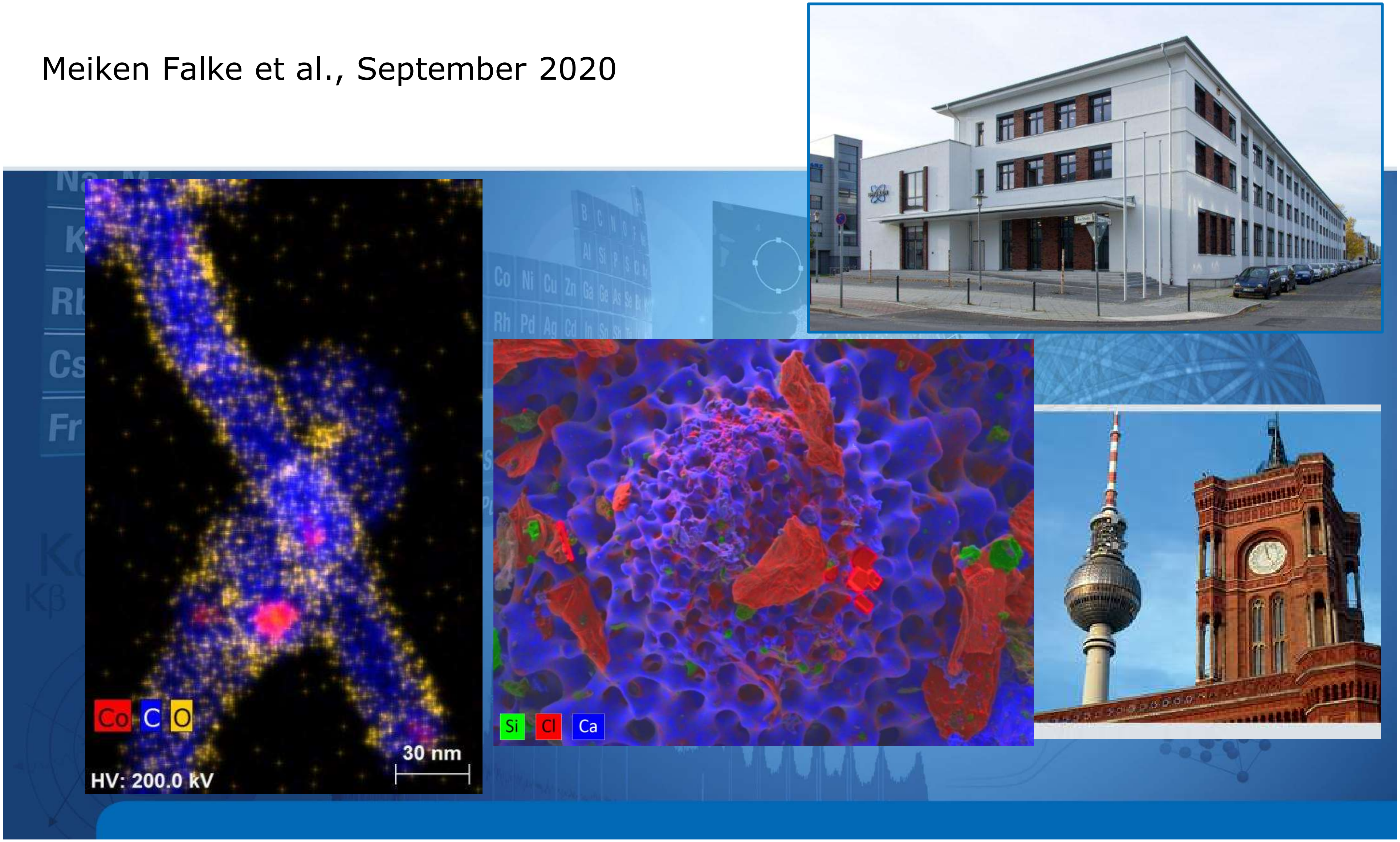
Element Mapping for Life Science; EDX Analysis of Organic and Soft Materials and their Interface to Minerals in the Electron Microscope

Meiken Falke et al., September 2020



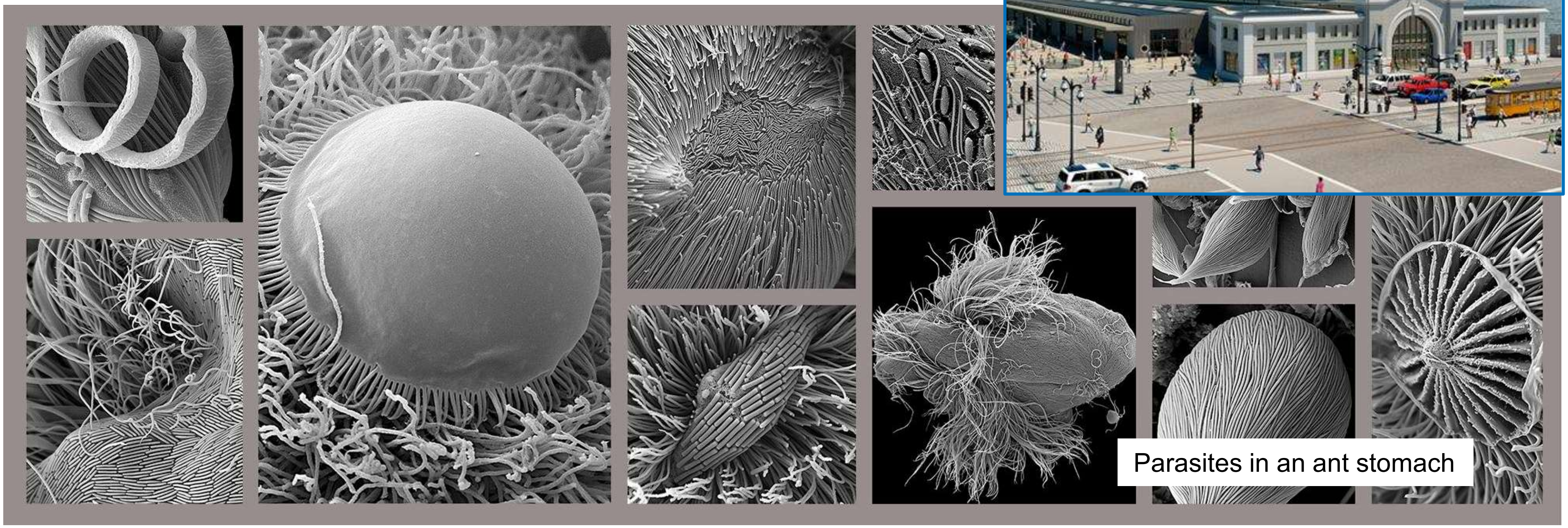
Element Mapping for Life Science; EDX Analysis of Organic and Soft Materials and their Interface to Minerals in the Electron Microscope

Dr. Meiken Falke

Product Manager TEM-EDS, Bruker Nano Analytics

Kevin J. Carpenter, SEM-Microbe Exhibit for Exploratorium

kevinjcarpenter.com



Kevin J. Carpenter, SEM-Microbe Exhibit for Exploratorium

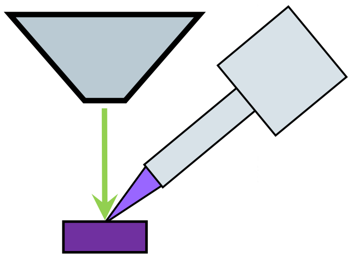
kevinjcarpenter.com

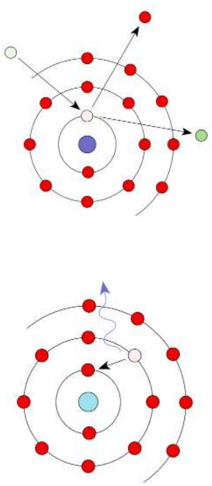
Bruker:
Add colour using element sensitive detectors!

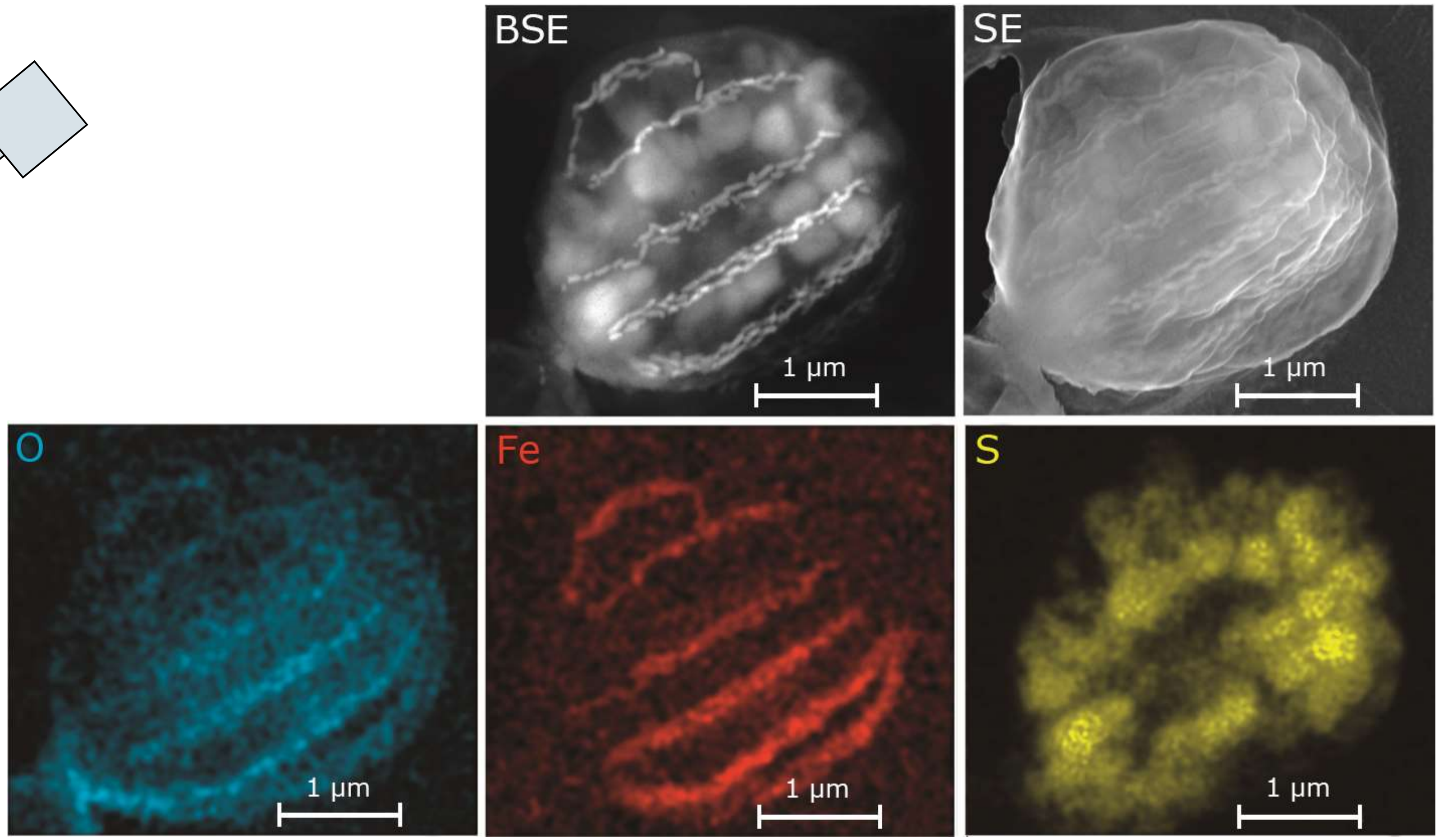
In SEM and TEM …

SEM: EDS-Double Detector System; Electron microscopy in color:

Magnetotactic Bacteria





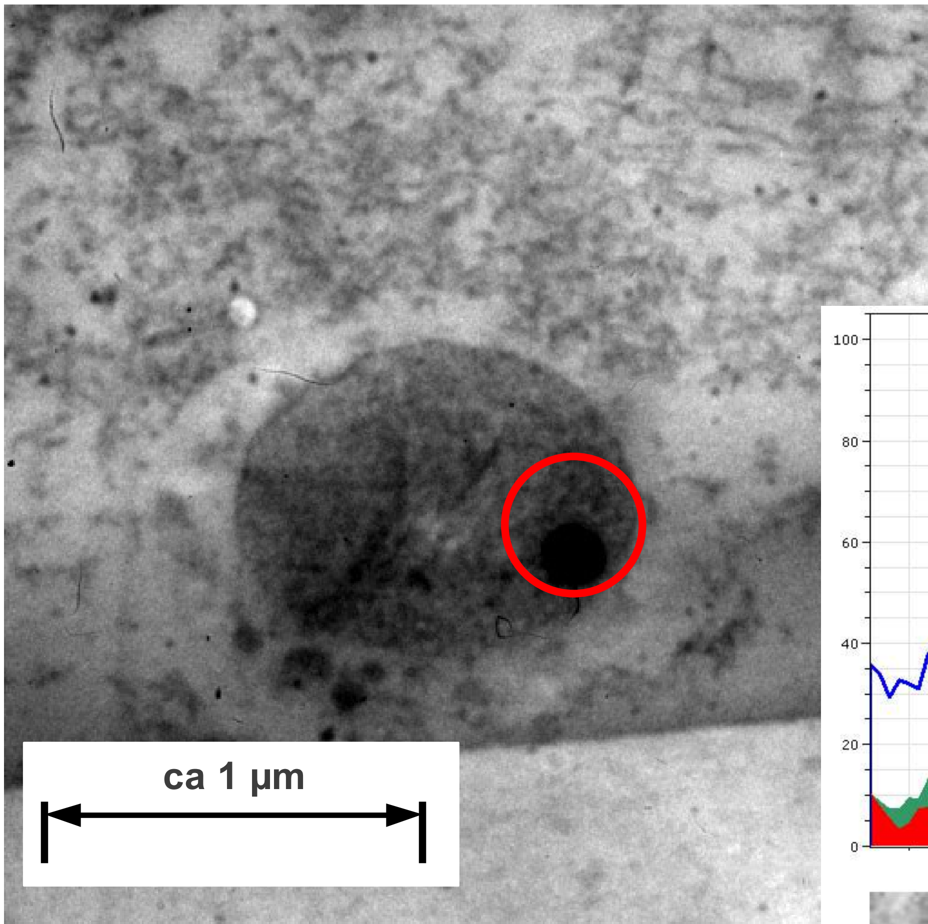


SDD-EDS in (S)TEM

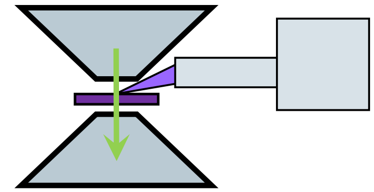
Fe oxidising bacteria ~0.1sr

Universität Regensburg

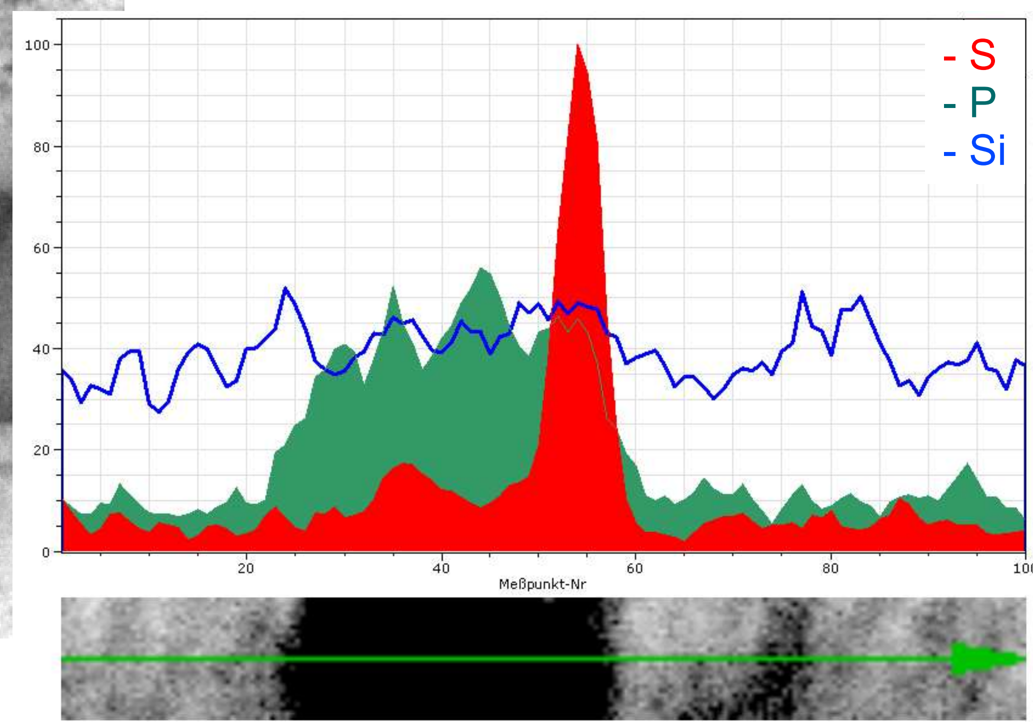
Uni Regensburg



TEM/EDS: Prof. J. Zweck, University of Regensburg
Sample: Thomas Heimerl
Group Prof. Rachel



Fe particle in Phosphorus close to cell membrane, with sulfur. Work in progress



Outline

- Intro:
  - EDS for life science?
  - EM: TEM, STEM and T-SEM EDS
- Available Technology
- EDS Data Acquisition, Quantification and Display of Results
  - Various examples demonstrating ESPRIT implementation of qualitative and quantitative analysis for different specimen types
- Element Mapping in Liquid, Ice, *in-situ*
- Complementary Techniques

EDS for Life Science; Suitable Specimens

- Bio-mineralization and related:
  - Bio-minerals (bone, teeth, Fe etc. in tissue, magnetotactic bacteria, crustaceans, egg shells, …)
  - Bio-mimetics (sea urchin, spider web, wood, collagen … tissue engineering)
  - Nanoparticles > nanotoxicity vs
  - Useful nanoparticles; cell uptake (drug delivery)

  - Distinguish immunolabels, labels for CLEM

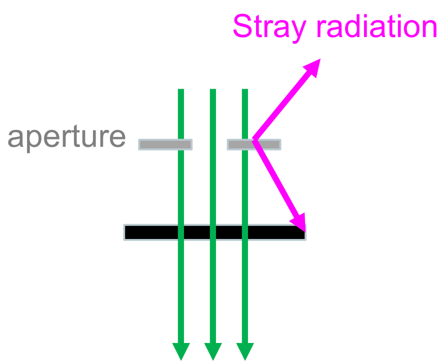
- SDDs have become so sensitive in the low energy region, that the detection of small amounts of bio-relevant light elements (N, P, S, O, …) is no problem anymore!

- Mapping of element and light element distribution in cells and organelles >
- Mapping of nearly the whole periodic system!

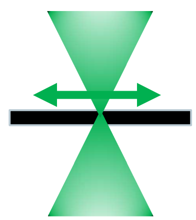
- CLEM (labels), in liquid cells or ice?

Electron Microscopy

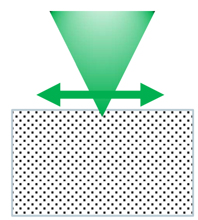
TEM



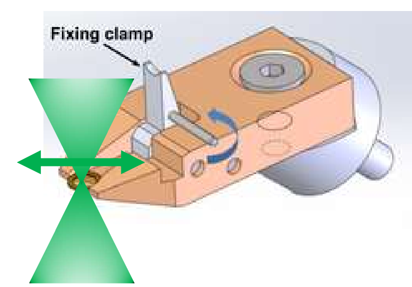
STEM
Scanning TEM



SEM
Scanning EM



SEM: „T-SEM“



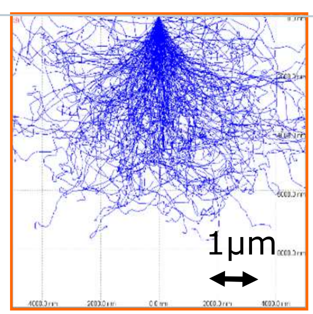
- TKD patented holder
- Commercial STEM holders
- Home made versions

Spatial Resolution and Cs-correction

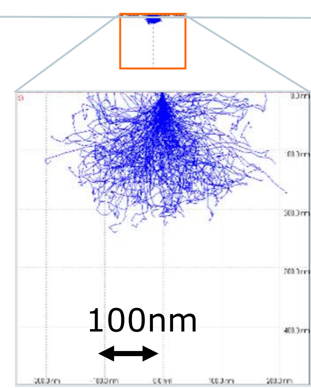
SEM: bulk

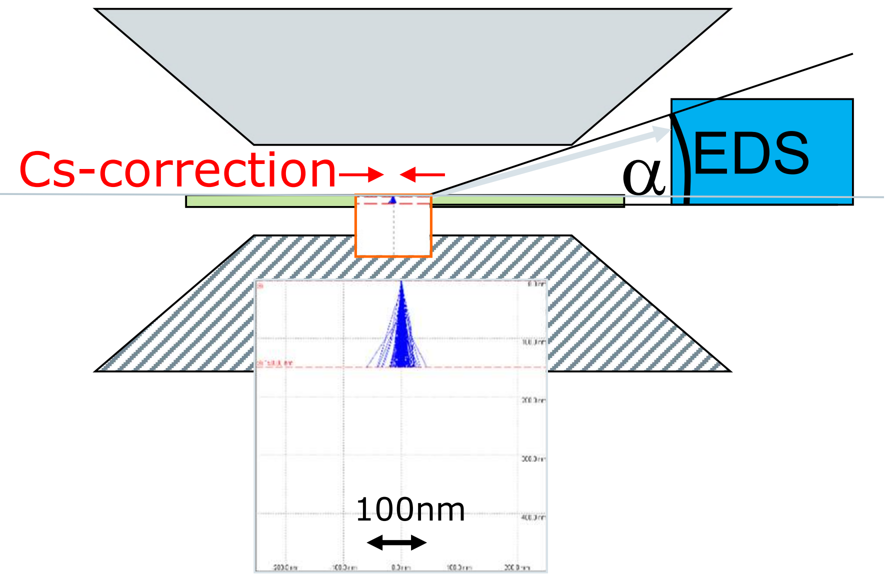
S/TEM, T-SEM: thin specimen, small probe

High voltage
30kV



Lower voltage
4kV

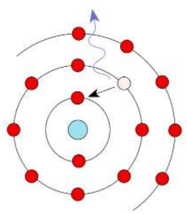




(TEM) EDS Quantification; R. Egerton

1994, line intensity for a particular element line / transition

$$I_x = N_A \,\sigma_A\, \omega_A\, (\Omega/4\pi)\, \varepsilon\, N_e = n_A\, t\, \sigma_A\, \omega_A\, (\Omega/4\pi)\, \varepsilon\, N_e$$

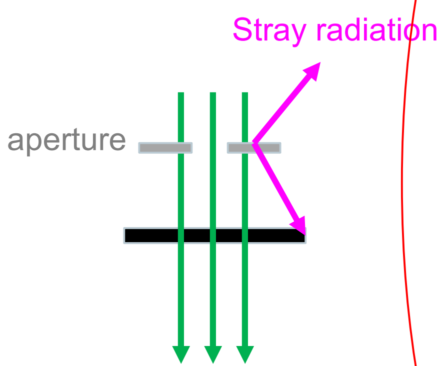


I_x	number of X-ray photons in a characteristic peak of species A
N	number of atoms per unit volume
$n\ t$	number of atoms per unit area times thickness
σ	ionization cross section (Casnati et al., 1982, Bote et al., 2009)
ω	fluorescence yield (Hubbell et al., 1994, Krause, 1979)
$\Omega/4\pi$	solid angle / geometrical collection efficiency
ε	detection quantum efficiency (window: SLEW or no window or other)
N_e	number of incident electrons

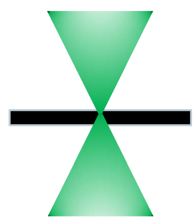
+ absorption, fluorescence, other effects…

Electron Microscopy

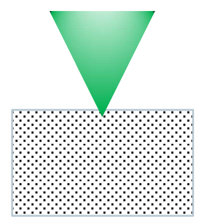
TEM



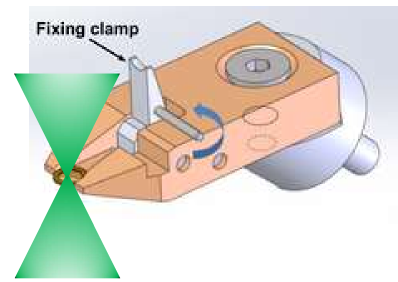
STEM
Scanning TEM



SEM
Scanning EM



SEM: „T-SEM“

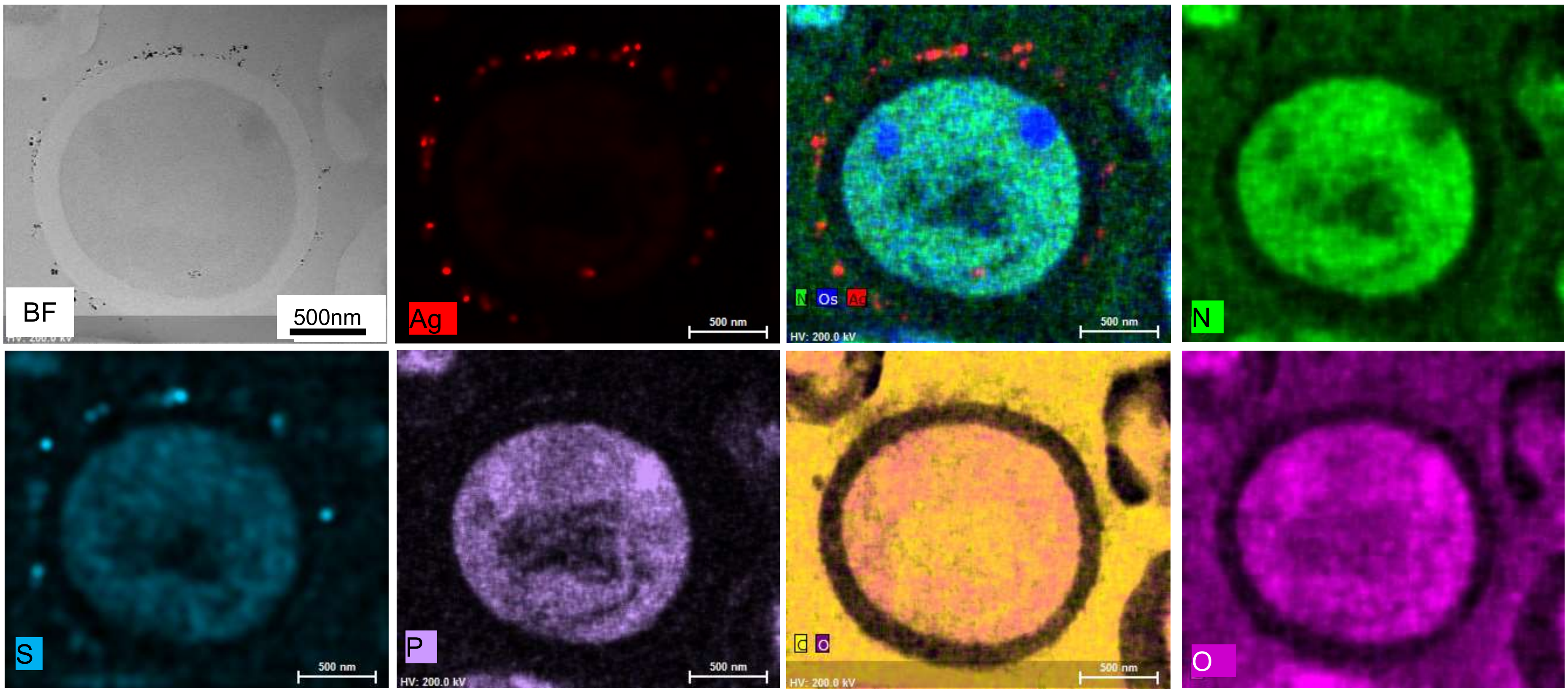


- TKD patented holder
- Commercial STEM holders
- Home made versions

TEM EDS for Life Science

Yeast cell: Element mapping of protein (Ag) labels and light (N, O) and heavy (Os, Ag) elements

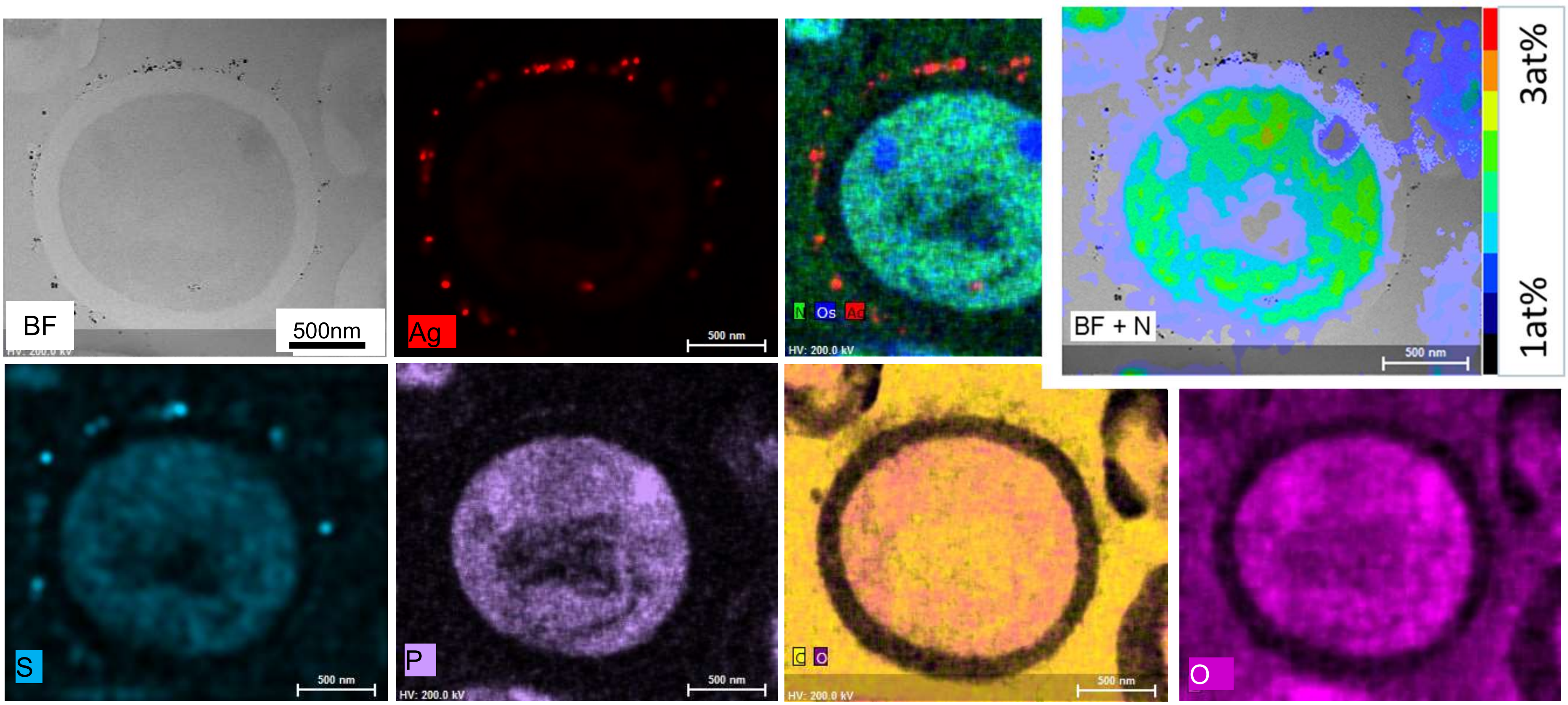
30 mm^2, 0.12 sr (Standard EDS); Conventional STEM

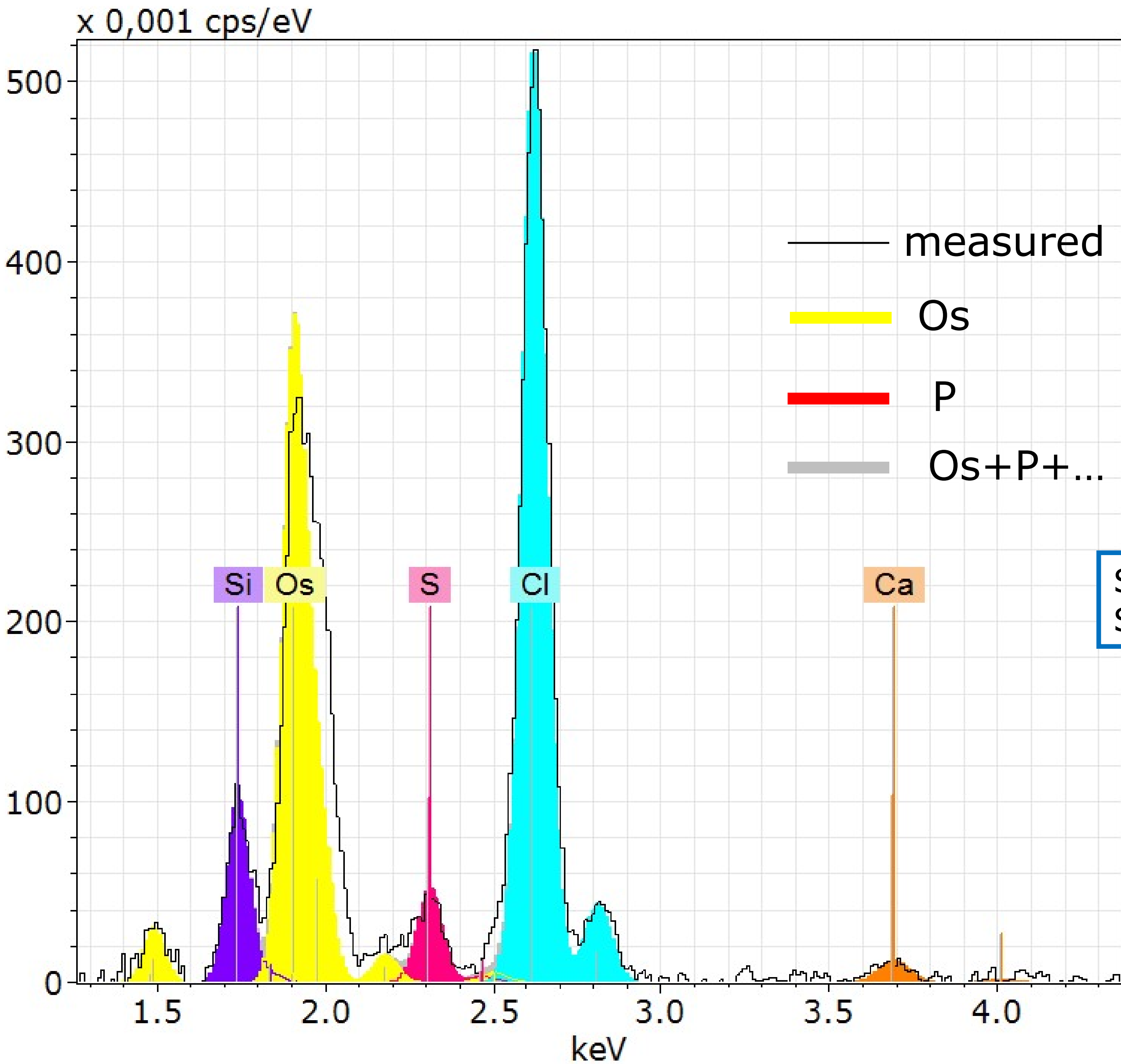


TEM EDS for Life Science

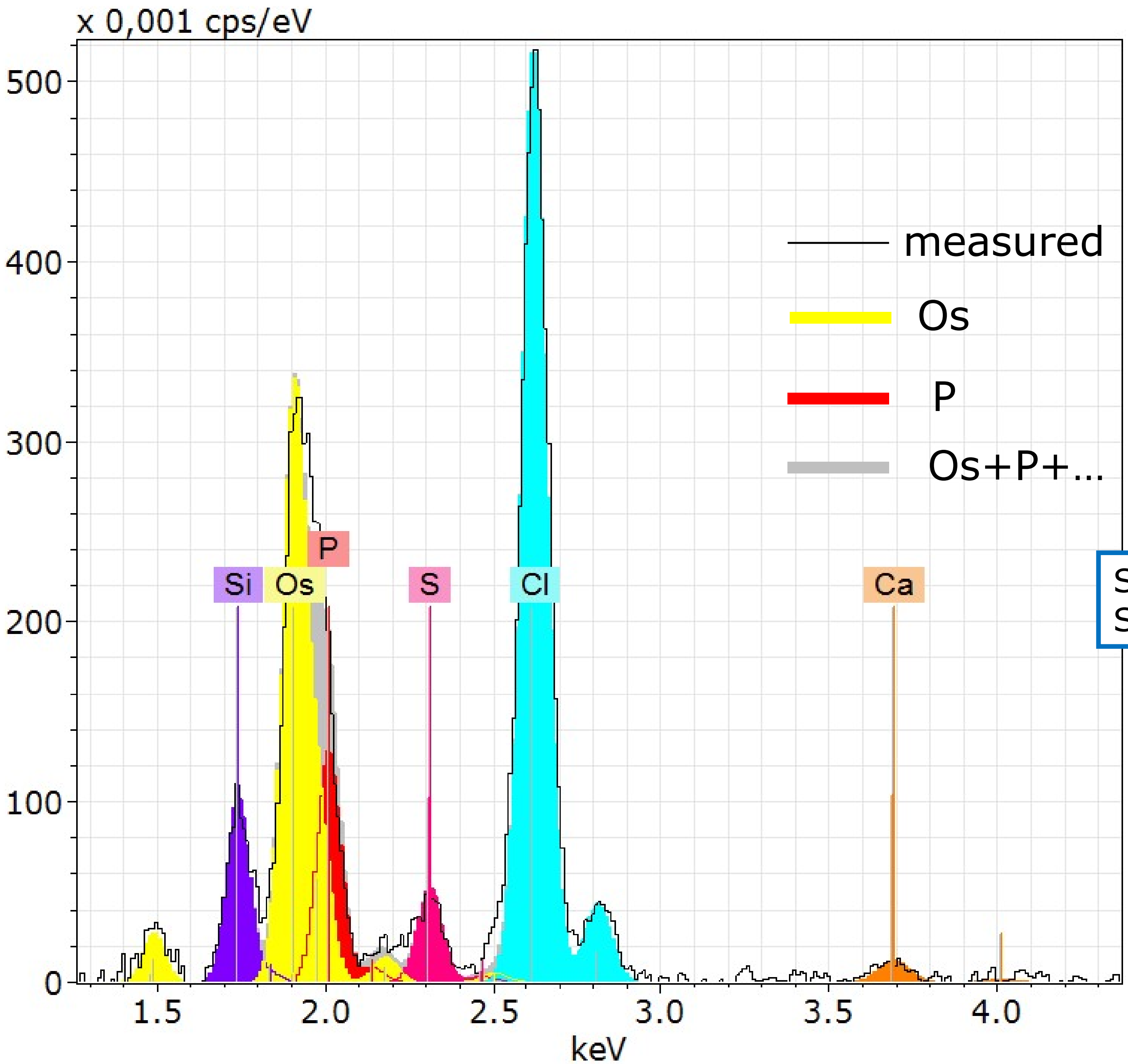
Yeast cell: Element mapping of protein (Ag) labels and light (N, O) and heavy (Os, Ag) elements quantitatively

30 mm², 0.12 sr (Standard EDS); Conventional STEM





Standard EDS,
Standard STEM



Standard EDS,
Standard STEM

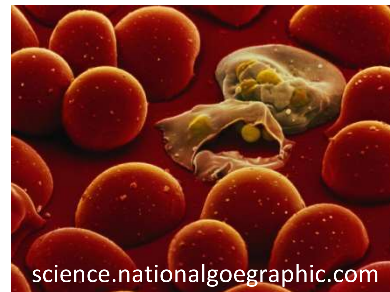
TEM-EDS for cell analysis at 0.1sr

Malaria parasite: *Plasmodium* in erythrocyte (red blood cell) treated with Chloroquine

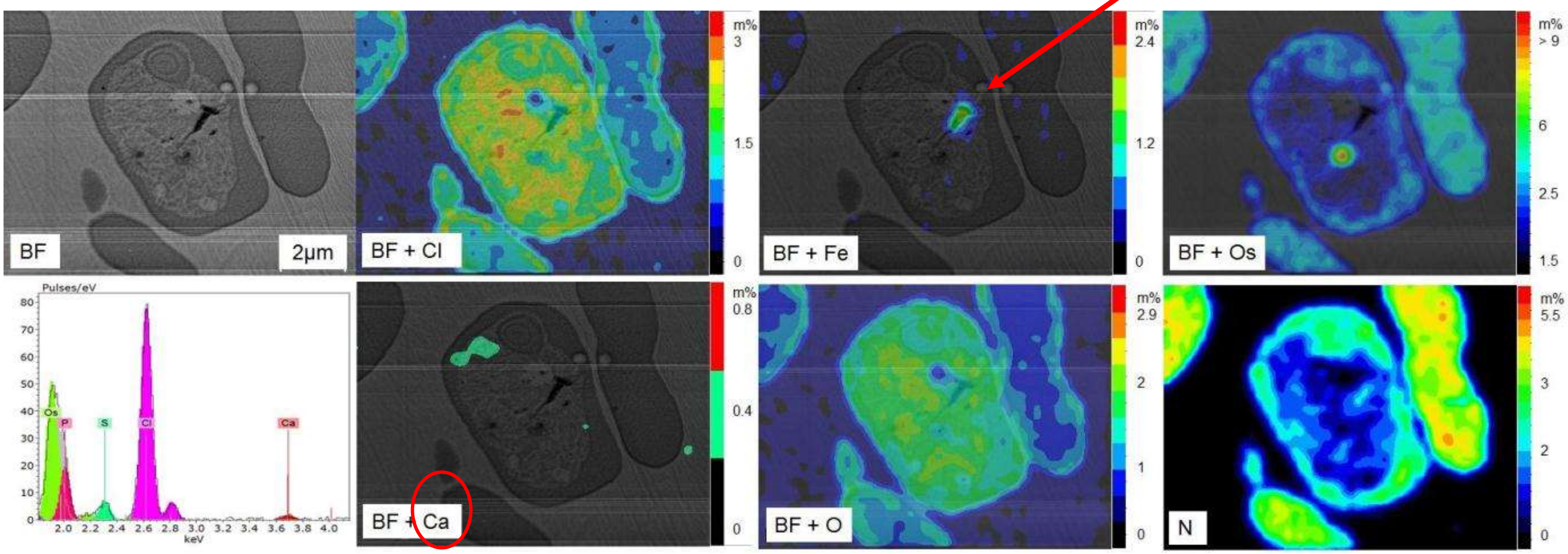
Standard EDS, Standard STEM

Anopheles mosquito, spreader of *Plasmodium falciparum*, the cause of Malaria

The parasite multiplies by destroying red blood cells

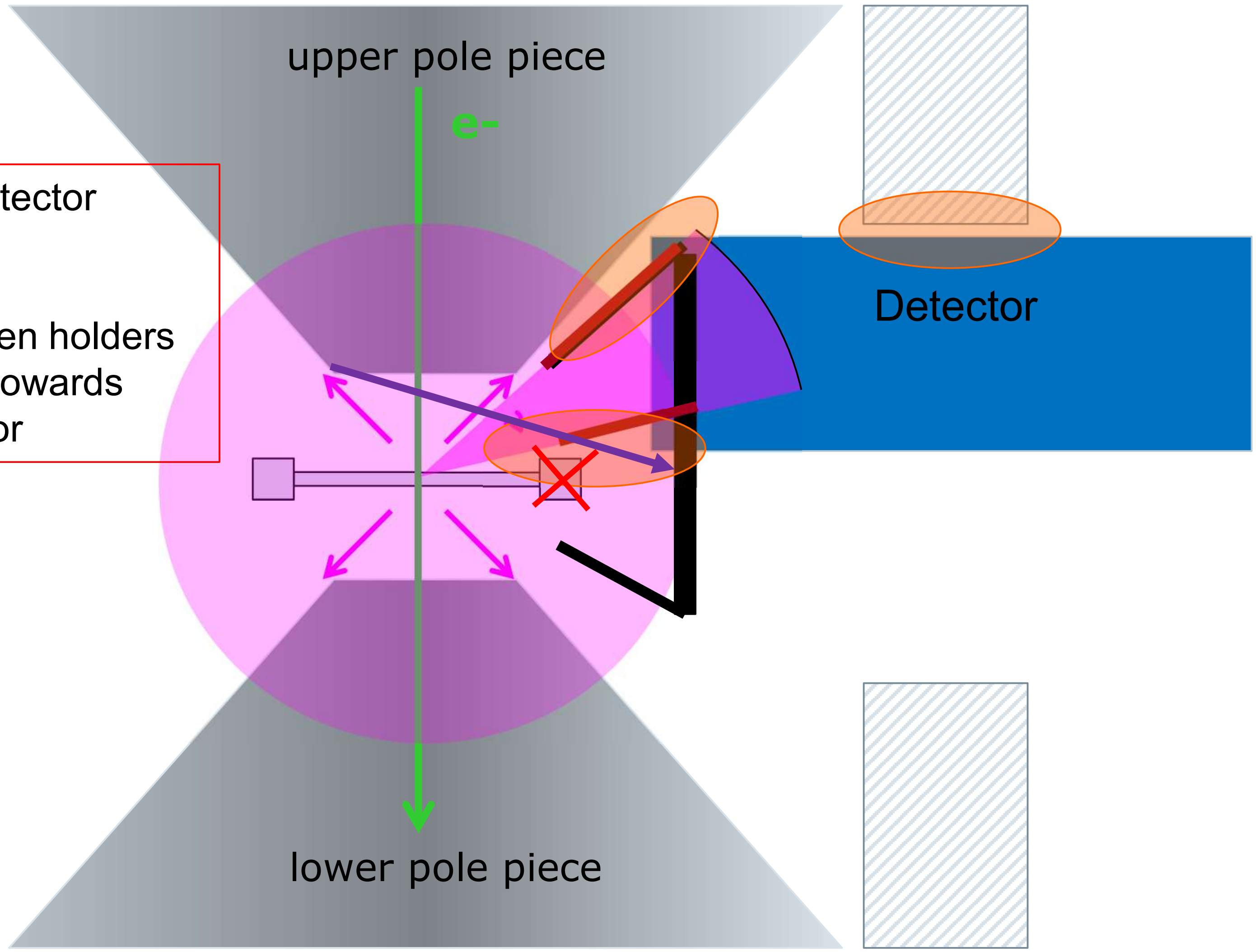


The parasite digests haemoglobin, hence the iron intake in its food vacuole

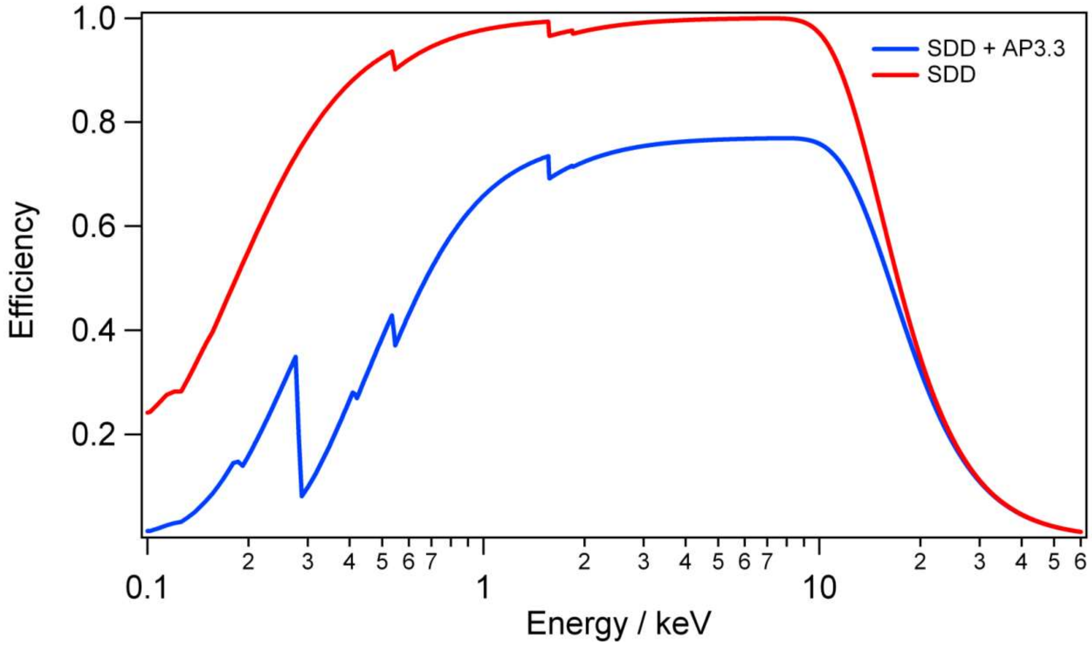


Geometric Limitations

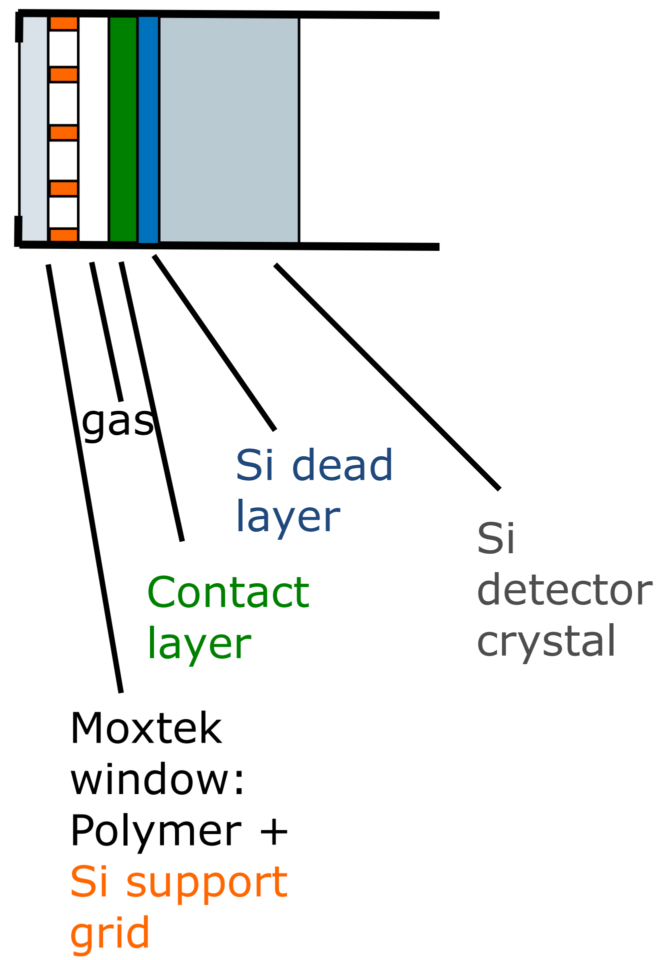
- Optimize detector position
- Use specimen holders with cutout towards EDS detector



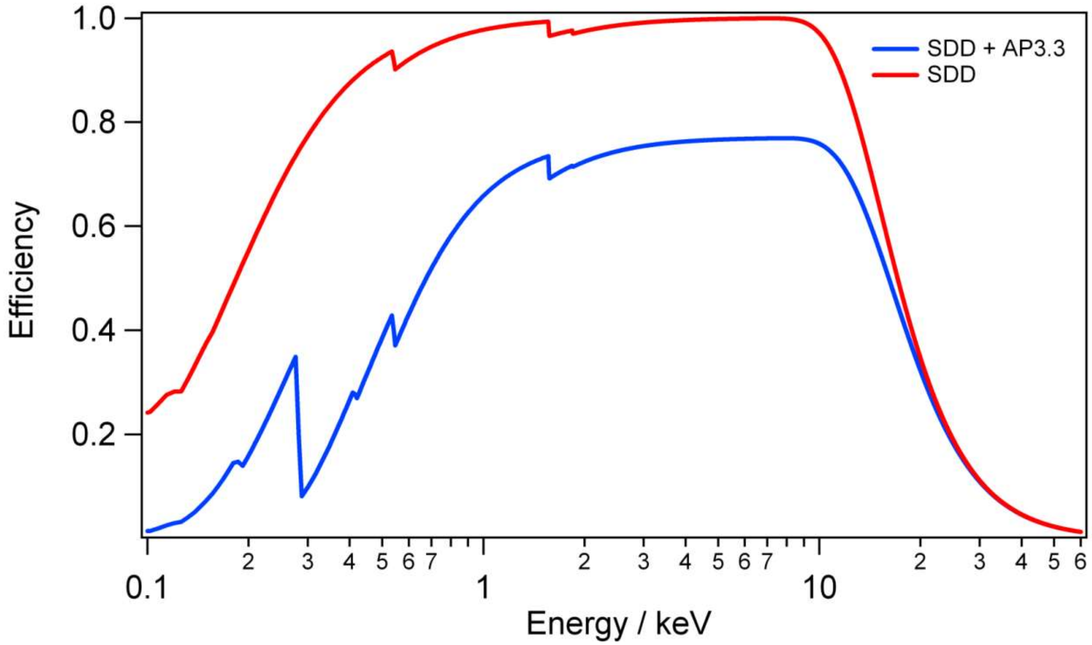
Detector (window) quantum efficiency and windows of in situ reaction cells



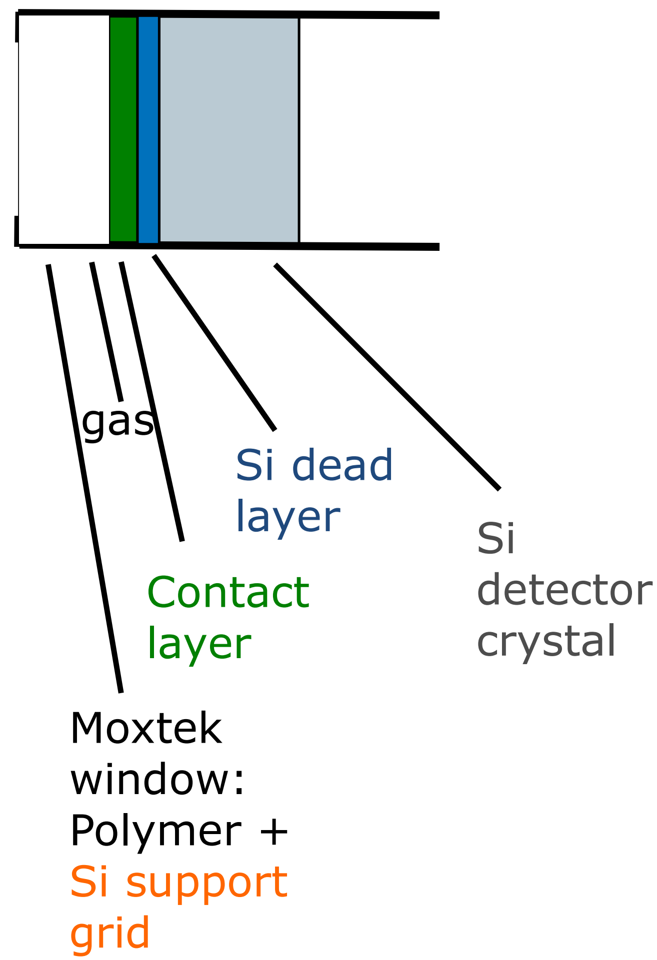
Detector w/wo window

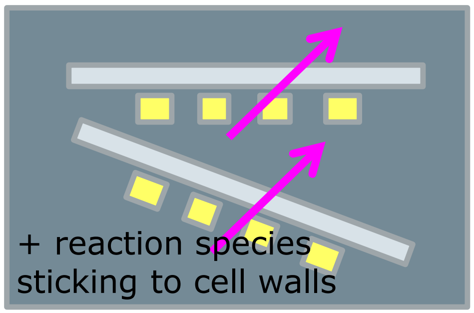


Detector (window) quantum efficiency and windows of in situ reaction cells



Detector w/wo window

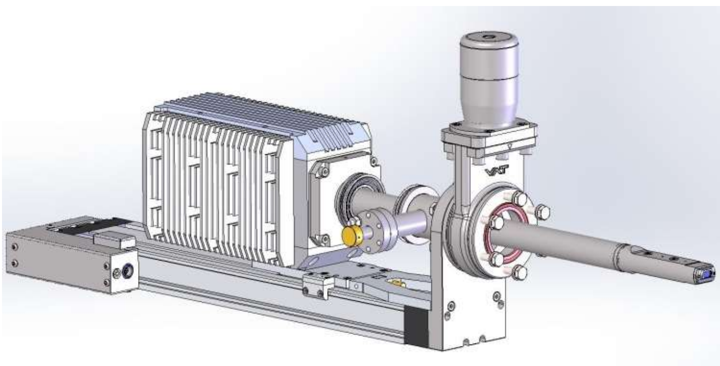




Window of reaction cell

EDXS with 100 mm² windowless oval detector area;

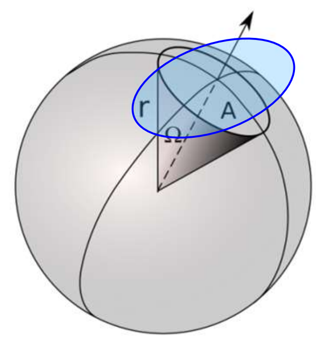
Nion UltraSTEM, Cs-corrected, high brightness source



EDXS at ~0.7 sr. This is the real solid angle for a flat SDD (see wiki below).

Wrong: $100\text{mm}^2 / (10.5\text{mm})^2 = 0.91\text{sr}$.

TOA: 13.4°

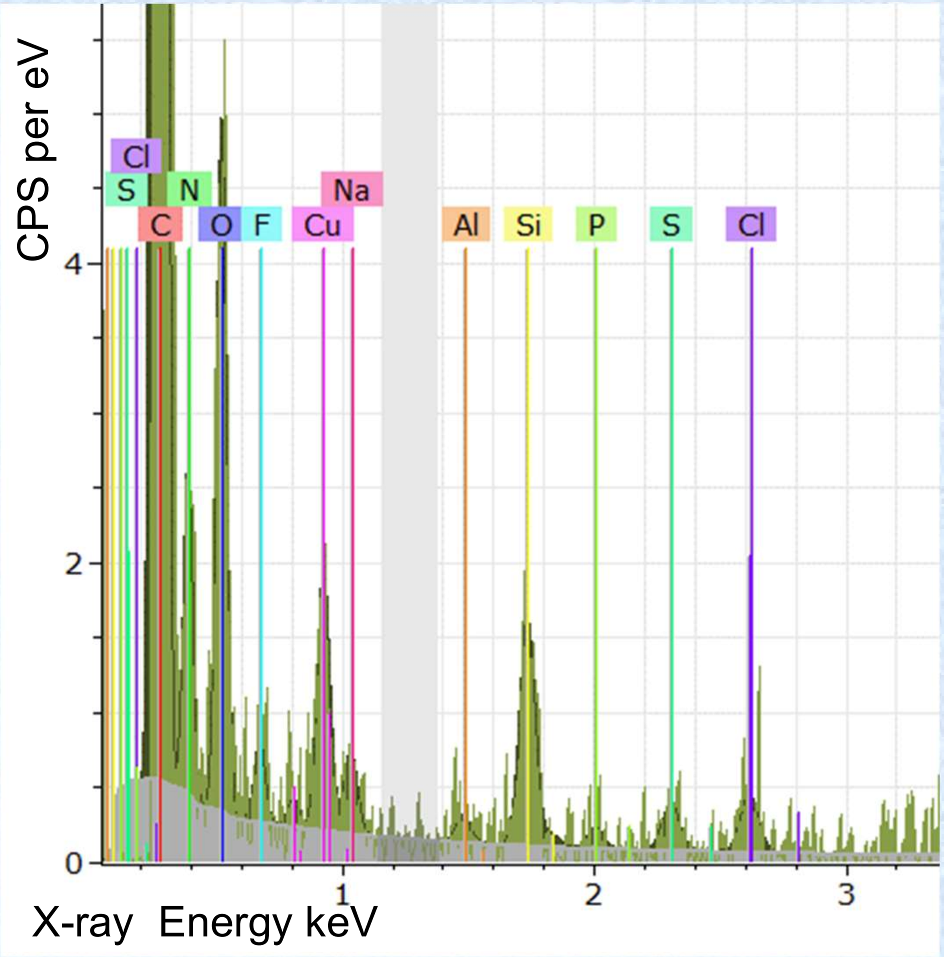


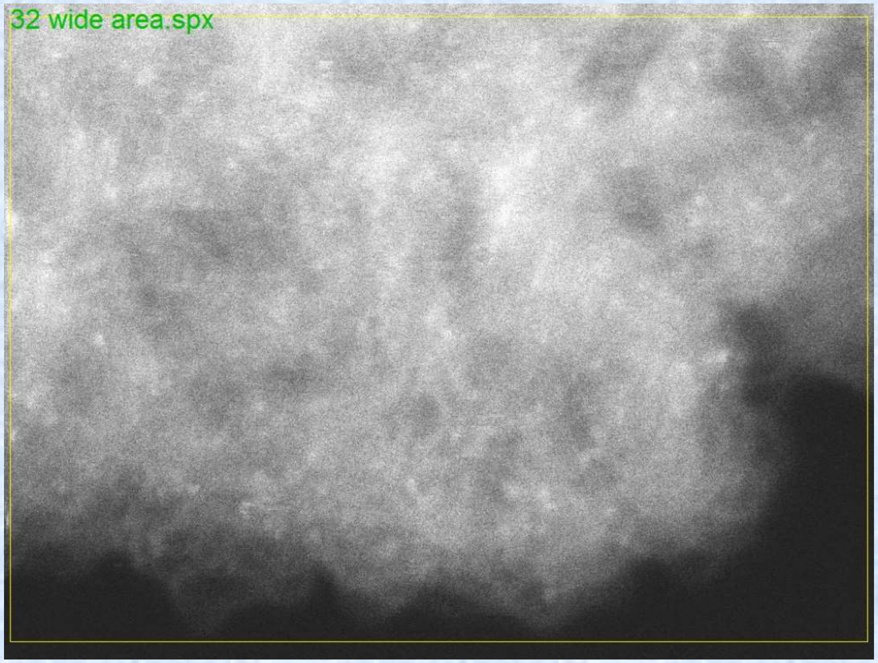
wikipedia

Used to analyse dust from space!
See next slides …

EDXS detects single atoms *and* concentrations ~0.01%

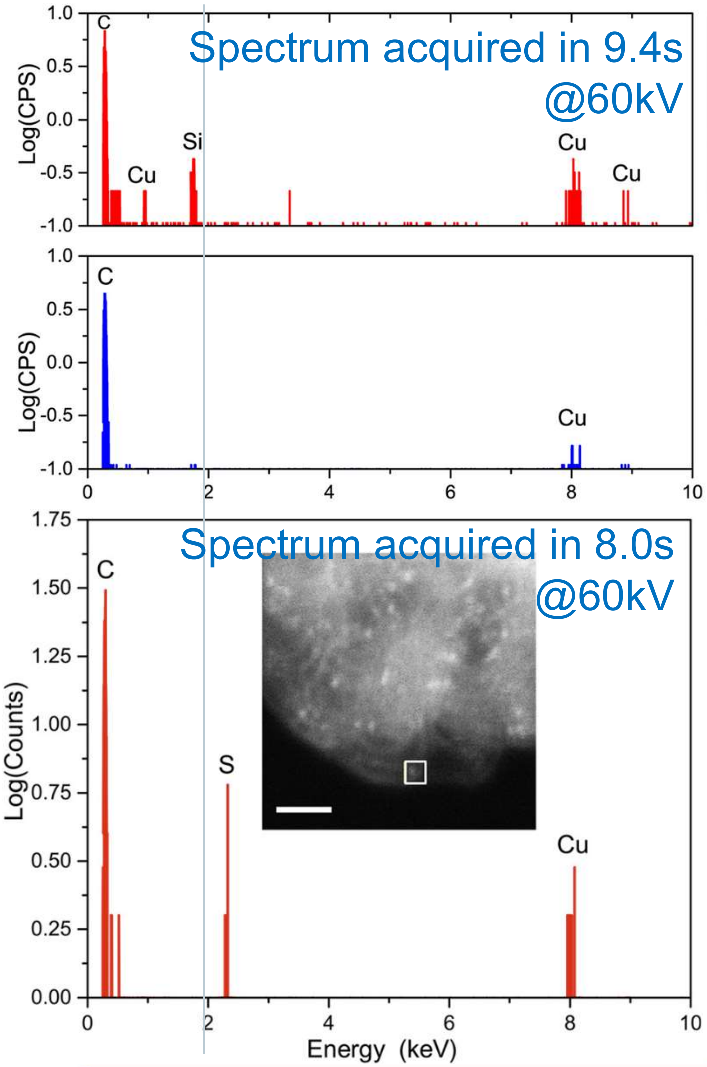
C (at.%)	N	O	F	Cu	Na	Al	Si	P	S	Cl
96.75	0.84	1.45	0.16	Sys.	0.11	0.05	0.4	0.04	0.08	0.11





NRL UltraSTEM200 with Bruker X-flash detector, 60 kV. Concentrations as low as 0.01 atomic % can be explored. *Courtesy Rhonda Stroud, NRL.*

STEM: <u>single atom ID</u> in carbonaceous material, here: nano-diamonds from space; 0.65sr using 100mm^2 oval detector



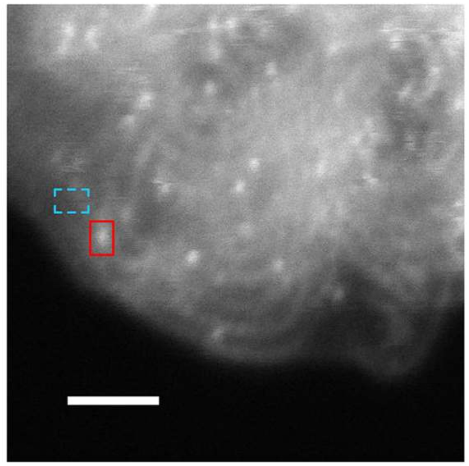
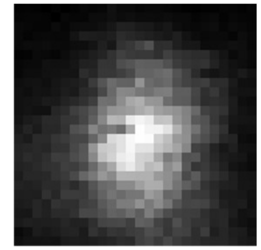


FIG. 1. High-angle ADF image and EDX spectra on (red solid line box) and off (blue-dashed line box) an Si atom on amorphous carbon. The scale bar is 1 nm.

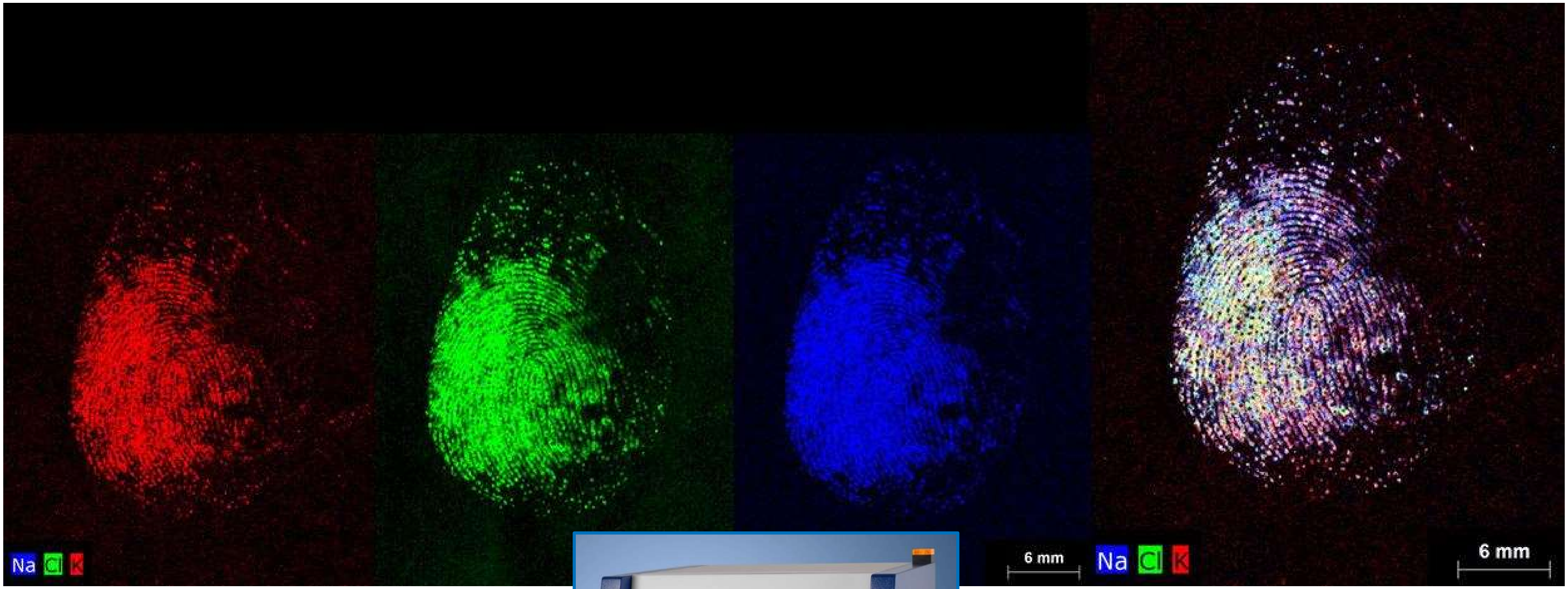
FIG. 2. High-angle ADF image and EDX spectrum of an S atom on amorphous carbon. The scale bar is 1 nm.



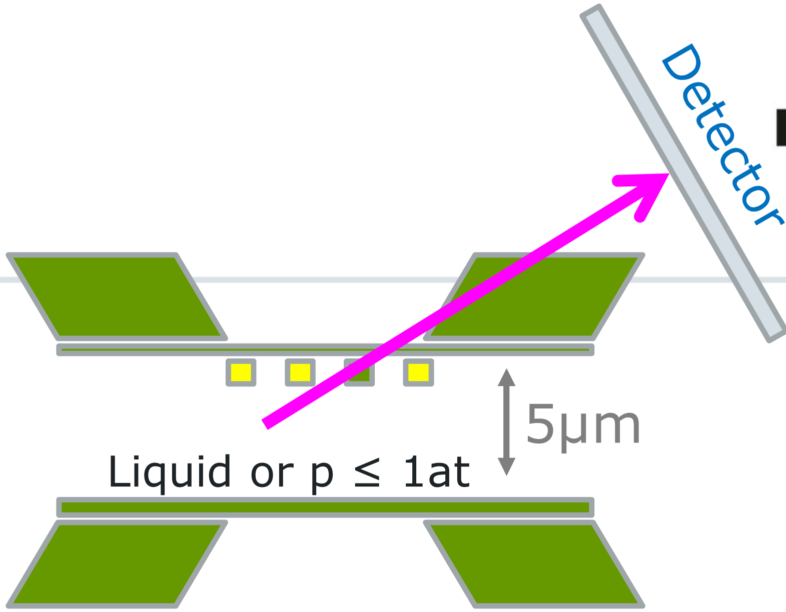
Trackingwindow for EDS of single atom.

*R. M. Stroud et al., APL **108**, 163101 (2016) open access*
*T. C. Lovejoy et al., APL **100**, 154101 (2012): 30mm^2, 0.1sr*

Sources of foreign signals? Fingerprint on Paper; X-Ray fluorescence in the M4 Tornado



Reaction Cells

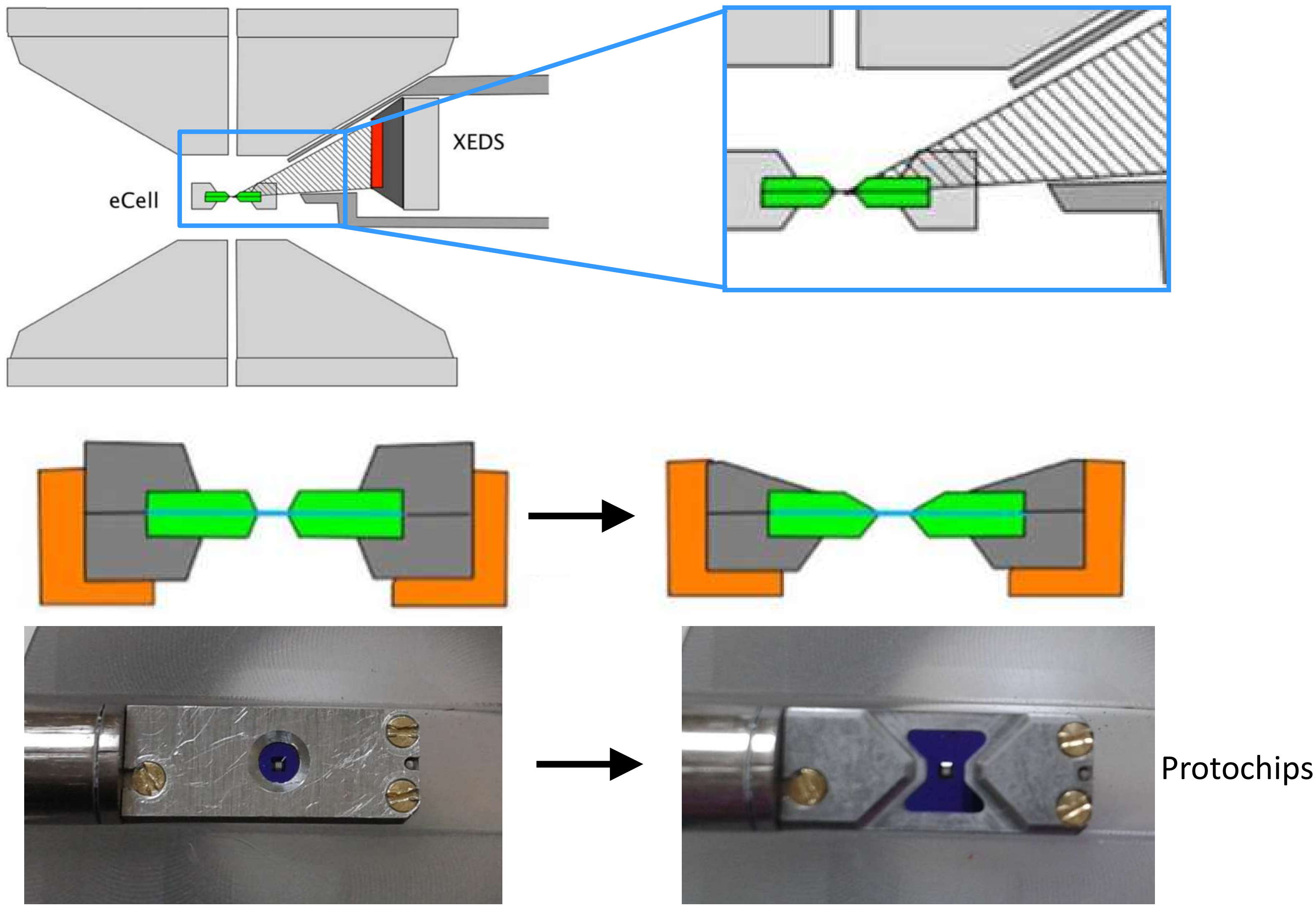


- Shadowing
- Gas, liquid, + heat:
- Windows:
  - Graphene, Si3N4 or alpha SiNx
  - Thickness,
  - Support (grid): Si, polymer film, SiC (heating), Au spacers O-rings
  - Al film (light tight))
- Reaction species sticking to cell walls

- Effects:
  - Windows etc. > Reduced transmission / quantum efficiency
  - Cell Materials > System peaks
  - Heat > enlarged peak width and spectrum background / noise

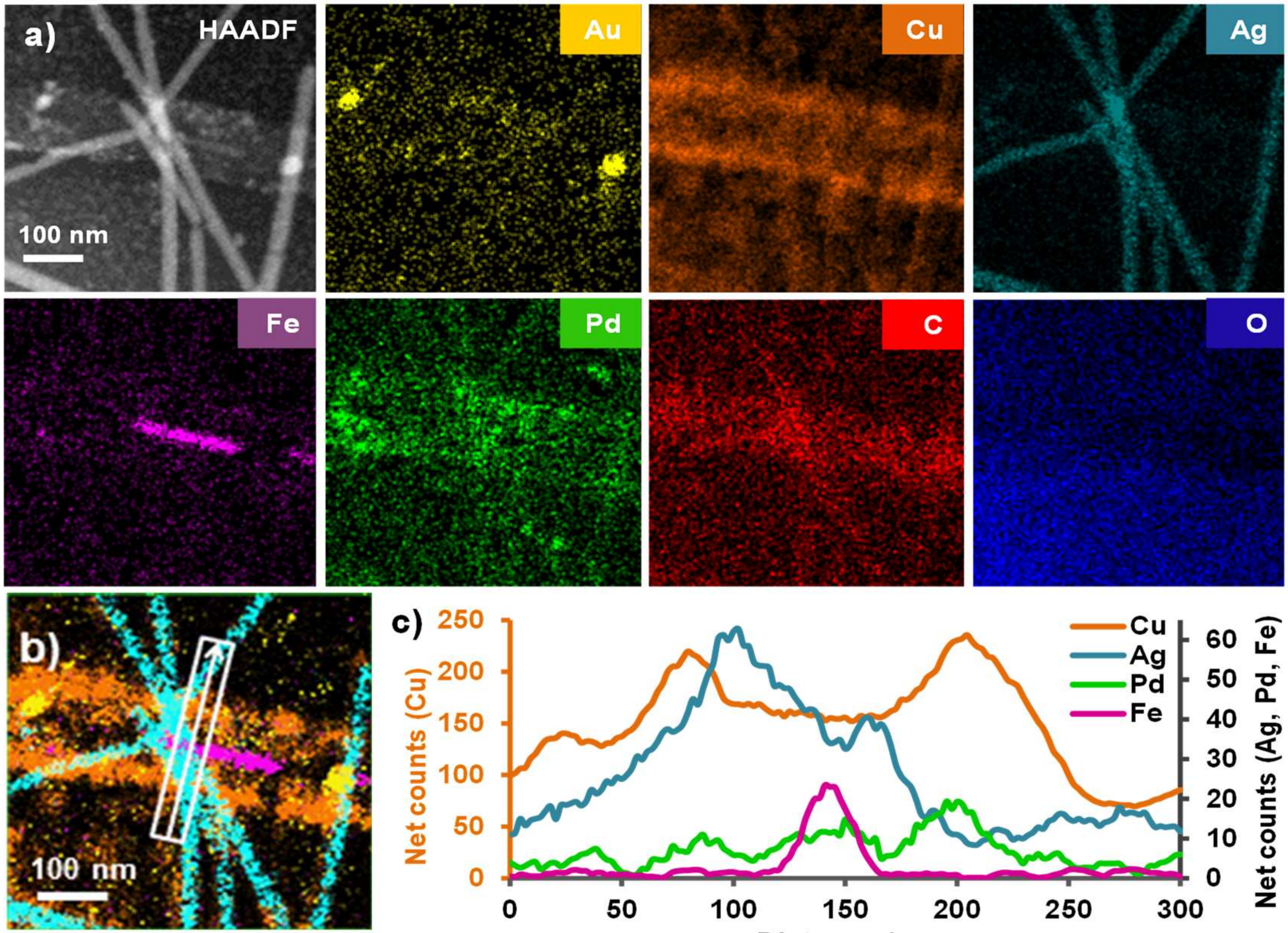
calculate transmission depending on thickness/materials here:
http://henke.lbl.gov/optical_constants/filter2.html

EDXS compatible environmental cell design



Zaluzec *et al.* Microsc. Microanal. (2014) 20 p. 323

EDXS Elemental mapping in liquids



Lewis et al Chem Comm (2014) 50, 70, 10019-22

Cryo-STEM-EDS: M&M 2018 with N.J. Zaluzec and TFS

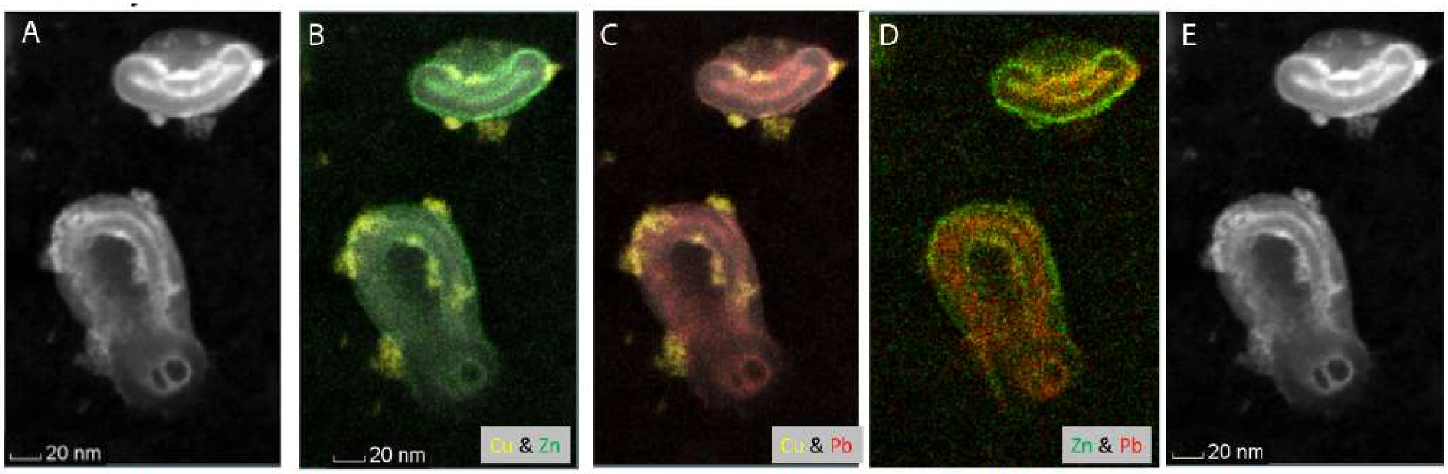


Figure 1.) Hyperspectral images of site specific heavy metal localization to folds of polyamide film.
A) HAADF preXEDS, B) Cu & Zn, C) Cu & Pb, D) Zn & Pb, E) HAADF postXEDS measurement (333 Frames)

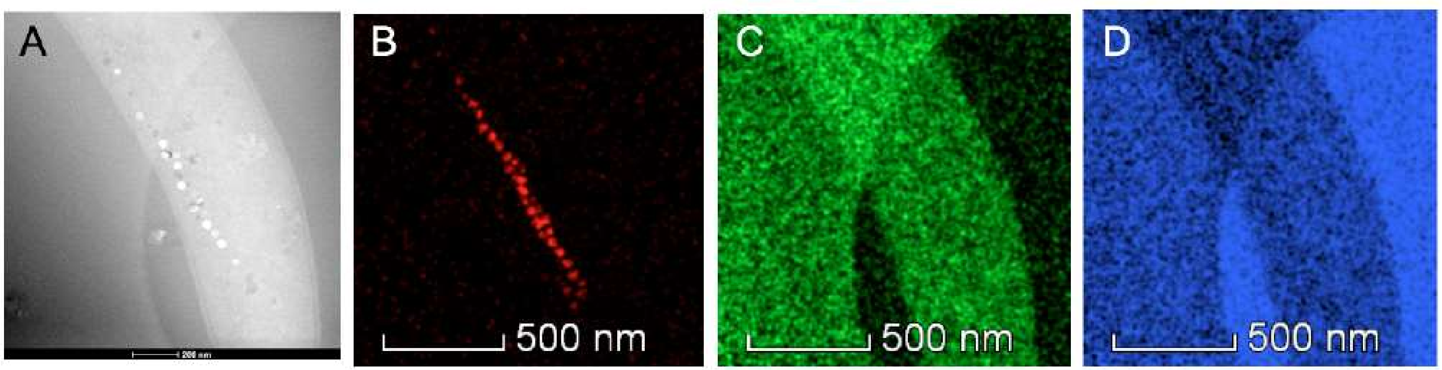


Figure 2.) Hyperspectral images of frozen magnetotactic bacteria, Eo:200 kV, 5 pA, Dual X detector.
A) HAADF image, B) Fe , C) Carbon, D) Oxygen elemental distributions.

Cryo-STEM-EDS

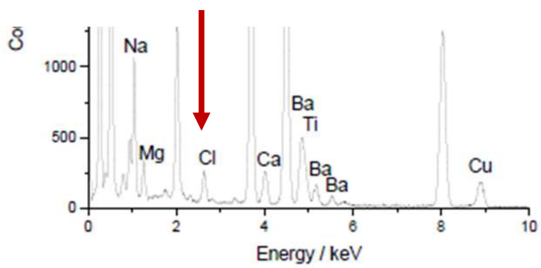
Drying artefacts for drop casted NP in CCCM,
overcome by plunge-freezing, confirmed by EDS.

IOP Conf. Series: Journal of Physics: Conf. Series **902** (2017) 012006

Cryo-STEM-EDX spectroscopy for the characterisation of nanoparticles in cell culture media

M Ilett[1], F Bamiduro[1], O Matar[1], A Brown[1], R Brydson[1] and N Hondow[1]

[1] School of Chemical and Process Engineering, University of Leeds, Leeds LS2 9JT, UK



Drop Casting

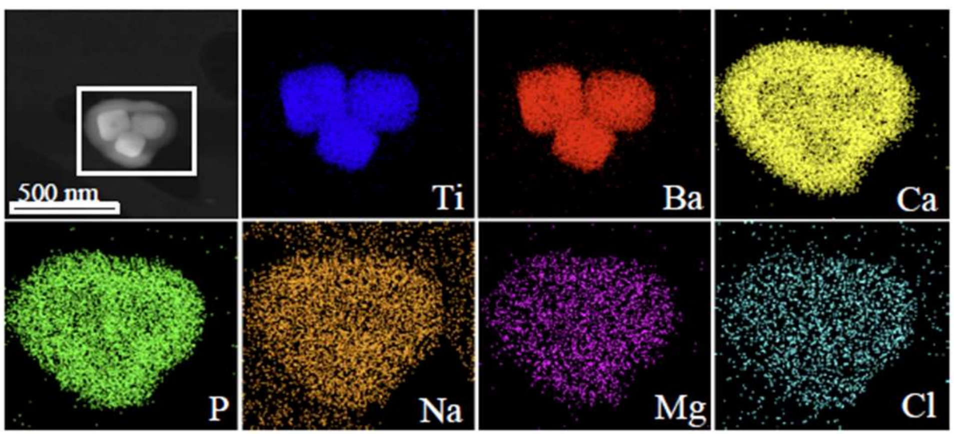


Figure 2: STEM-EDX data of DC BT in CCCM. In the EDX spectrum Ca and P are the most prominent peaks but also present were Cl, Mg and Na, all of which spatially match to the coating imaged around the BT nanoparticles.

Cryo-STEM-EDS

Drying artefacts for drop casted NP in CCCM,
overcome by plunge-freezing, confirmed by EDS.

IOP Conf. Series: Journal of Physics: Conf. Series **902** (2017) 012006

Cryo-STEM-EDX spectroscopy for the characterisation of nanoparticles in cell culture media

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[1] Schoo
UK

Plunge-Freezing

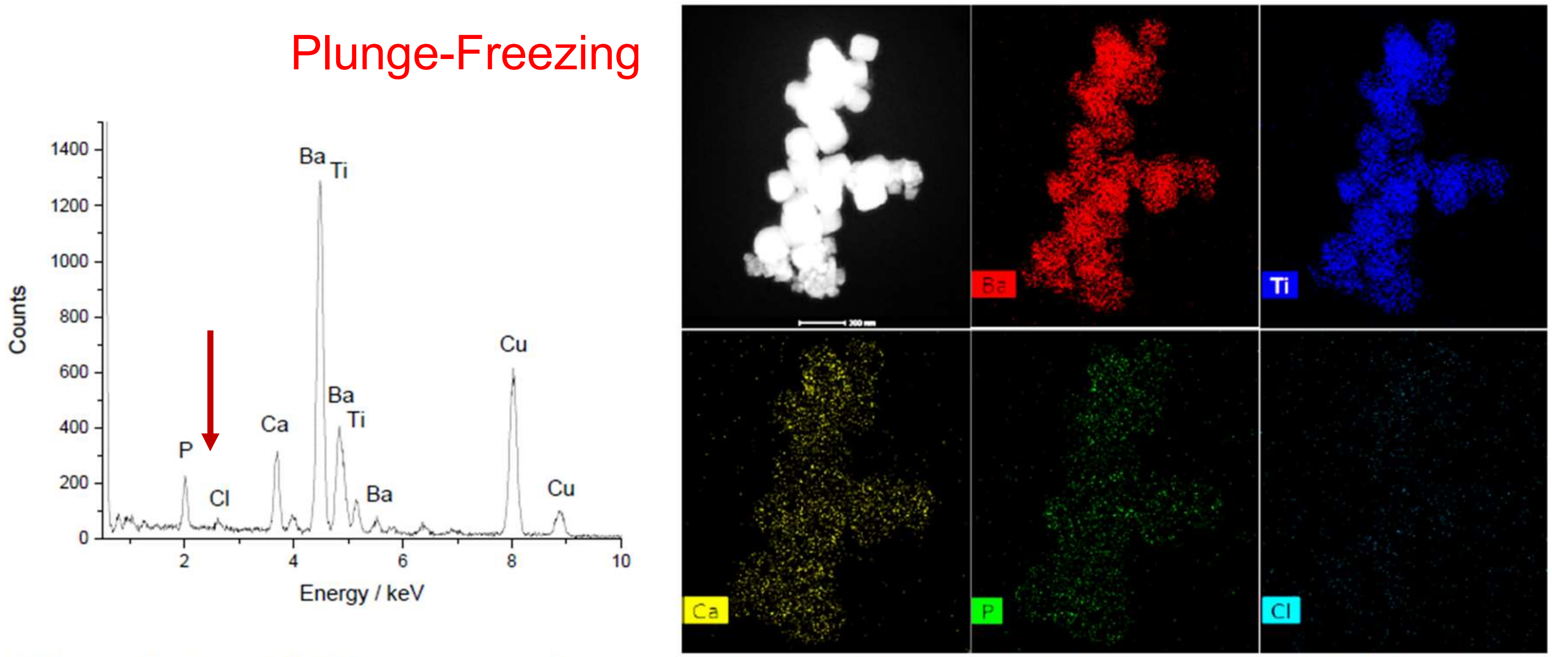
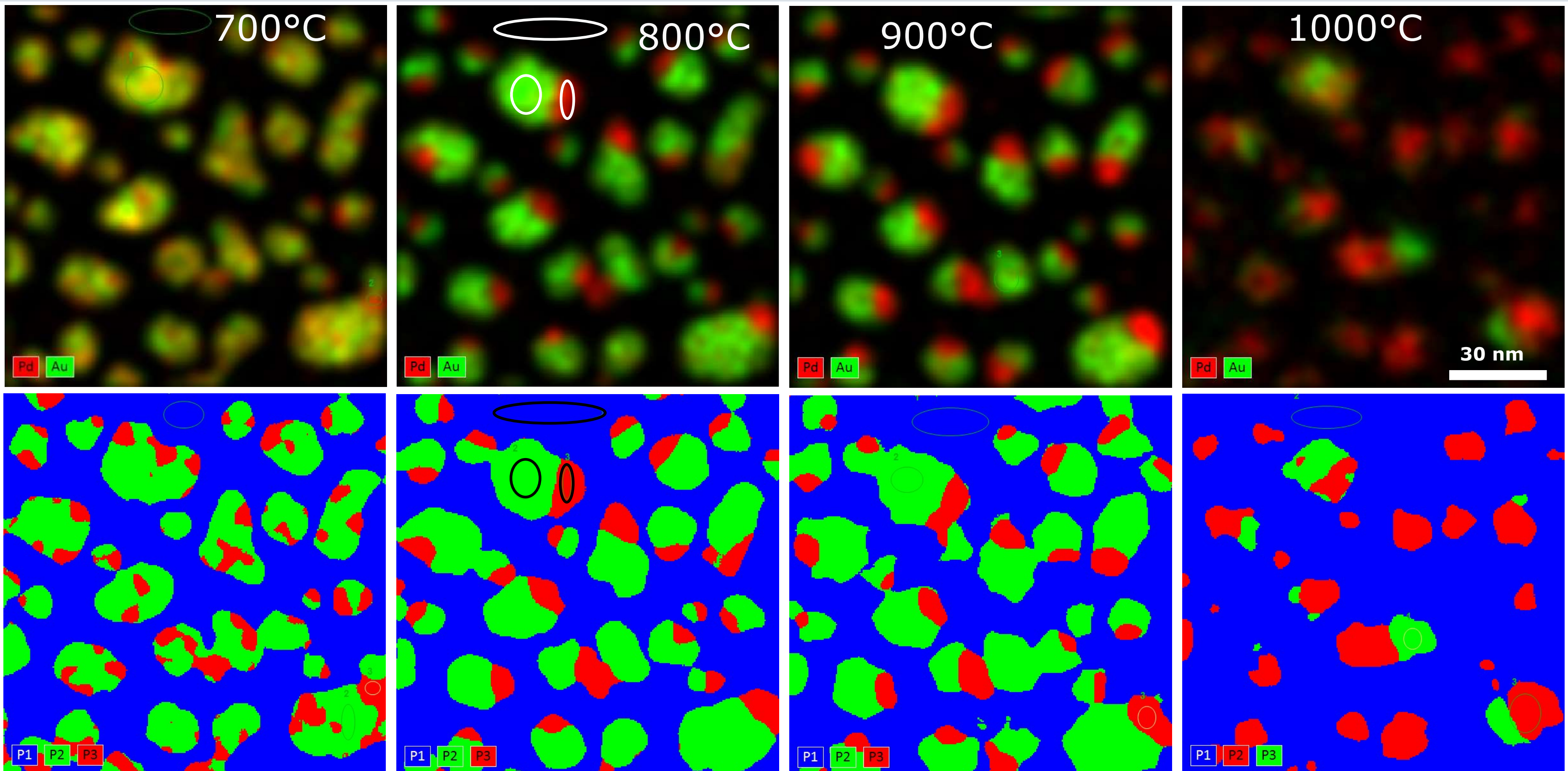


Figure 3: Cryo-EDX spectrum and corresponding EDX maps for BT dispersed in CCCM. Ca and P are present in the spectrum but through cryo analysis no visible Na and Mg peaks were present and the Cl peak was only just distinguishable. Analysis was carried out using a probe current of 40 pA.

Phase Analysis (PCA-based) and (*in situ*) monitoring of particles



	Counts/Pixel	Area
P1		59,2 %
P2	Au	30,5 %
P3	Pd	10,3 %

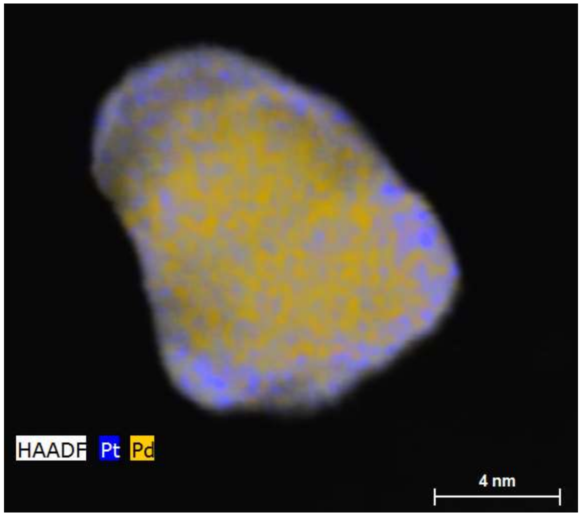
	Counts/Pixel	Area
P1		58,1 %
P2	Au	32,2 %
P3	Pd	9,7 %

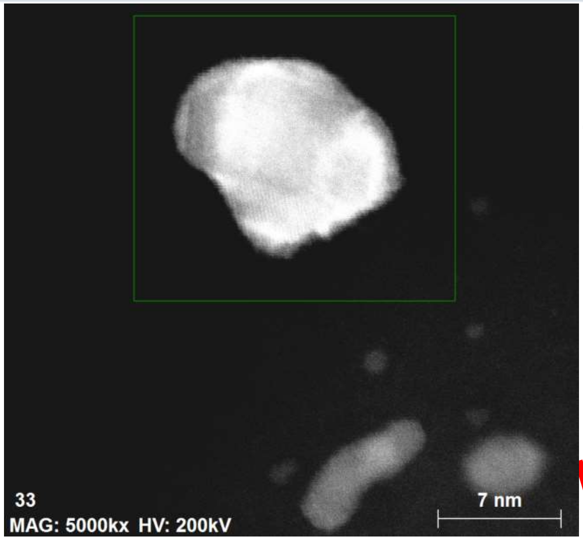
	Counts/Pixel	Area
P1		57,1 %
P2	Au	33,6 %
P3	Pd	9,3 %

	Counts/Pixel	Area
P1		82,6 %
P3	Pd	13,8 %
P2	Au	3,7 %

EDS for Catalysis, Quantification
Pt-Pd Core Shell Particles

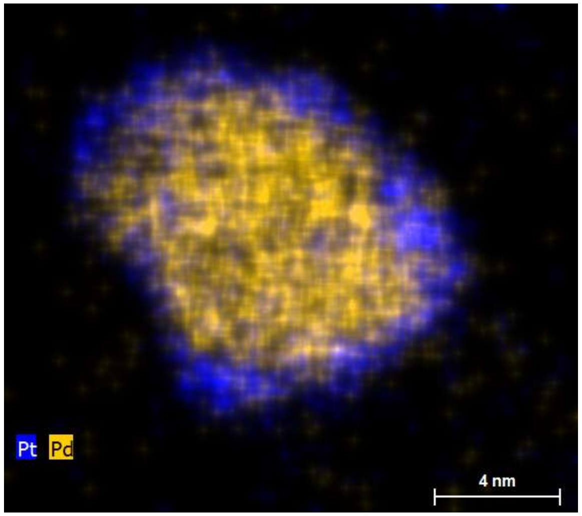
mass%, 30 mm², 0.12 sr (Standard EDS); Cs-corr. STEM

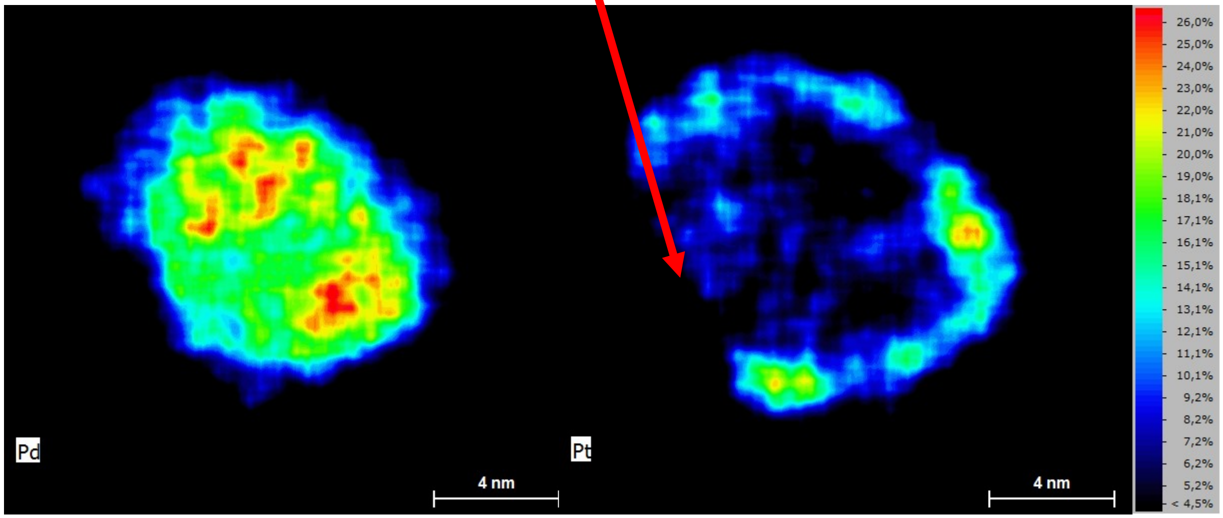




Data courtesy:Dogan Ozkaya, Johnson Matthey Technology Center

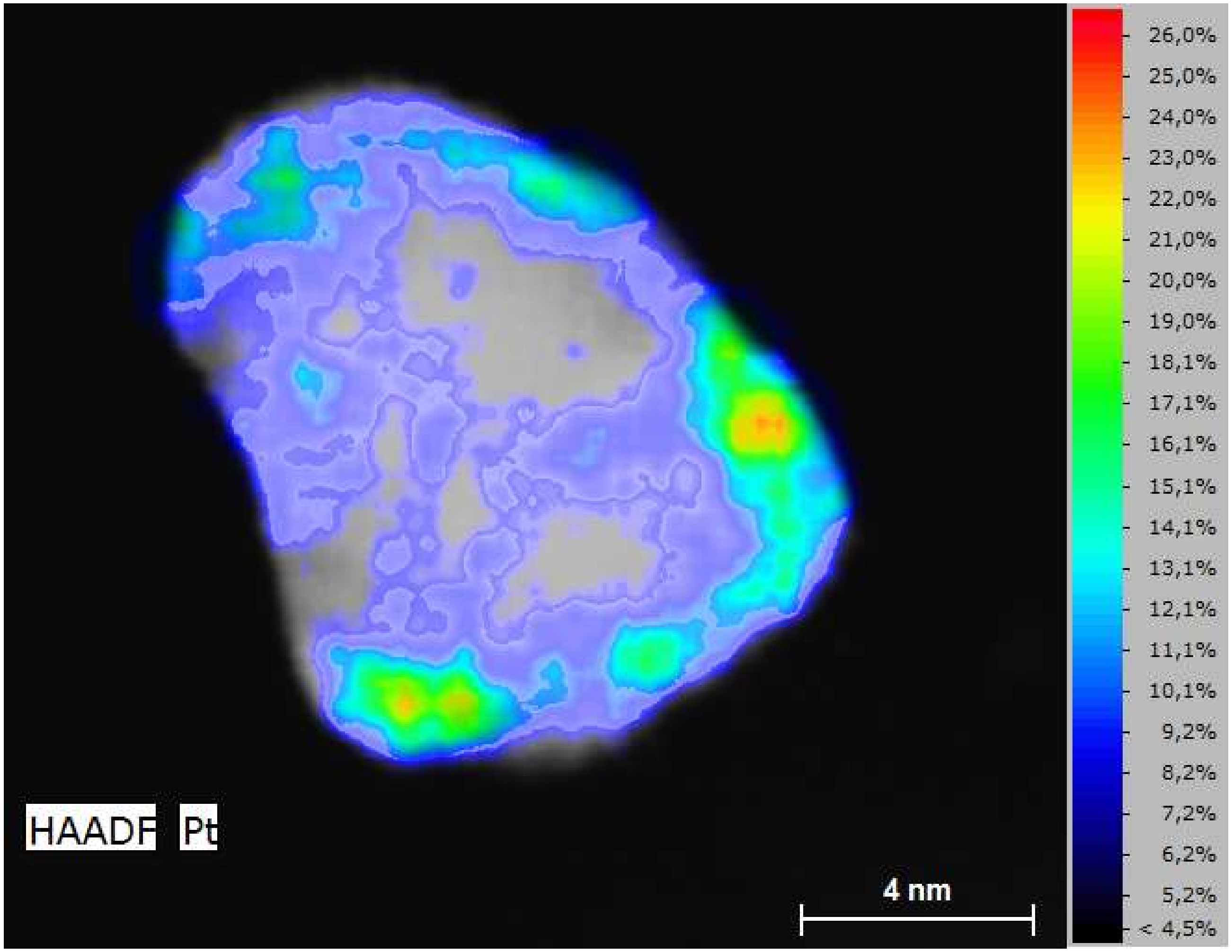
Pt shell not closed
due to fabrication procedure





EDS for Catalysis, Quantification
Pt-Pd Core Shell Particles

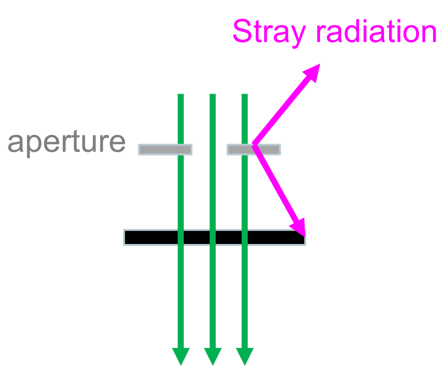
mass%, 30 mm², 0.12 sr (Standard EDS); Cs-corr. STEM



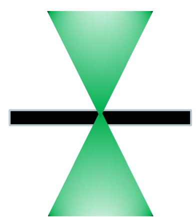
Data courtesy:
Dogan Ozkaya,
Johnson Matthey
Technology Center

Electron Microscopy

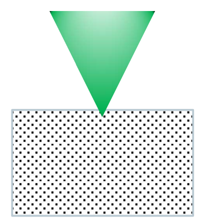
TEM



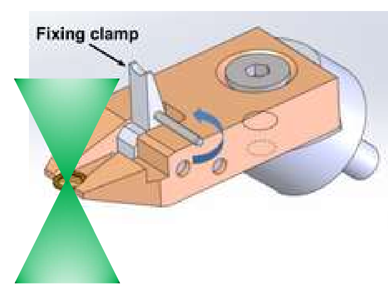
STEM
Scanning TEM



SEM
Scanning EM

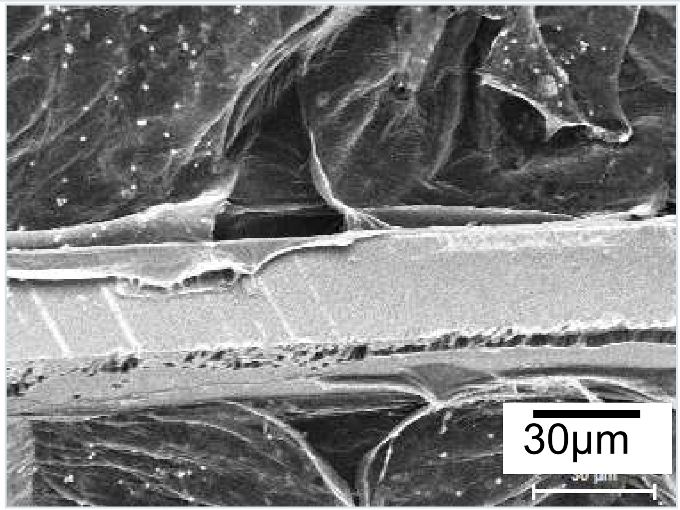


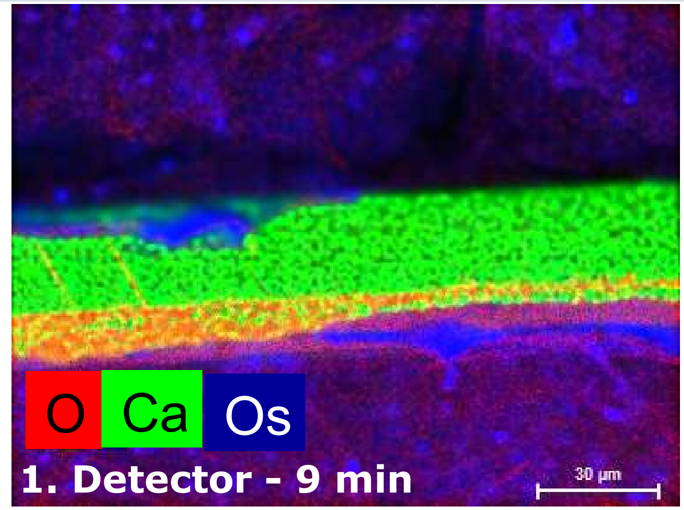
SEM: „T-SEM“



- TKD patented holder
- Commercial STEM holders
- Home made versions

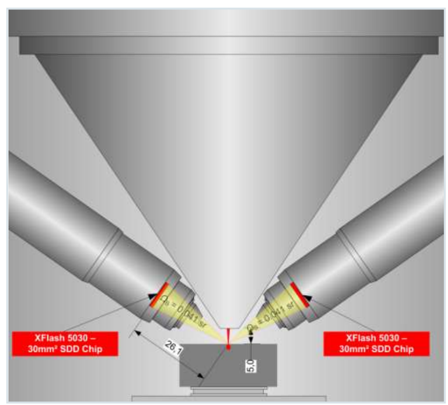
Detector geometries for EDS
Agave Leaf

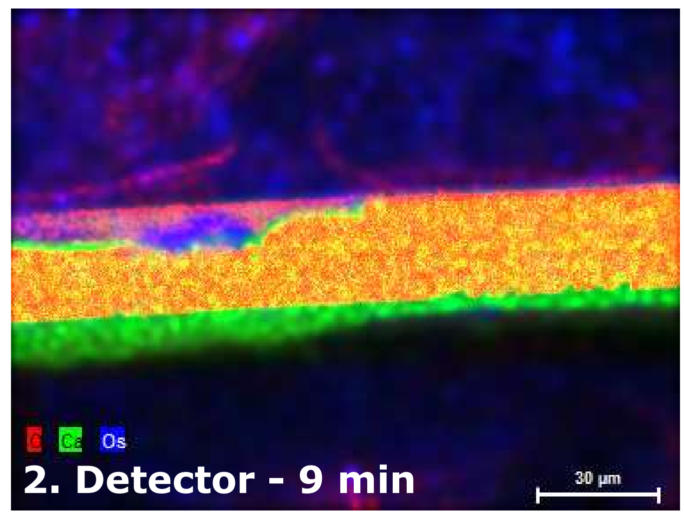




<u>Agave:</u>

Ca-oxalate (CaC_2O_4)
styloid crystal, iching





<u>Analytical conditions:</u>

- 8 kV
- ~0.5 nA
- ~3-5 kcps (each detector)
- 400 x 300 pixel

Detector geometries for EDS
Agave Leaf

Agave:

Ca-oxalate (CaC_2O_4) styloid crystal, iching

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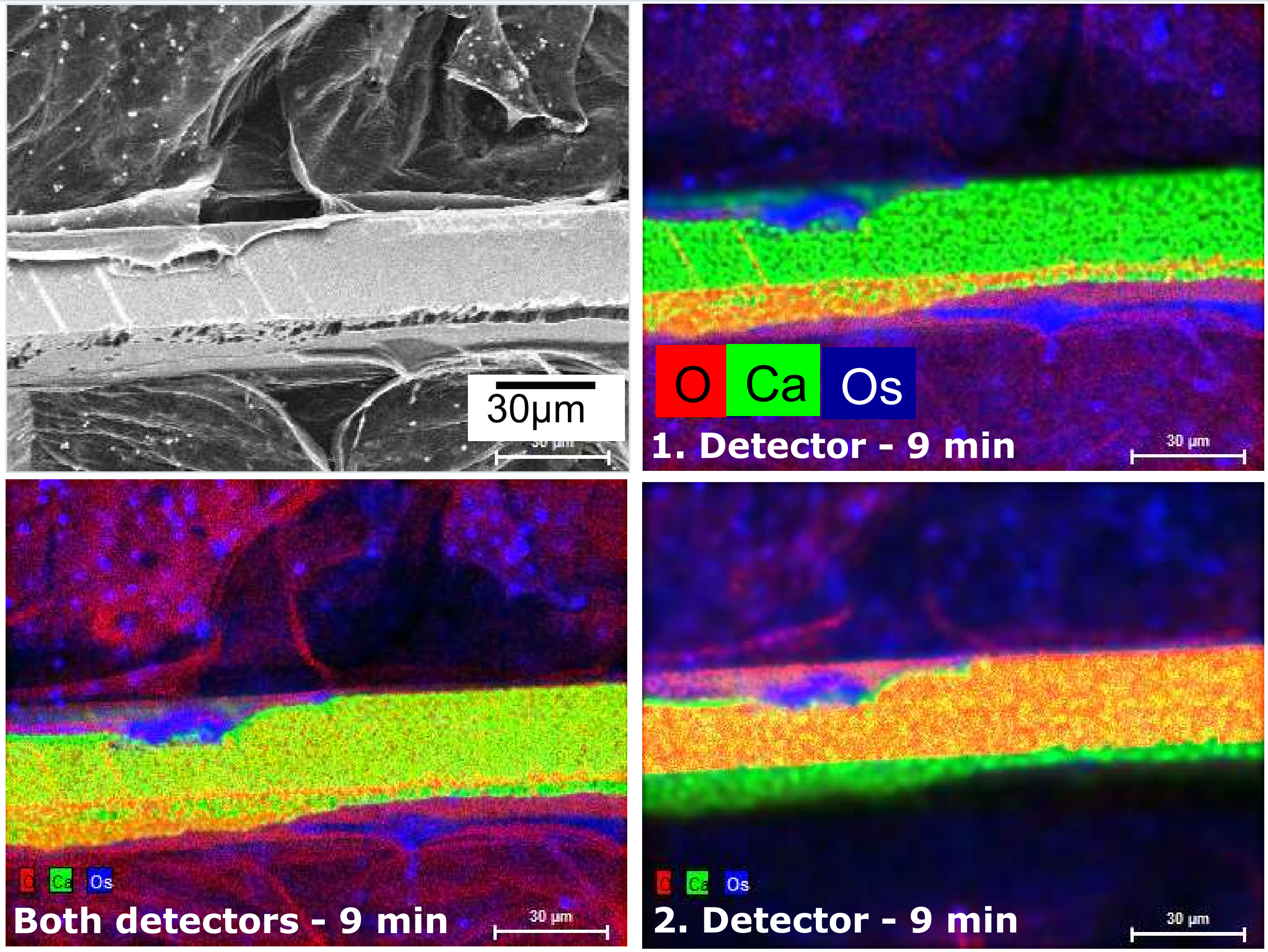
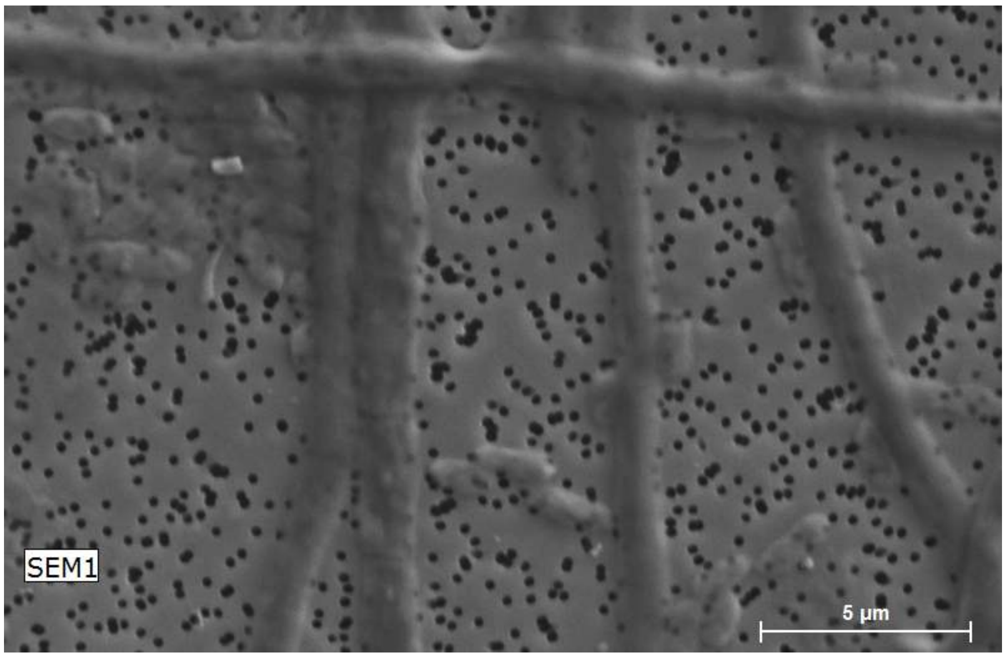
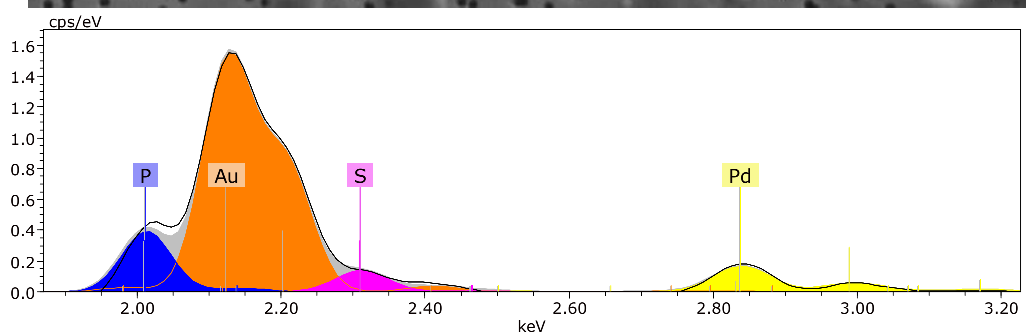


Double detector system

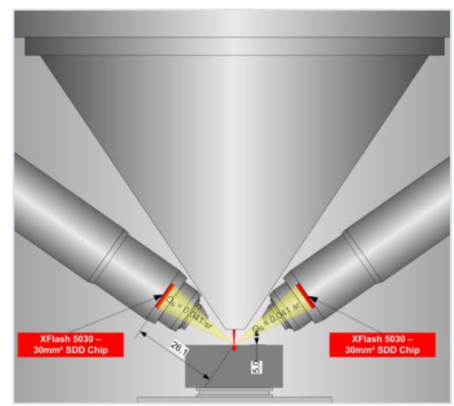
Cell analysis: *Beggiatoa alba* on Au/Pd coated polycarbonate filter

2x30mm^2, 5 kV, 415 pA, 12 kcps, 86 min





- Accumulation of nitrate in massive vacuoles
- Oxidize organics to CO_2 for biosynthesis
- Store sulfur
- Peak deconvolution reveals distribution of S and P



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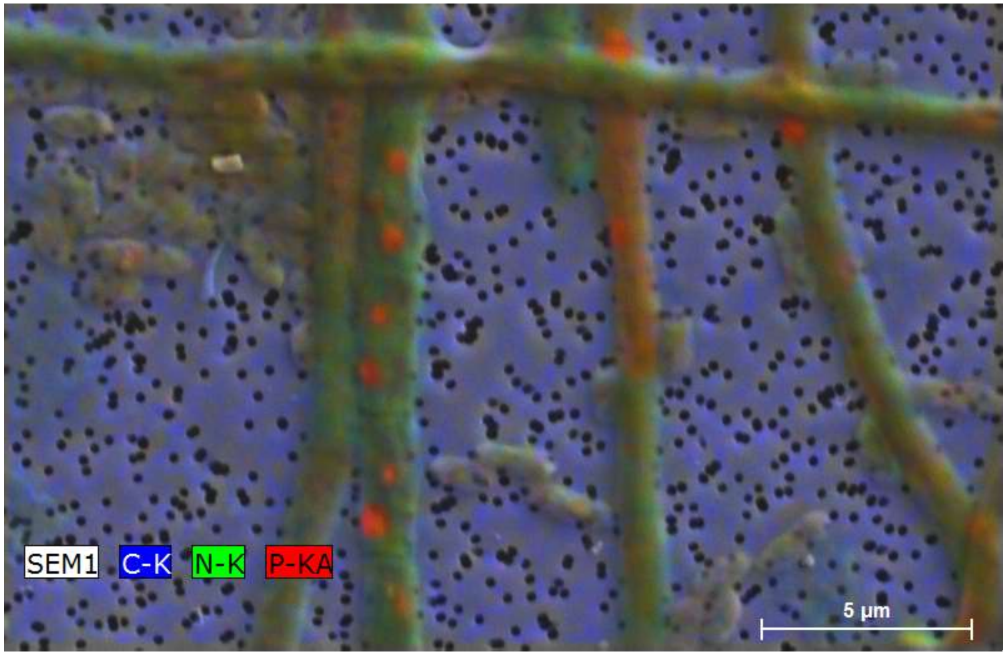

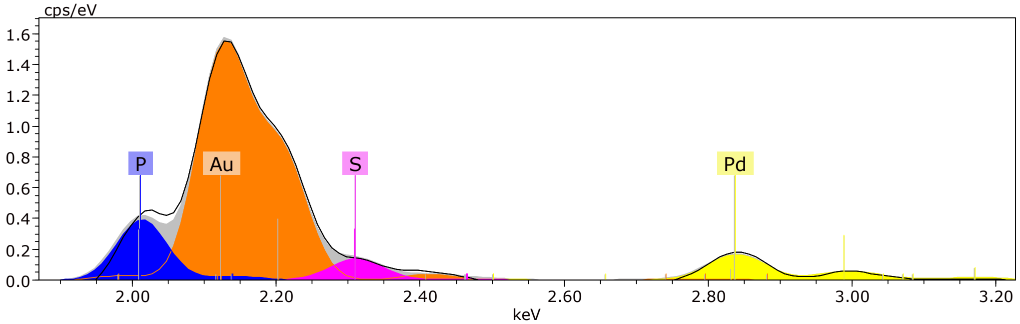

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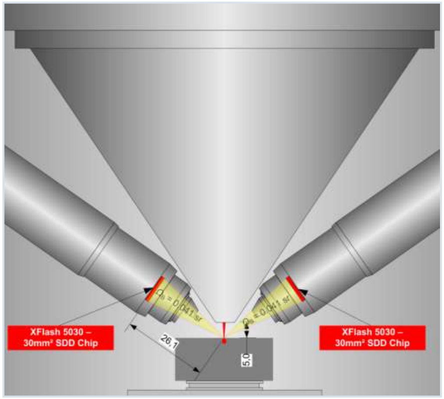

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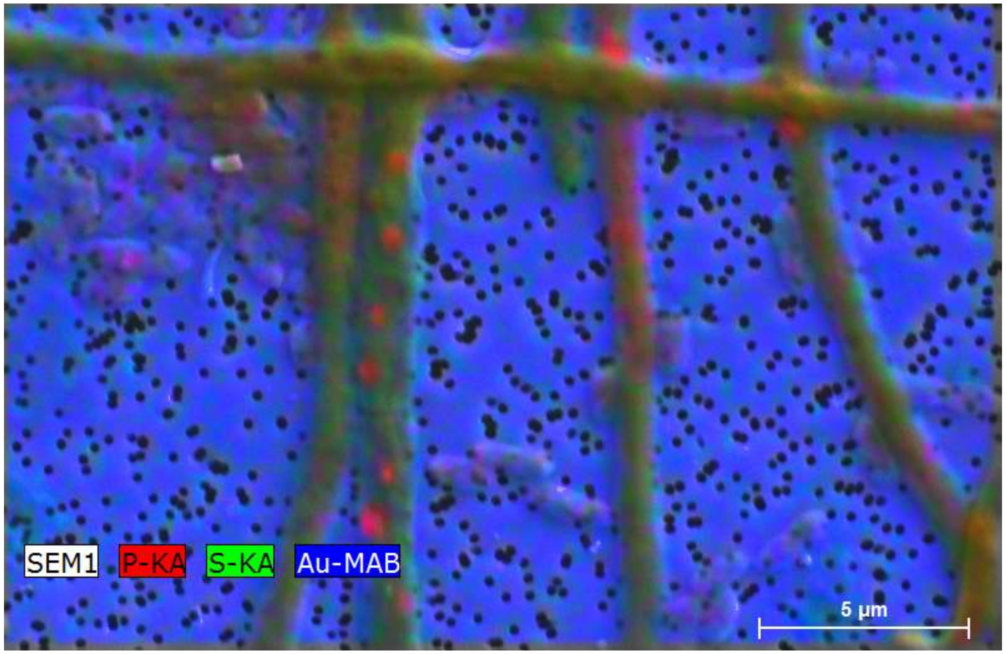

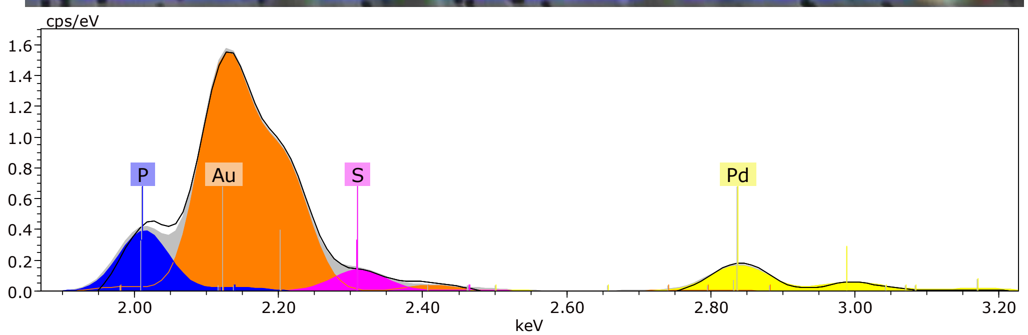

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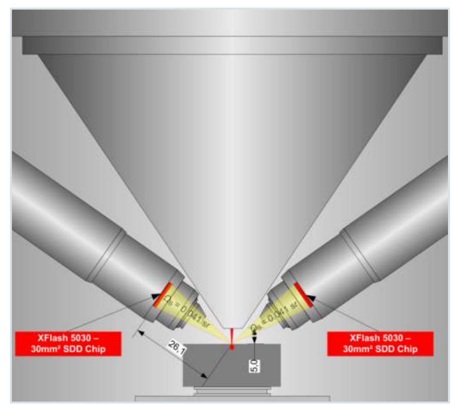
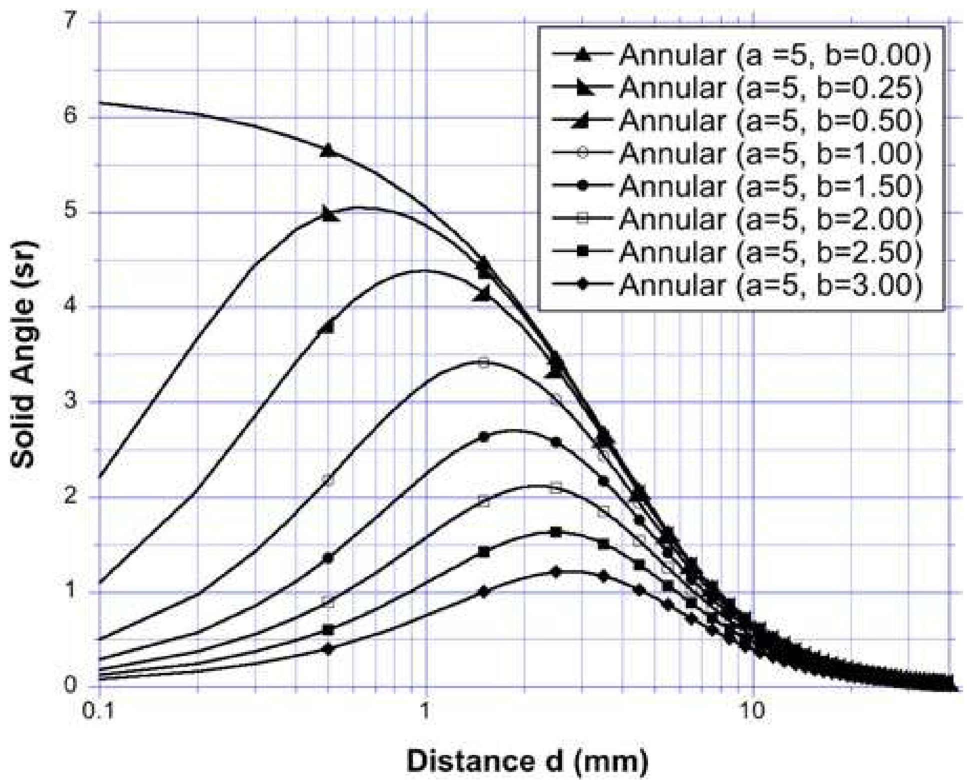
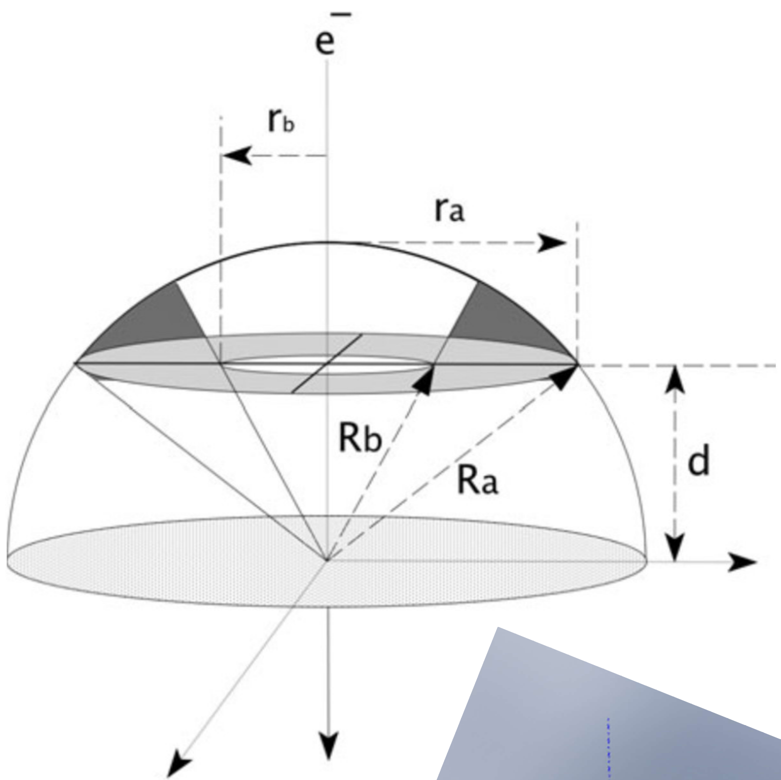
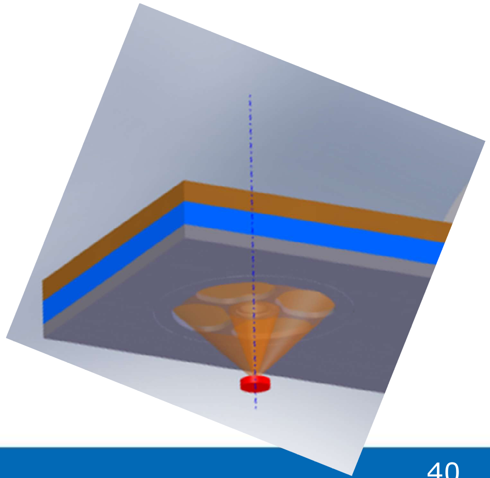


Annular EDS for T-SEM
Flat Quad XFlash® 5060FQ





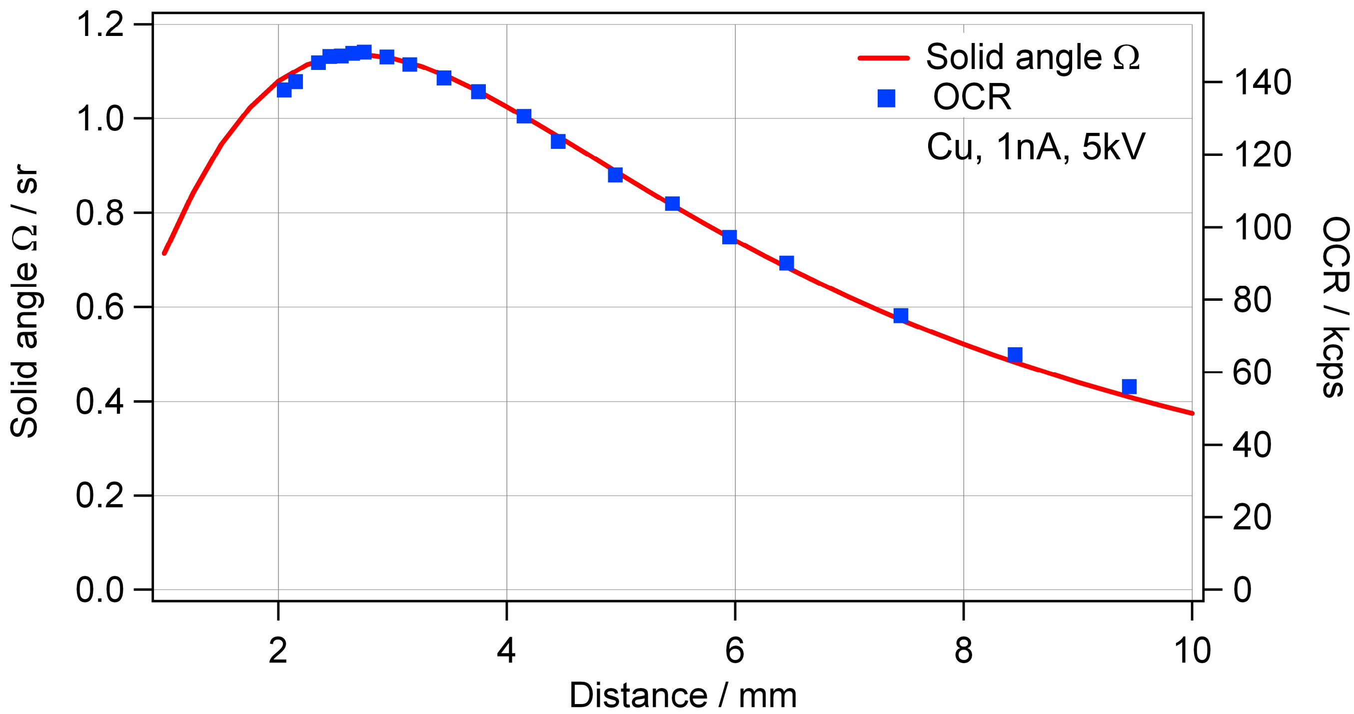


N. J. Zaluzec, Detector solid angle Formulas for use in EDS, Microsc. Microanal., 15 (2009) 93

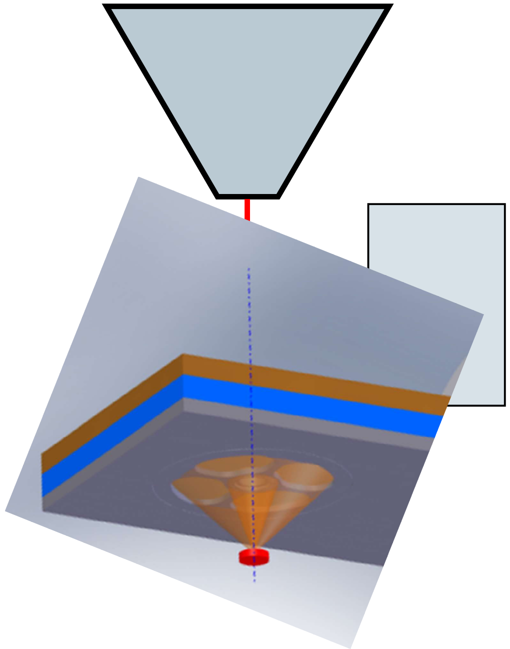
http://tpm.amc.anl.gov/NJZTools/XEDSSolidAngle.html

Annular EDS for T-SEM
Flat Quad XFlash® 5060FQ

Solid angle and OCR vs distance d

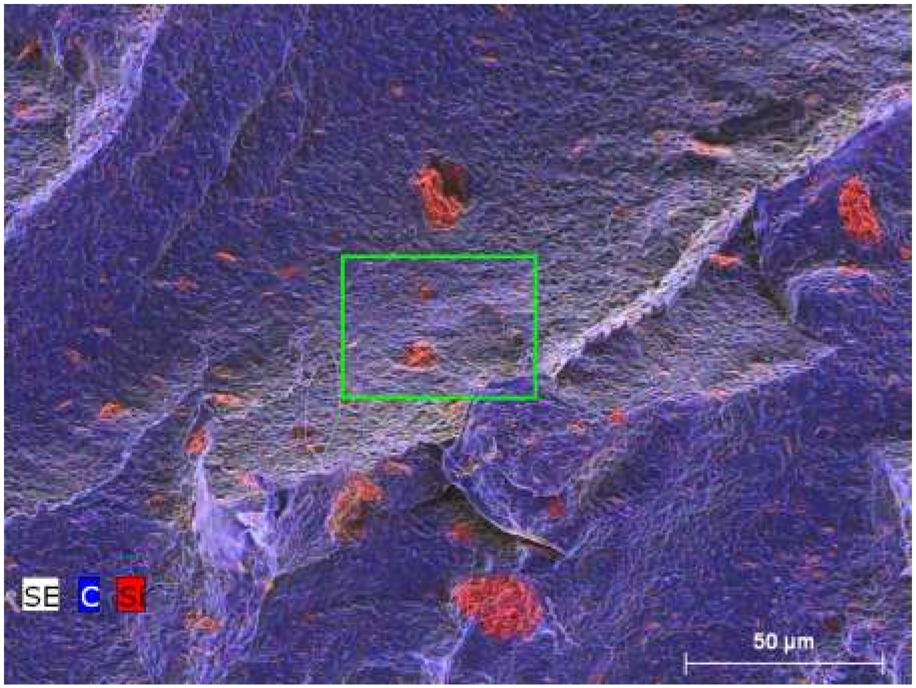


max solid angle at d = 2.5mm: Ω > 1.1 sr

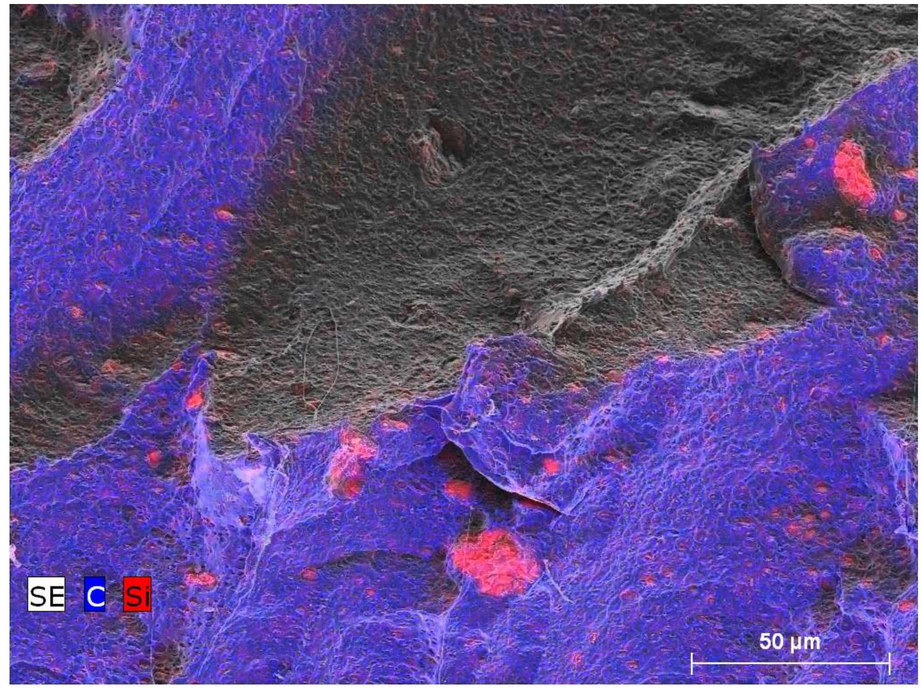


XFlash QUAD vs Single detector in SEM:

Polymer composite containing organo clay



XFlash Flat QUAD detector
3 kV, 220pA, 10 kcps,
320 s, 1024x768 pixel

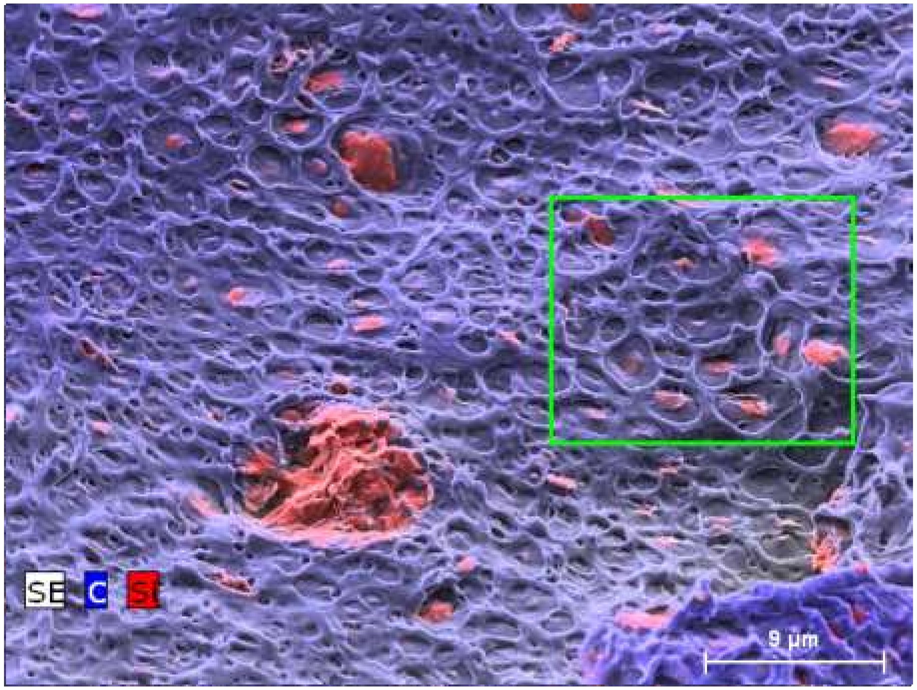


Single 30mm² XFlash
3 kV, 220pA, 0.8 kcps,
320 s, 1024x768 pixel
Shadow effects due to rough surface

Sample courtesy by Dalto et al., Universidade Federal do Rio de Janeiro,
Data courtesy T. Salge (Bruker / NHM, London);

XFlash QUAD vs Single detector in SEM:

Polymer composite containing organo clay



XFlash Flat QUAD detector
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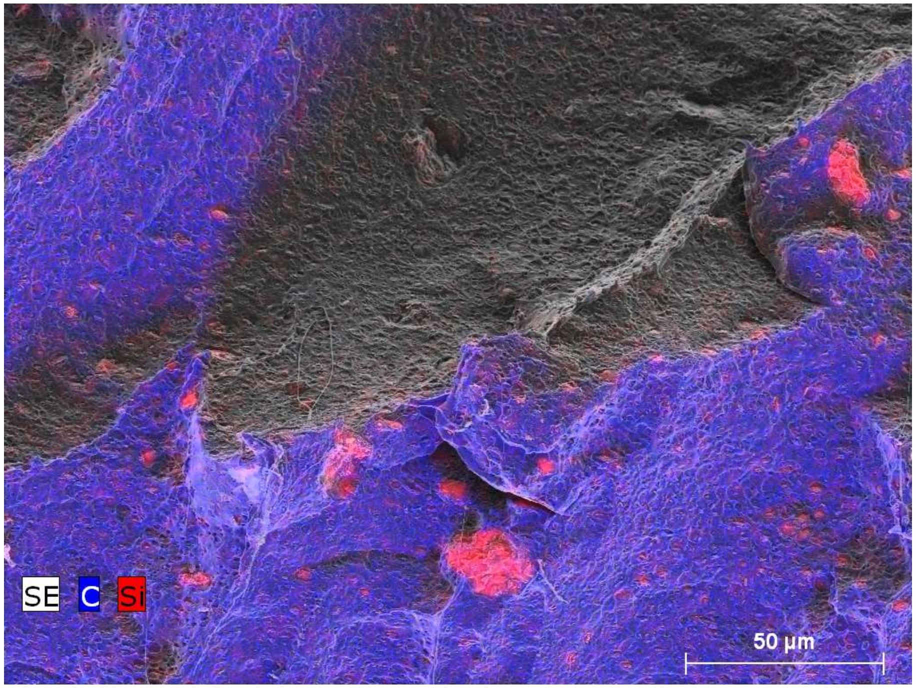
320 s, 1024x768 pixel

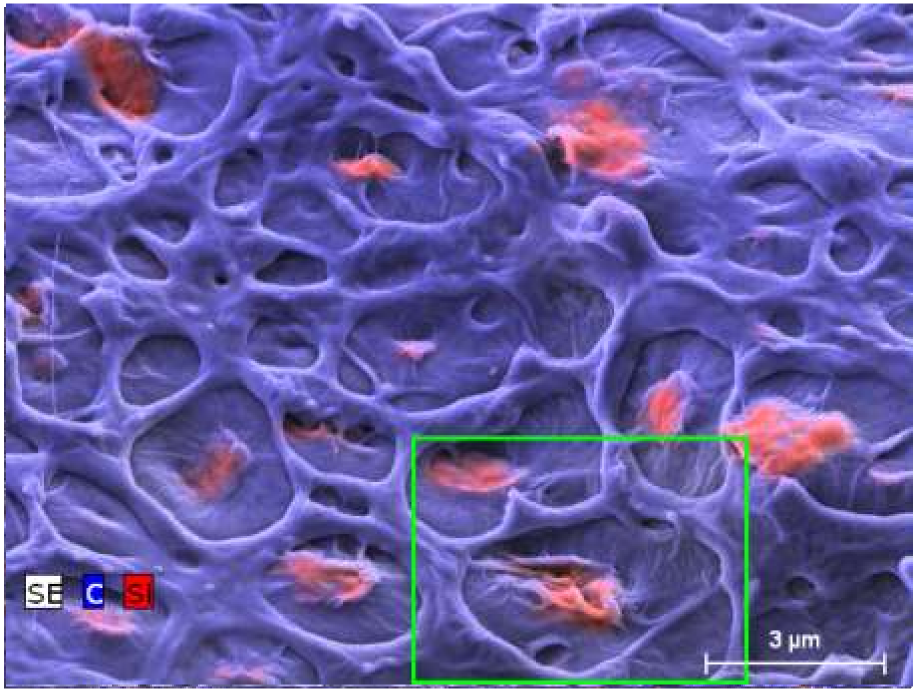


Single 30mm^2 XFlash
3 kV, 220pA, 0.8 kcps,
320 s, 1024x768 pixel
Shadow effects due to rough surface

Sample courtesy by Dalto et al., Universidade Federal do Rio de Janeiro,
Data courtesy T. Salge (Bruker / NHM, London);

XFlash QUAD vs Single detector in SEM:

Polymer composite containing organo clay



XFlash Flat QUAD detector
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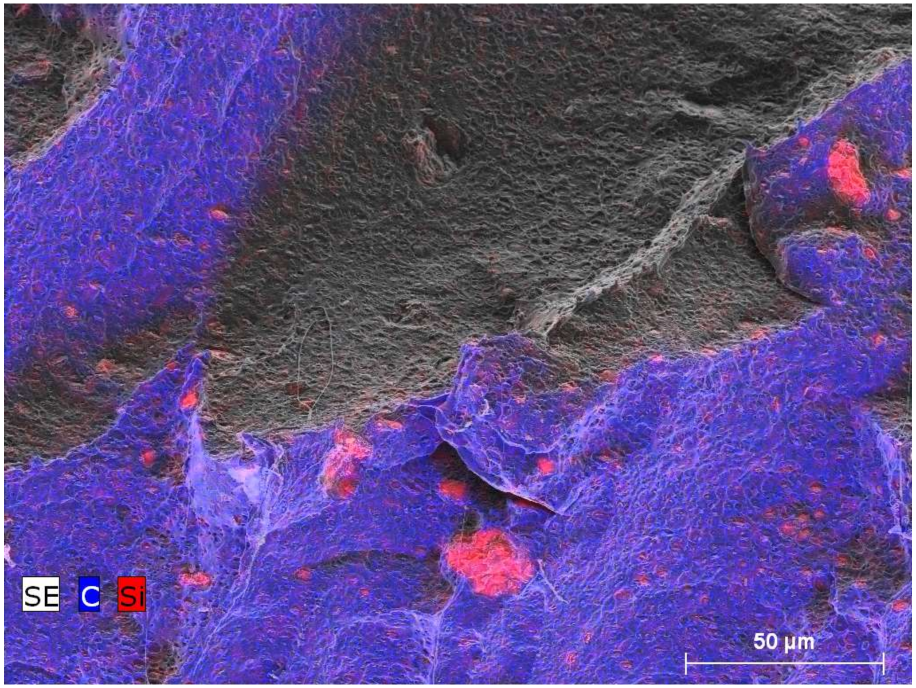

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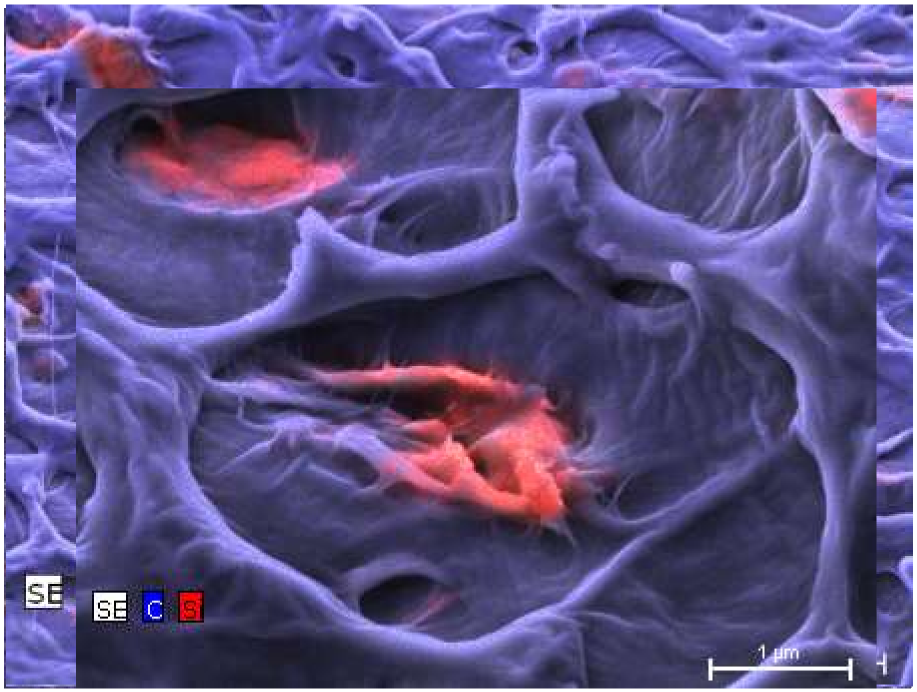

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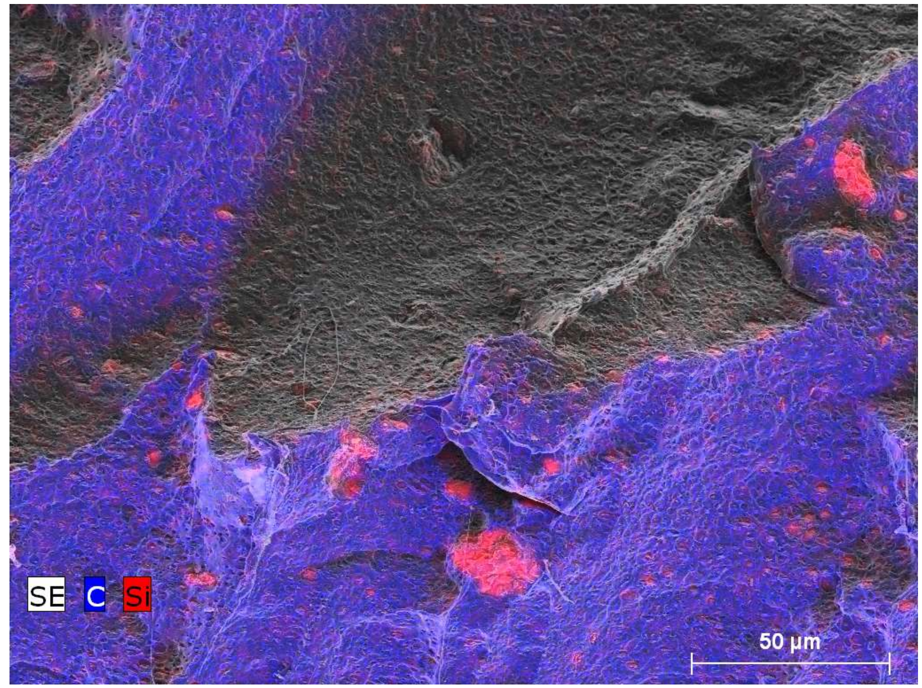

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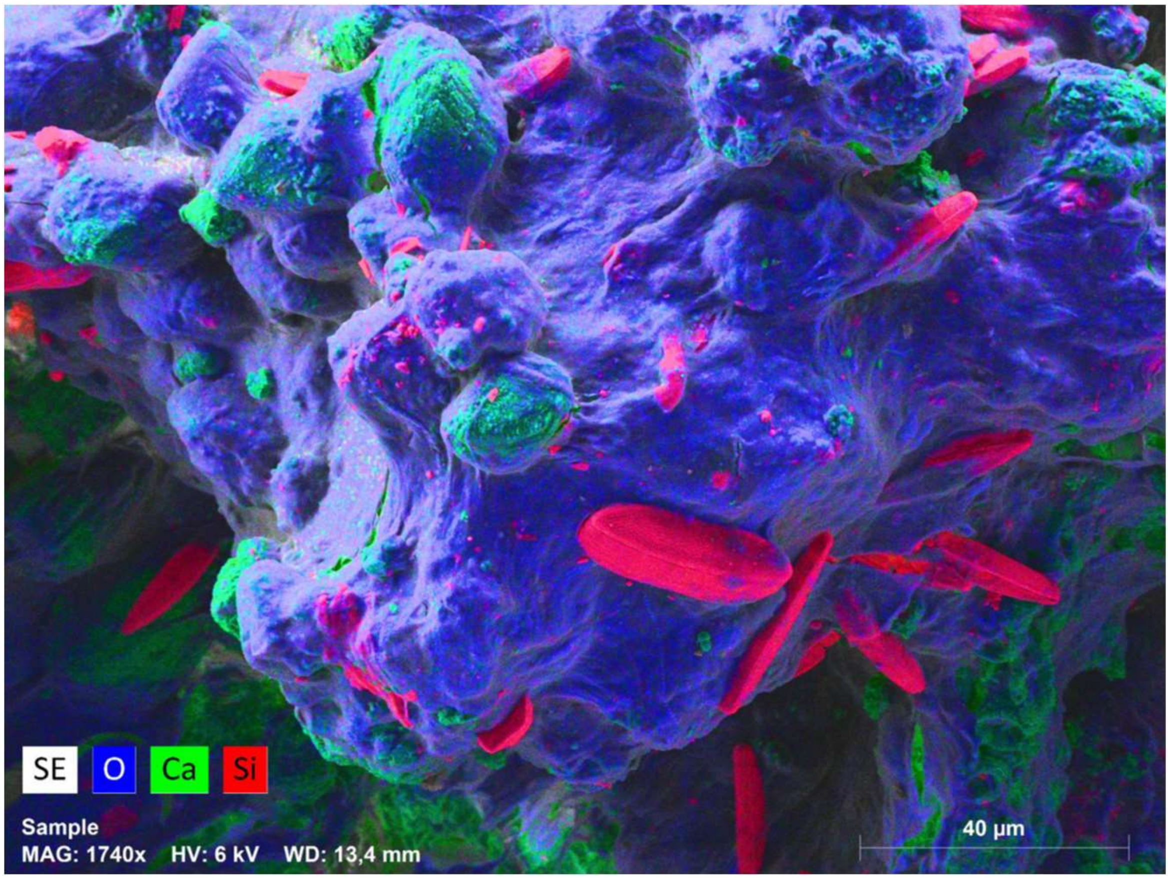
Microbial mat with diatoms, Specimen taken from Hot spring



Reddish ovals are diatoms sticking to the bluish biofilm surface

Carbonates (green) are embedded within the biofilm matrix

Microbial mat with diatoms, Specimen taken from Hot spring

Reddish ovals are diatoms sticking to the bluish biofilm surface

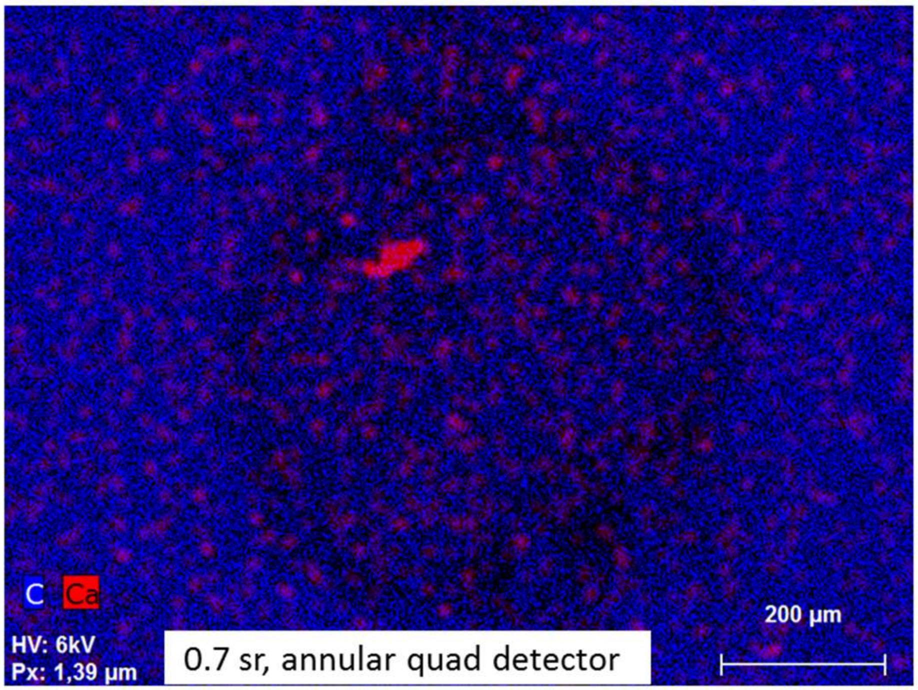
Carbonates (green) are embedded within the biofilm matrix

Low vacuum analysis
Parasitoid wasp
Monolexis fuscicornis

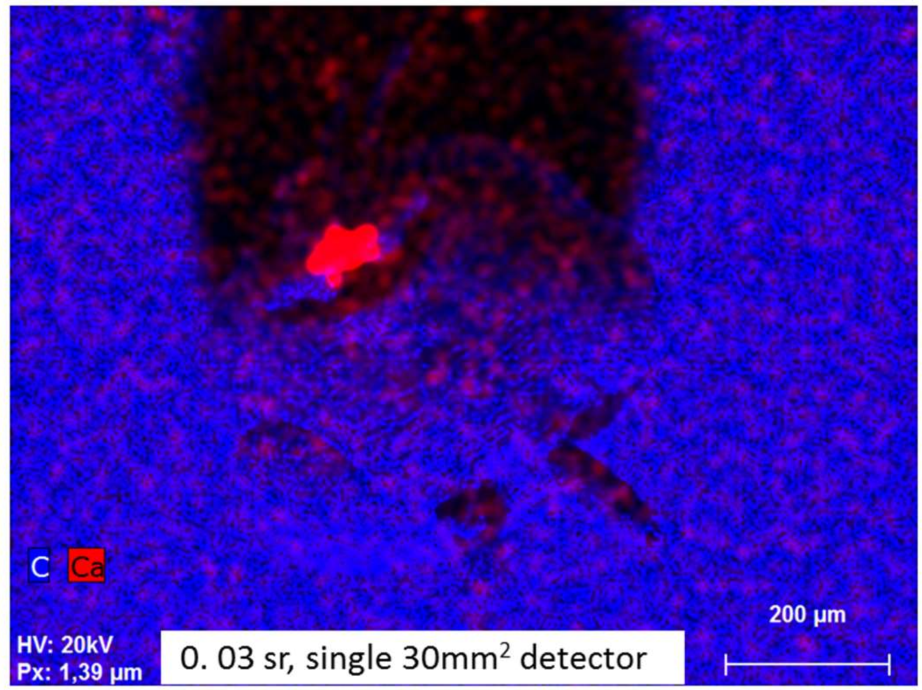
1.2sr

Wasps head with tooth

Flat Quad (**annular**)



Single 30mm^2 detector



Low vacuum (20 Pa), 6kV, 240 sec, 800 x 600 pixel

Low vacuum analysis
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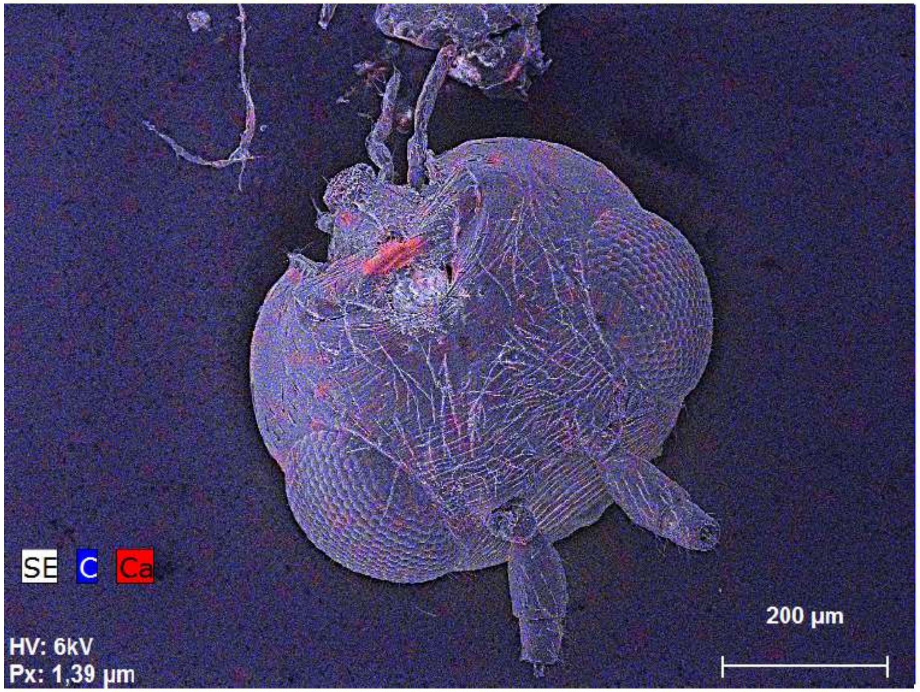
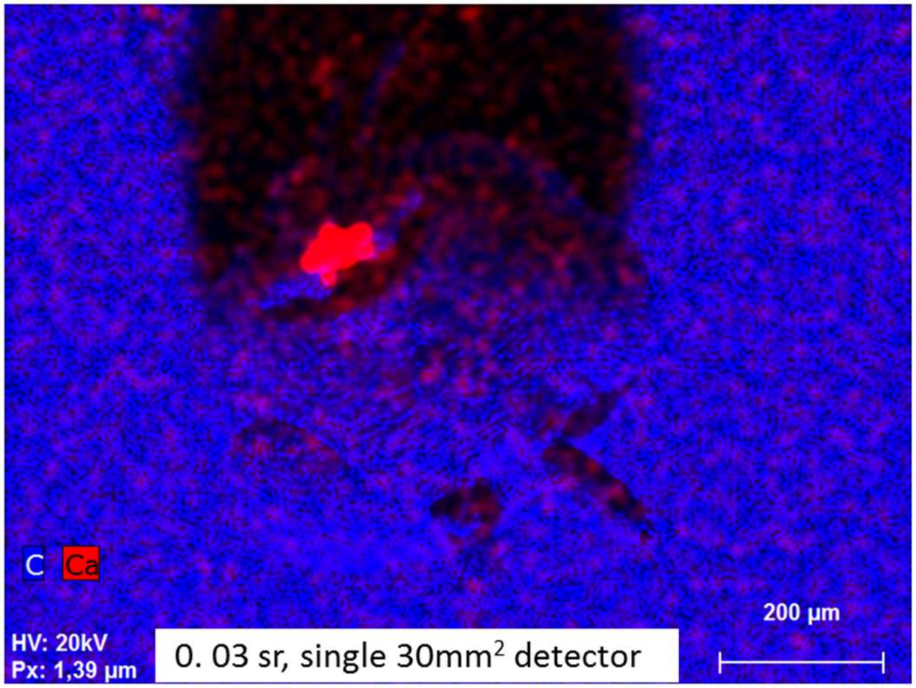


Single 30mm² detector



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Monolexis fuscicornis

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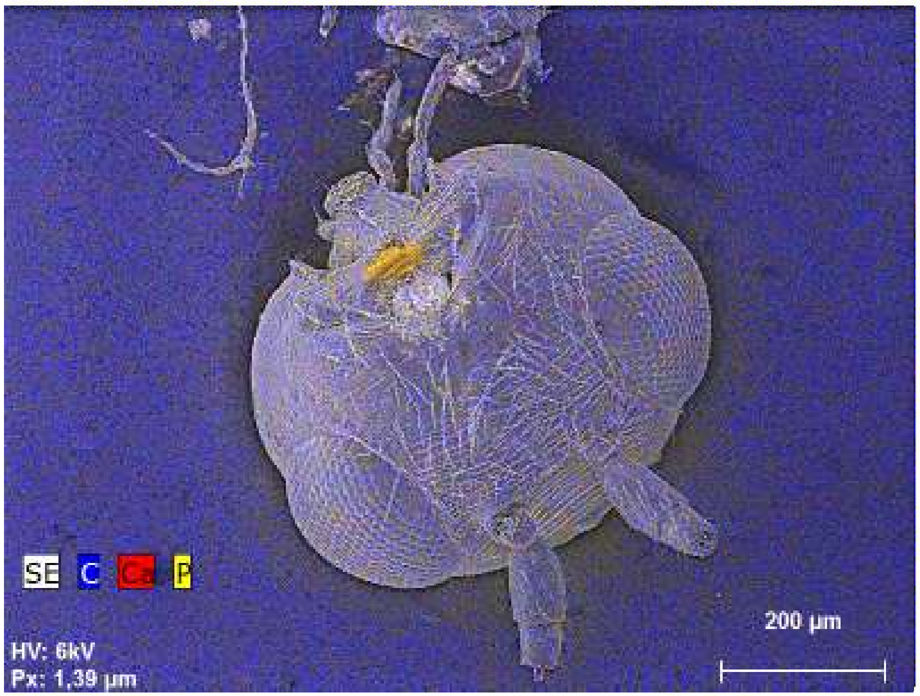
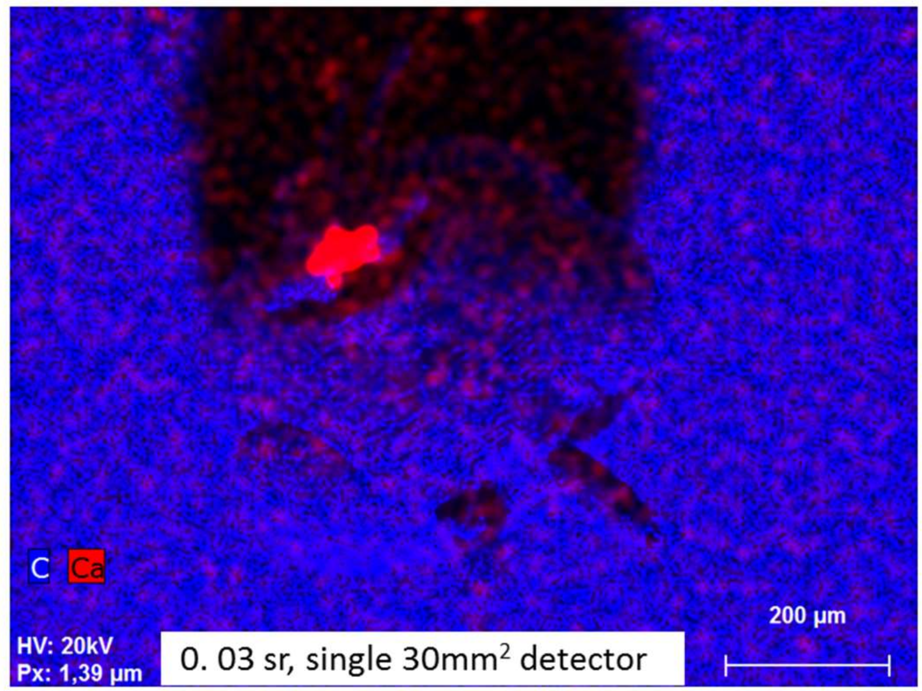
1.2sr

Wasps head with tooth

Flat Quad (**annular**)



Single 30mm^2 detector



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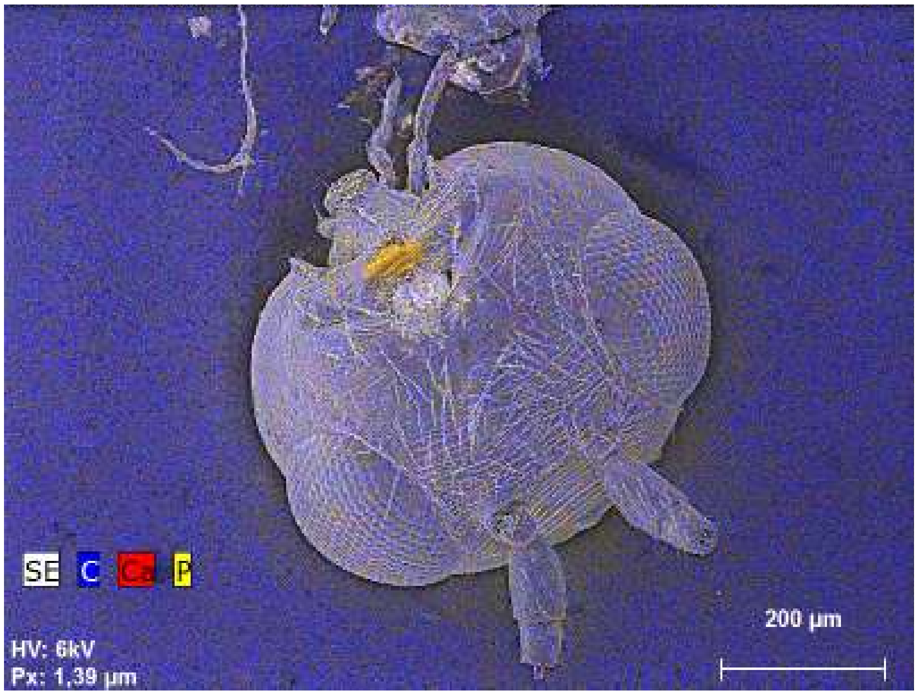
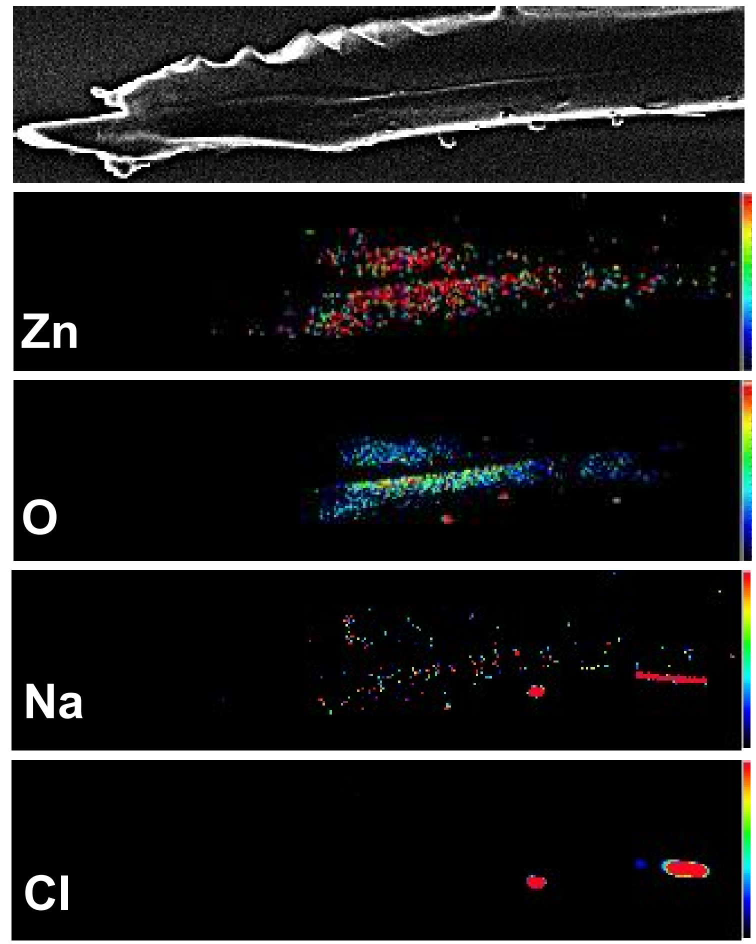
Parasitoid wasp
Monolexis fuscicornis

1.2sr

Head



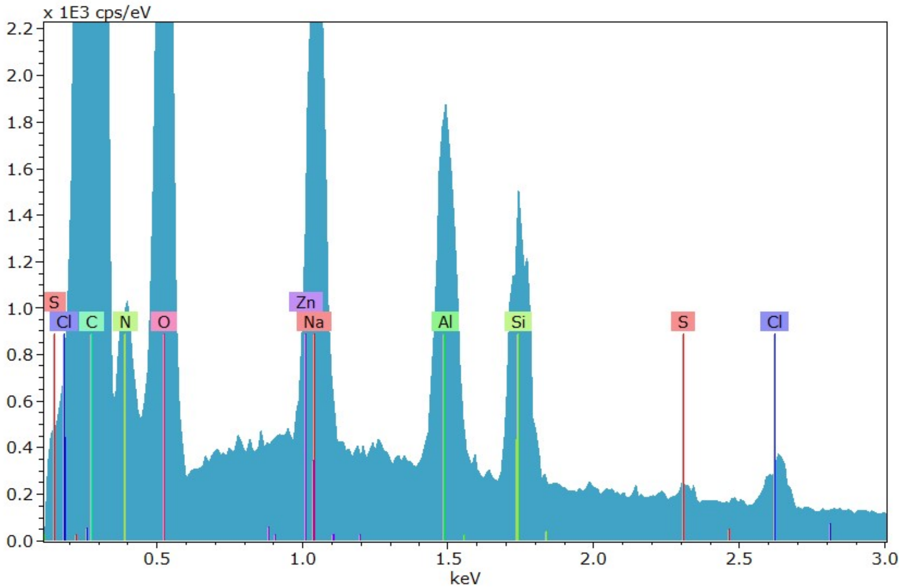
Ovipositor (sting and egg-layer)

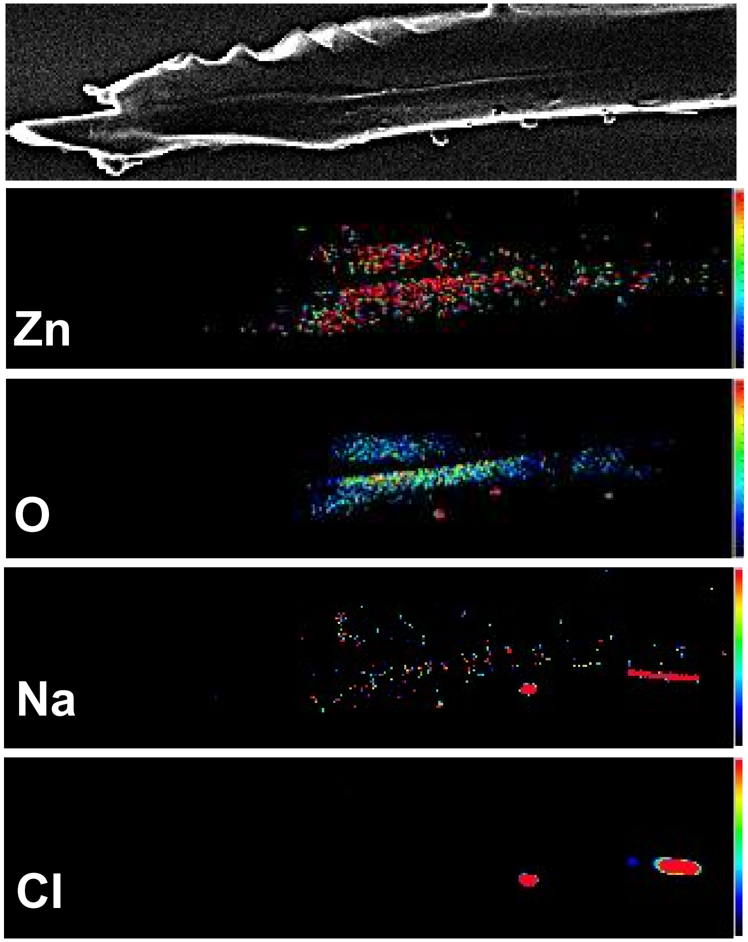


Low vacuum analysis
Parasitoid wasp
(*Monolexis fuscicornis*)

Ovipositor
(sting and egg-layer)

- Low vacuum (20 Pa),
  5kV, 30 min, 320 x 240 pixel





Low vacuum analysis
Parasitoid wasp
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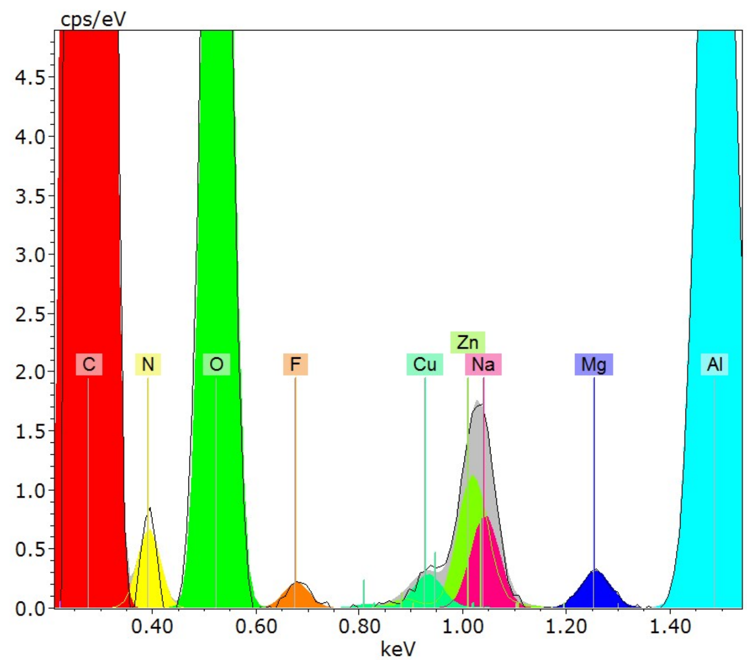

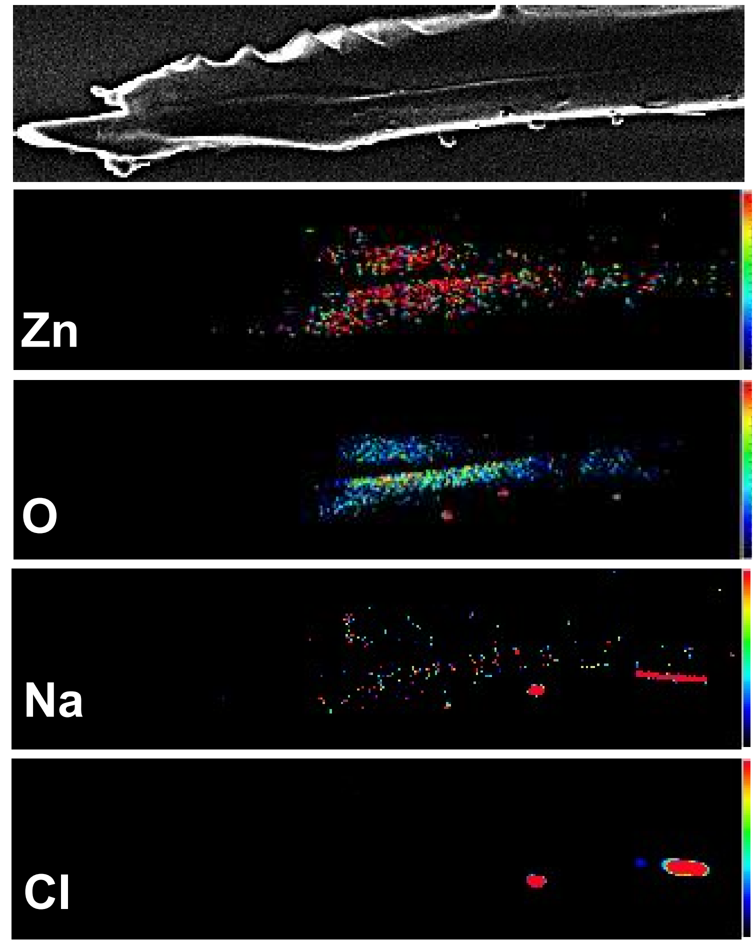

In cooperation with: A. T. Kearsley & G. R. Broad (Natural History Museum, London)

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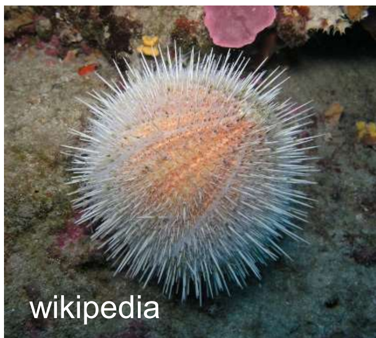
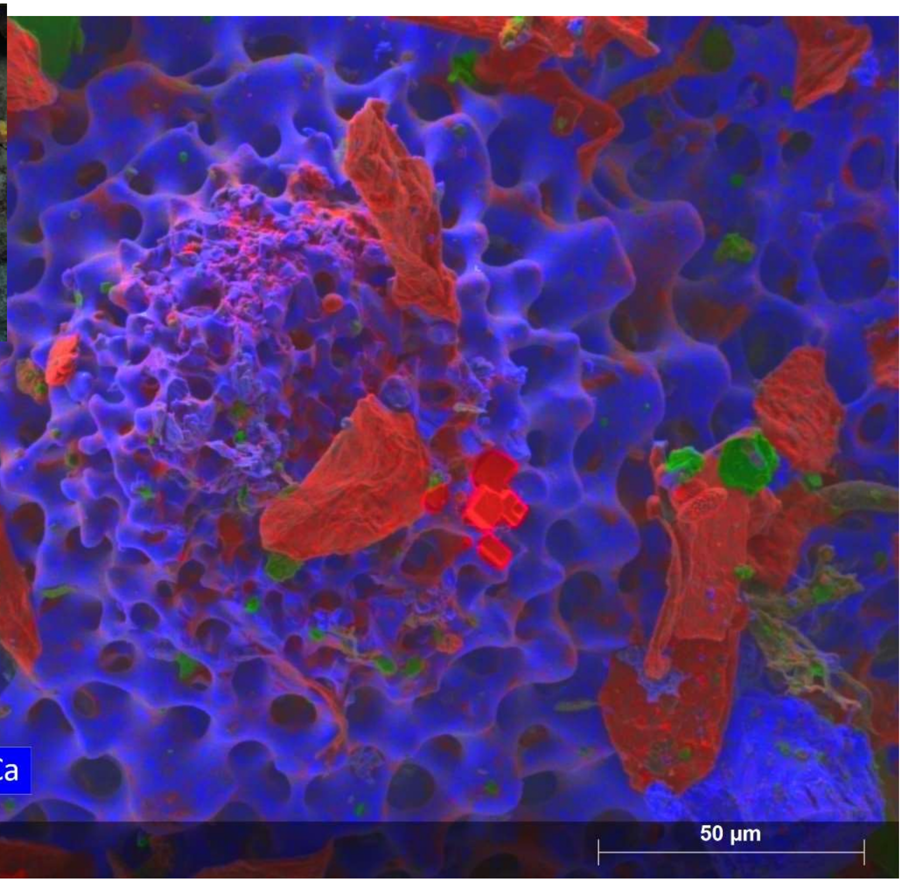
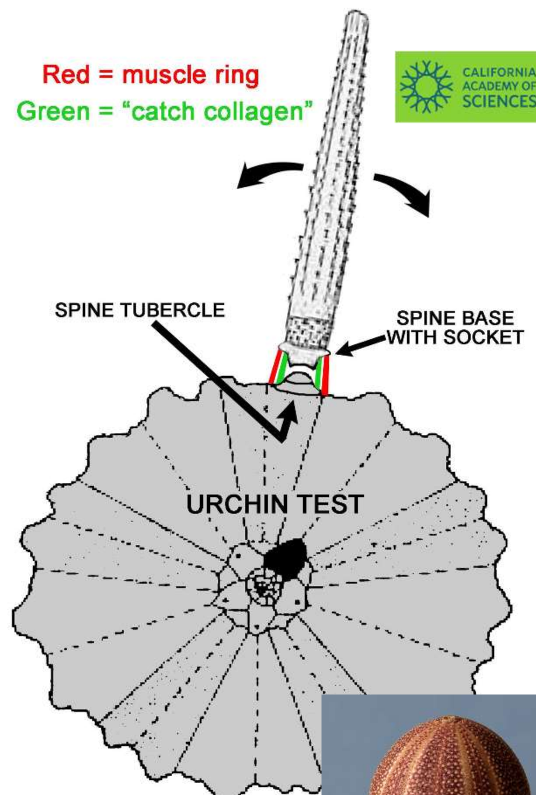
A Sea Urchins Spine Tubercle: bio-mimetics / bio-inspired construction

T. B. Grun, J. H. Nebelsick, PLOS, Sep. 27, 2018, https://doi.org/10.1371/journal.pone.0204432

Lightweight hirachical construction ensures stiffnes, heavy load bearing and flexibility







A Sea Urchins Spine Tubercle: bio-mimetics / bio-inspired construction

https://www.calacademy.org/blogs/2011-philippine-biodiversity-expedition/when-it-comes-to-echinoderm-collagen-there-is-always-a

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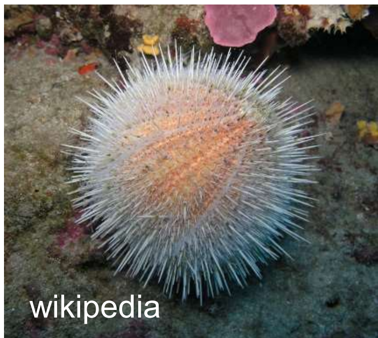

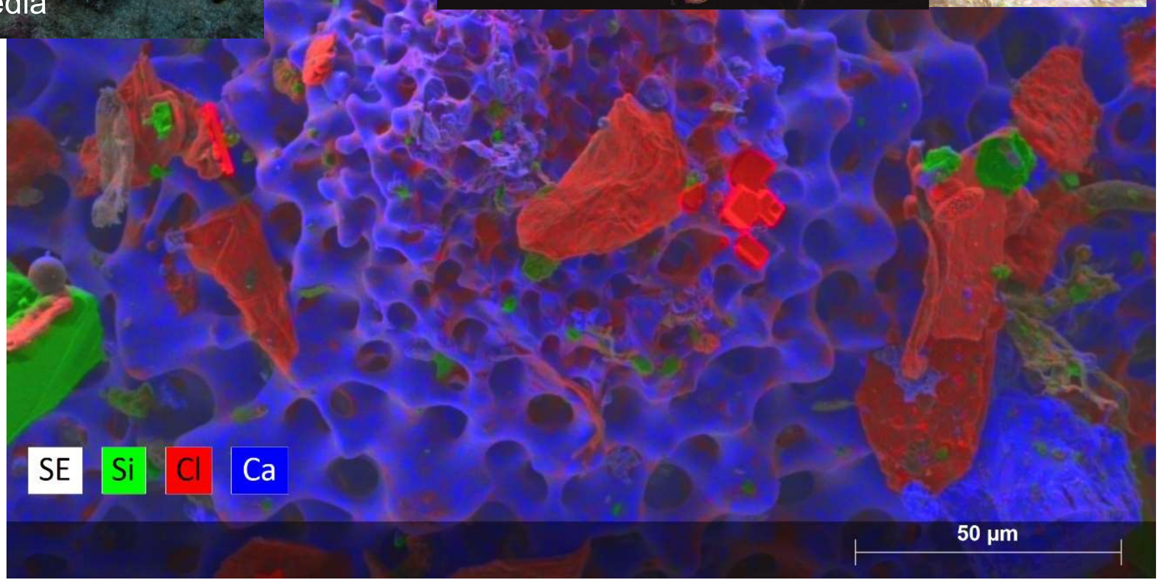

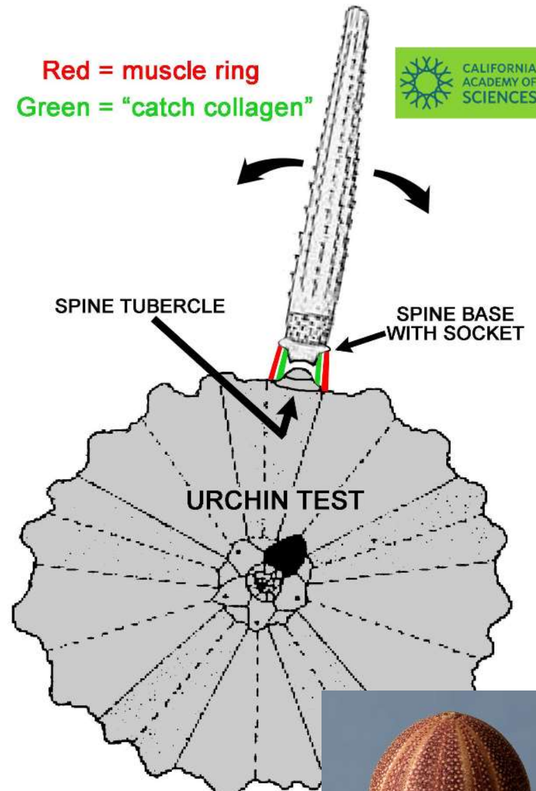

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BRUKER

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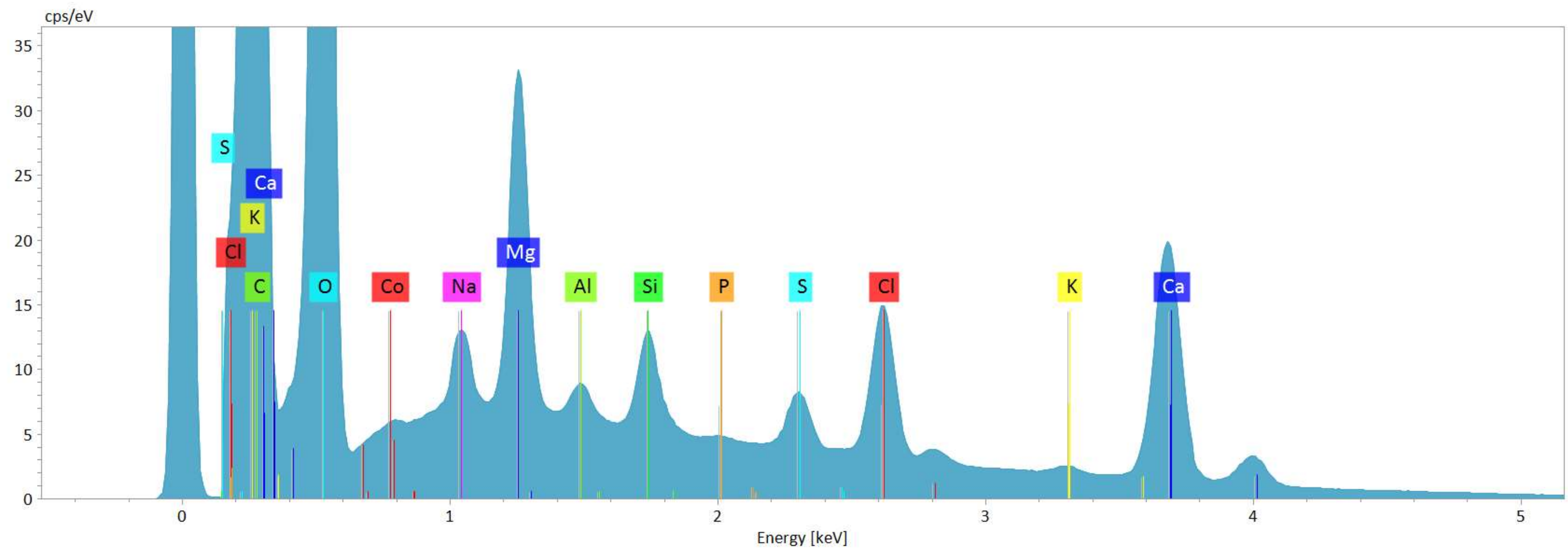
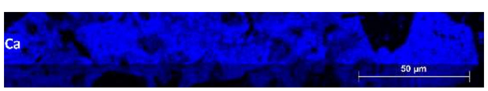
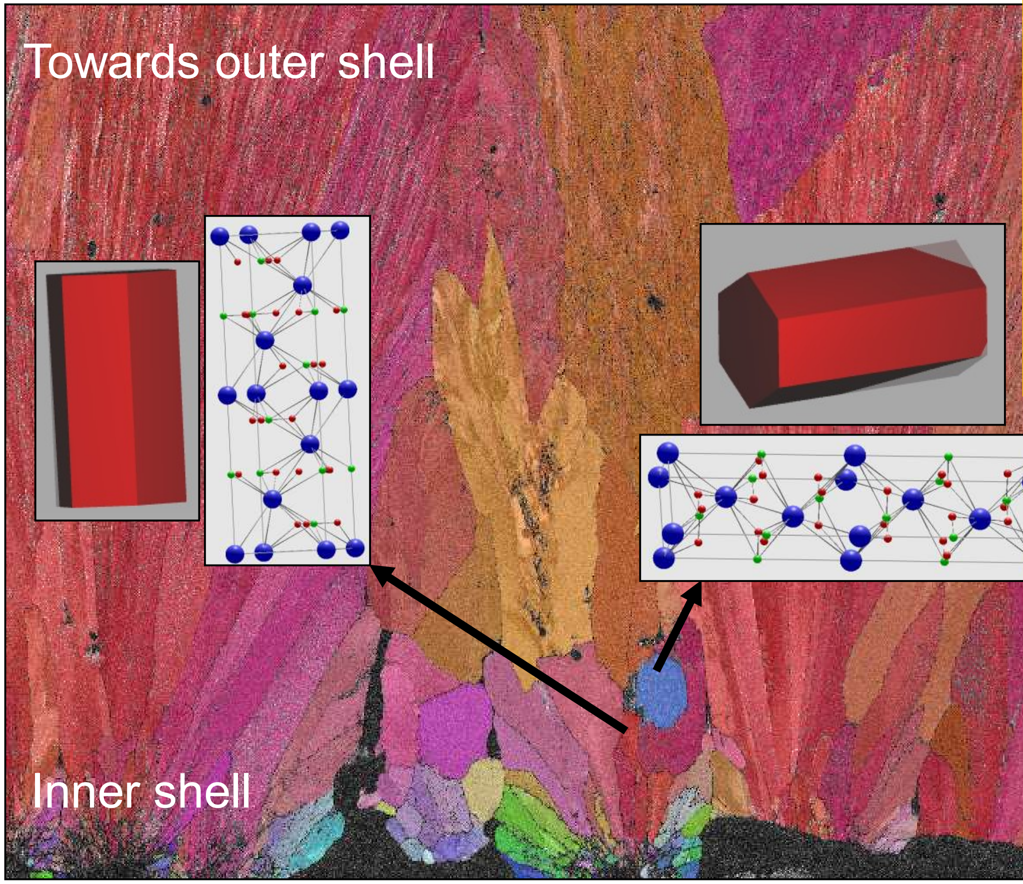
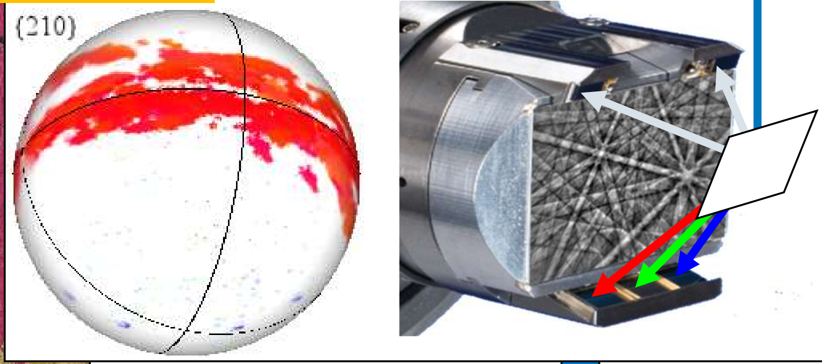




EBSD for bio (bone, teeth, crustaceans,…): Egg shells; Ostrich: strongest texture but micro-cracks and disorder for chick to get out





Color coded FSDs for just qualitative orientation imaging

Crystallographic orientation distribution map of the

Cross section of an Ostrich egg shell (inner part)

Understanding the early evolution of nervous systems including vision

Ma et al., 2015, Current Biology 25, 2969–2975
http://dx.doi.org/10.1016/j.cub.2015.09.063
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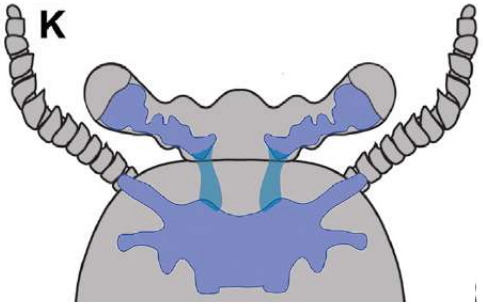
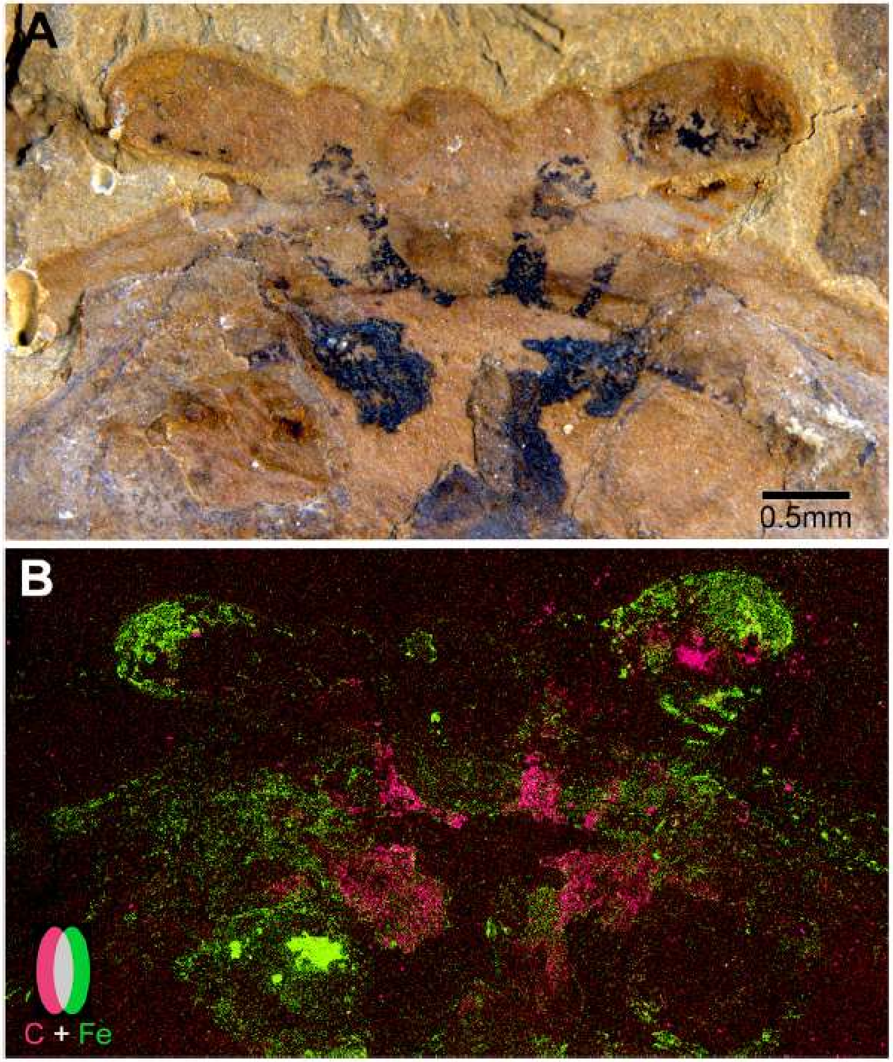


K: interpretive drawing of a Cambrian arthropod fossil

A: Direct illumination, C-traces of preserved neural tissue are shown as black

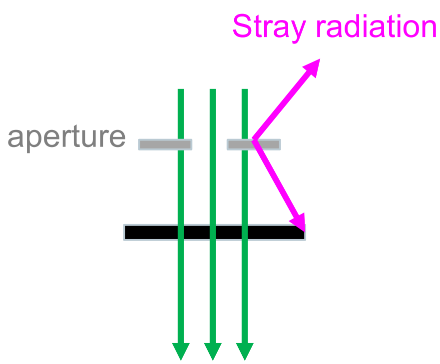
B: Merged C and Fe EDX maps resolve carbon and iron as entirely non-overlapping



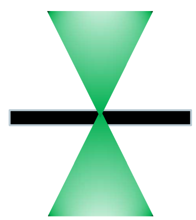
- VP-mode: 30Pa, 10kV
- Stiching 63 fields of view
- each frame 15min, 923nm/p,
- > 500kcps OCR

Electron Microscopy

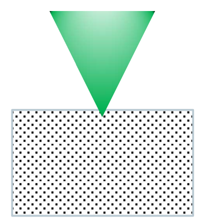
TEM



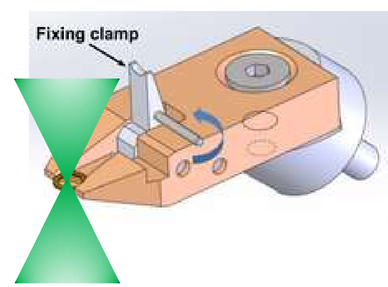
STEM
Scanning TEM



SEM
Scanning EM

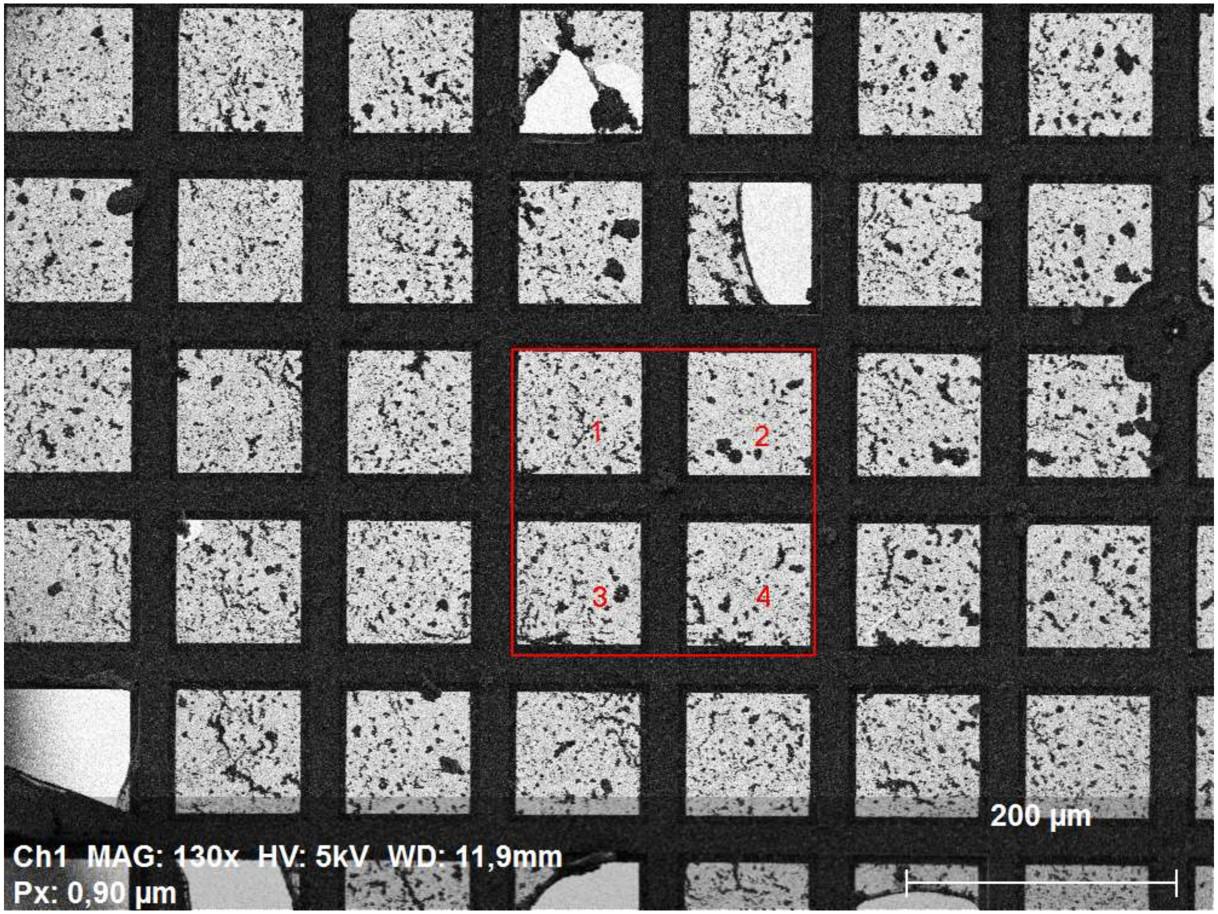


SEM: „T-SEM“



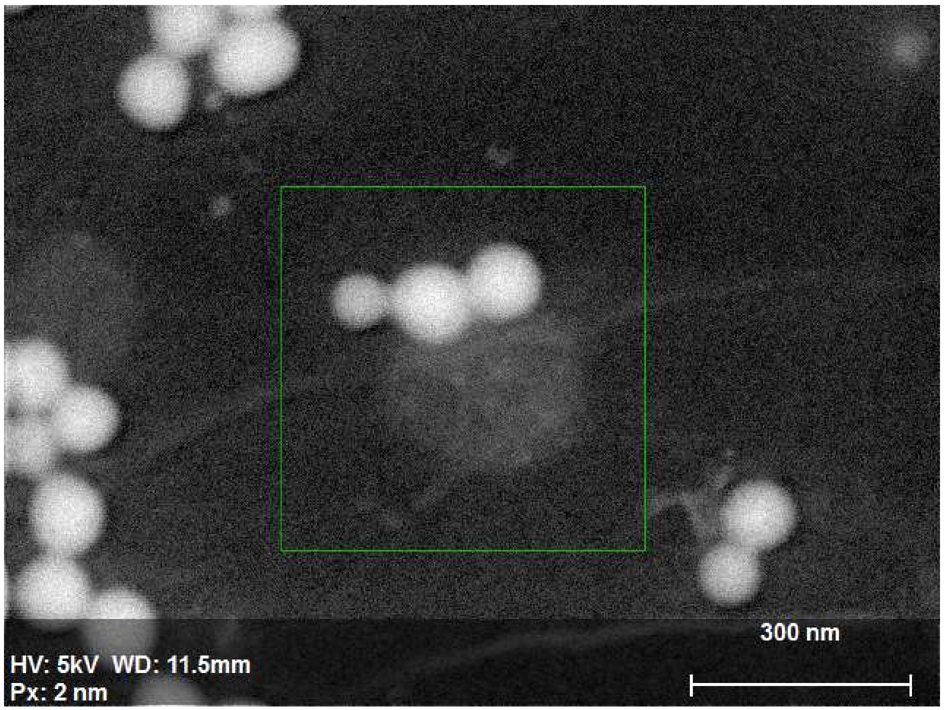
- TKD patented holder
- Commercial STEM holders
- Home made versions

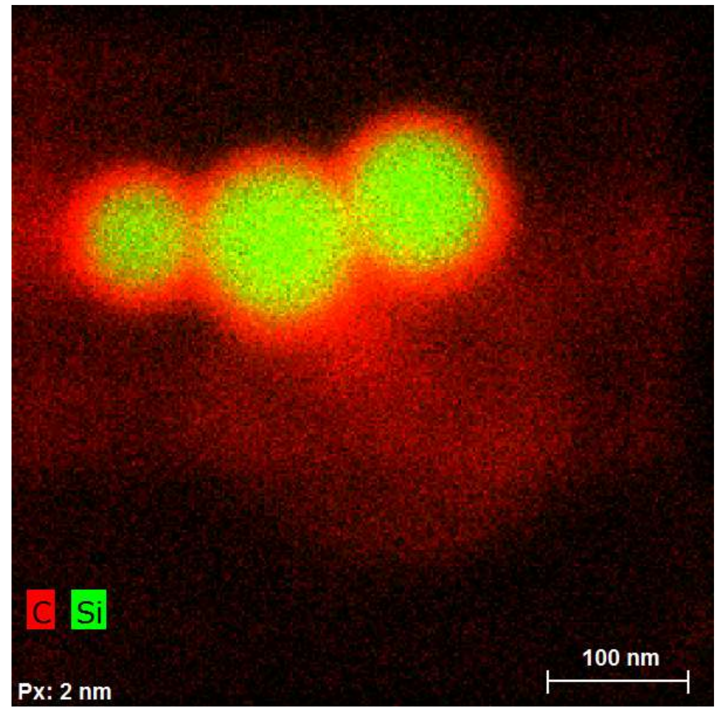
T-SEM-EDS of NP
Typical Overview



T-SEM-EDX of
fluorescent core shell NP;
Silica nanoparticles Alexa® dye coated

XFlash FlatQUAD, 5 kV, 520 pA , 22.5 kcps, 250x250 pixel, 2 nm pixel size, 377 s



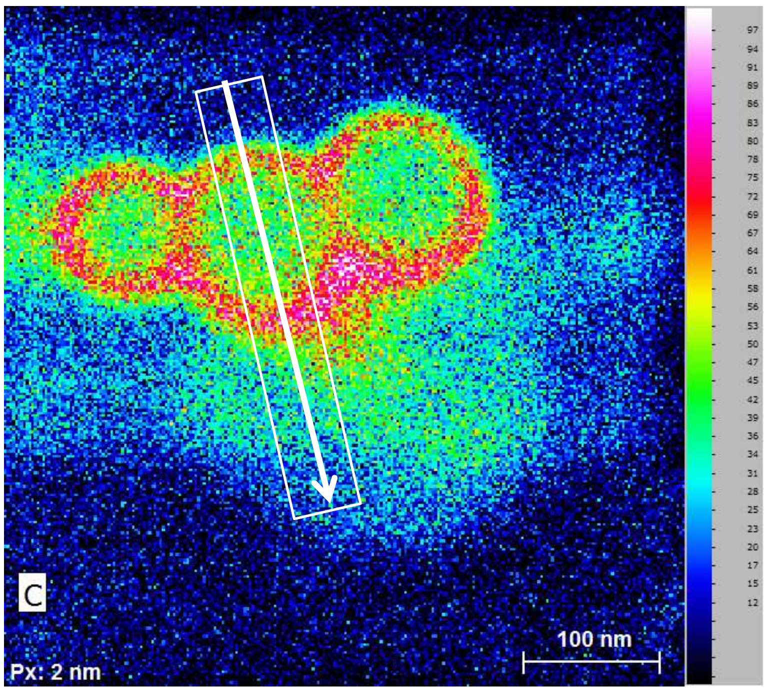


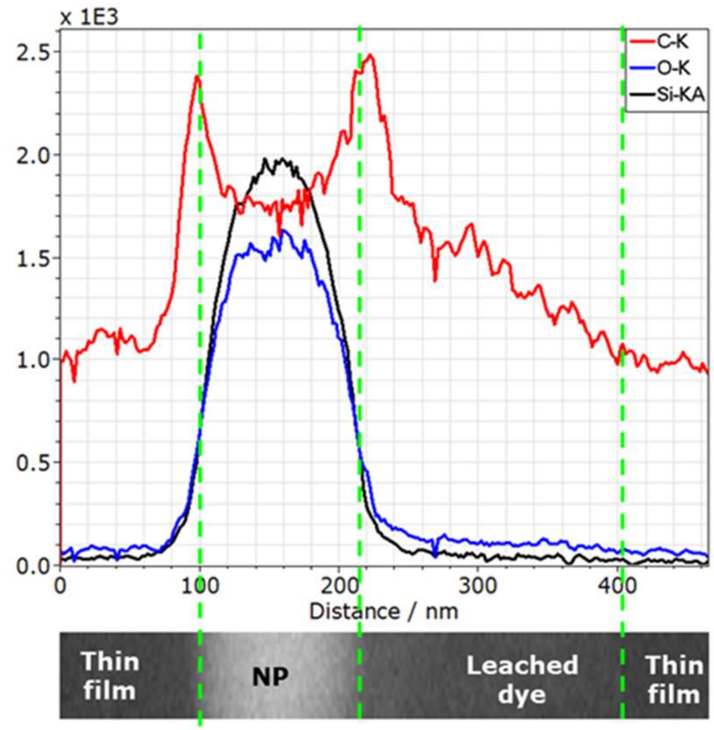
K. Natte, T. Behnke, G. Orts-Gil, C. Würth, J. F. Friedrich, W. Österle and U. Resch-Genger, J Nanopart Res, 2012, 14, 680

T-SEM-EDX of fluorescent core shell NP;

Silica nanoparticles Alexa® dye coated

XFlash FlatQUAD, 5 kV, 520 pA , 22.5 kcps, 250x250 pixel, 2 nm pixel size, 377 s



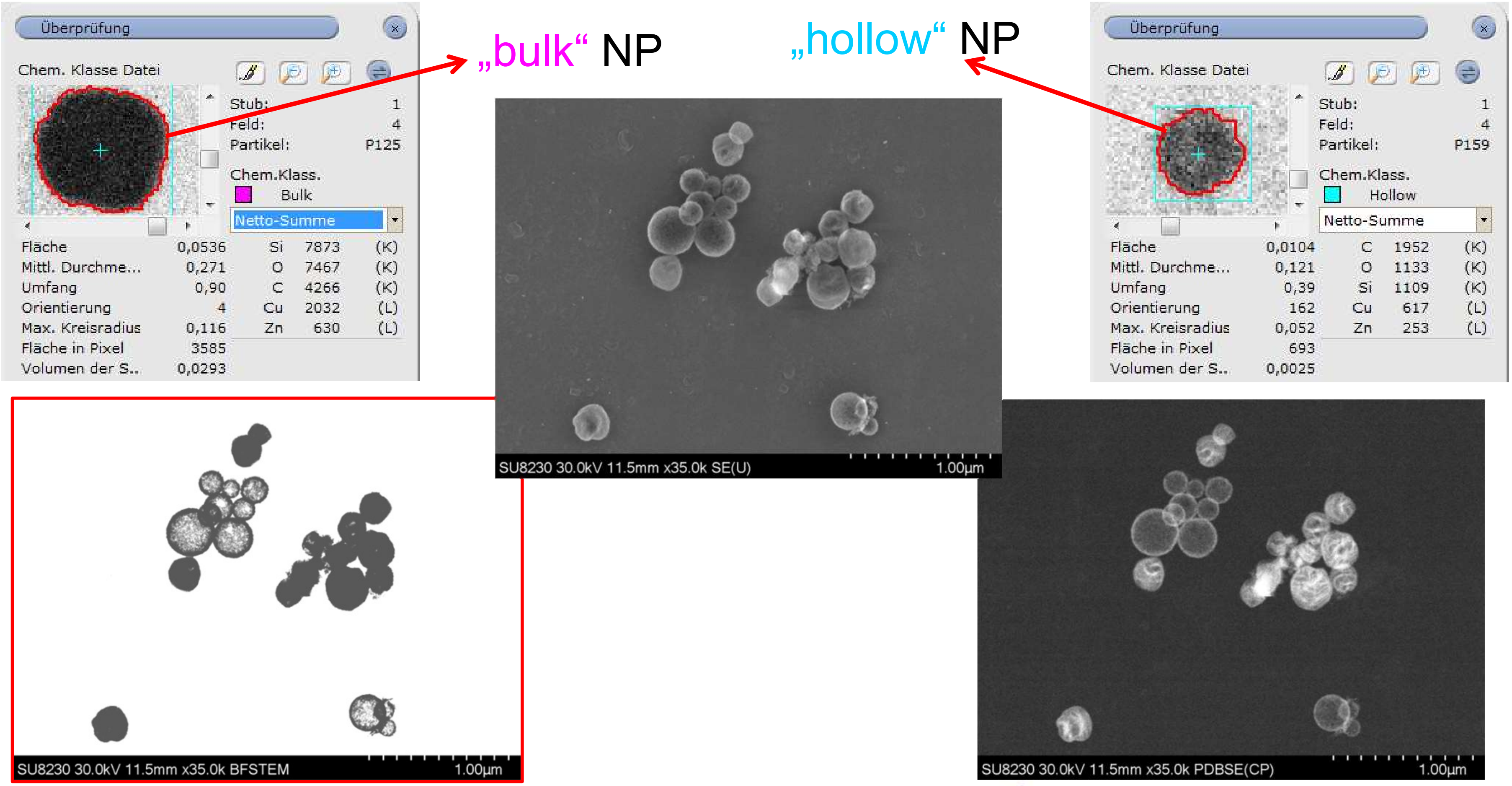


K. Natte, T. Behnke, G. Orts-Gil, C. Würth, J. F. Friedrich, W. Österle and U. Resch-Genger, J Nanopart Res, 2012, 14, 680;

Analysis: T. Salge, M. Falke; Hitachi SEM

T-SEM-EDX of NP

Classification using Si/C ratio

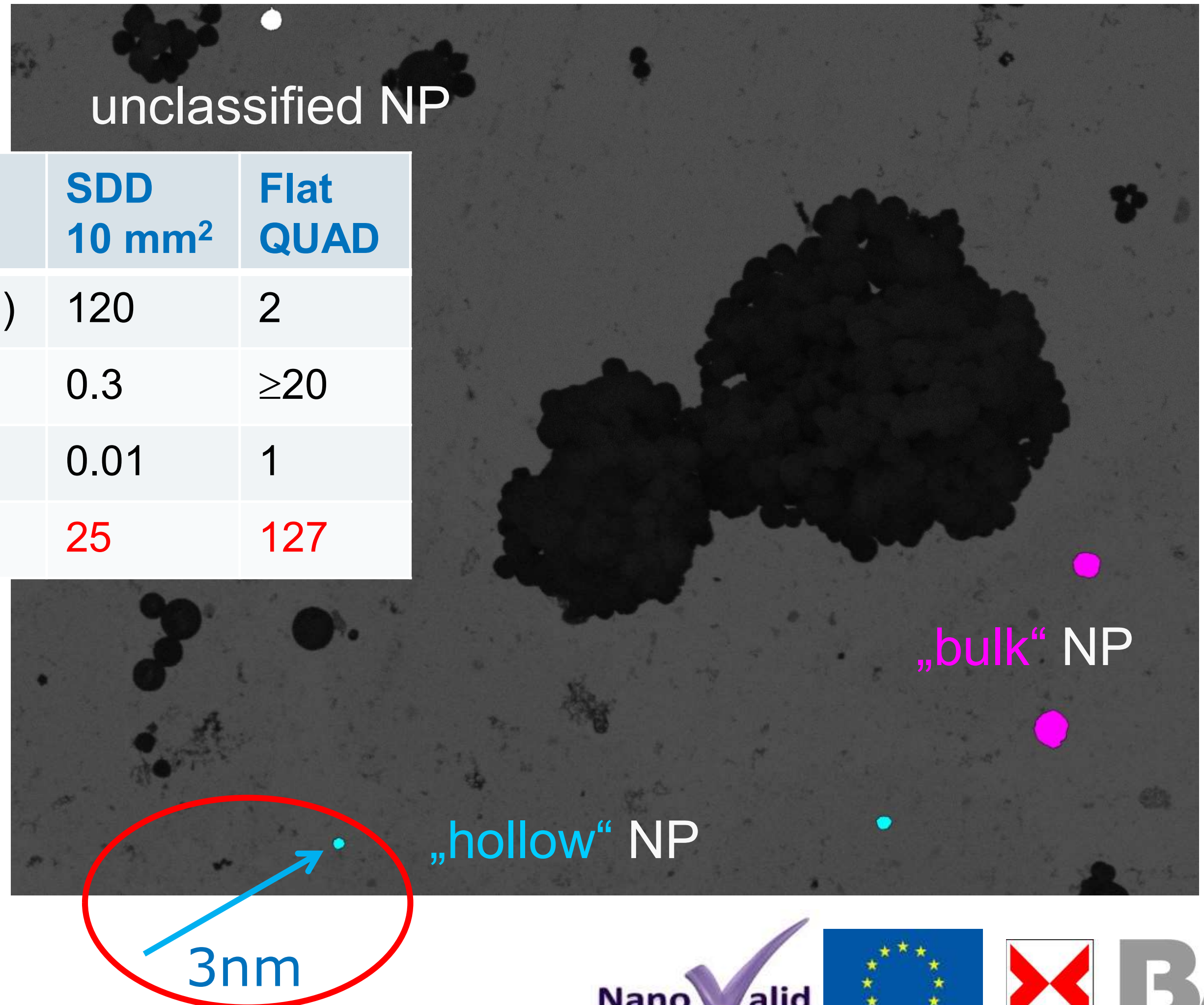


Analysis: T. Salge (Bruker/NHM)

T-SEM-EDX of SiO_2 NP;

PA: Classification, Statistics

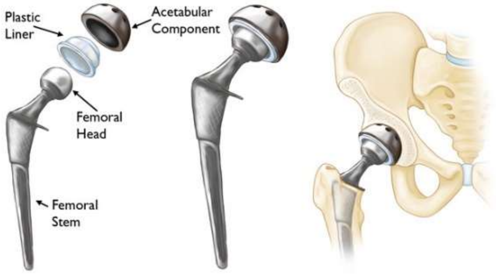
	SDD 10 mm²	Flat QUAD
Acq time (s per NP)	120	2
ICR (kcps)	0.3	≥20
Solid angle (sr)	0.01	1
NP identified	25	127



Analysis: T. Salge (Bruker/NHM)

SEM-EDX of Au-NP on TiO_2 Sponge-like coating for implants
Overview > Particle Statistics!

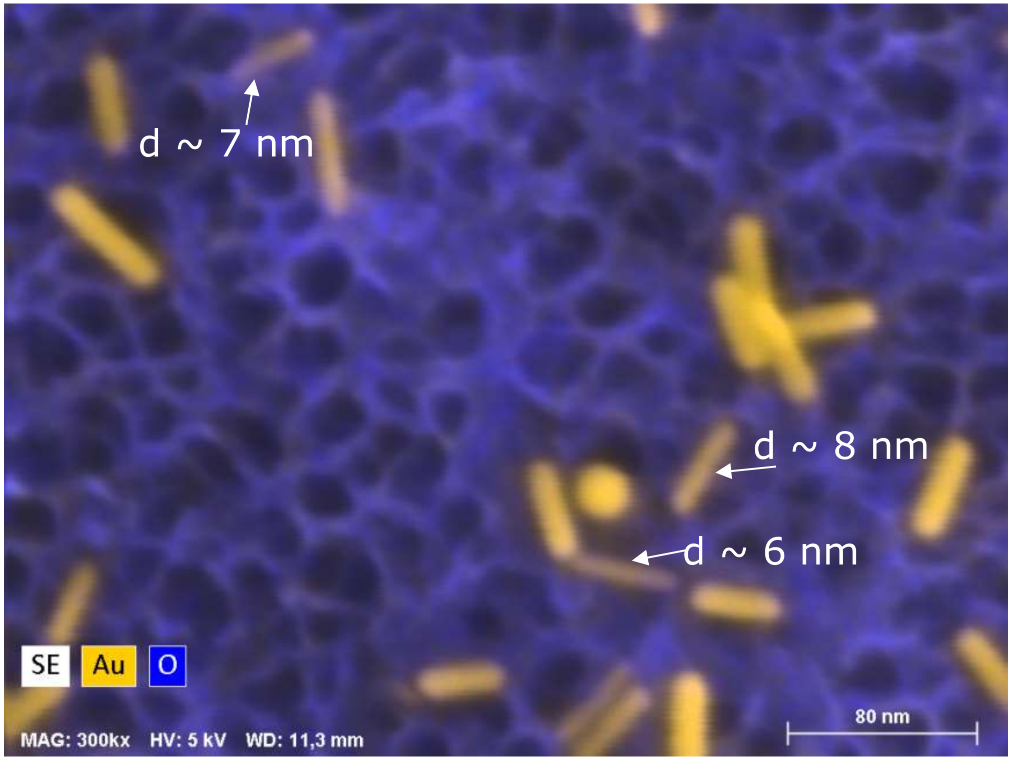
Nanotoxicity



Au-NP to avoid inflamation around implant:
NP change surface potential,
Settling bacteria get „electrocuted".

For successful tissue growth it is very important to judge the distribution of the NP and compare it to fluorescence light microscopy.

> Statistical analysis!



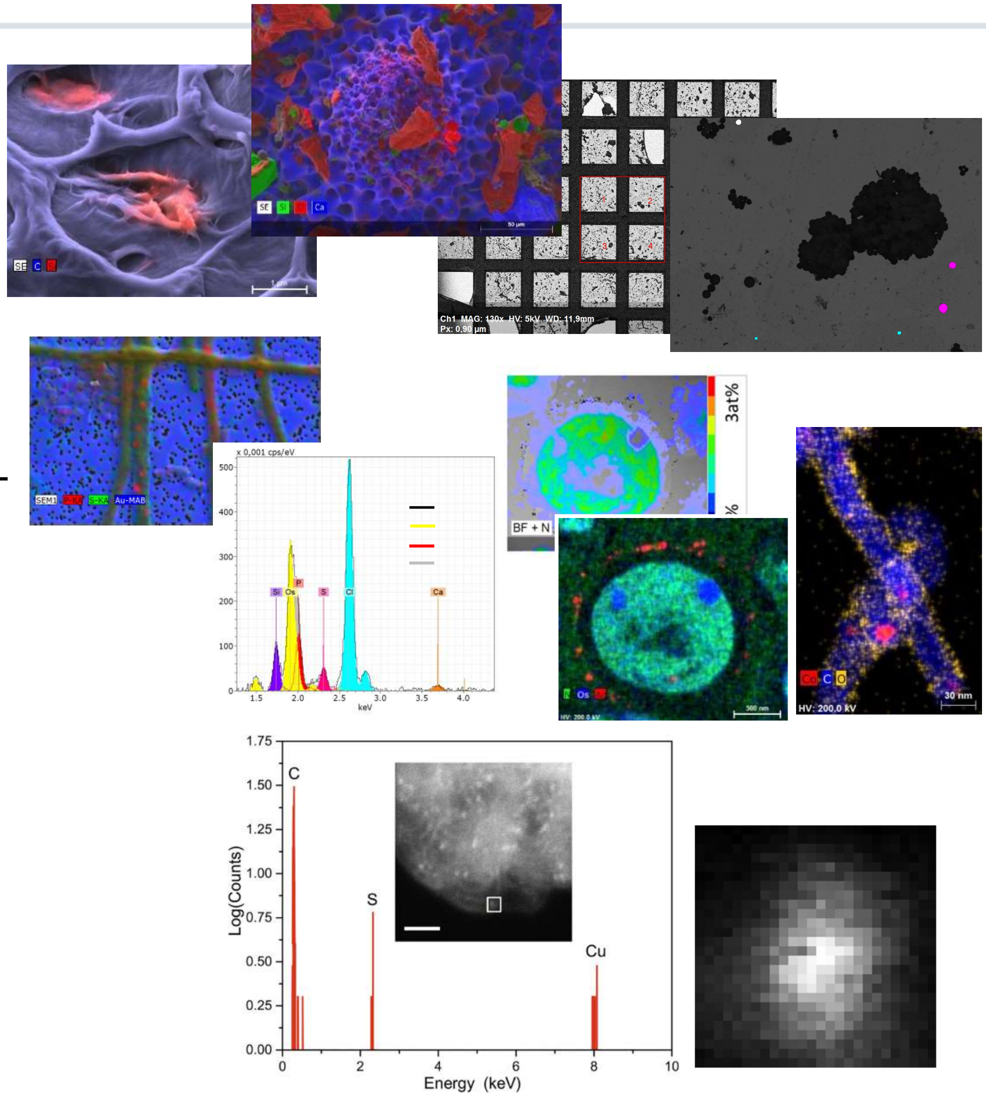
T. Yang et al., Colloids and Surfaces B: **145**, 597 (2016).

EDS Characterization; Possible Steps:
From mm via nm to single atoms

- SEM/T-SEM
> Overview / embedding/ statistics from mm to nm scale, Low vacuum,
> Use multiple/annular detectors
> Combine with other analysis techniques on SEM (EBSD/TKD, µXRF)

- Standard / Cs-corrected SEM/STEM + Standard EDS
➤ Q-Mapping in at% at nm spatial resolution, in-situ, liquids, ice … force

- Cs-corr. STEM + high brightness + high Ω EDS
> Single atoms
> Combine with EELS, CL, diffr. …
> in situ (liquids, ice, gases, temp., force)



Innovation with Integrity