

# **Imagerie, EDS et EBSD en mode environnemental: études de matériaux non-conducteurs, humides, gras et aussi les très sensibles échantillons archéologiques**

*Dr. Egle Conforto*

*Responsable de la Plateforme de Microscopie Electronique*

*LaSIE UMR 7356 CNRS-ULR, La Rochelle Université, UFR Sciences  
Avenue Michel Crepeau, 17042 La Rochelle, France*

# La Rochelle Université

- Créée en 1993, c'est la plus jeune université française
- Recherche thématique: Littoral Urbain Durable Intelligent ("LUDI")
- Recherche au **LaSIE** : matériaux traités ou recouverts de films de protection pour résister aux conditions littorales\*, pour la construction civile et navale, bâtiments bas carbone, béton recyclé, etc..

\*aussi basé sur la connaissance des mécanismes de transformation des matériaux qui ont interagi avec l'environnement littoral pendant des siècles

# OUTLINE

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## Introduction

- Analyses of non-conductive, dry, humid and fatty samples: which interest?
- Difficulties of using conventional SEM
- Alternative: Environmental SEM – Exemples of ESEM use in the materials research
- Environmental EDS and EBSD?
- Goal of this talk

## Materials and Methods

Results in archaeological materials by EDS and EBSD

Conclusions and Perspectives

Analyses of non-conductor, humid and fatty materials.  
Which interest?

Materials as:

- Oxidized and hydrided metals
- Minerals
- Polymers
- Materials for civil construction
- Pharmaceuticals
- Cosmetics
- Supplementary food
- Biological
- etc..

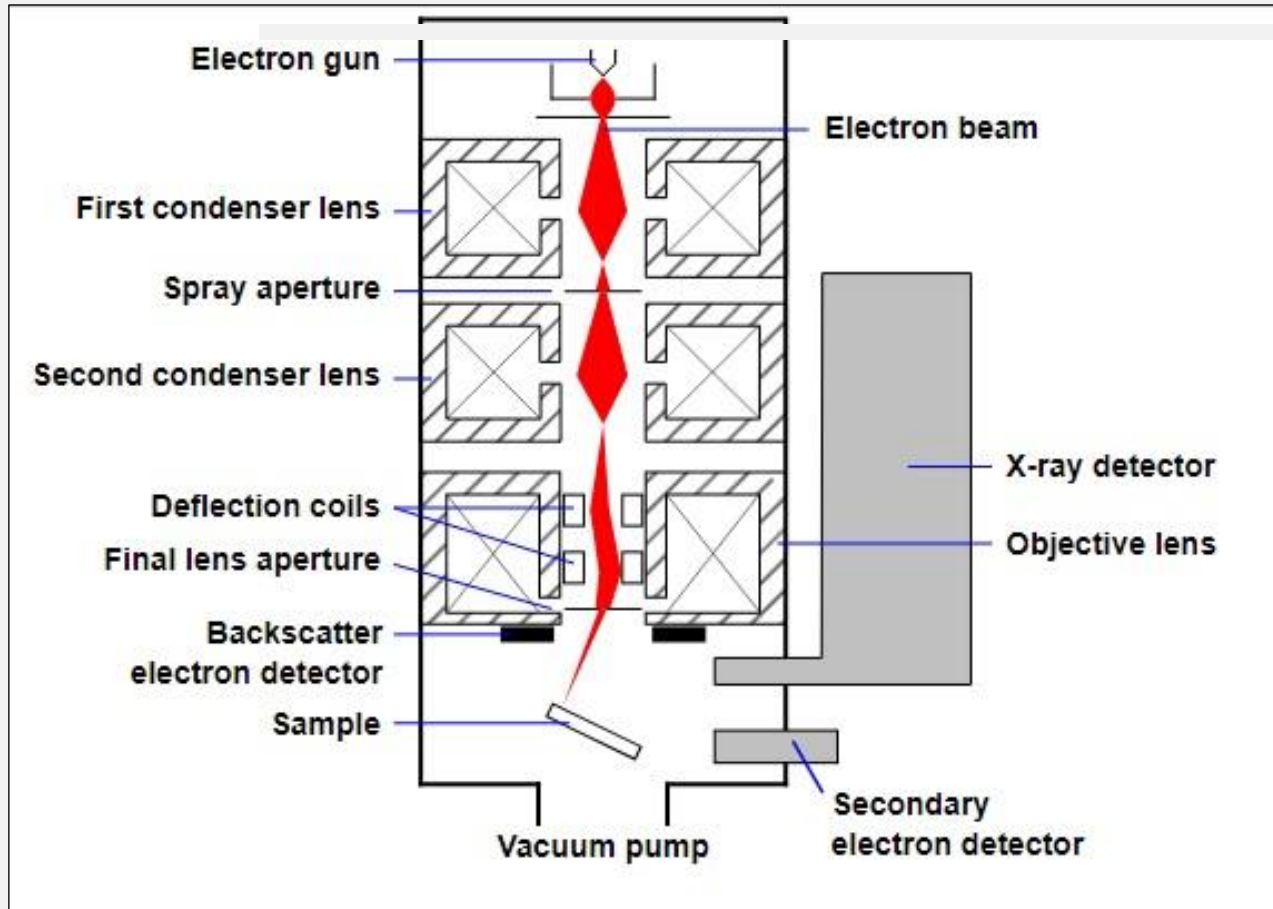
Object of Research and R&D for  
an incalculable number of  
applications

Present in all-day life worldwide.

But their original surface cannot be analyzed by SEM/EDS/EBSD in High vacuum mode.

Why?

# High-Vacuum Scanning Electron Microscope ( $10^{-4}$ - $10^{-6}$ Torr):



- Only specimens which are good electrical and thermal conductors can be observed
- Humid, fatty samples are not allowed in the specimen chamber.
- For dry, stable but slightly resistive samples, a metallic layer (carbon, gold, etc.) can be deposited on the sample surface to make it conductor.
- The vacuum system is unique for the whole column, constituted of a mechanical and a turbomolecular pump.

# Metallic Coating vs. Original surface characteristics

## Artefacts introduced by preparation methods

- During film deposition:
  - Vacuum can cause damage (deformation/collapse) to humid, soft samples
  - Carbon evaporation can cause damage to heating-sensitive surfaces
- Film thickness : very important parameter which has to be carefully controlled because:
  - Risk of thickness inhomogeneity creating artificial roughness
  - Risk of masking completely or partially the small porosities and roughness of the original sample surface
  - Risk of misevaluation of porosity size and distribution over the surface in samples where their role is determinant, as in supplementary food and cosmetics

**= RISK OF MISINTERPRETATION !!!!**
- Film deposited on humid or fatty samples:
  - Risk of water evaporation from the sample and damage to the surface and film
  - Risk of fat heating under the metal coverage during observation, followed by its expansion in volume and “explosion” :  
**“pop-corn effect”**
- **Alternative: Using Environmental Scanning Electron Microscope (ESEM)**

# ESEM Examples: LaSIE Research

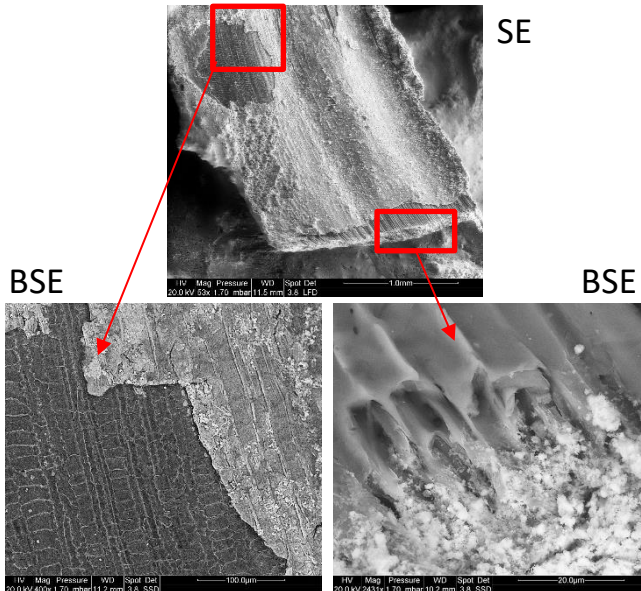
## Materials for Civil Construction



Clay, SE

Mortars, BSE

## Paroi de Bâtiment écologique: Ciment + balles de riz

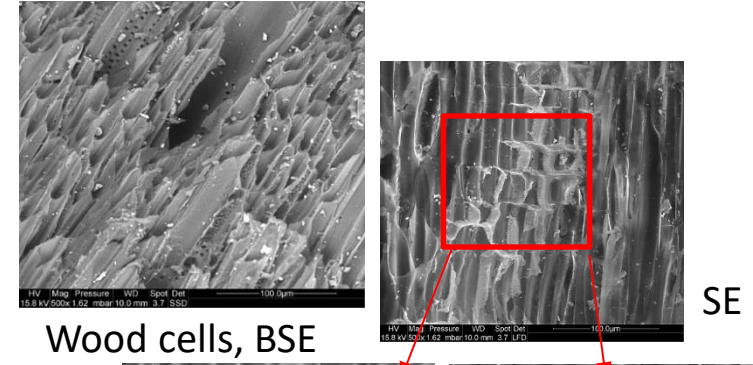


SE

BSE

BSE

## Wood fragments dried at high temperature and pressure



Wood cells, BSE

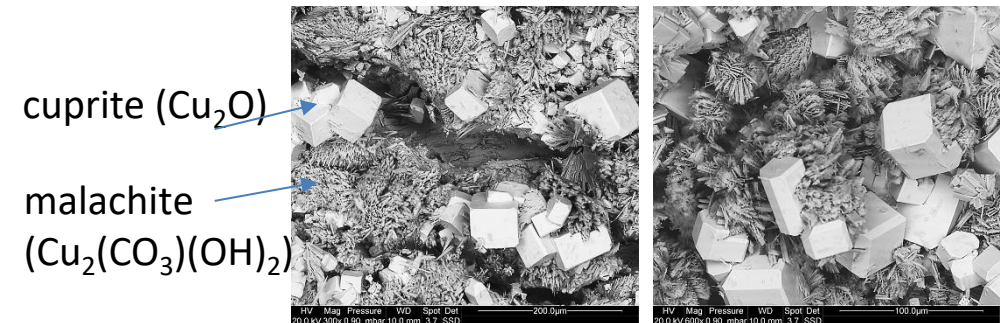
SE



Detail, SE

Same detail, BSE

## Analyse de défaillance d'un réseau en cuivre d'eau chaude sanitaire en milieu hospitalier

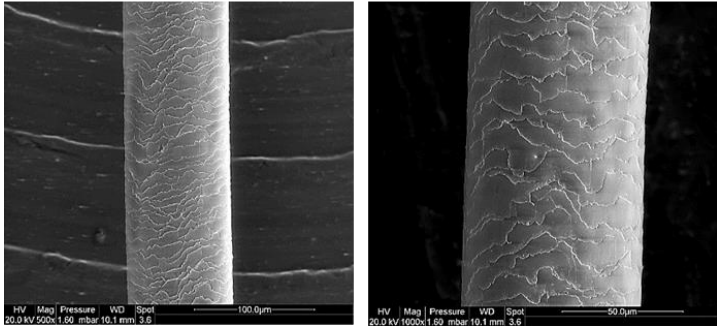


cuprite (Cu<sub>2</sub>O)

malachite (Cu<sub>2</sub>(CO<sub>3</sub>)(OH)<sub>2</sub>)

# R&D with enterprises partners

Quality of Human Hair after treatment with organic shampoos

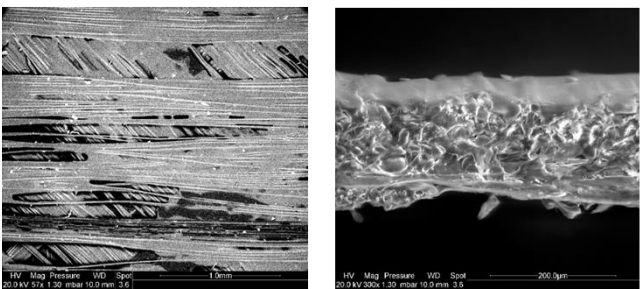


20 kV; 1.6 mbar

Resin sails for worldwide sailing ships competition

Planar view, BSE

Cross-section, SE

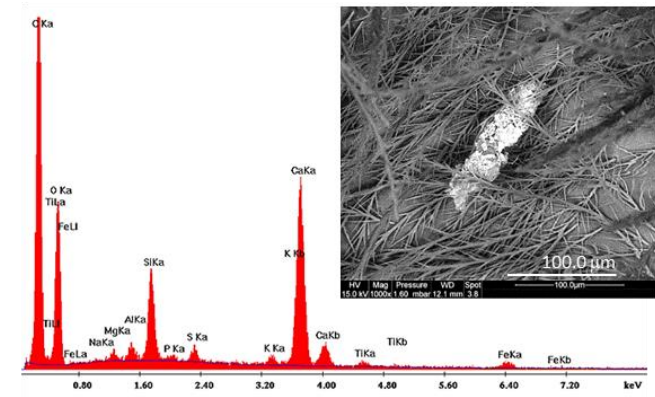


20 kV; 1.3 mbar

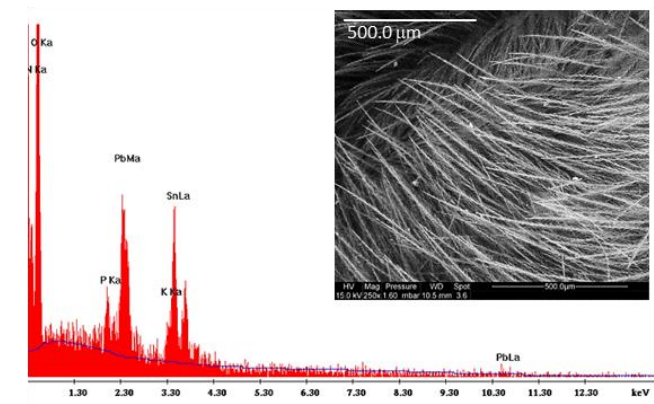
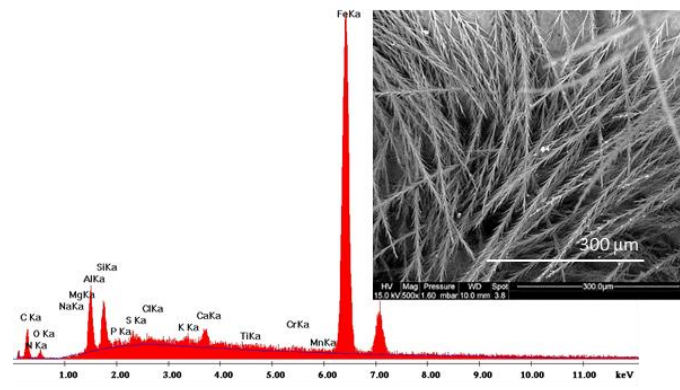
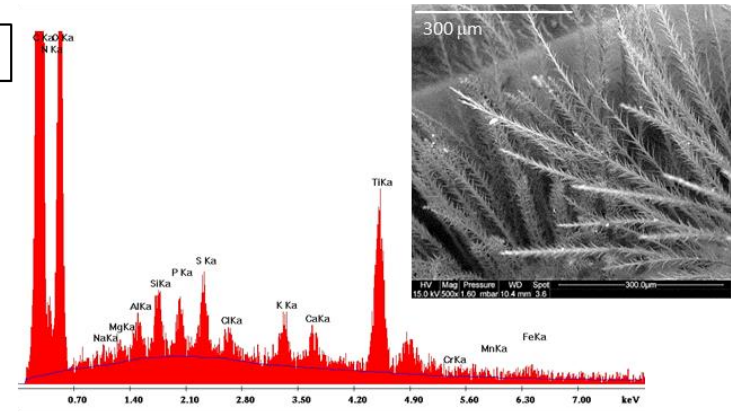
Survey of the environment contamination through the EDS analyses of particles retained in the body of bees

BSE images

Found: metallic contaminants (Pb, Sn, Ti, Fe) and cement and also particles coming from the environment (sand, alumino-silicates, etc)



15.0 kV; 1.6 mbar



# Environmental SEM

## What is the difference?

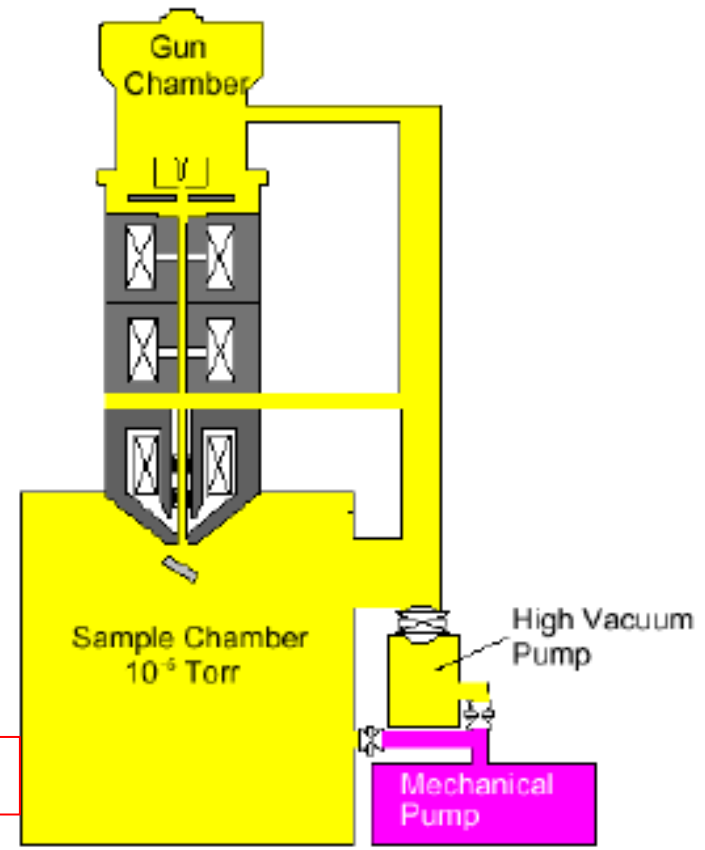
### Environmental (ESEM):

- All kind of solid samples, also non-conductors, humid, fat, can be analyzed without any preparation or surface transformation. Possibility of performing in-situ dynamic tests.
- Advantages:
  - no artefacts are introduced on the sample surface, which keeps its original roughness, porosities and chemical composition
  - charges in the specimen surface are neutralized by positive ions generated by the ionization of the gas or water vapor
- Special detectors for environmental mode
- Double vacuum system: the gun is maintained at  $10^{-9}$  Torr by 2 ionic pumps, meanwhile the vacuum in the specimen chamber can be varied between ( $10^{-6}$  – 20 Torr) by the introduction of water vapor. The pressure is then maintained in the specimen chamber by using a dedicated mechanical pump.

# Vacuum system

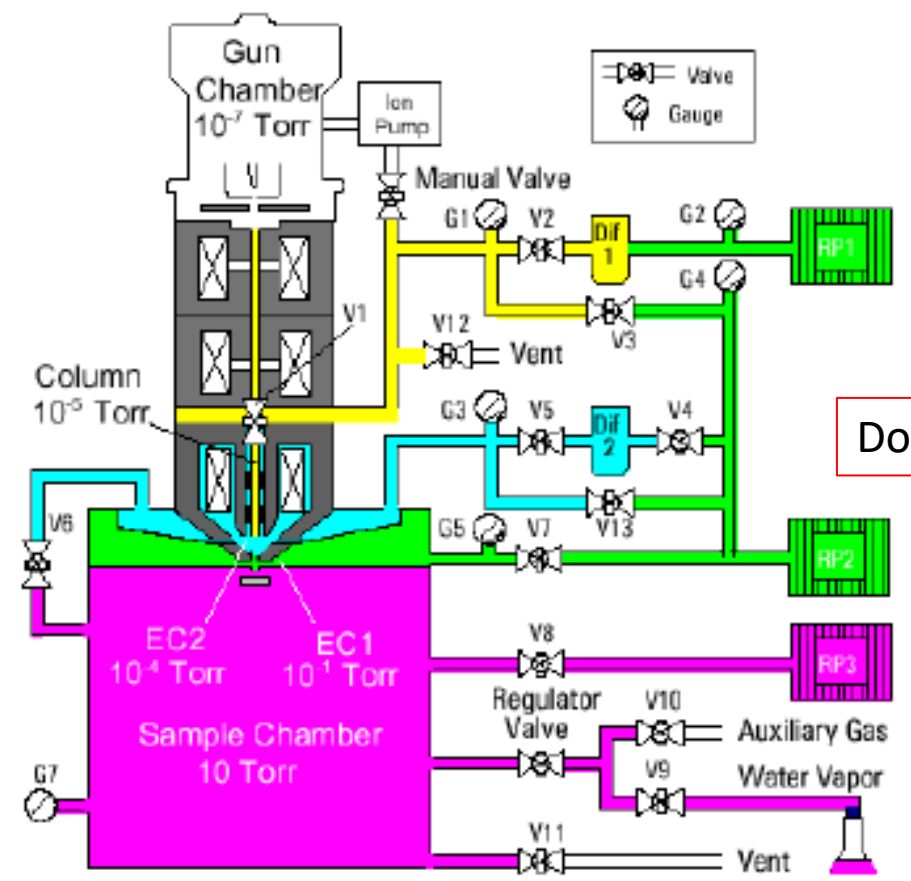
## Conventional SEM "(C)SEM"

Also «Low-Vacuum SEM» :  $P_{max} < 0.45$  Torr



## Environmental SEM "ESEM"

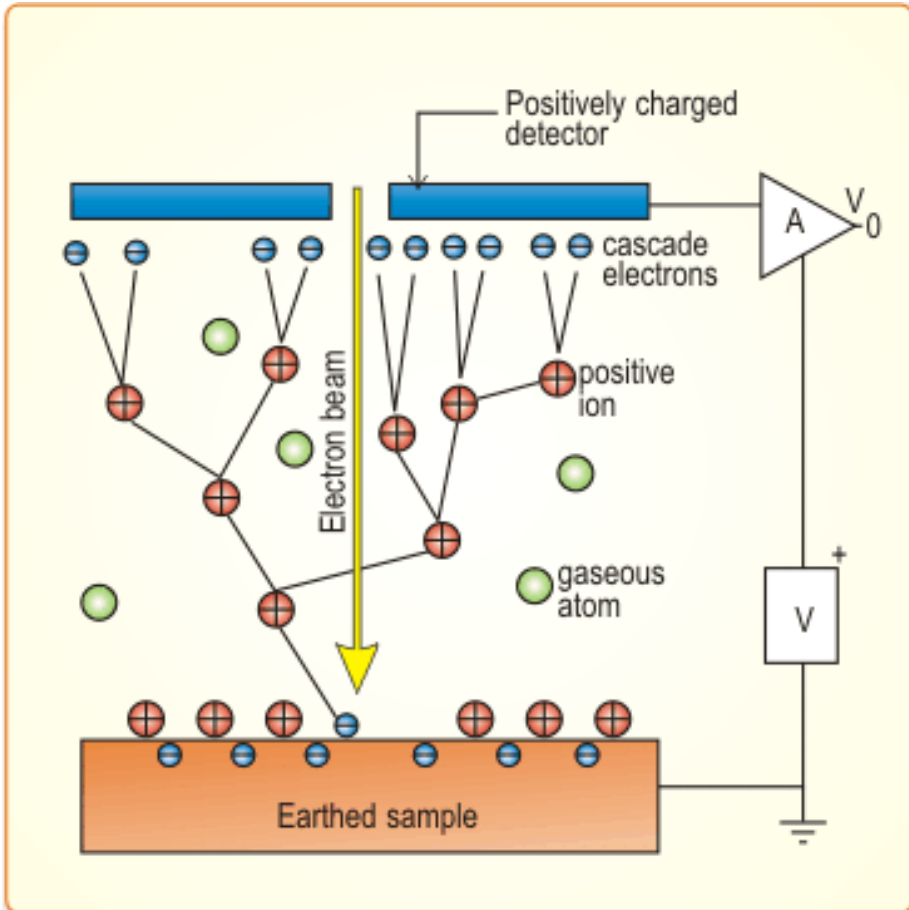
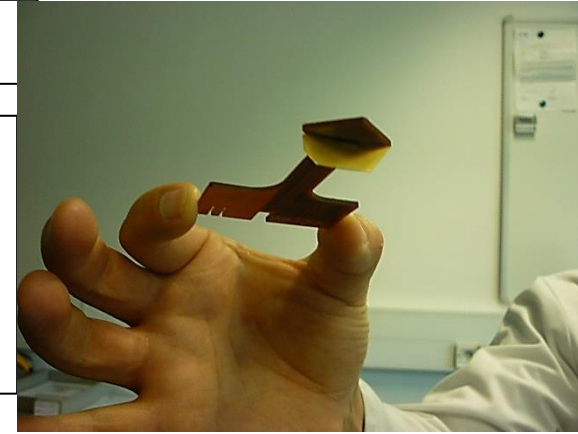
$10^{-6}$  Torr  $< P < 20$  Torr  
Water vapor or gaz



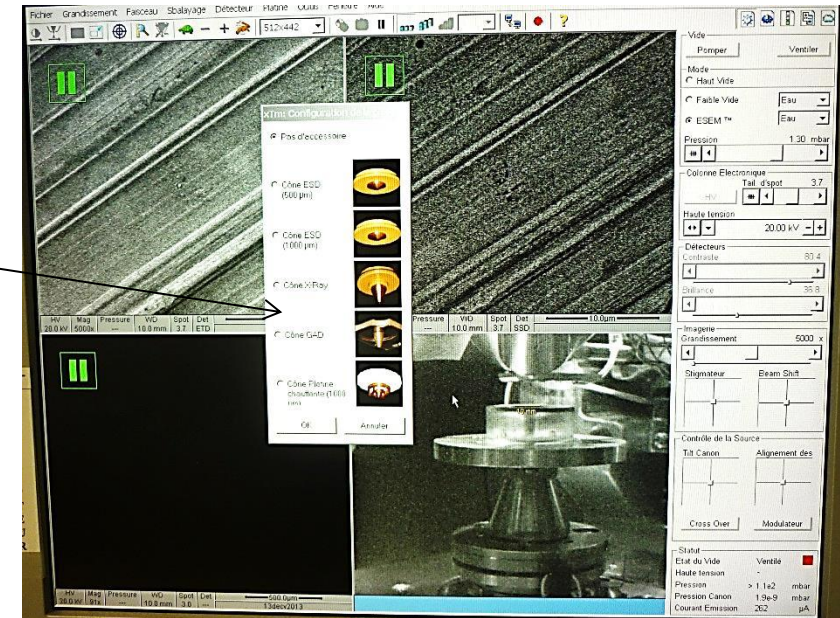
# How does ESEM works?

Secondary electrons: Low energy ( $\approx 50$  eV)

The Environmental Secondary Electron Detector uses the gas surrounding the sample to amplify the secondary electron signal

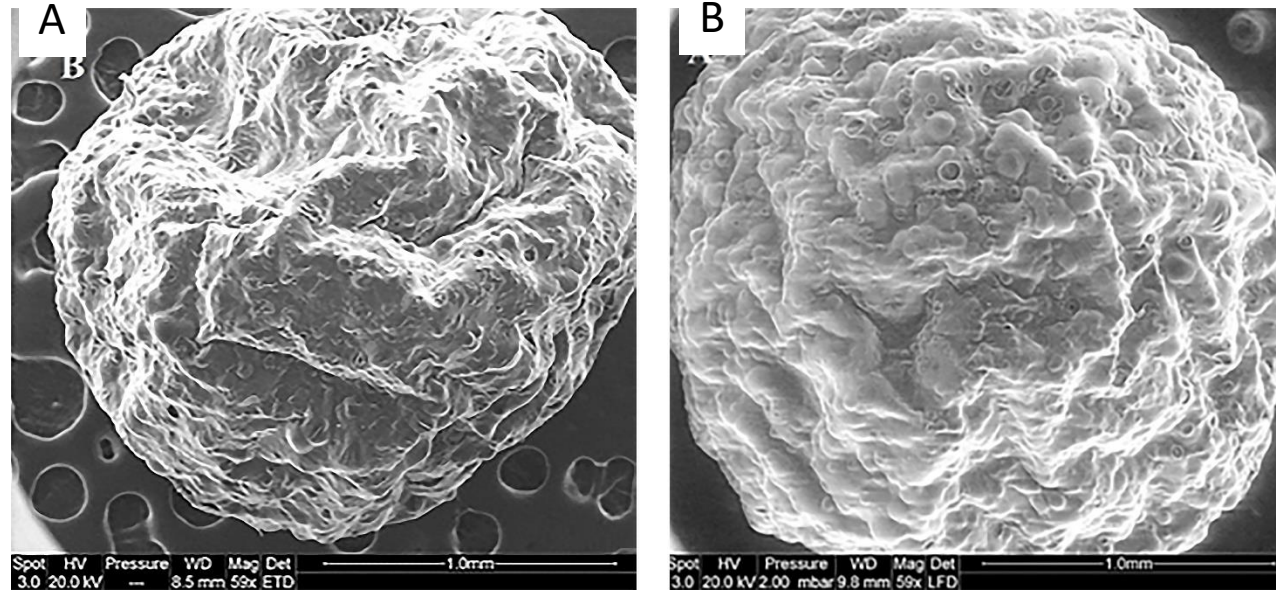


Other detectors used in Environmental mode



# Damage due to the vacuum during sample preparation and SEM observation

## Reticulated alginate beads



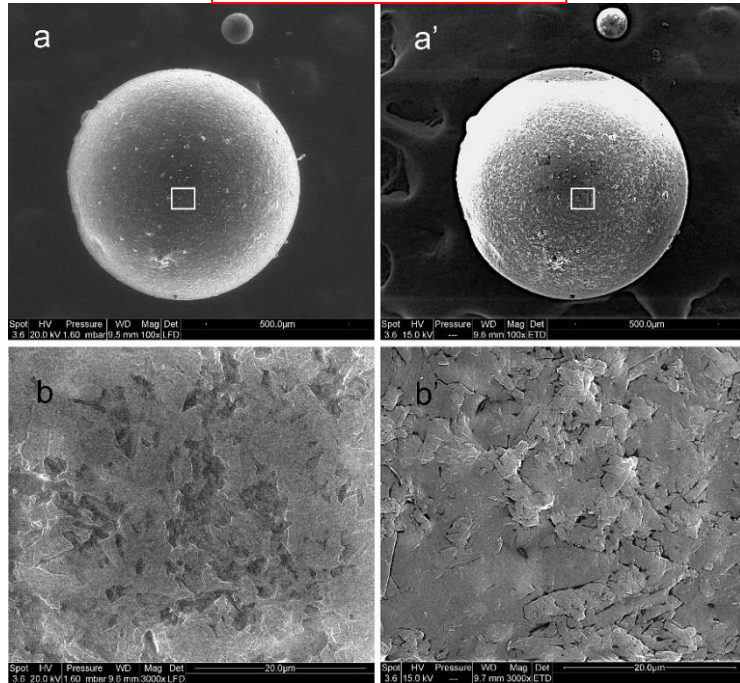
- Deposition of a gold film under vacuum
- Observation in high vacuum mode (20 kV)
- No preparation
- Observation in ESEM mode
- Water vapor pressure: 2 mbar (20 kV)

- Bead walls collapse under vacuum
- The shape of alginate beads is preserved in ESEM mode

# Surface roughness masked by metallization

Aromatic plant extract coated by hydrogenated vegetable oil

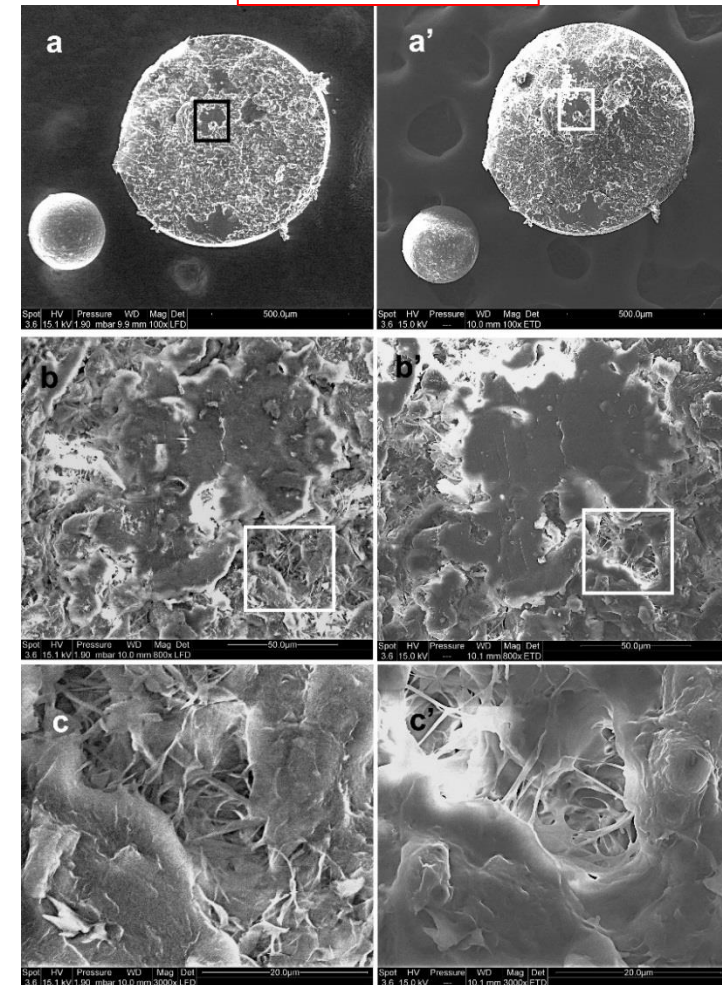
External surface



ESEM mode

Metallization +  
High vacuum mode

Cross-section



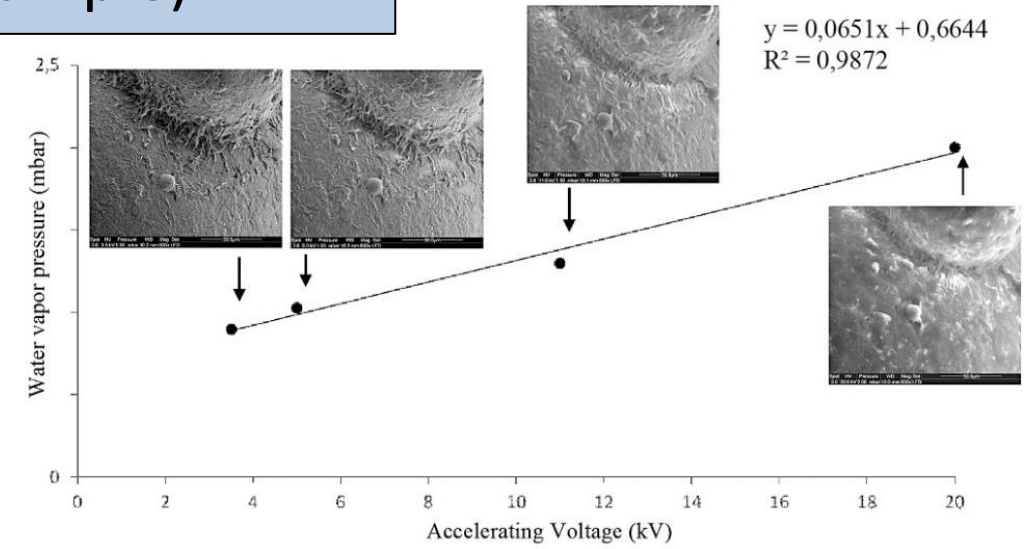
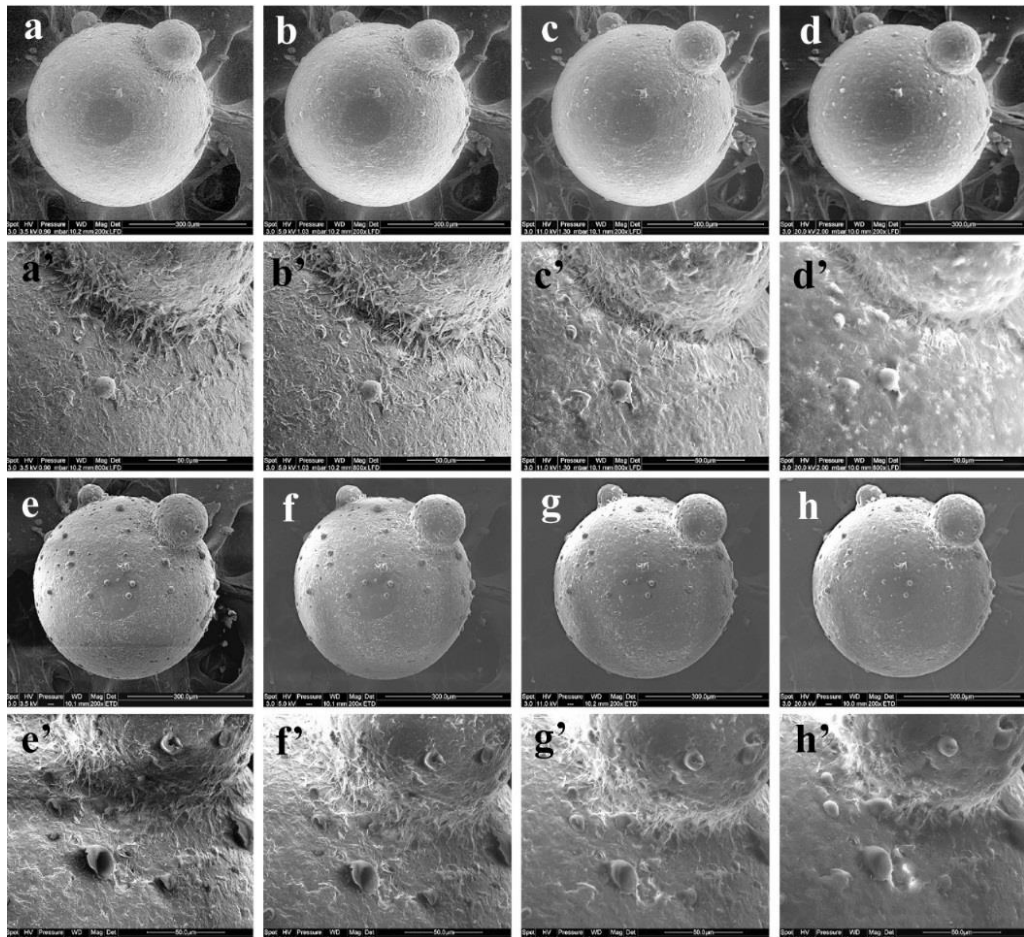
ESEM mode

Metallization +  
High vacuum mode

**\*Published in:** Egle Conforto et al. « An optimized methodology to analyze biopolymer capsules by environmental scanning electron microscopy », *Mat. Sci. Eng. C* 47 (2015) 357–366.

# Comparison of fatty coatings observations : ESEM vs High vacuum SEM (gold covered sample)

Aromatic plant extract coated by hydrogenated vegetable oil



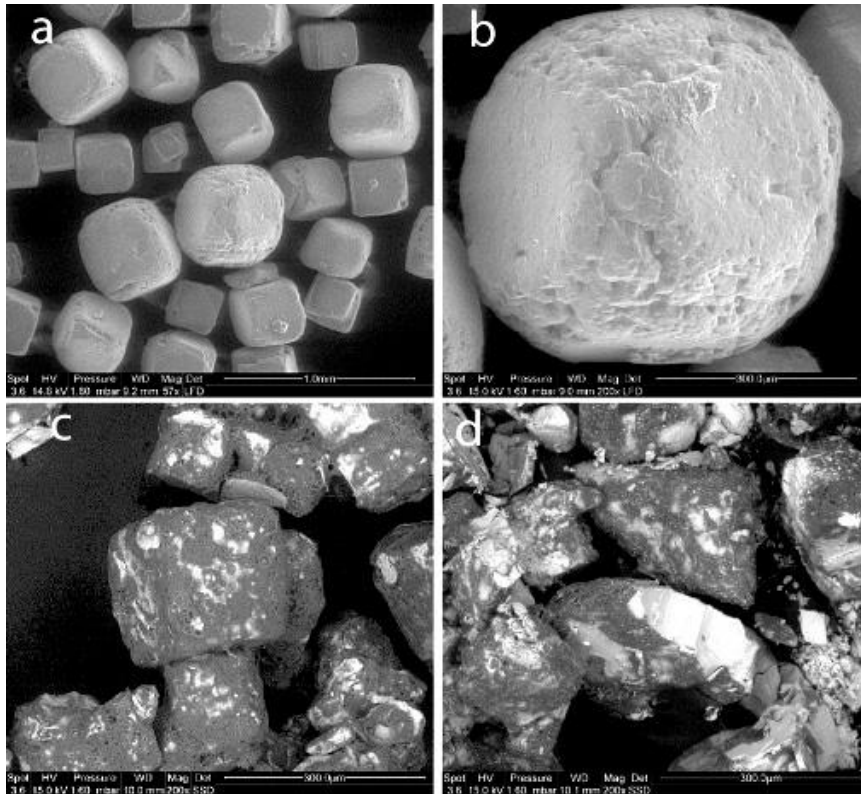
Fatty-coated capsules observed by ESEM in various voltages/water vapor conditions: No transformation of the original surface

Same sample, covered with gold prior to the observation in high vacuum SEM in the same voltages:

- The sample surface is modified;
- damage appears during observation.

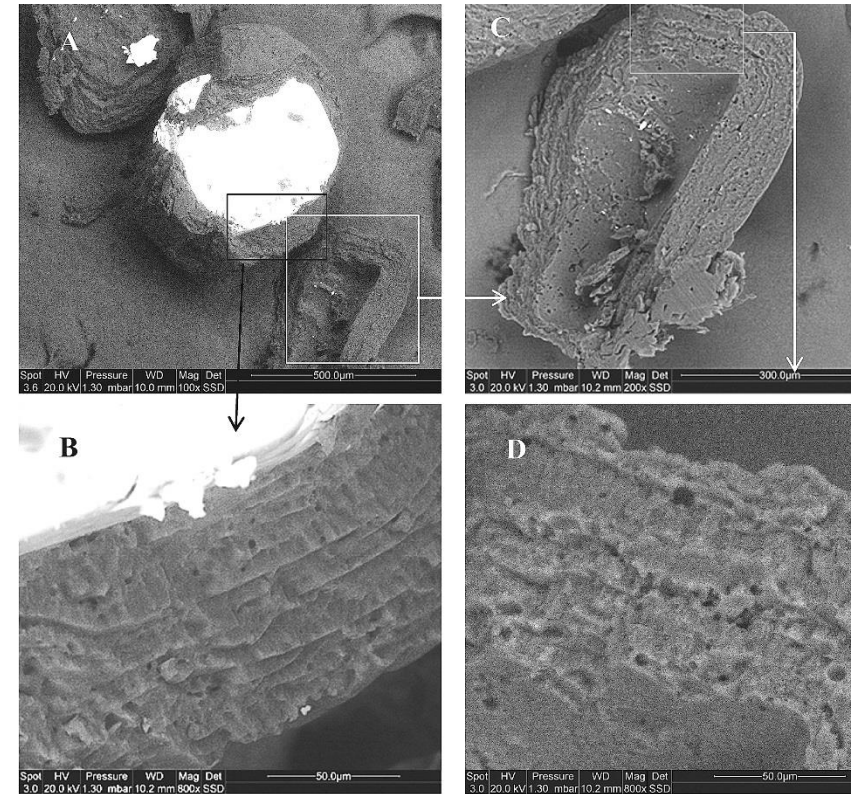
# Supplementary Food Capsules by ESEM: Original surface roughness and core/shell chemical contrast

Sodium chloride particles encapsulated with gelatin



- a, b: unencapsulated sodium chloride (SE)
- c, d: gelatin-encapsulated sodium chloride: chemical contrast (BSE)

Sodium chloride particles encapsulated with hydrogenated vegetable oil

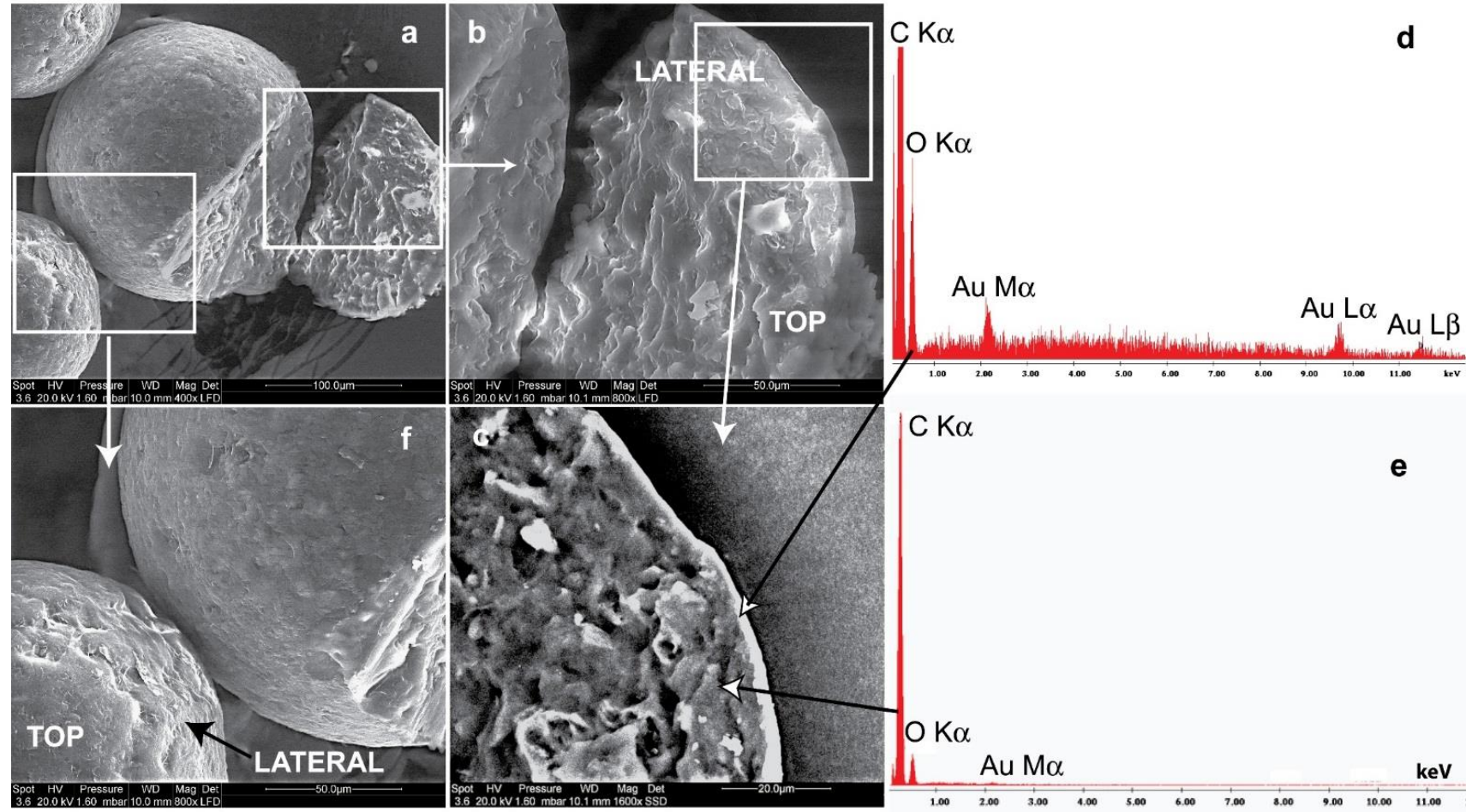


- Capsule in cross-section
- Core/shell in chemical contrast
- Details of the fatty layer structure

# Characteristics of the metal coverage and the artefacts it introduces

Aromatic plant extract encapsulated with hydrogenated vegetable oil

- Metallized first, then cut
- Uncovered capsule core observed and analyzed by EDS in cross-section in ESEM mode



- Inhomogeneous gold layer thickness on spherical surfaces
- Artefacts (steps) introduced on the lateral surface
- Gold layer detectable by EDS - Au peak could overlap with those of sample elements

## EDS analyses of non-conductor samples: which possibilities?

### - In High vacuum mode:

1) coverage with metallic films.

**Problem:** Au-L peaks can overlap with K-peaks of Ge, As, Se (9.7 – 11.5 keV);

Au-M peaks overlap with K-peaks of P and S

Or

2) Using low voltage (< 10kV).

**Problem :** only low-energy peaks can be detected

L and M peaks of medium and heavy elements superimposed to K-peaks of light elements → difficult to deconvolute peaks, loss of accuracy in quantification

### - In Environmental Mode:

1) No film deposition needed

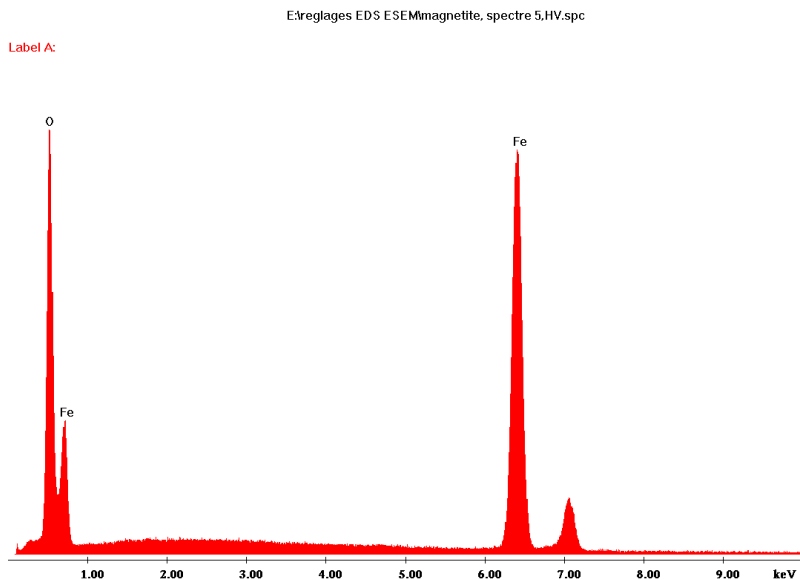
2) Possibility of using the same SEM parameters (voltage, current) as those used for metals in high vacuum mode;

3) No charge accumulation; no damage to the sample;

**Question:** Are water vapor (or gas) molecules surrounding the sample surface interfering with EDS analyses ?

# Comparison between semi-quantitative EDS in High- vacuum and ESEM mode

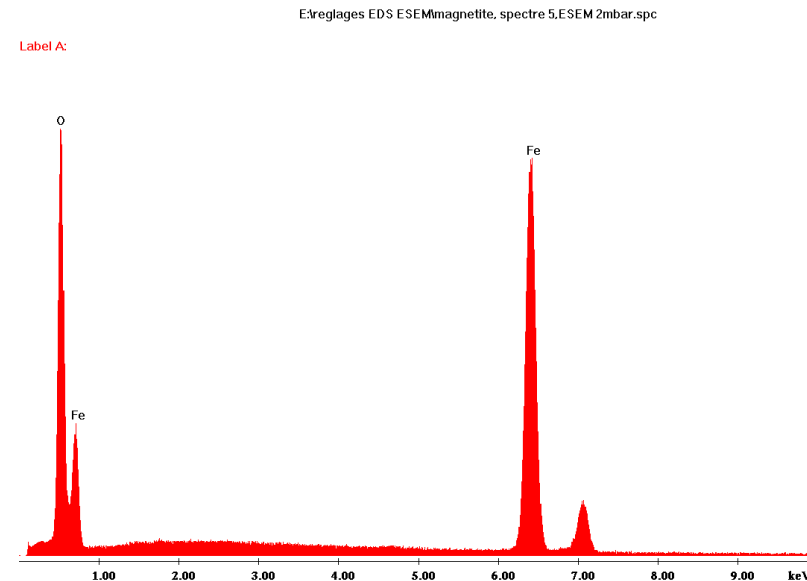
Sample: Standard of purified mineral magnetite  $\text{Fe}_3\text{O}_4$



**Magnetite in High Vacuum Mode (20 kV)**

	O Wt%	O At%	Fe Wt%	Fe At%
region 1	27.63	57.13	72.37	42.87
region 2	27.34	56.77	72.66	43.23
region 3	27.22	56.63	72.78	43.37
region 4	27.32	56.75	72.68	43.25
region 5	26.96	56.30	73.04	43.70
<b>Average</b>	27.29	56.72	72.71	43.28

$[\text{Fe}]_{\text{At}} / [\text{O}]_{\text{At}} = 0.76$  (instead of 0.75)



**Magnetite in ESEM mode.  
Water vapor pressure: 2 mbar (20 kV)**

	O Wt%	O At%	Fe Wt%	Fe At%
region 1	27.77	57.30	72.23	42.70
region 2	27.50	56.97	72.50	43.03
region 3	27.55	57.04	72.45	42.96
region 4	27.73	57.26	72.27	42.74
region 5	27.75	57.28	72.25	42.72
<b>Average</b>	27.66	57.17	72.34	42.83

$[\text{Fe}]_{\text{At}} / [\text{O}]_{\text{At}} = 0.75$

# Optimization of EDS analyses of fatty acids in ESEM mode

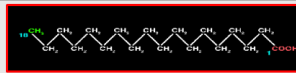
## Stearic Acid:

Saturated fatty acid, long chain

Formula:  $C_{18}H_{36}O$

Molar mass : 284,4772 g/mol

Crystallogr. Space Group:  $P2_1/a$

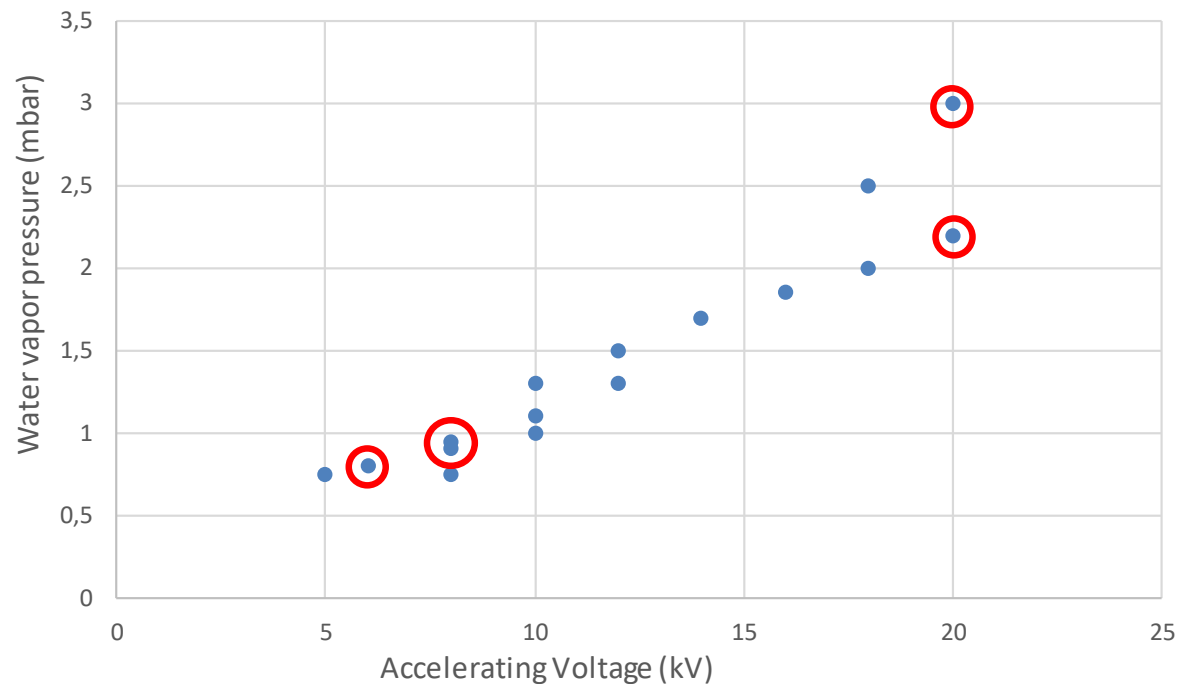


- White solid
- $T_{\text{fusion}} : 70\text{ }^{\circ}\text{C}$
- Applications: candles, soap

Stearic acid: Theoretical composition in wt% :

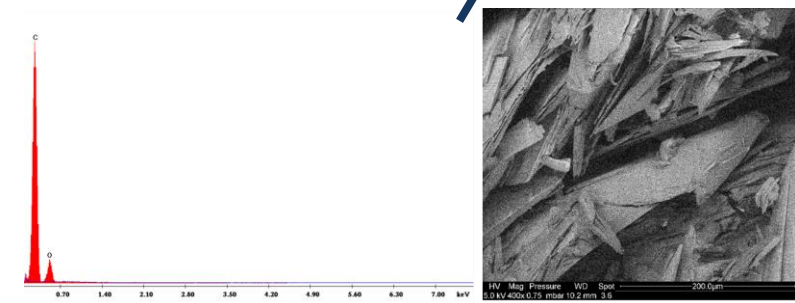
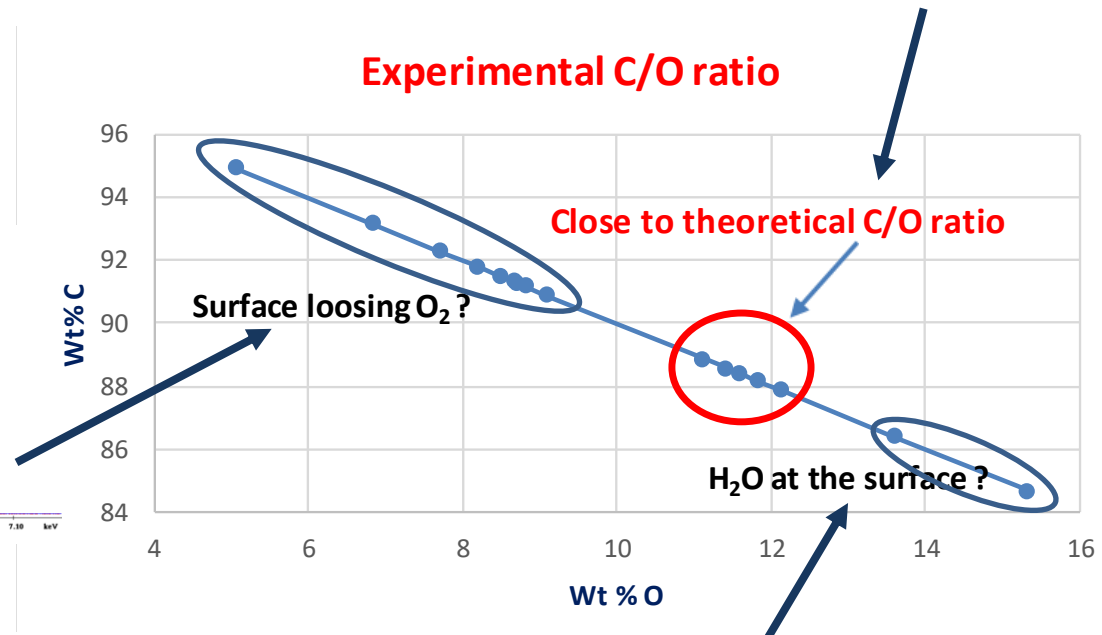
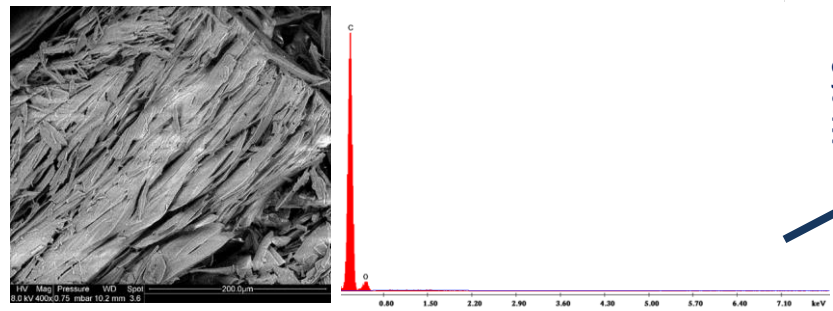
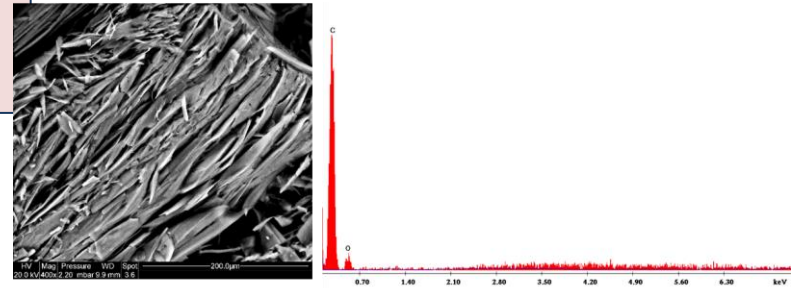
O: 11.25; H: 12.76; C: 76; C + H : 88.7

## EDS Stearic Acid Analysis - Experimental Conditions

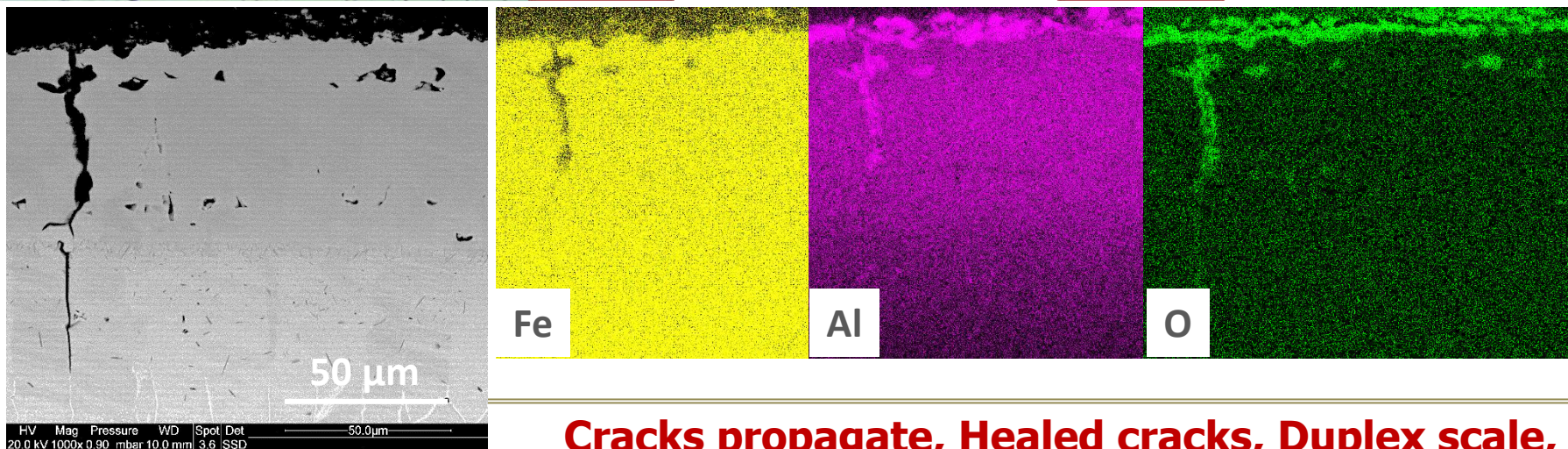
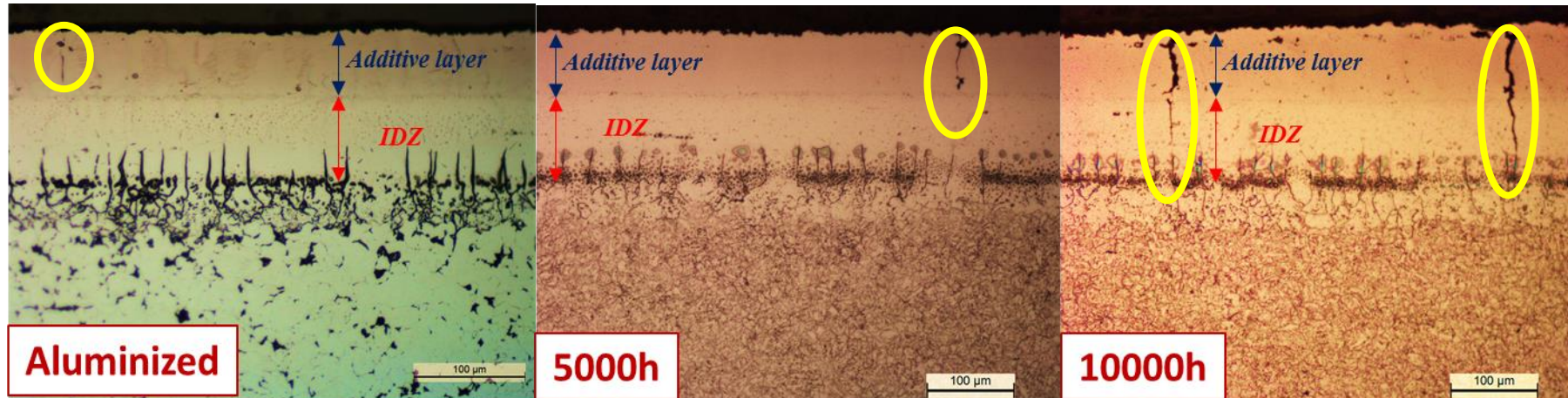


Accelerating Voltage (kV)	Water vapor pressure (mbar)	Wt% O	Wt% C
14	1,7	5,05	94,95
10	1	6,82	93,18
16	1,85	7,7	92,3
10	1,1	8,18	91,82
12	1,3	8,5	91,5
18	2	8,67	91,33
12	1,5	8,69	91,31
8	0,75	8,81	91,19
10	1,3	9,09	90,91
20	2,2	11,12	88,88
8	0,9	11,41	88,59
6	0,8	11,58	88,42
20	3	11,82	88,18
8	0,95	12,13	87,87
18	2,5	13,6	86,4
5	0,75	15,33	84,67

Experimental conditions which provide "Wt% O" close to the theoretical C/O ratio value allows obtaining Gaussian curves with the correct profile and BSE images of a good quality.



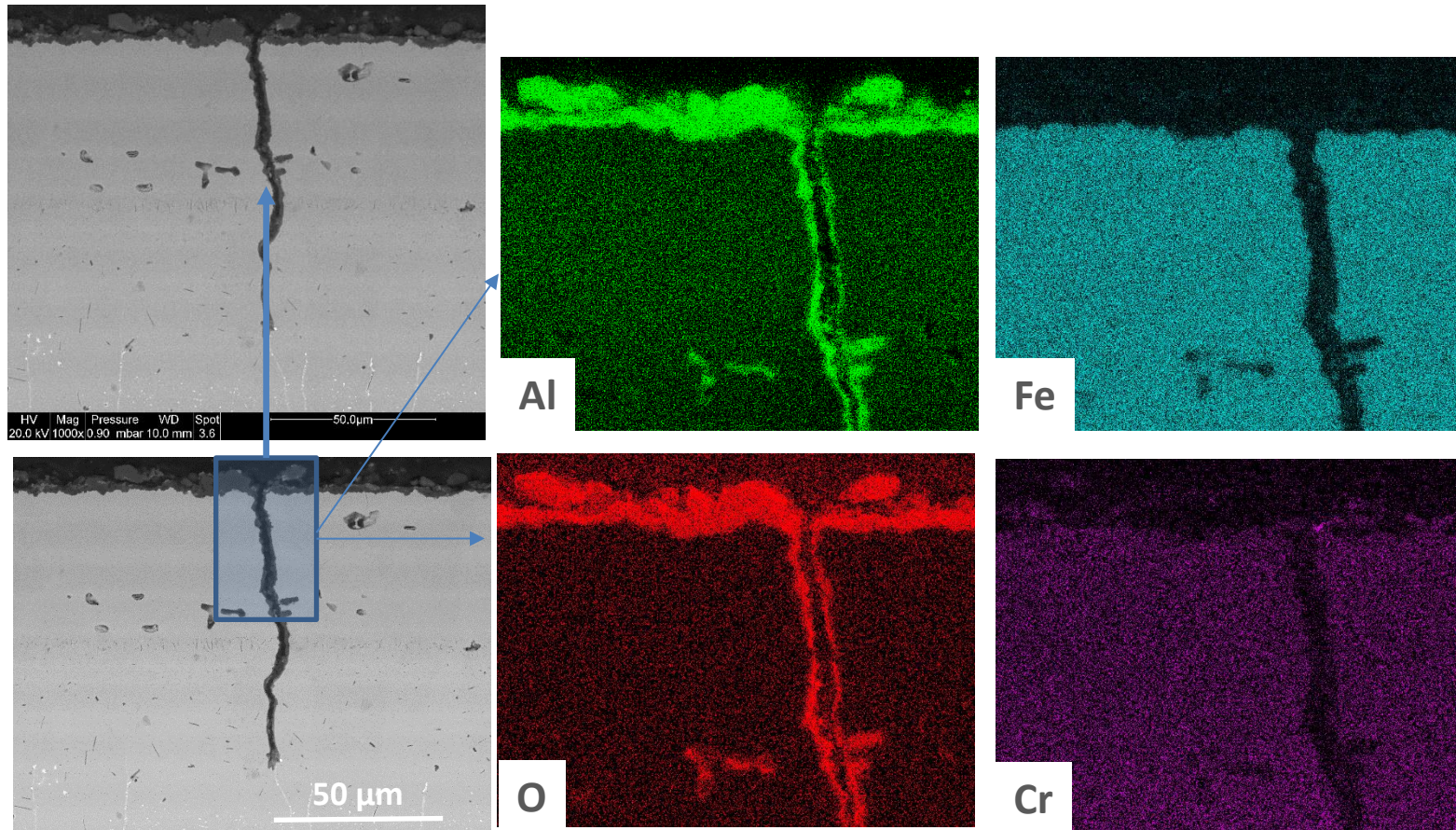
Epoxy-mounted cross-section, no metallic coverage, 20kV; 0.9 mbar of water vapor pressure,  
Spot Size: 3,7, K-peaks for all elements



**Cracks propagate, Healed cracks, Duplex scale,  
Inner  $\alpha\text{-Al}_2\text{O}_3$ , Outer  $\alpha\text{-Al}_2\text{O}_3$  + Clusters Fe-rich**

*Boulesteix et al.,  
Corros. Sci. (2018) in press*

**EDS: same conditions as previous** (20kV; 0.9 mbar of water vapor pressure, K-peaks for all elements )



- **Main oxide scale:  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> + growth of platelets. Onset of Fe+Cr**
- **Cracks partially healed + blunted at IDZ. SIMILAR TO STAGNANT**

Hydrogen-charged  $\alpha$ -Zr samples:  
 FCC  $\delta$ -ZrH<sub>1.66</sub> precipitates in  
 OR2\* at the surface

Resin-mounted cross-section under  
 0.6 mbar of water vapor pressure

(a) Phase map;

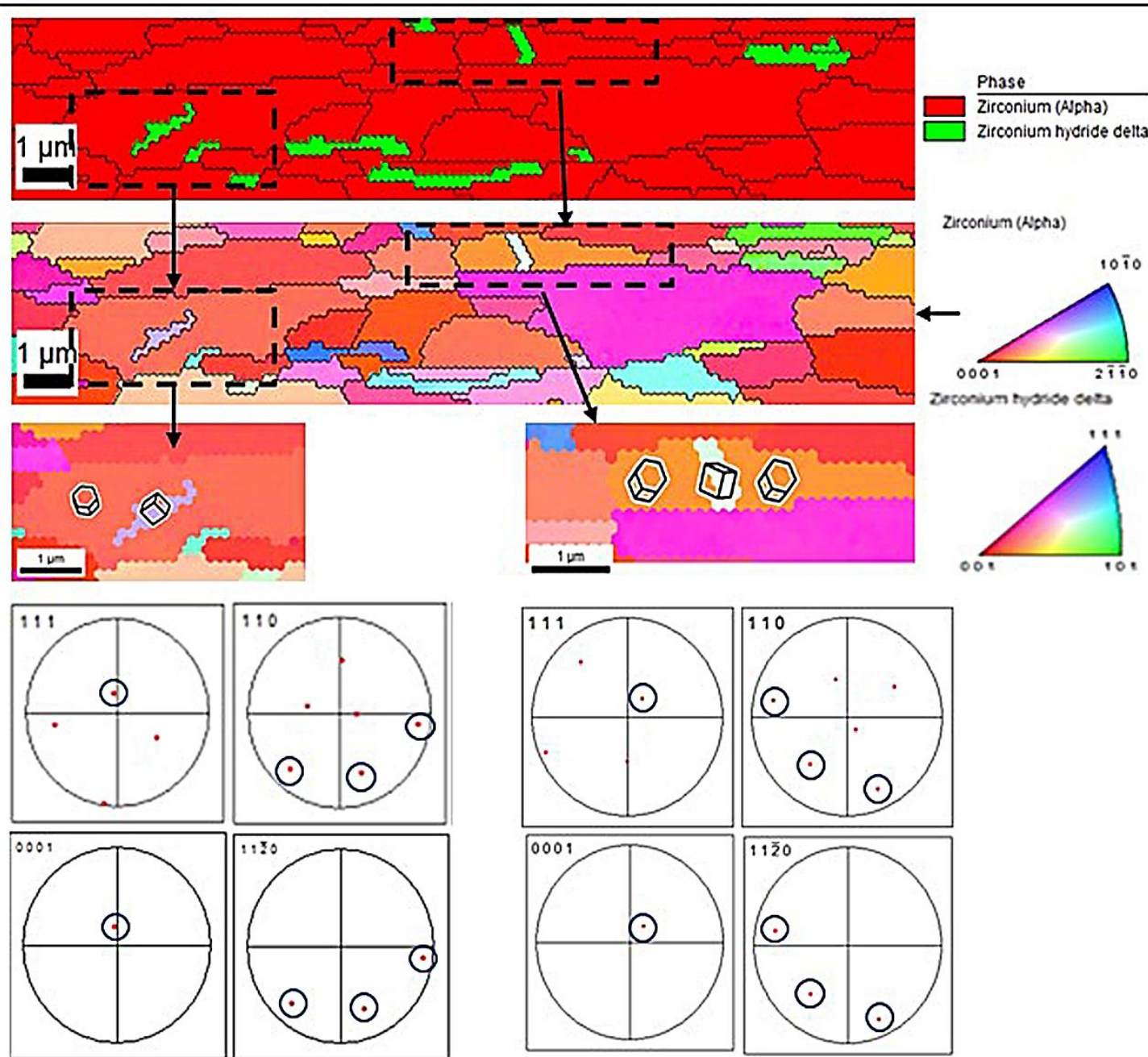
(b) crystallographic orientation map;

(c) and (d) details of two regions in (b)  
 containing hydride precipitates;

(e) and (f) stereographic projections  
 showing the orientation relationship  
 between hydrides and the substrate: ~  
**OR2\*** : (0001)//(111) and (11 $\bar{2}$ 0) // (110).

**Published in:** Conforto E. et al., Minerals,  
 Metals and Materials Series Part F6, 2017

\*for details about OR's, see: E. Conforto and  
 D. Caillard, Acta Mat. 2007



## EBSD on a Environmental SEM under water vapor

- Allows studying non-conductors, metal oxides and hydrides crystals minimizing risks of sample transformation during scanning
- Organic crystals can in principle be studied. However samples are very soft and often hydro-soluble (impossible to polish) - **trials with Riboflavin (B2 Vitamin) in progress**
- The water vapor absorbs part of the diffracted beam, decreasing the intensity of the detected signal
- Compromise between SEM parameters, water vapor pressure and EBSD camera sensitivity
- Gaseous (or LFD) detector is not in front of the sample !! better use FSD detector, if available, to define the area to be scanned.

### Hydrogen-charged samples:

- Easy and spontaneous hydrogen diffusion in hexagonal (Ti, Zr and their alloys)
- Surface heating due to the incident beam leads to hydrogen desorption from hydride layer at the surface or from hydride precipitates
- An equilibrium between hydrogen absorption/desorption has to be found at the surface – use of the appropriate water vapor pressure !
- However: The presence of oxygen can influence the kinetics and the stability of the reaction

### Archaeological objects excavated from anaerobic medium:

- Stable crystalline phases were successful analyzed but some corrosion products grew in iron parts due to the oxygen contained in water vapor. Better to analyze them under dry inert gas ?

- **Goal of this study:**

- to identify the provenance career of stones used in roman monuments by EDS;
- to observe and analyze ageing/transformations in sensitive archaeological samples after centuries of interactions with the environment through images, EDS and EBSD.

- **Materials:** stones from roman monuments; corrosion products, deposited layers and precipitates on shipwrecks; remaining metallic material in roman and medieval nails

- **Methods:**

- ESEM : FEI Quanta 200 ESEM/FEG equipped with EDAX-Genesis EDS and EDAX OIM-EBSD
  - Accelerating voltage: in the 3.5 – 20 kV range
  - (Distilled) Water vapor pressure: varied between 0.3 and 2.25 Torr ( = 0.4 and 3 mbar).
  - Detectors used for images in ESEM mode:
    - Large-Field Detector (LFD) for secondary electrons (SE) images
    - Solid-State Detector (SSD) for Backscattered Electrons (BSE) ones.
- EDS: Si:Li detector; spectra quantification using ZAF corrections
- EBSD : Digiview-4 CCD camera and OIM-5 softwares (Collection and Analysis )

# Stones from Archaeological monuments: The gallo-roman river port town of BARZAN (The Gironde estuary, French West Coast)

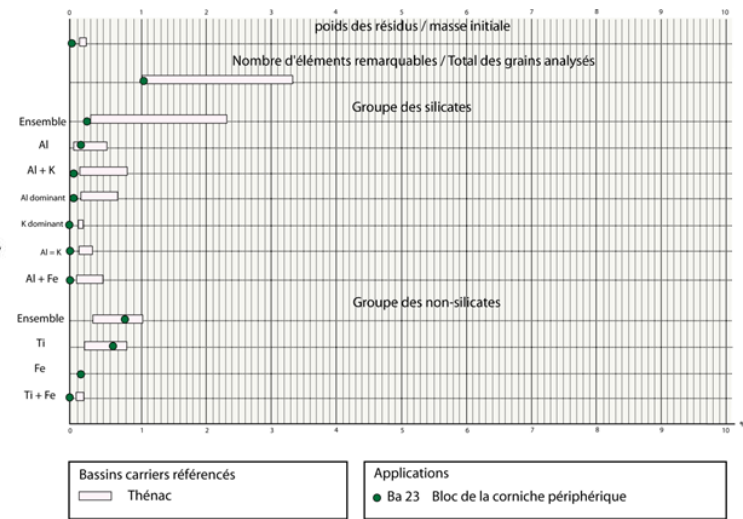
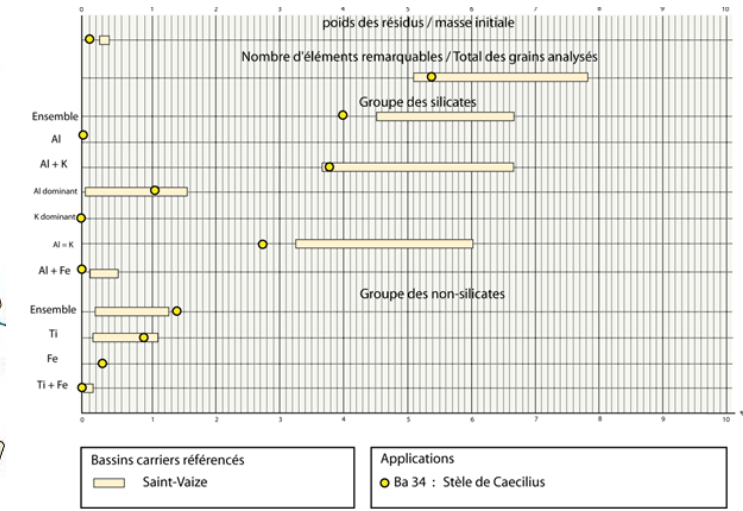
Provenance of stones?

Comparative EDS analyses between\*:

- the chemical composition of the stone used to built the monument and
- the typical chemical composition of stones from 14 different existing careers (in activity or abandoned) in the region, constituting a database built on the basis of the EDS analyses.
- Six of them were correlated to ancient sites, allowing making hypothesis about ancient stone trade routes.



Graphical synthesis of Saint-Vaize and Thenac Careers references and samples which fit with



\*For details of this methodology, see:  
J. Gaillard, E. Conforto et al., *Aquitania*, Vol. 30, 2014

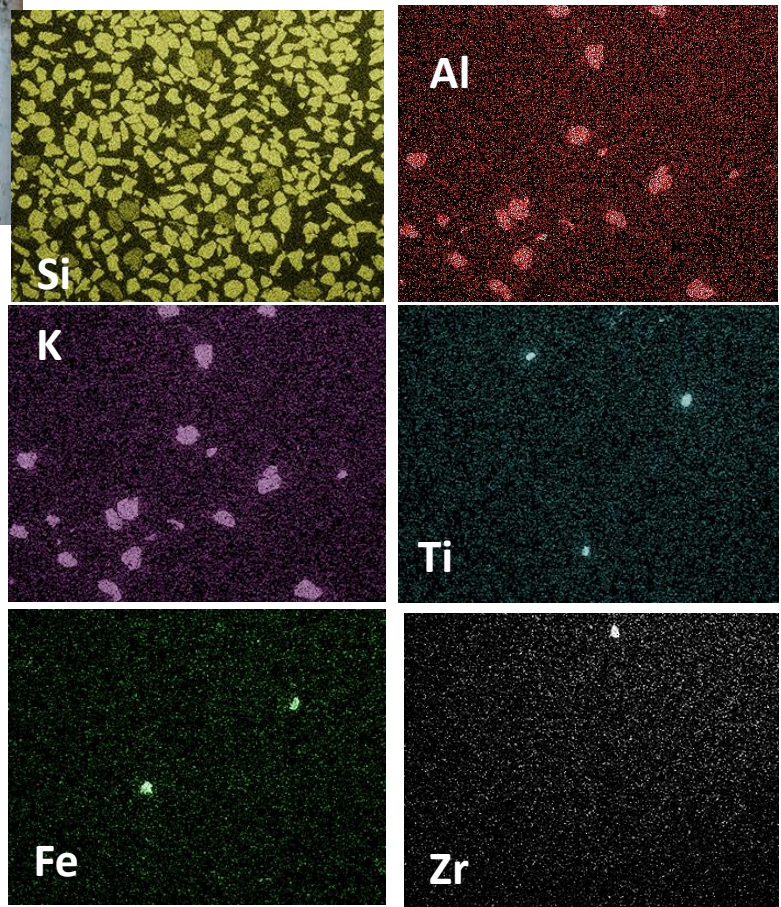
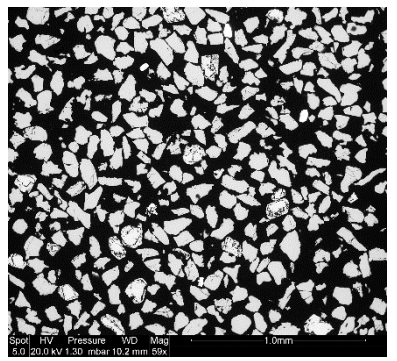
See also: <http://pierre-et-carriers.haute-saintonge.org/la-pierre-et-son-identification>

# Stele of Caecilius, Saint-Vaize career

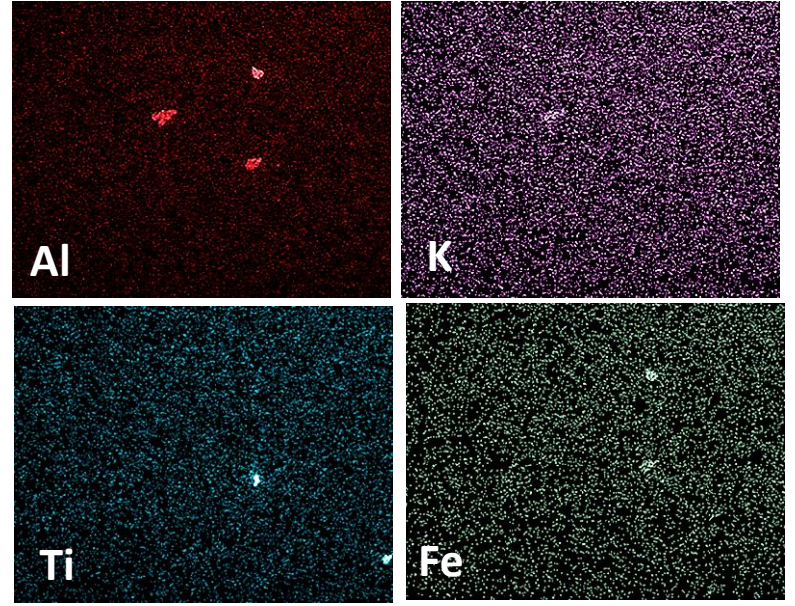
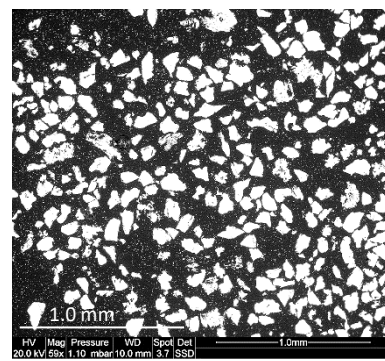
20 kV; 1.1 – 1.2 mbar



1.0 mm



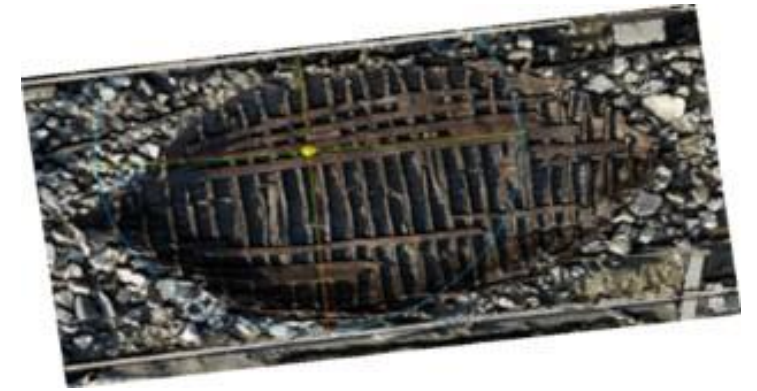
«Grand Théâtre»  
pilaster, from  
Thénac career



# SHIPWRECKS



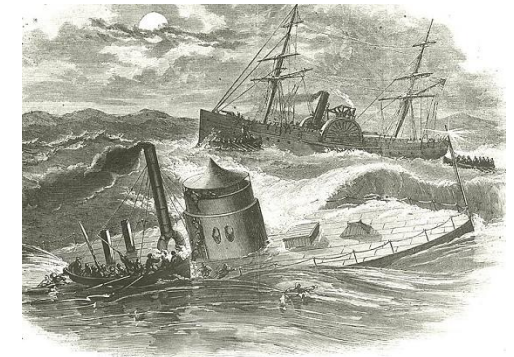
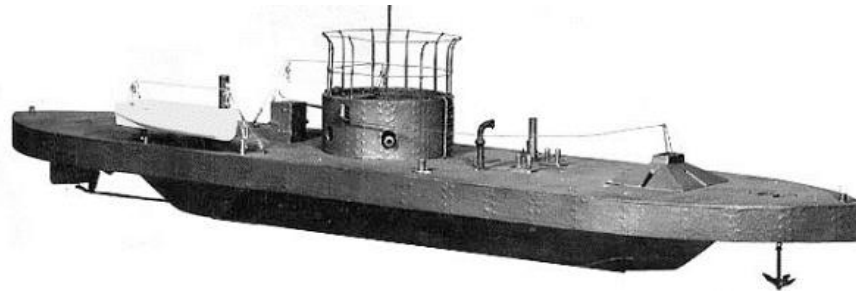
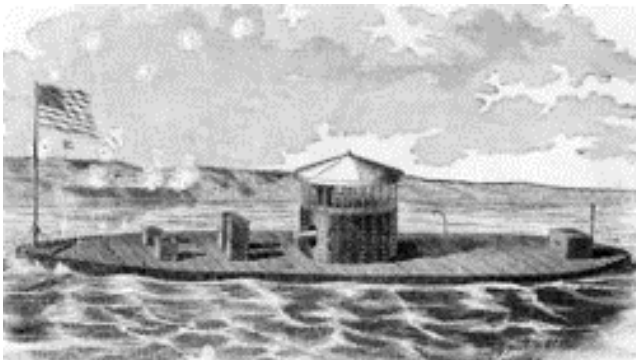
**Lyon Saint-Georges wreck n°4 (3<sup>rd</sup> century):** one of the 16 wrecks excavated in a site planned for the building of an underground parking along the Saône river (Lyon, France).  
**Soon:** the wreck, restored will be exposed in the Lugdunum Museum.



**Castelou-Mandirac wreck:** excavated on the roman port of Narbonne, nowadays inland.

## *USS Monitor*

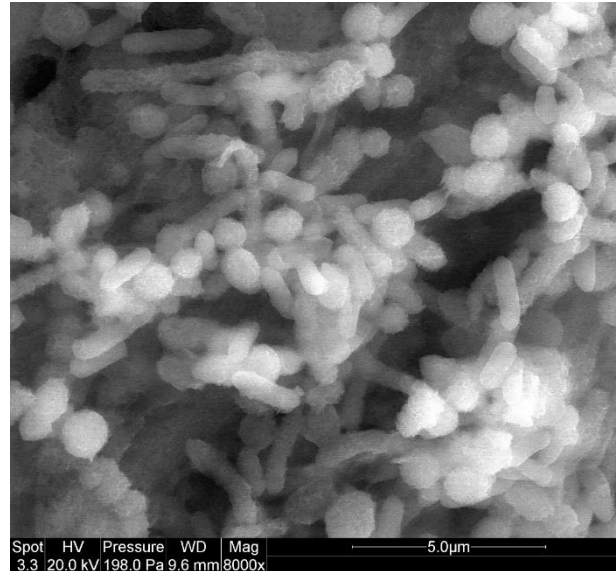
The first world iron-clad ship, sank in the Atlantic Ocean during the American Civil War (1862). The region it sank and surroundings were declared a first American Marine Sanctuary. Many objects were recovered.



# Iron sulfide crystals grown on wood parts of 2000-years shipwrecks:

## Shipwrecks:

- Constituted of pieces of wood assembled with iron fasteners (nails) and maintained in wet and anoxic conditions for long periods
- Presence of Sulfideproducing Bacteria (SPB) in the environment of shipwrecks:



Sulfideproducing Bacteria in the rope of the “La Natière” (Saint Malo) shipwrecks. Observed without any preparation; still alive in the very beginning of the observation.

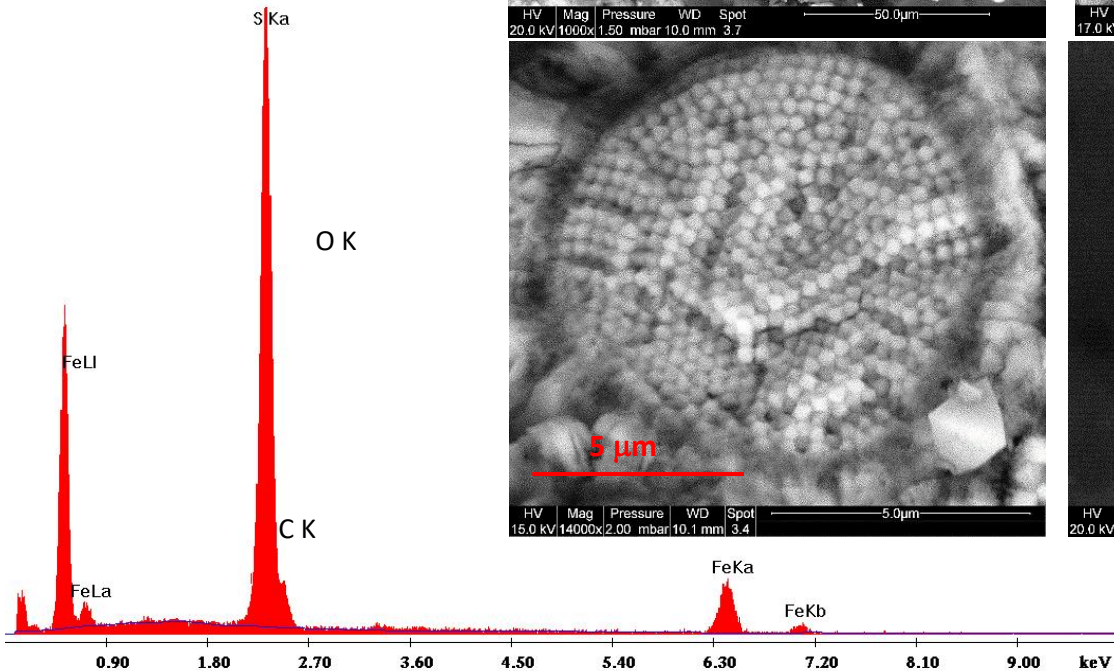
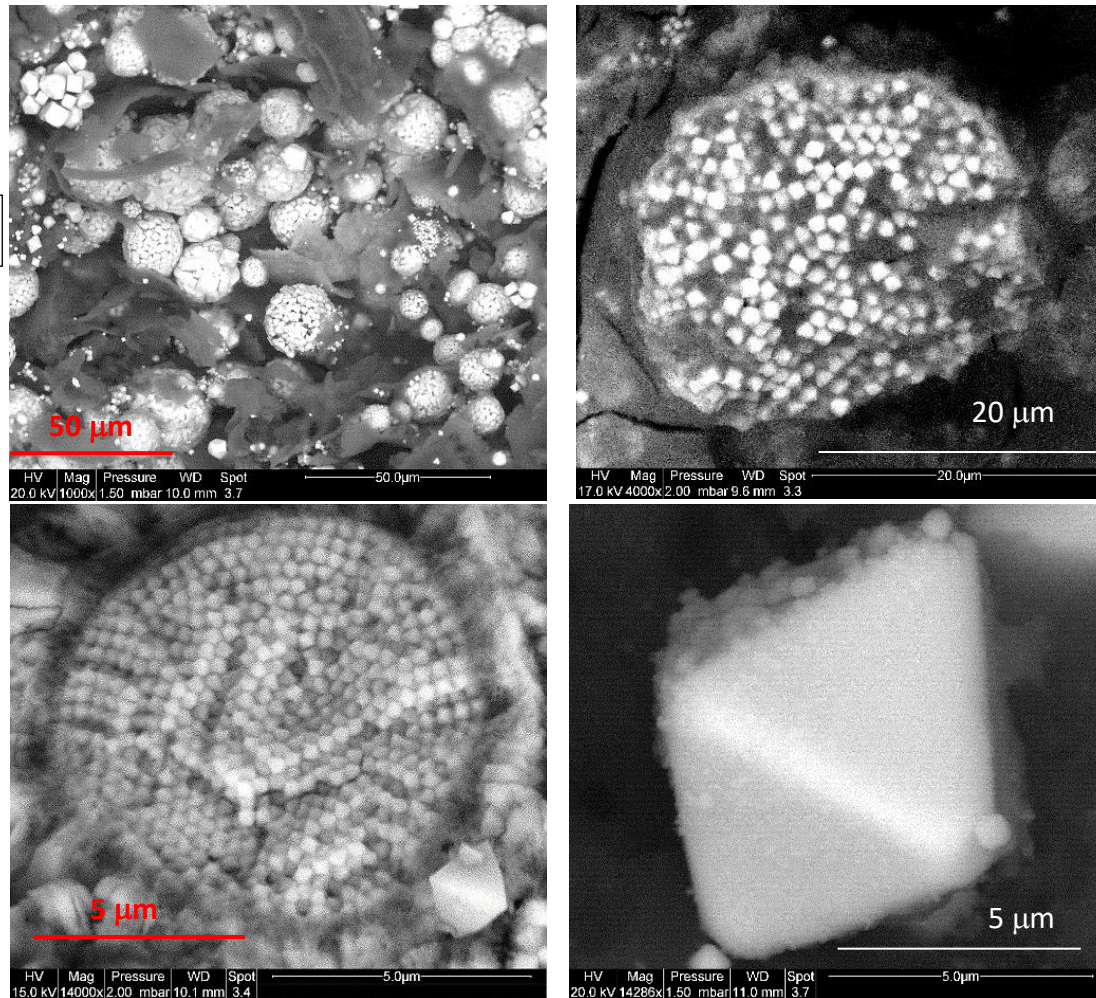
20 kV; 1.98 mbar

- SPB metabolic activity is responsible for the microbiologically influenced corrosion (MIC) of the metal
- SPB can use the oxygenated species of sulfur (sulfates, thiosulfates, sulfites...) present in the environment as electron acceptors and convert them to sulfides
- Sulfides easily react with the metal

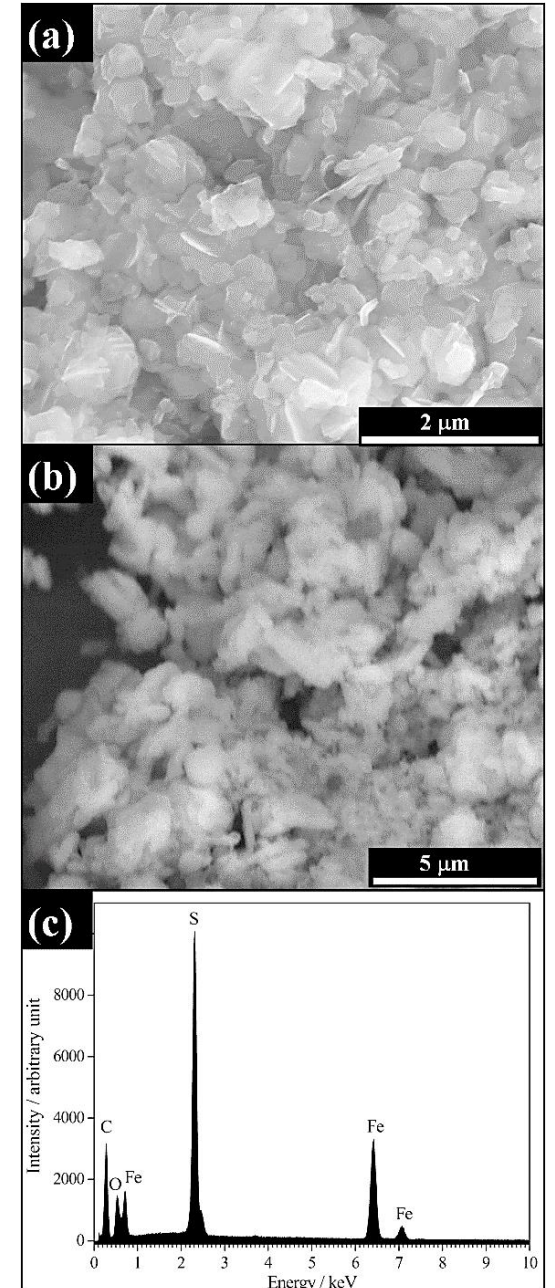
# Iron sulfide crystals grown on wood parts of 2000-years shipwrecks\*:

Framboidal Pyrite  
 $\text{FeS}_2$  crystals

15 – 20 kV; 1.50 – 2.00 mbar



Greigite  $\text{Fe}_3\text{S}_4$

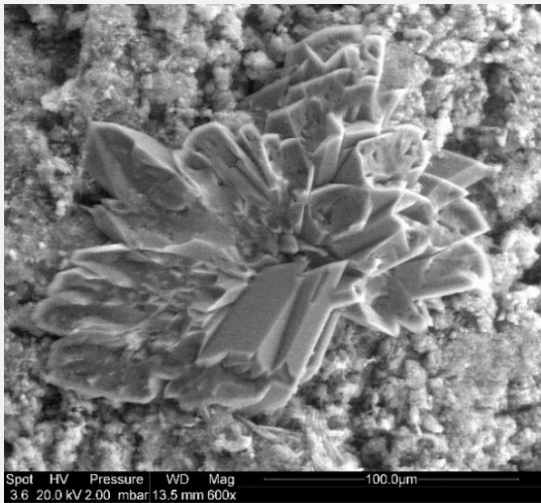


\*C. Remazeilles, E. Conforto et al., Microchemical J. 148 (2019) 10-20 ; Studies in Conservation, *in press*

**SHIPWRECKS** - Gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) monoclinic crystals

(15 – 20 kV; 2.00 mbar)

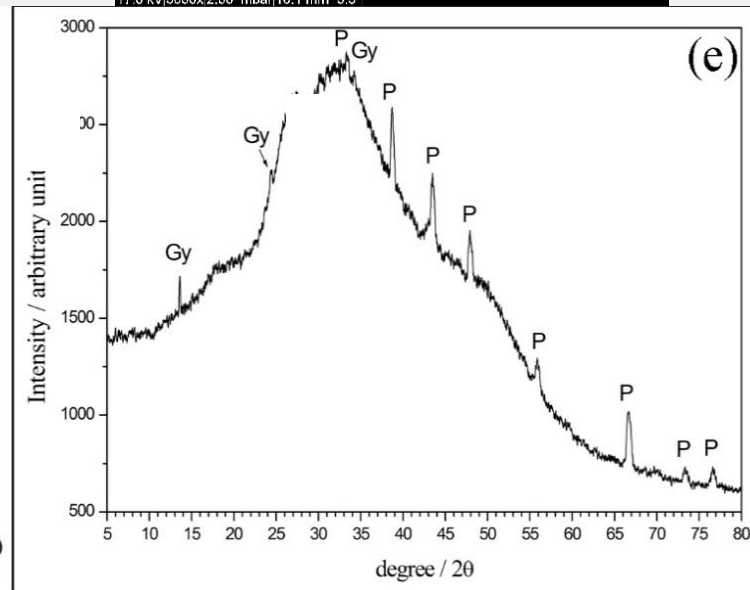
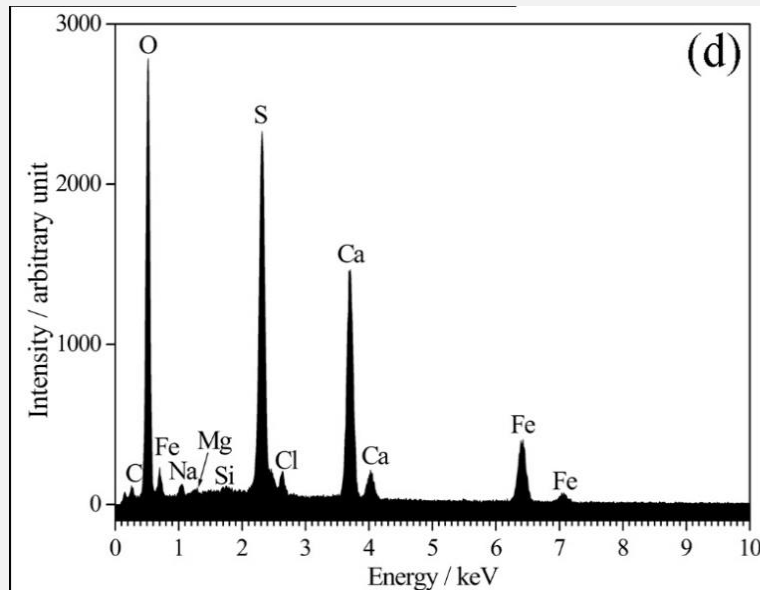
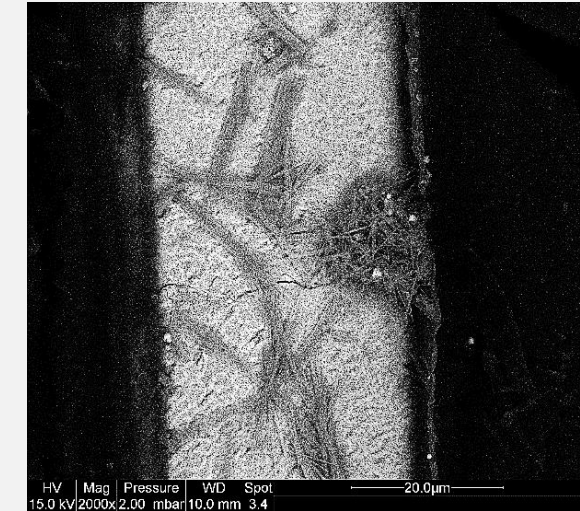
Grown on the wood parts of the  
*USS Monitor*



On the wreck of  
Castelou/Mandirac



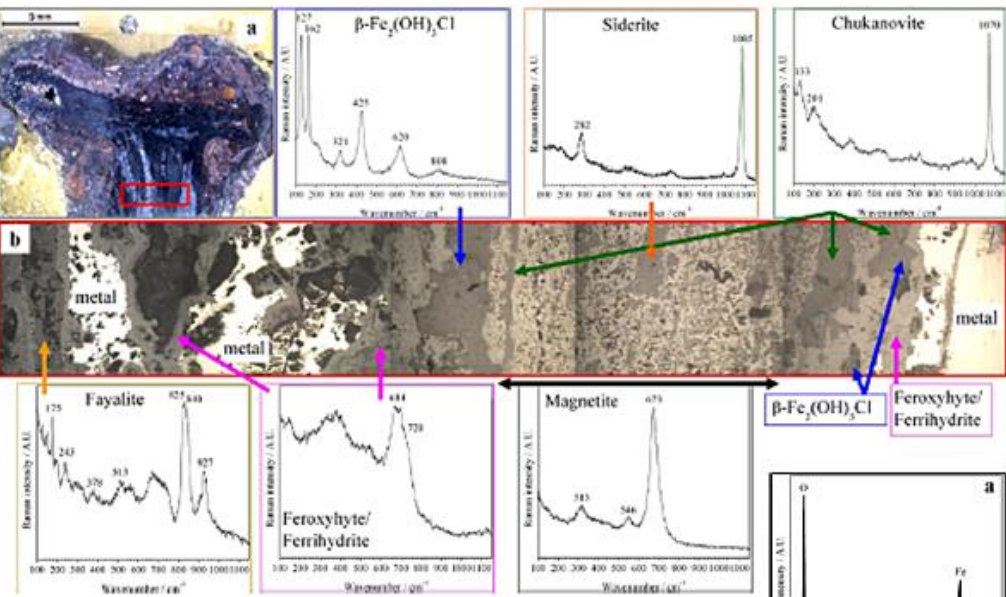
On the Lyon Saint-Georges wreck n°4



EDS spectrum and X-rays diffractogram showing the presence of gypsum crystals (Gy) and Pyrite  $\text{FeS}_2$  (P) in the wood of the 3 samples.

# Study of corrosion products in ancient nails by EBSD

## Raman spectra



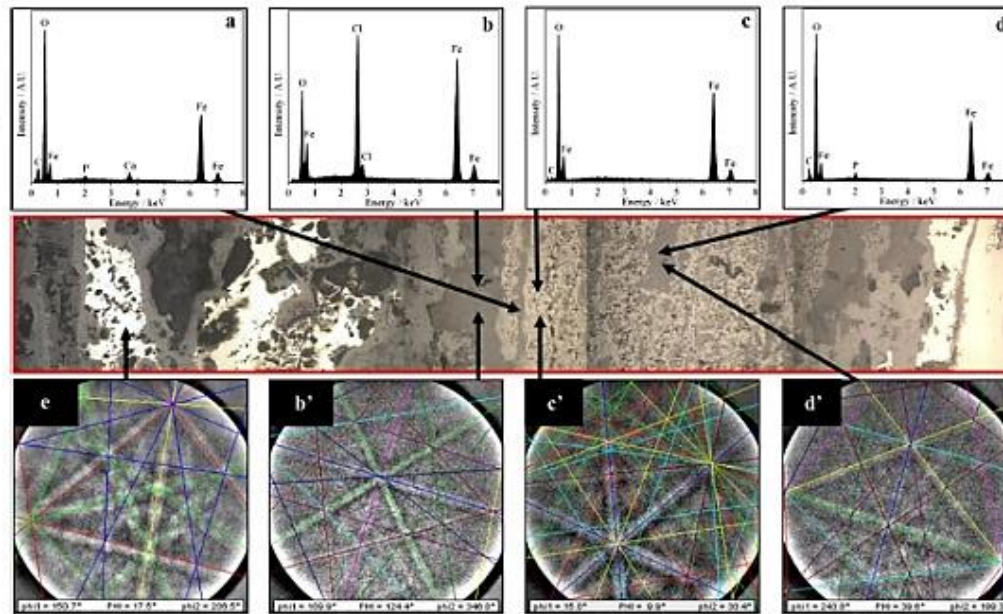
## Epoxy-mounted cross-section of a Nail:

- Gallo-roman period
- Extracted from an excavation site at Angoulins-sur-mer (Charente Maritime)

16.7 kV; 0.4 mbar

Interest: metal transformations after 2000-years exposition to the environment

Samples are too delicate and brittle to be unmounted !!!

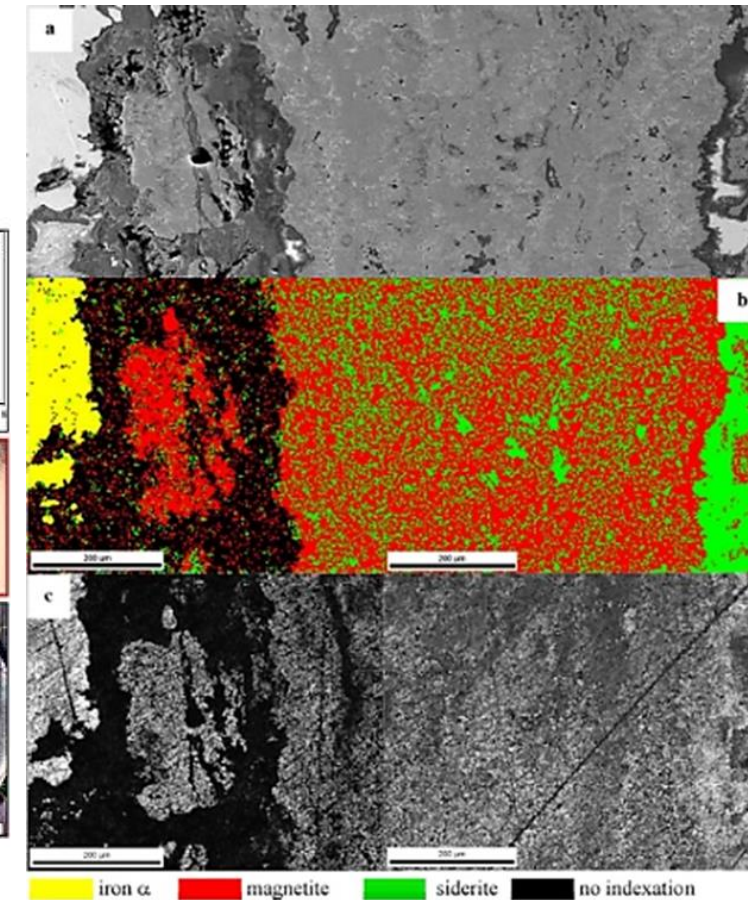


Chukanovite  
 $\text{Fe}_2(\text{OH})_2\text{CO}_3$   
monoclinic

$\beta\text{-Fe}_2(\text{OH})_3\text{Cl}$   
rhombohedral

Magnetite  
 $\text{Fe}_3\text{O}_4$   
FCC

Siderite  
 $\text{FeCO}_3$   
rhombohedral



I. Azoulay et al., Appl Phys A (2013) 110:379–388

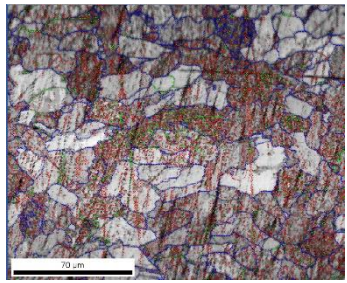
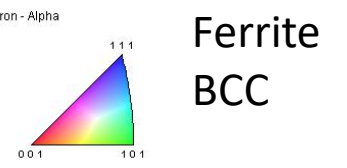
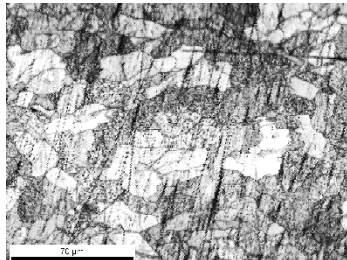
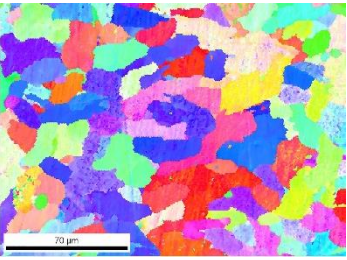
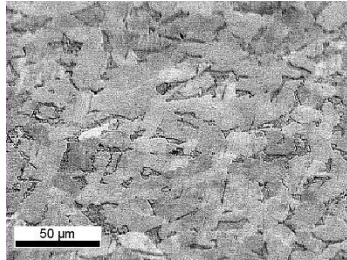
# Nails from terrestrial excavations in Spain: ancient metallurgical process revealed by the metal microstructure \*

Epoxy-mounted cross-sections of nails from:

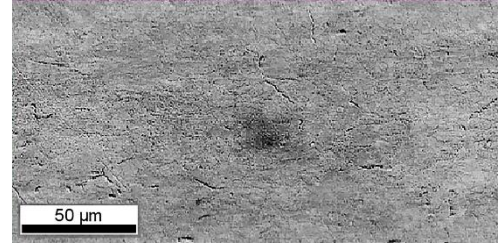
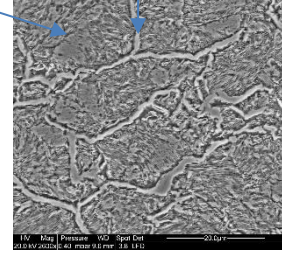
20 kV; 0.3 – 0.4 mbar

*Reinosa* : roman period

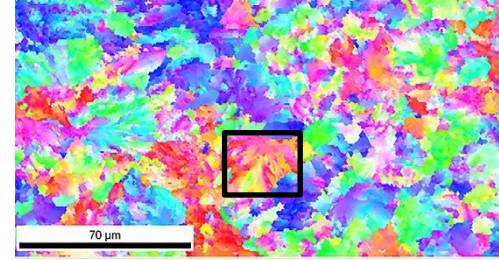
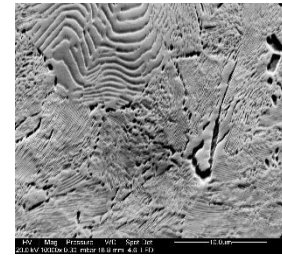
*Oiola*: Middle Age



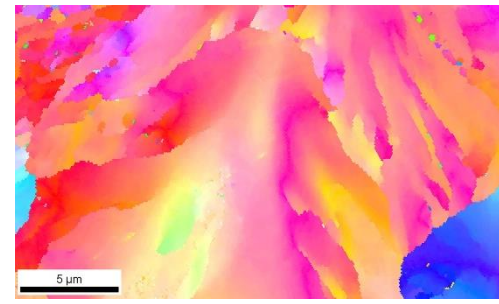
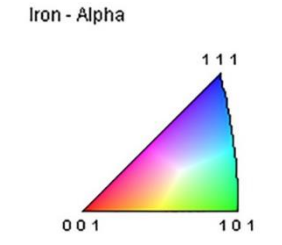
cementite  
perlite



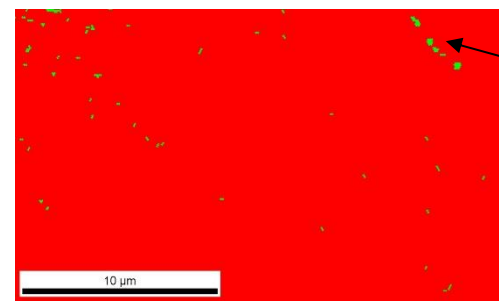
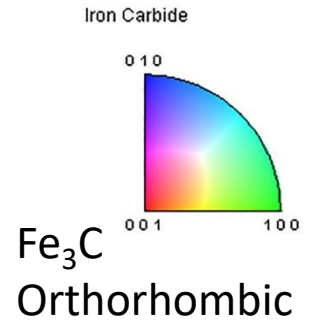
Scanned zone



IFP



IFP detail



cementite

Phase map

Boundaries: Rotation Angle				
Min	Max	Fraction	Number	Length
2°	5°	0.604	30660	1.42 cm
5°	15°	0.113	5718	2.64 mm
15°	180°	0.283	14352	6.63 mm

**Roman period:** very low carbon, only ferrite

**Middle Age:** carbon added (< 0.6 %?)  
Ferritic-pearlitic steel → improvement of mechanical properties

\*Haizea Portillo, PhD work (unpublished)

# Conclusions and Perspectives

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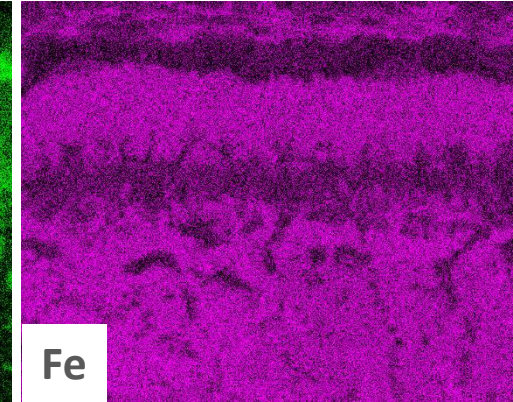
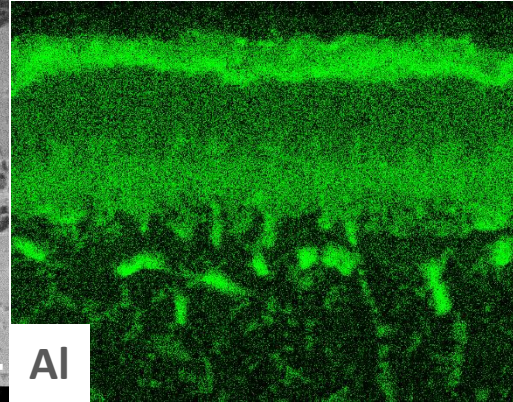
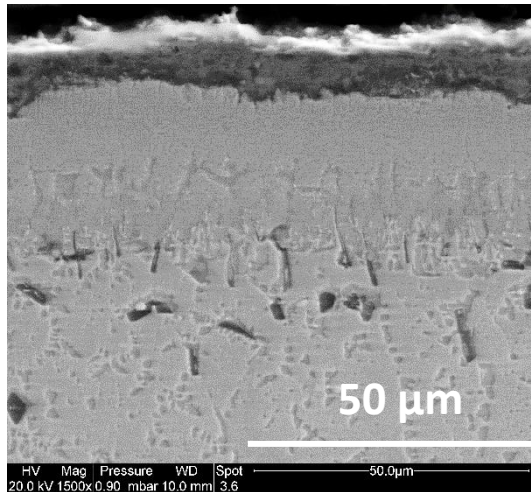
The advantages of performing observations, EDS and EBSD analyses under water vapor pressure in ESEM mode (without any preparation) on non-conductor, humid, fatty and sensitive samples respect to the observation under high vacuum after metallization are:

- Data is obtained from the original surface without any artefact due to vacuum and/or to the metallization;
- Possibility of using the same SEM parameters (voltage, current) as those used for metals in high vacuum mode;
- Neutralization of the charge accumulated on non-conductive surfaces (no thermal drift) - Protection of sensitive surfaces against transformation due to vacuum, charge and heating during analyses;
- The oxygen from water vapor is not detected in EDS spectra; the quantification of elements present at the surface is not modified; The EDS signal is not attenuated by the water vapor;
- EBSD under low water vapor pressure is suitable to maintain the hydrogen stability of hydride layer/precipitates during scan and to preserve very sensitive archaeological samples from damage;
- Some attenuation of the EBSD signal can be observed for pressures higher than 0.4 - 0.6 mbar;
- Further tests on unmounted archaeological iron objects\*\* and organic crystals will be performed using an EBSD EDAX/Pegasus system under N<sub>2</sub> on a ZEISS CrossBeam 340 VP (Gemini column).

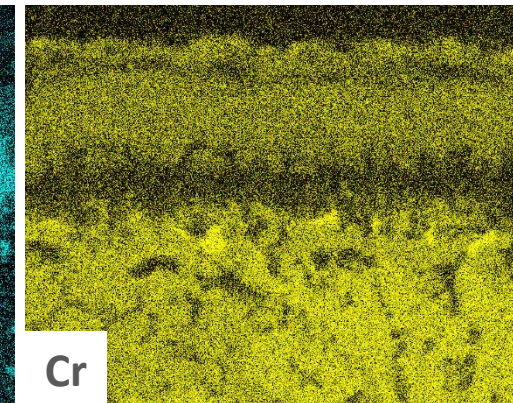
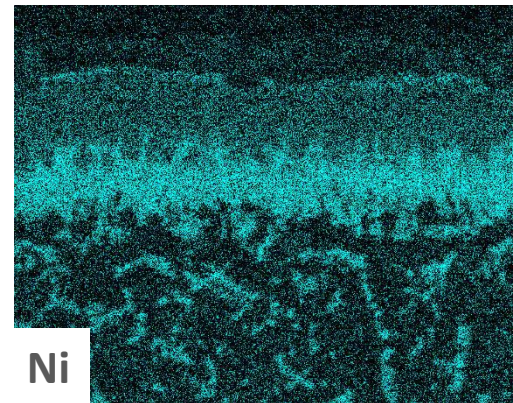
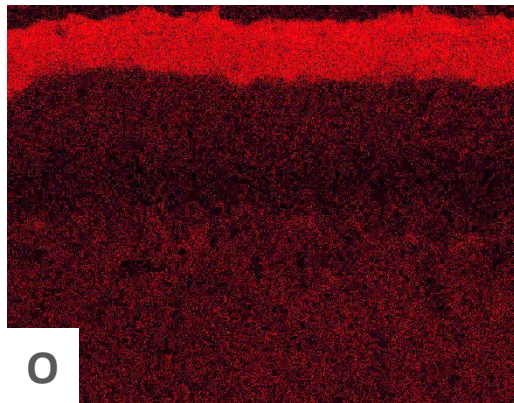
\*\* polishing and analyses using special sample holders manufactured for purpose

THANK YOU

EDS: same conditions as previous



**1 bar,  
2000 h**



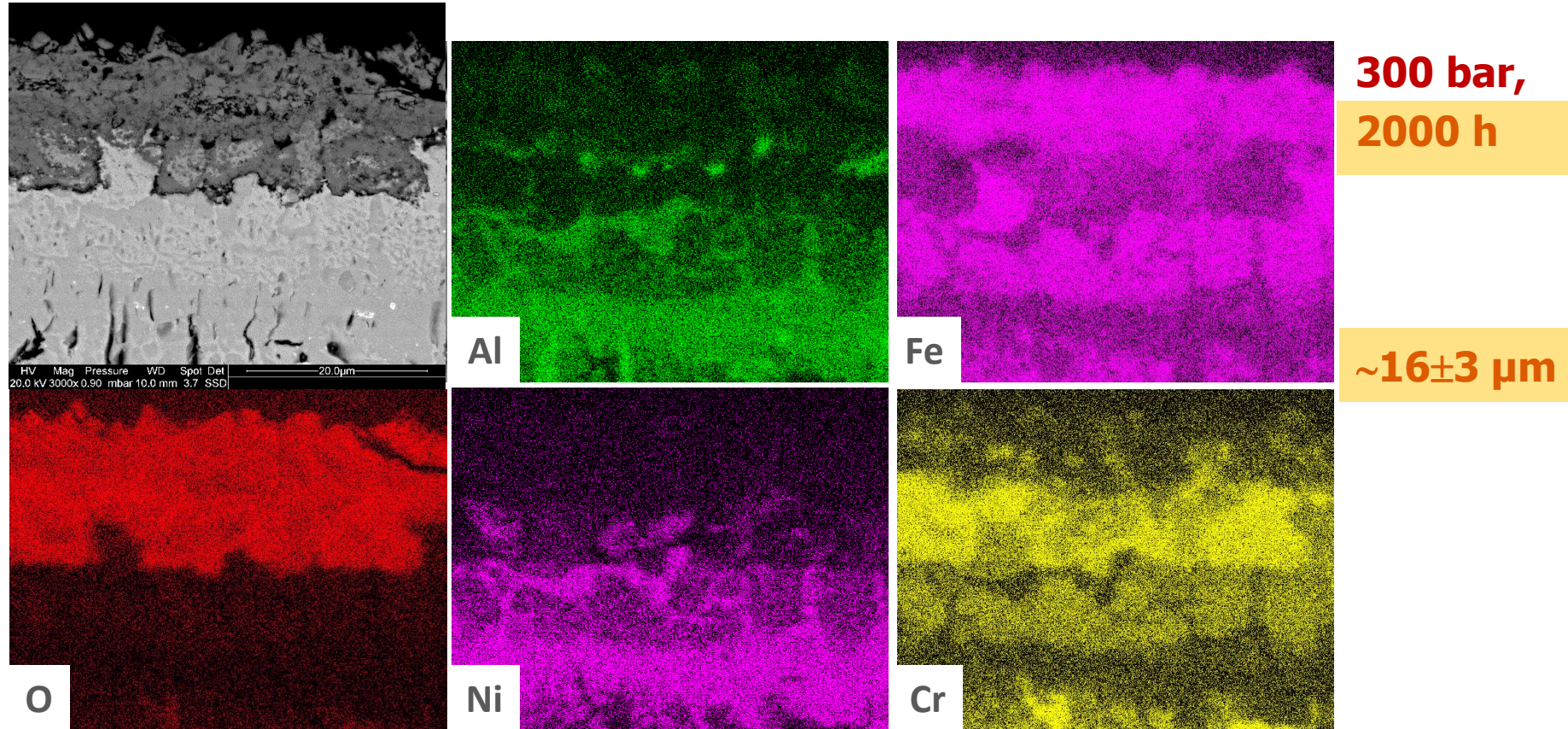
**~ 7±1 μm**

→ Main inner  $\alpha$ - $\text{Al}_2\text{O}_3$  scale, central  $(\text{Al,Cr,Ni})_x\text{O}_y$ , outer  $\text{Fe}_2\text{O}_3$  ( $\text{Fe}_3\text{O}_4$ ?)  
→ Upward flow of Fe (mostly), of Cr (mixed with Al) and of some Ni.

**HRC.AI 700°C**

**flow of 3.2 cm/s steam, 300 bar**

EDS: same conditions as previous

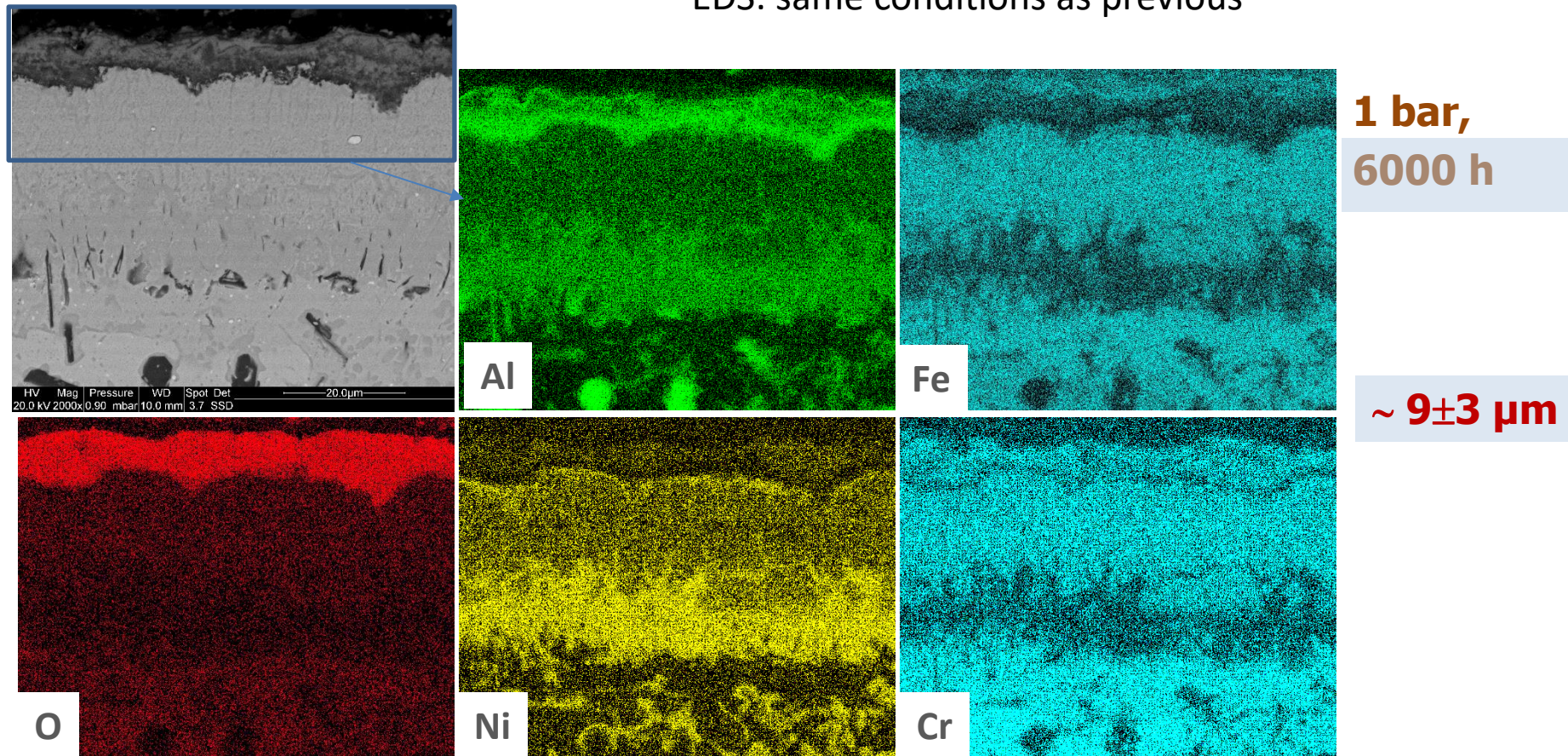


- **300 bar: 2-fold thicker scales in 3-times less exposure (6000 vs. 2000h)**
- **Much more (Fe & Cr) scales + porosity + "dissolution" of  $\alpha\text{-Al}_2\text{O}_3$**

HR3C.AI

700°C, flow of 3.2 cm/s steam, 1 bar

EDS: same conditions as previous



- Enhanced Fe+Cr (and some Ni) diffusion but coating "stable"
- Scale similar to 2000 h but a bit thicker and more irregular