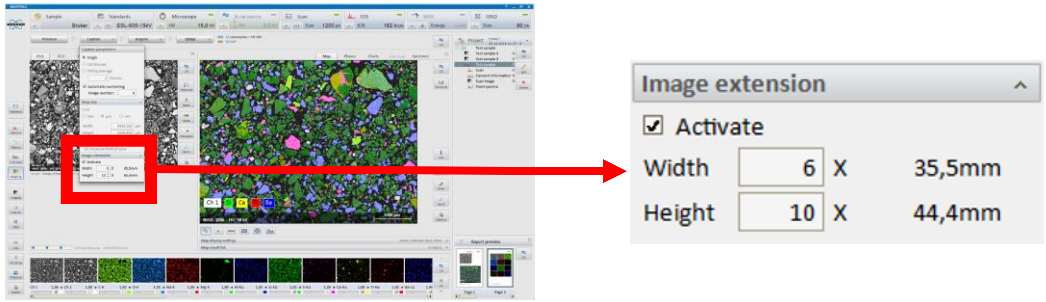






Increase sample measurment area with one click
Image Extension





- **Image Extension**
  - Use actual sample position as central Mapping position and define number of x/y frames around
  - Result: one Hypermap file
  - Image extension can be enlarged for a full sample map

Sample curtesy of University Bonn

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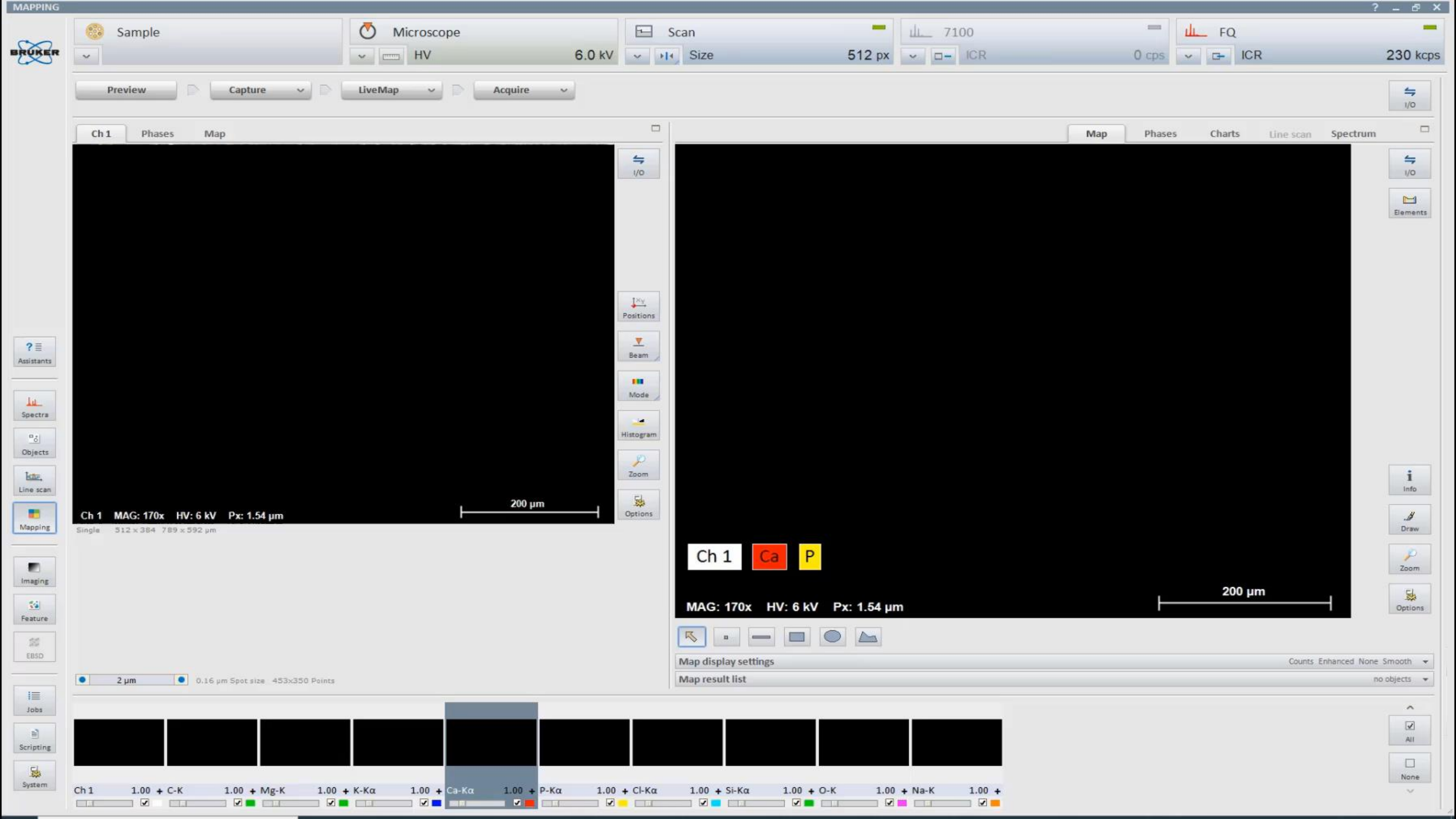
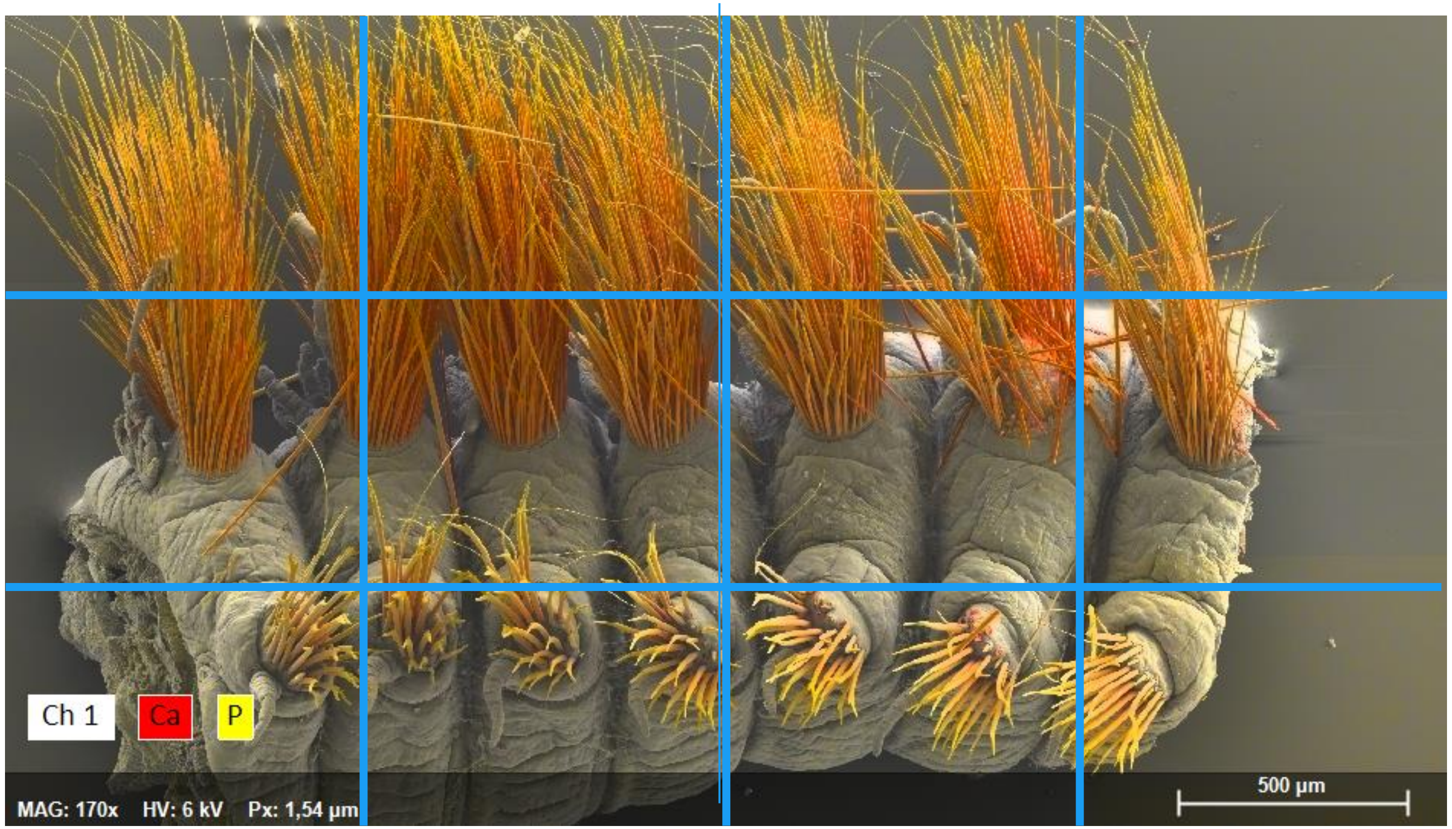

MAPPING
BRUKER
Sample
Microscope
HV 6.0 kV
Scan
Size 512 px
7100
ICR 0 cps
FQ
ICR 230 kcps
Preview
Capture
LiveMap
Acquire
Ch 1
Phases
Map
I/O
Positions
Beam
Mode
Histogram
Zoom
Options
Ch 1 MAG: 170x HV: 6 kV Px: 1.54 µm
200 µm
Single 512 x 384 789 x 592 µm
2 µm
0.16 µm Spot size 453x350 Points
Map
Phases
Charts
Line scan
Spectrum
Elements
Ch 1
Ca
P
MAG: 170x HV: 6 kV Px: 1.54 µm
200 µm
Info
Draw
Map display settings
Counts Enhanced None Smooth
Map result list
no objects
Assistants
Spectra
Objects
Line scan
Mapping
Imaging
Feature
EBSD
Jobs
Scripting
System
Ch 1 1.00
C-K 1.00
Mg-K 1.00
K-Kα 1.00
Ca-Kα 1.00
P-Kα 1.00
Cl-Kα 1.00
Si-Kα 1.00
O-K 1.00
Na-K 1.00
All
None

Firebristle worm (polychaeta)

Large area mapping over 16 fields in less then 3 minutes



- Mapping parameters:
  - 6 kV
  - 4x3 frames stitched together
  - Magnification: 170
  - Pixel spacing: 1,5 µm

- Polychaeta, with its mineralized bristles filled with a burning neurotoxin.
- The hollow, mineralized bristles, composed of calcium phosphate, play a crucial role in the worm's defense against predators.

Sample curtesy of University Bonn

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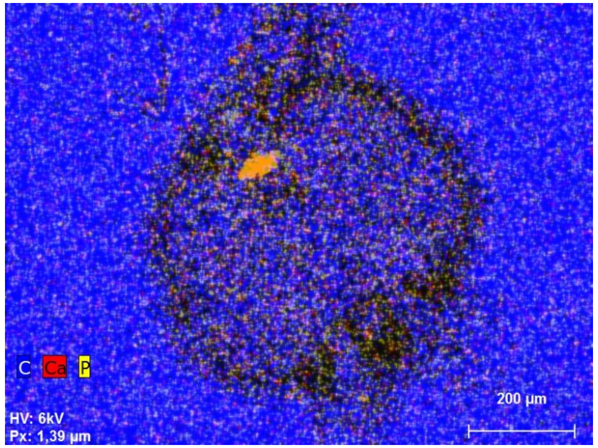
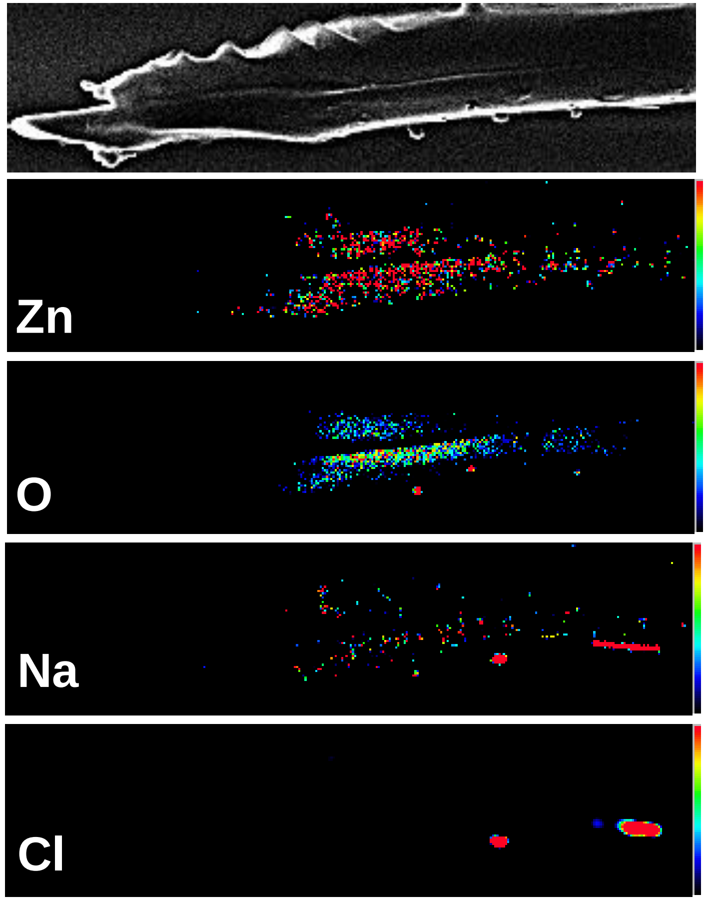
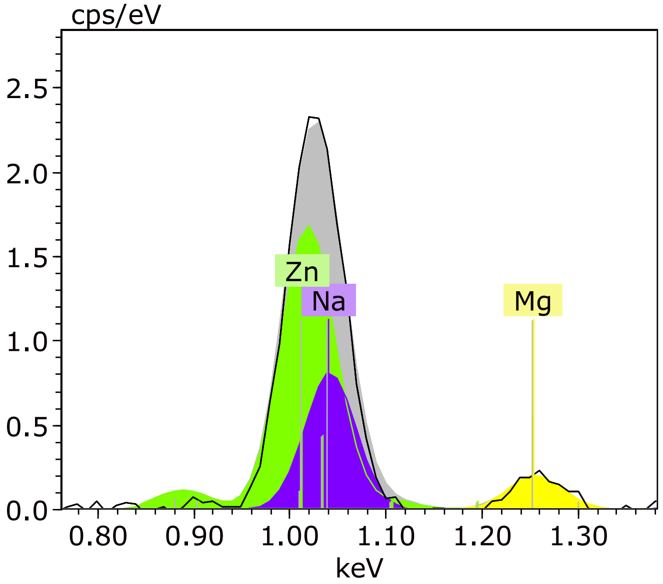
Low vacuum analysis
Parasitoid wasp (*Monolexis fuscicornis*)



- Head of the wasp with mineralized tooth(Ca & P)



- Singel element maps from Ovipositor sting and egg-layer

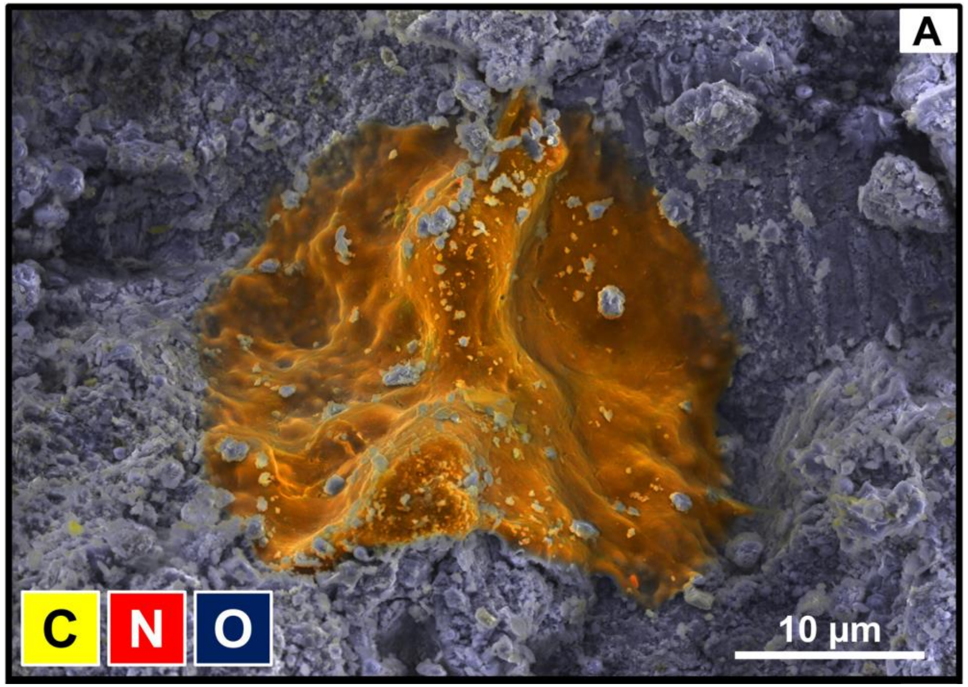


- Overlapping element lines (Zn-L and Na-K) were separated with automatic online deconvolution during the mapping

- 6 kV / 240 sec / 12,000 cps

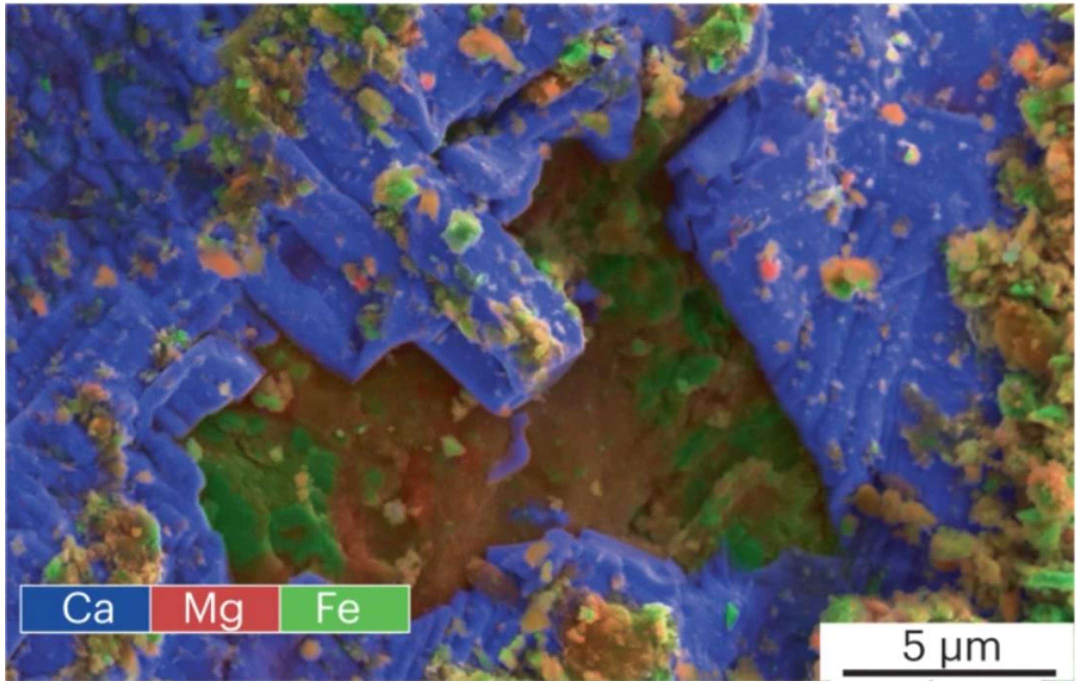
In cooperation with: A. T. Kearsley & G. R. Broad (Natural History Museum, London)

Winchcombe meteorite fall
Light element detection



- Carbon- and nitrogen-rich phases
- 6 kV / 158 pA / 38,000 cps

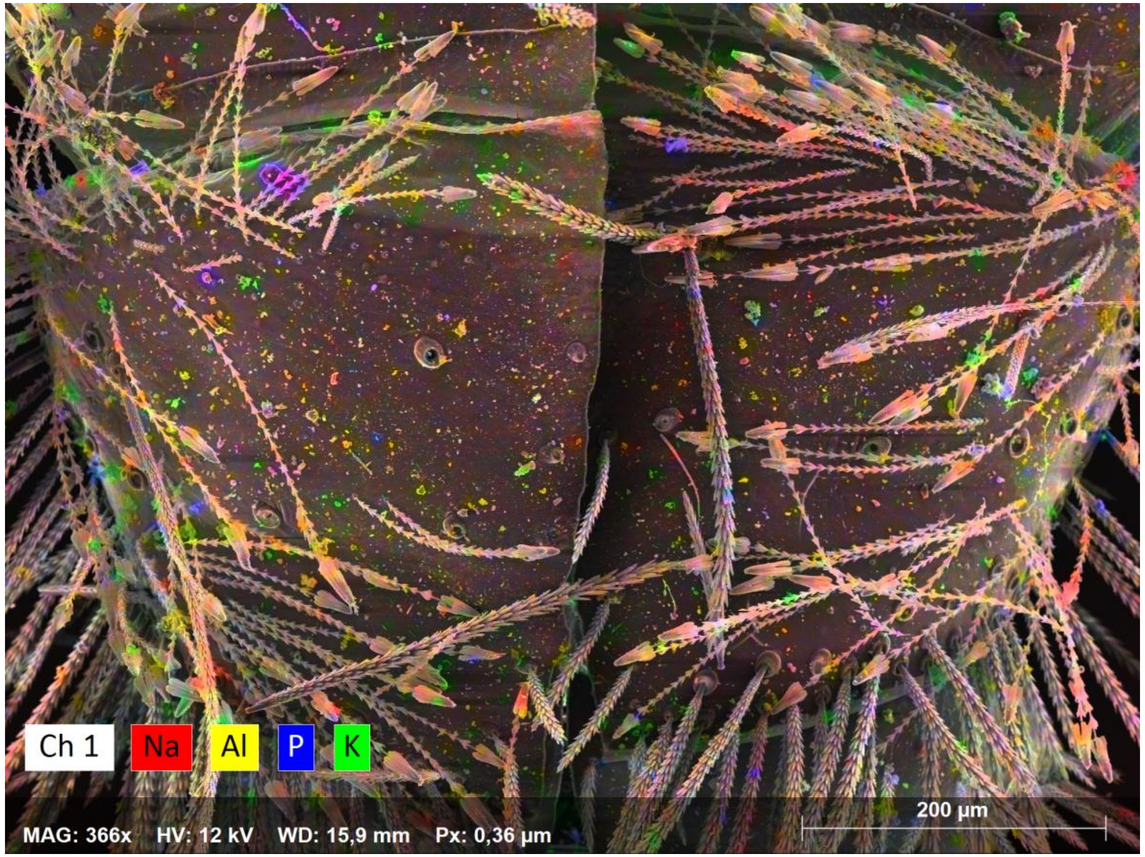
- King et al. Science Advances 2022
- *Image: Dr. T. Salge: ©Trustees of the Natural History Museum, London, Lizenz: „CC BY 4.0*

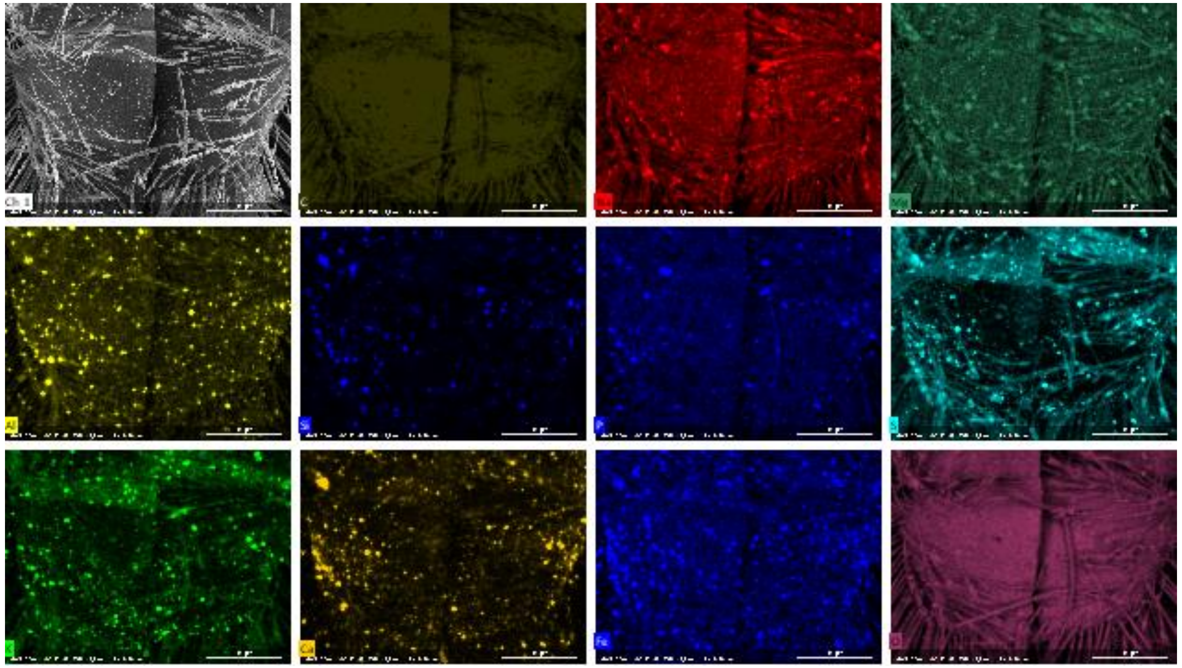


- Extraterrestrial Micrometre-sized calcite grains (blue) in the carbonaceous chondrite Winchcombe.

- *King et al., Science (2023). DOI:10.1126/sciadv.abq3925*
- *Image: Dr. Tobias Salge*

Molt of a bacon beetle
Preservation methods





- Molt of a bacon beetle
- 12 kV / 240 sec / 12,000 cps

- Bacon beetle

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Summary

The XFlash® FlatQUAD detector offers several key benefits for biological sample analysis:

- High Count Rate at Low kV: The detector maintains a high count rate even at low accelerating voltages (kV), allowing for detailed imaging and analysis of delicate biological samples without causing damage.
- High Sensitivity: The detector provides high sensitivity,
- High Signal-to-Noise Ratio: It produces a high signal-to-noise ratio, ensuring clear and precise imaging and analysis of biological structures and compositions.
- Minimized Shadowing Effects: It minimizes or eliminates shadowing effects, particularly beneficial for topographic samples with complex surface features.
- Large Field of View: With features like Image Extension, the detector enables the capture of a large field of view with a single click, facilitating comprehensive visualization and analysis of biological specimens.
- Elemental Mapping: Mapping of biological samples with the XFlash® FlatQUAD, providing insight into the distribution of elements and spectra information in each pixel.
- Drift Correction: Drift correction ensures precise pixel alignment when beam shift occurs.

Thanks to:

We are grateful to the University of Bonn's Institute for Organismic Biology for providing the sample used in this webinar.

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info.bna@bruker.com

https://www.bruker.com/bna