

Caractérisation ultrastructurale des oxydes de fer associés aux bactéries ectosymbiotiques de la crevette hydrothermale *Rimicaris exoculata*



Image M. Zbinden



présenté par :

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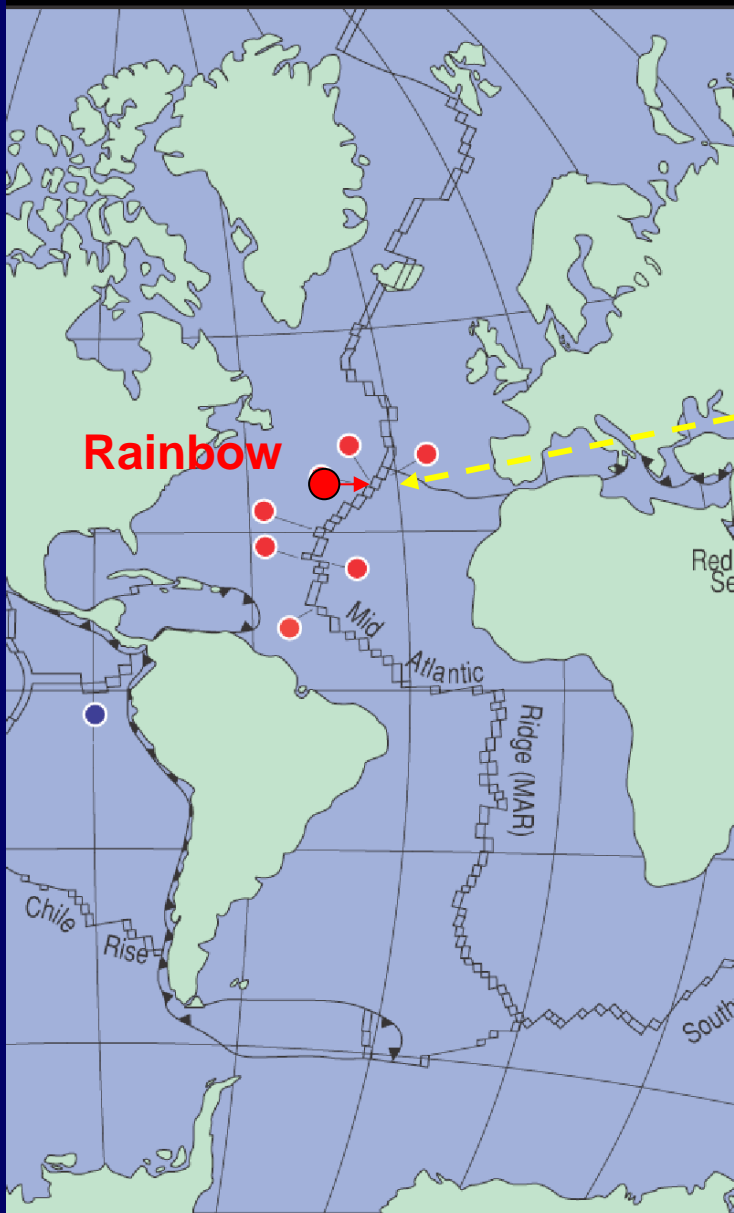
M.-A. Cambon-Bonavita (IFREMER-Brest)

B. Shillito (Université Pierre et Marie Curie – Paris)

F. Gaill (Université Pierre et Marie Curie – Paris)

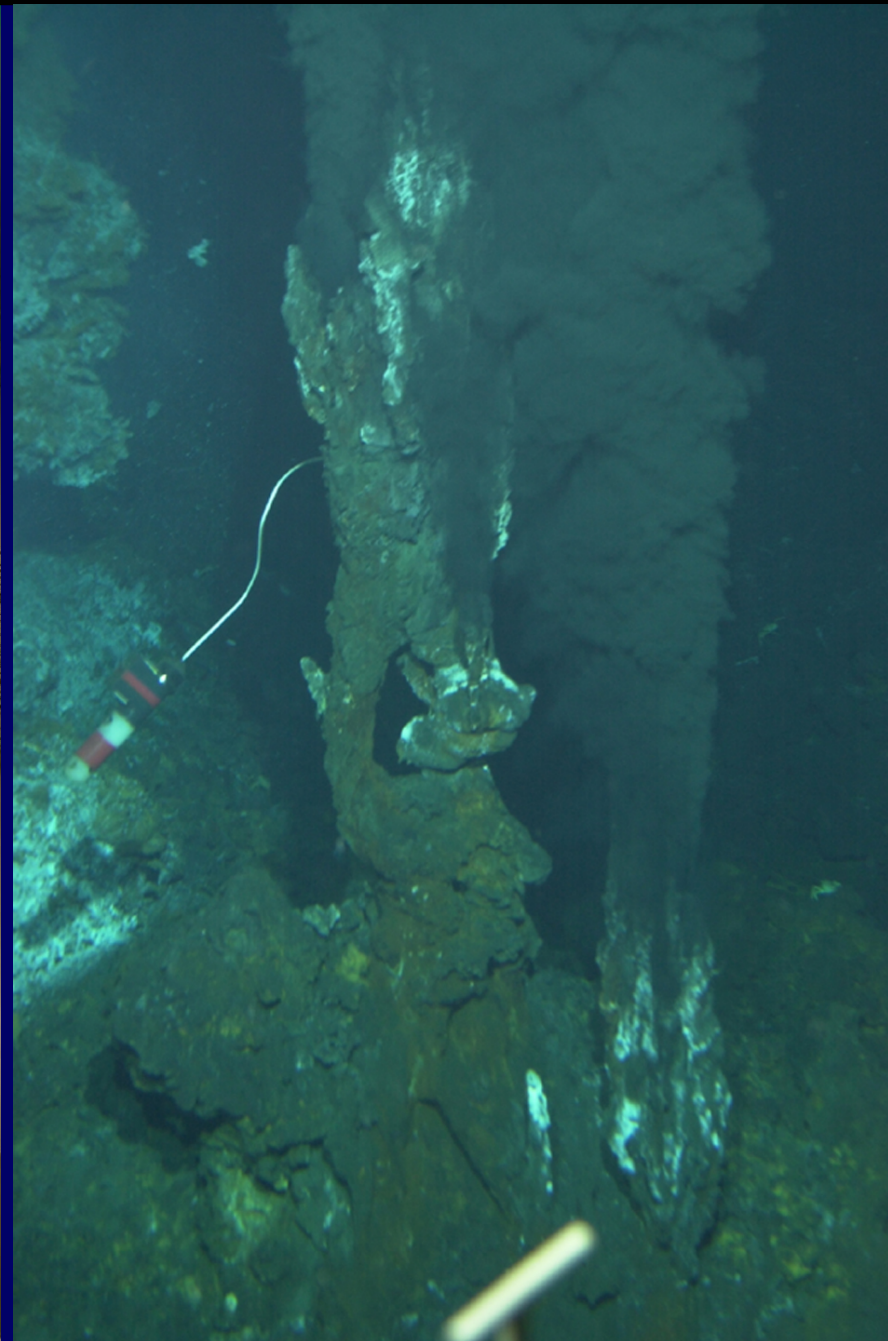
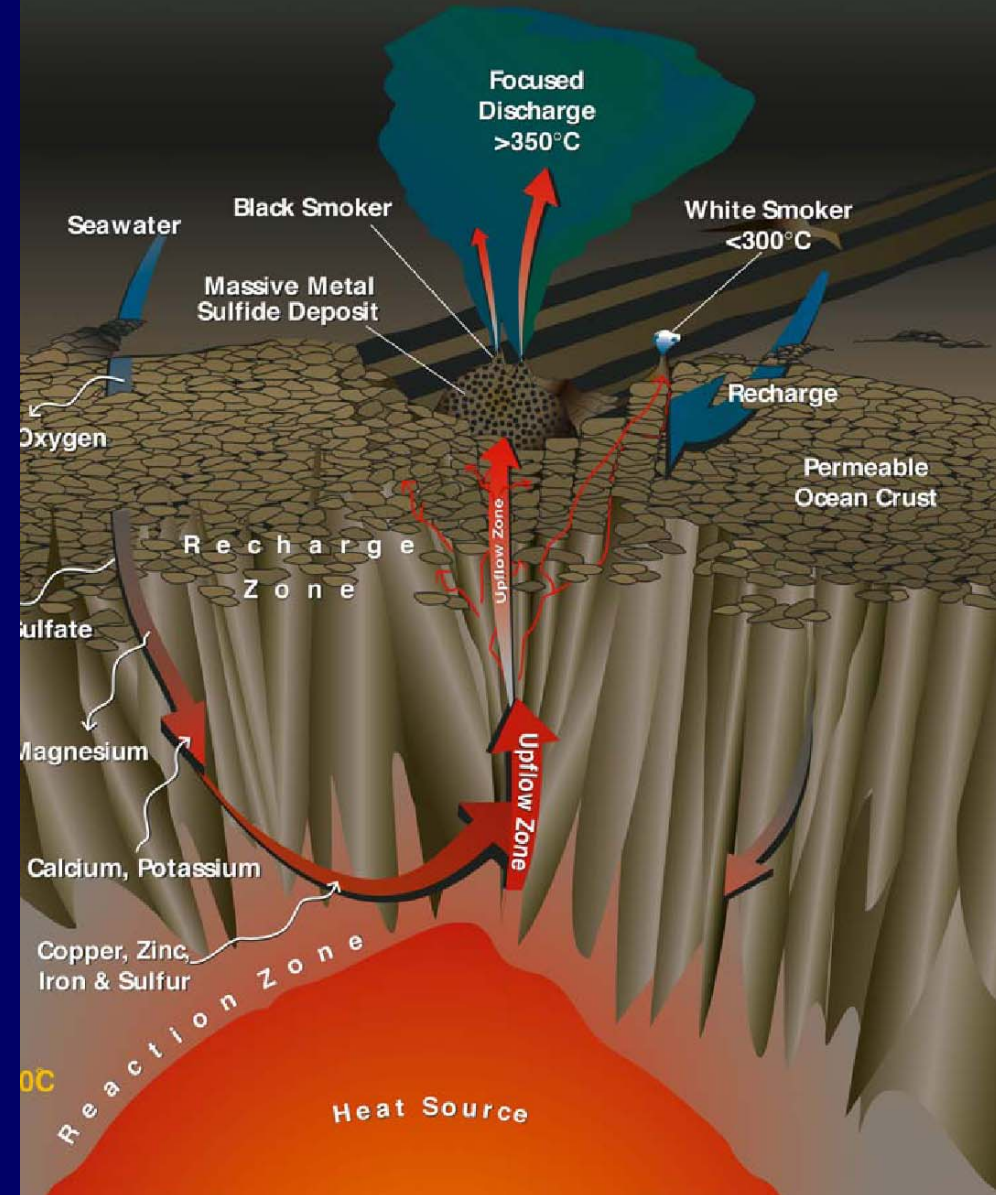
FNRS

Ifremer



Sources hydrothermales profondes

Fluide hydrothermal :
 H_2 , CH_4 , H_2S , Fe^{2+}



Essai de crevettes *Rimicaris exoculata*
Sur les parois d'un fumeur noir



Récolte à l'aspirateur !

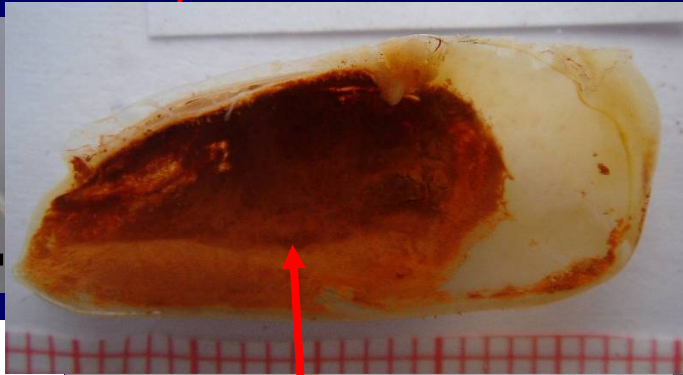


Crevettes Rimicaris exoculata

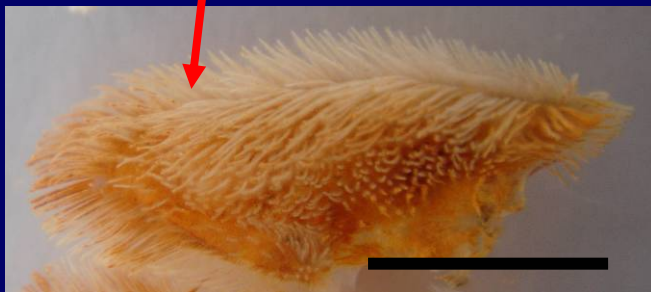
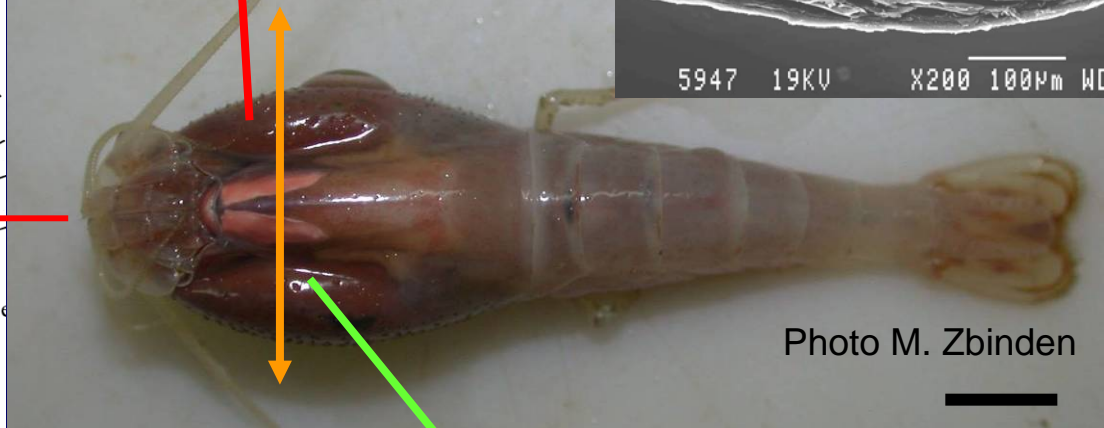
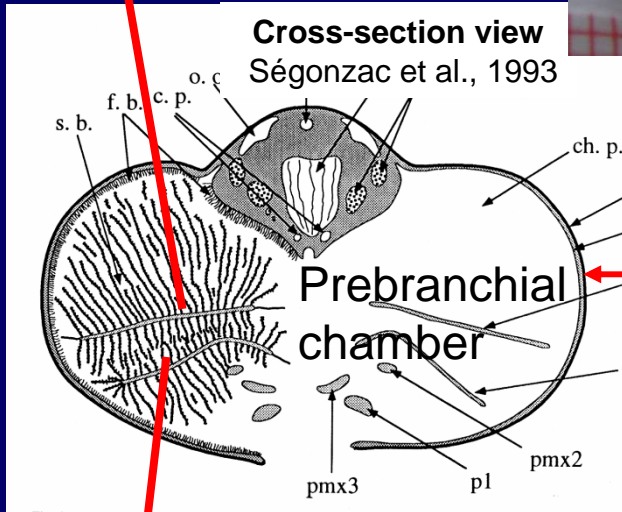
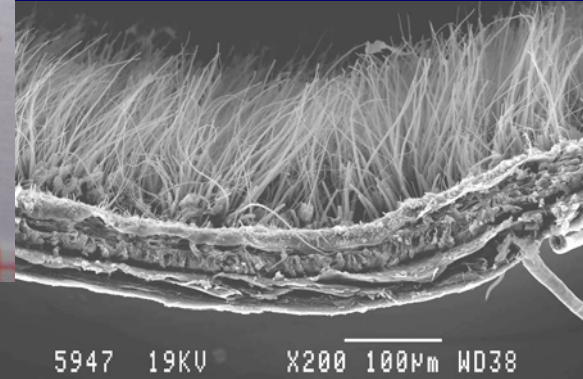


Chambre branchiale de la crevette

Scaphognathite (2e maxille)



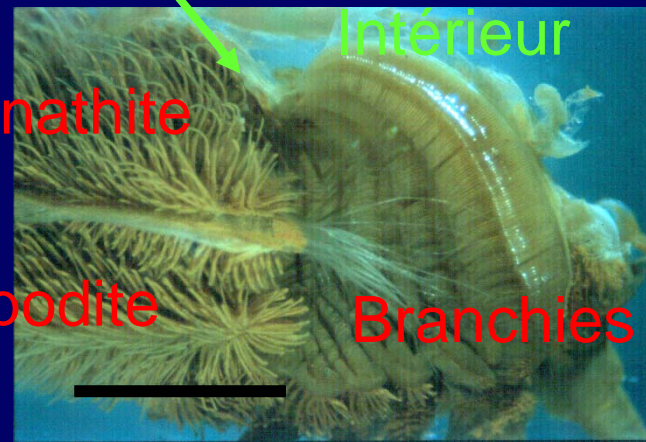
Branchiostegite



Exopodite (1e maxilliped)

Scaphognathite

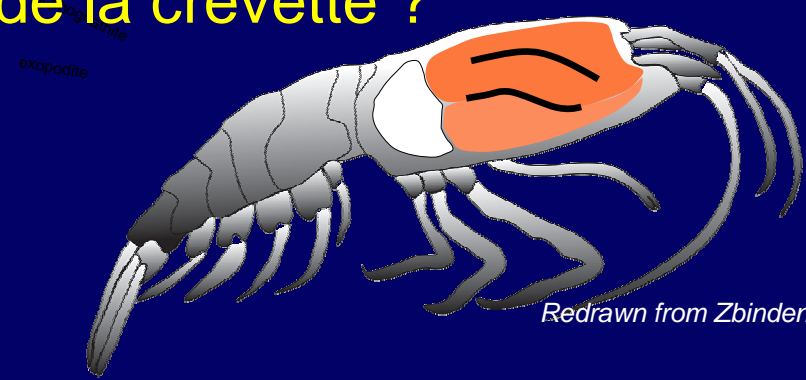
Exopodite



Branchies

Scale bars
= 1 cm

- Morphologie des bactéries et des oxydes de fer ?
- Distribution dans la chambre branchiale
- Relations avec le cycle de mue de la crevette ?



- Formation des oxydes de fer et relations avec les bactéries?
- Ultrastructure et composition des oxydes de fer ?

Why various shrimp colours ?





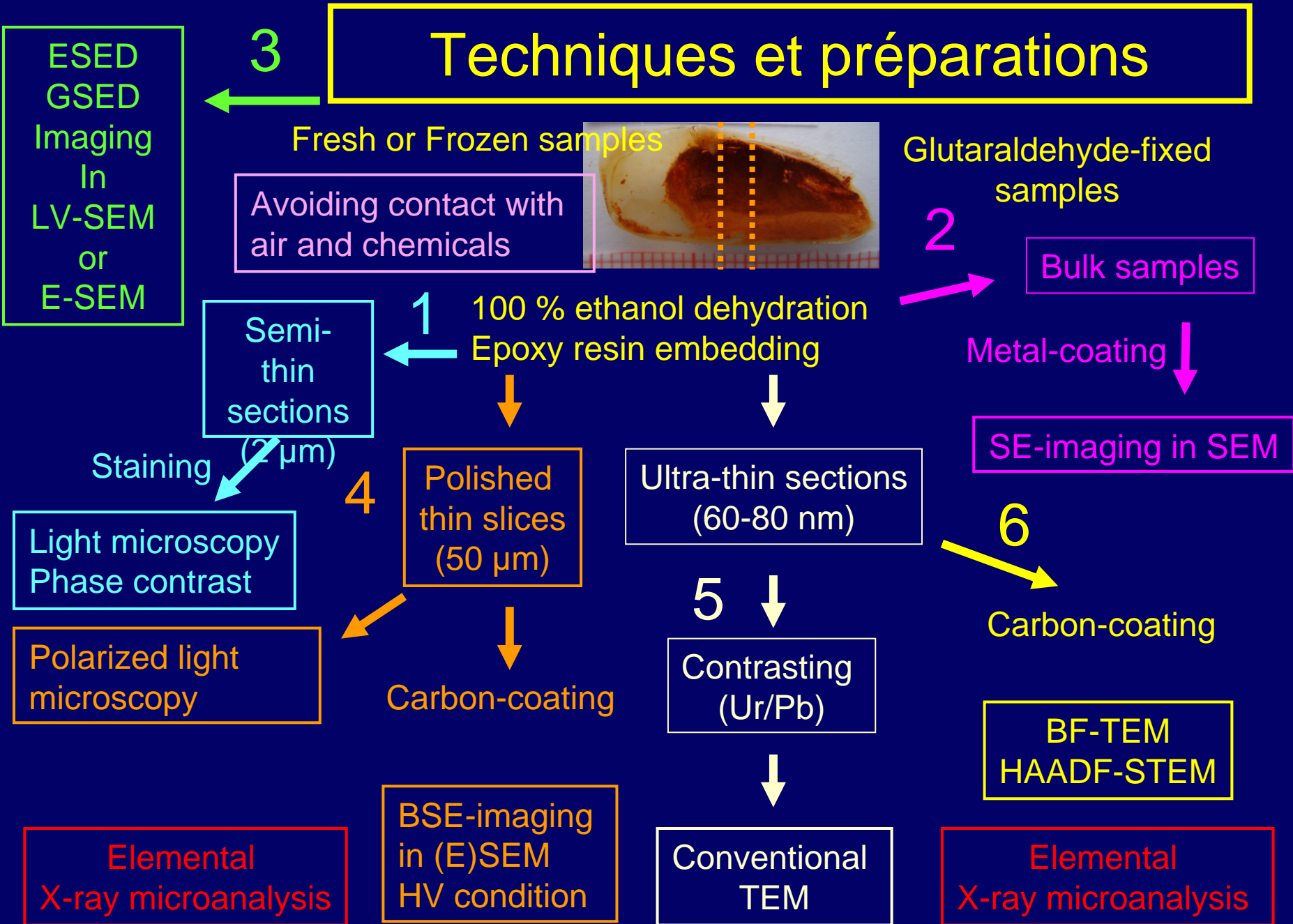
Cellule d'Appui Technologique en Microscopie



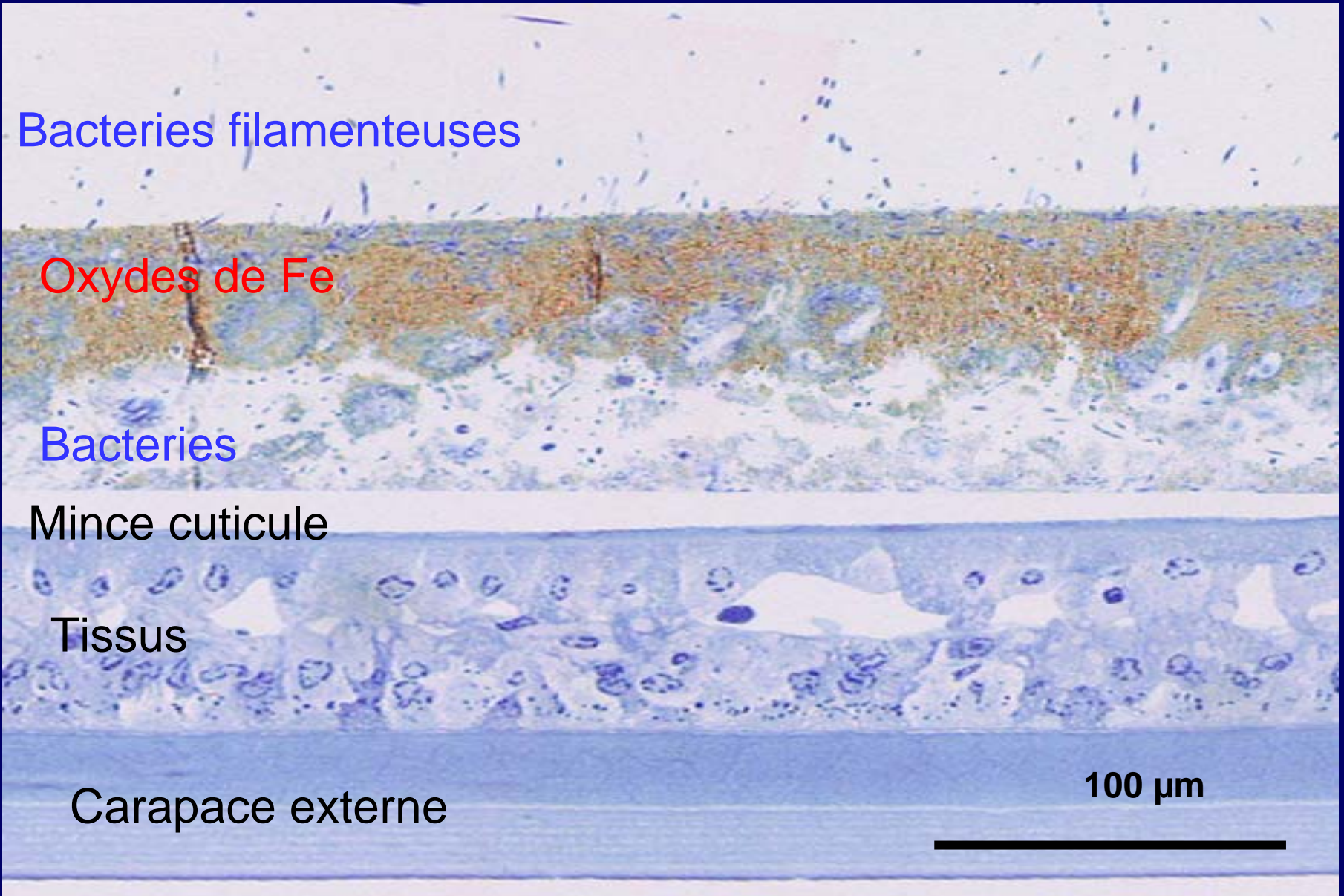
Plateforme technologique : Dir. Prof. R. Cloots
Services : C. Henrist, Ph. Compère

- **5 microscopes électroniques dont**
 - 2 SEM conventionnels (MEB) – Jeol et Hitachi
 - 1 ESEM (MEB « environnemental ») – FEI FEG-ESEM XL-30
 - 1 TEM conventionnel - Jeol
 - 1 TEM/STEM à haute résolution - Tecnai G2 twin
 - 2 Microanalyseurs de rayons X - EDAX
- **2 microscopes à force atomique**
- **2 microscopes confocaux**

Techniques et préparations



1. Microscopie optique: coupes semi-fines (2 μm)



Coupe horizontale

Particules d'oxyde de fer
1-2 μm

Bacteries

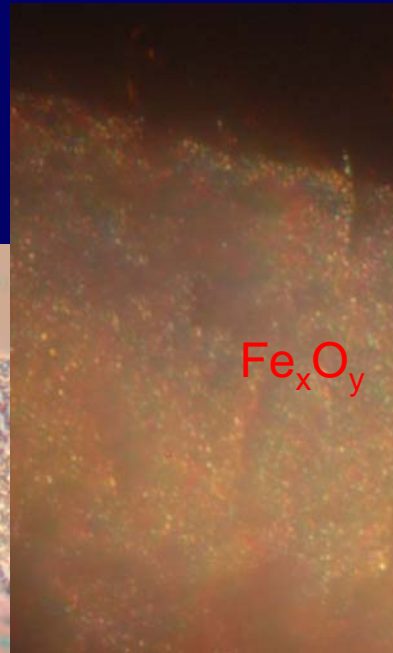
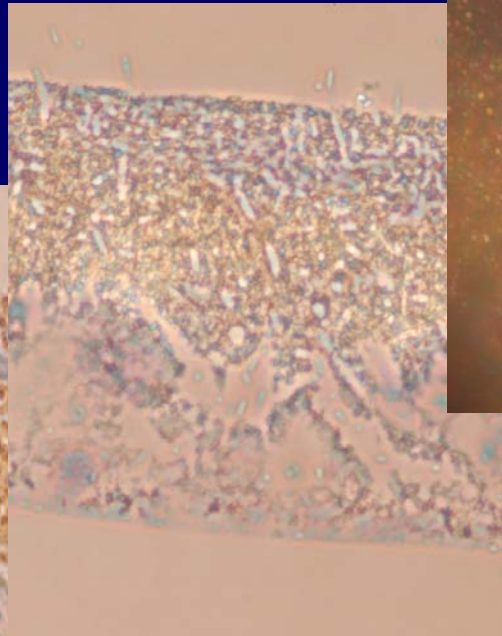
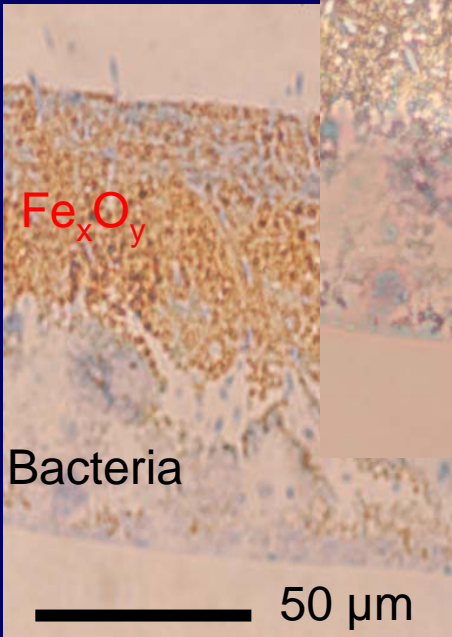


100 μm



Phase contrast

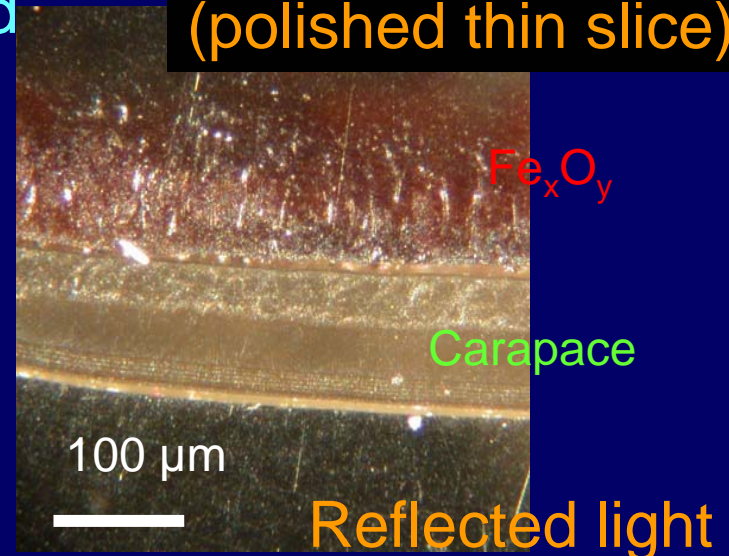
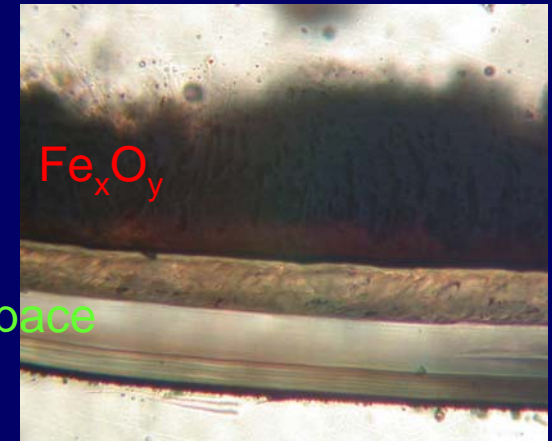
LM view



Bright field

1-2 μm -particles
of iron oxide

Polarized light



Le MEB à pression contrôlée ou « environnemental »

Environmental scanning electron microscope - ESEM

Modes :

Haut vide : 10^{-4} - 10^{-6} mbar

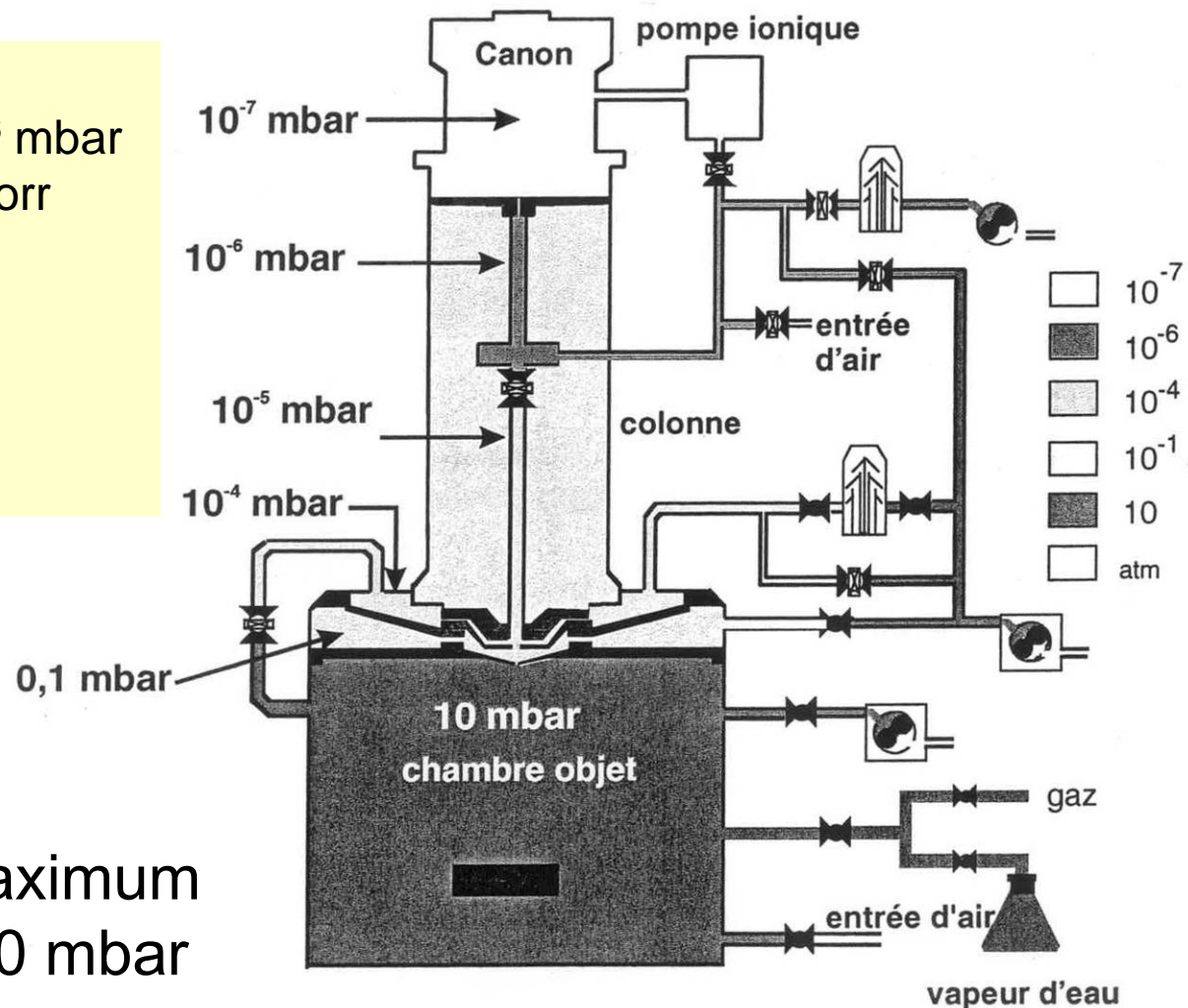
Bas vide : 0.1 – 5 Torr

Environnemental :

« wet sample »

: 3 - 15 Torr

+ contrôle de la température

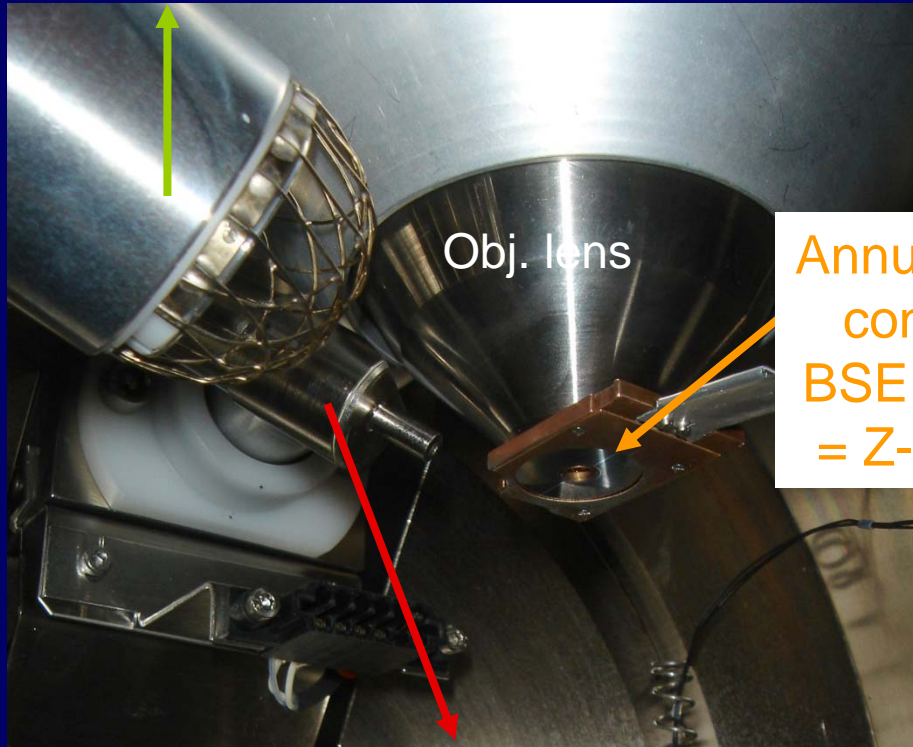


Maximum
50 mbar

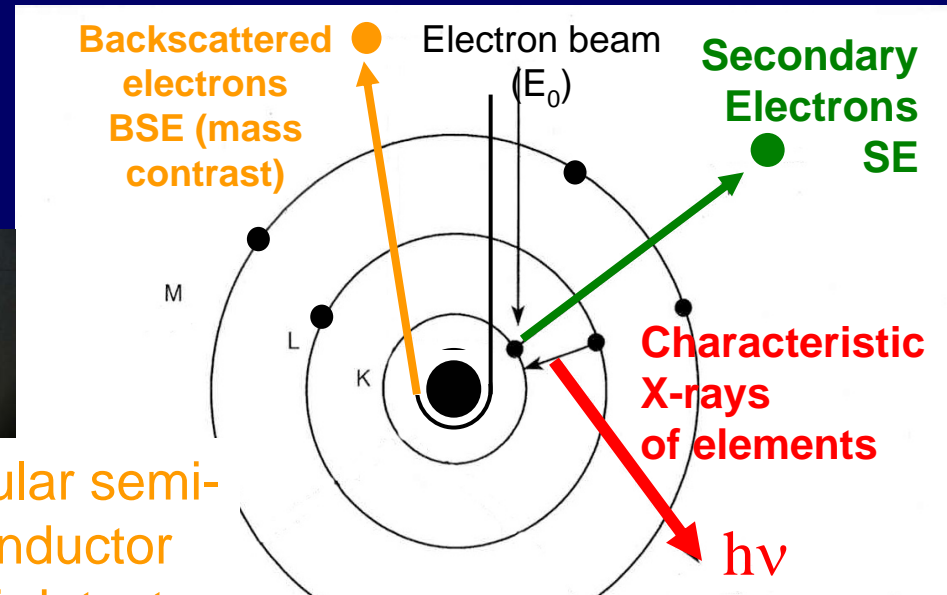
Figure 30 - Schéma d'un MEB à pression contrôlée montrant le différentiel de vide (document Electroscan)

FEI XL-30 ESEM-FEG in high vacuum mode

Everhard-Thornley detector
SE = relief contrast



Annular semi-conductor
BSE detector
= Z-contrast

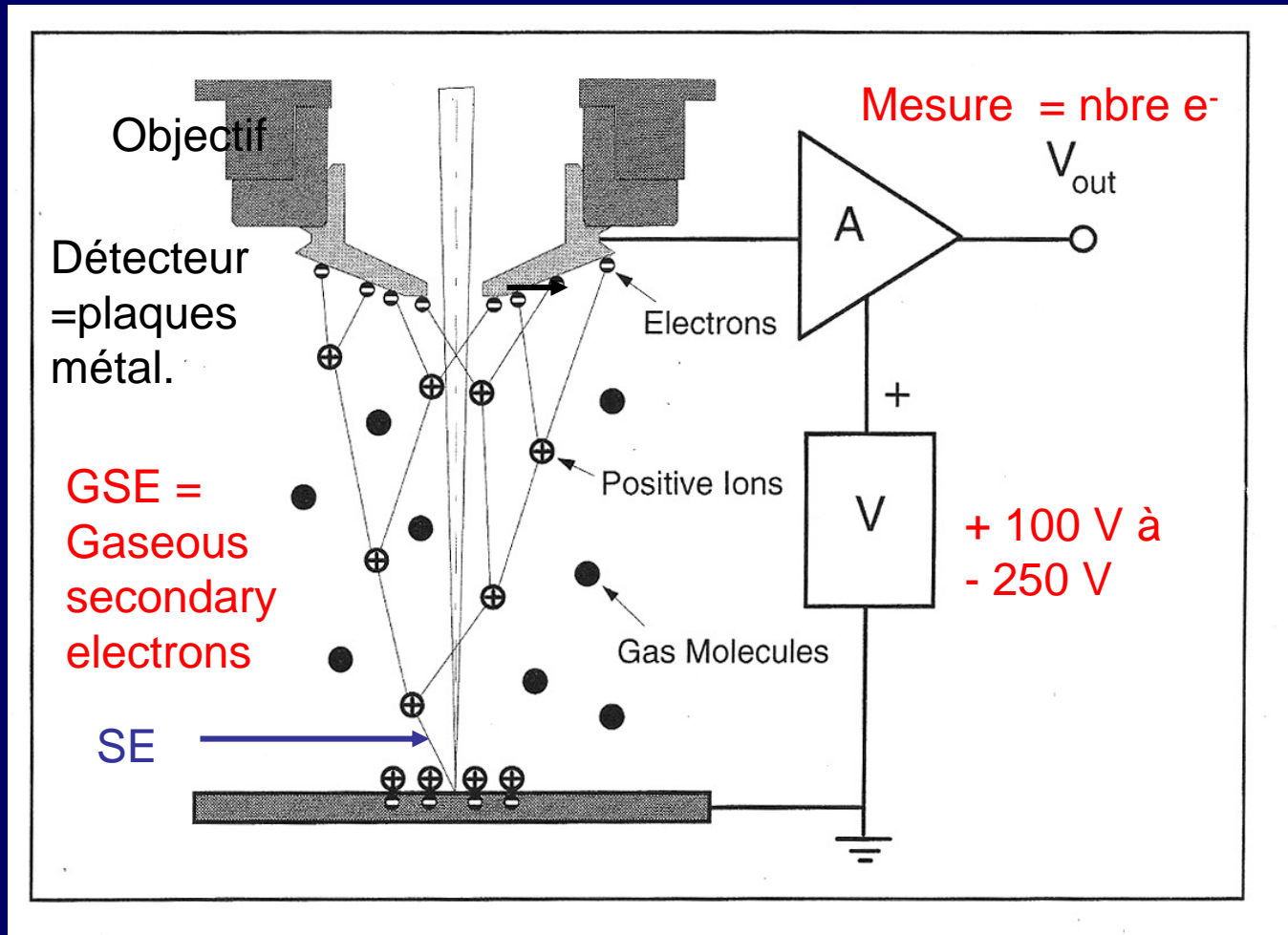


EDAX : Si(Li) detector with
SUTW for X-rays from light elements



Détecteurs d'électrons secondaires en bas vide ou en conditions environnementales

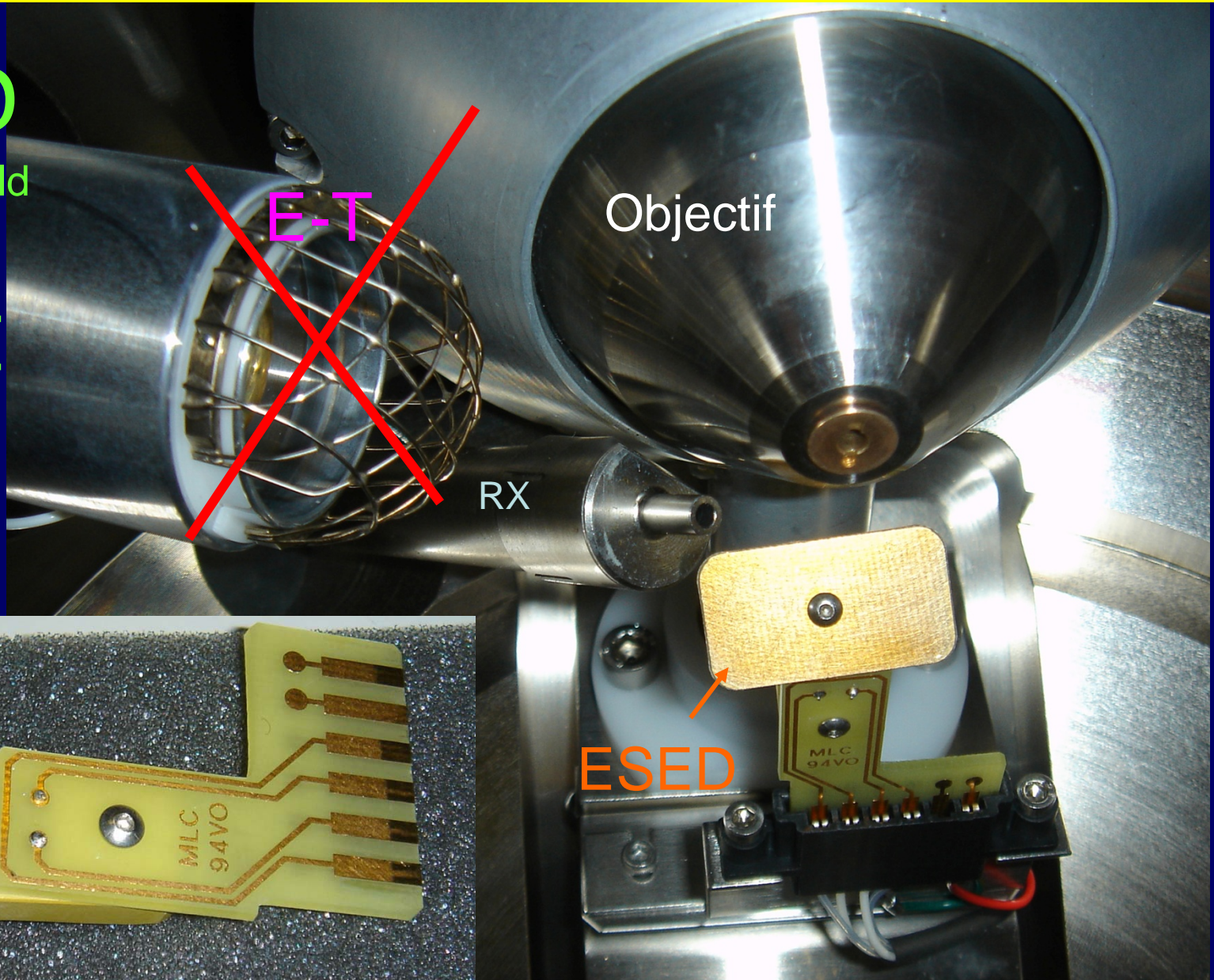
GSED = « Gaseous secondary electron detector »



Neutralisation des charges (-) sur l'éch. par les ions (+)
BSE moins amplifiés car trop énergétiques

Le MEB à pression contrôlée (LV-SEM) - max 1.5 Torr

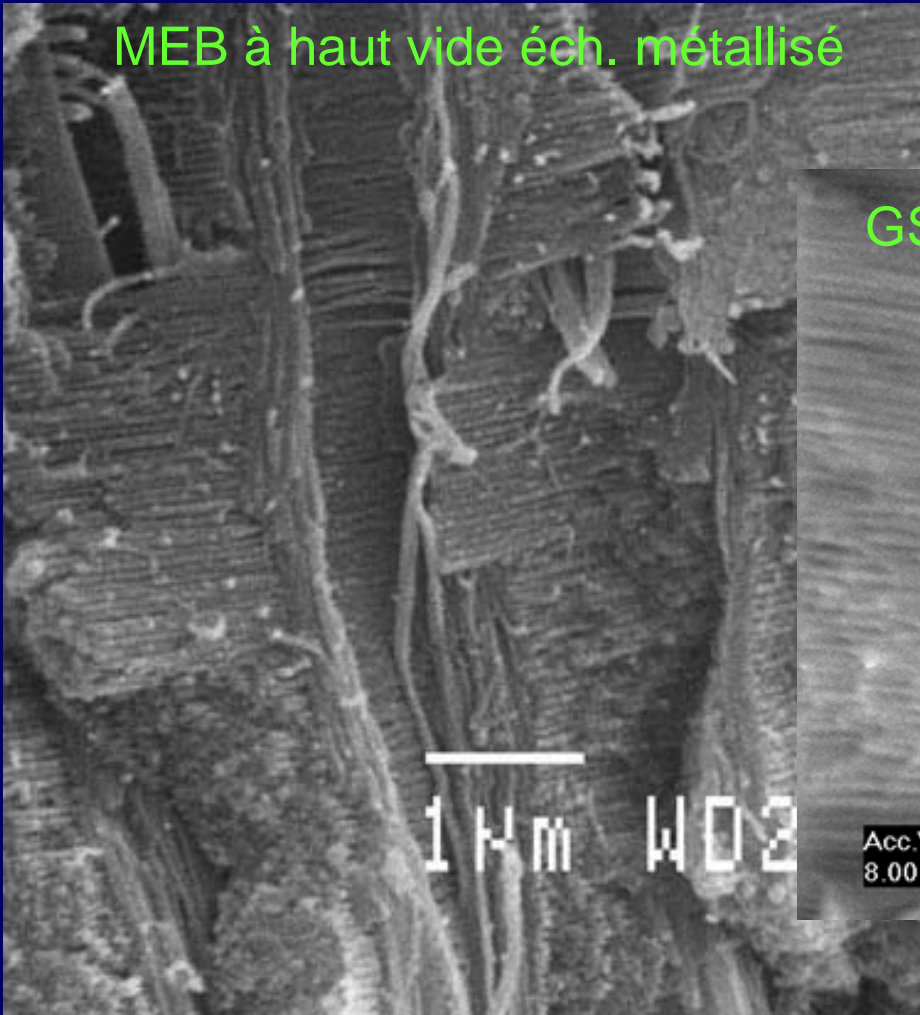
LFD
Large field
+
BSE



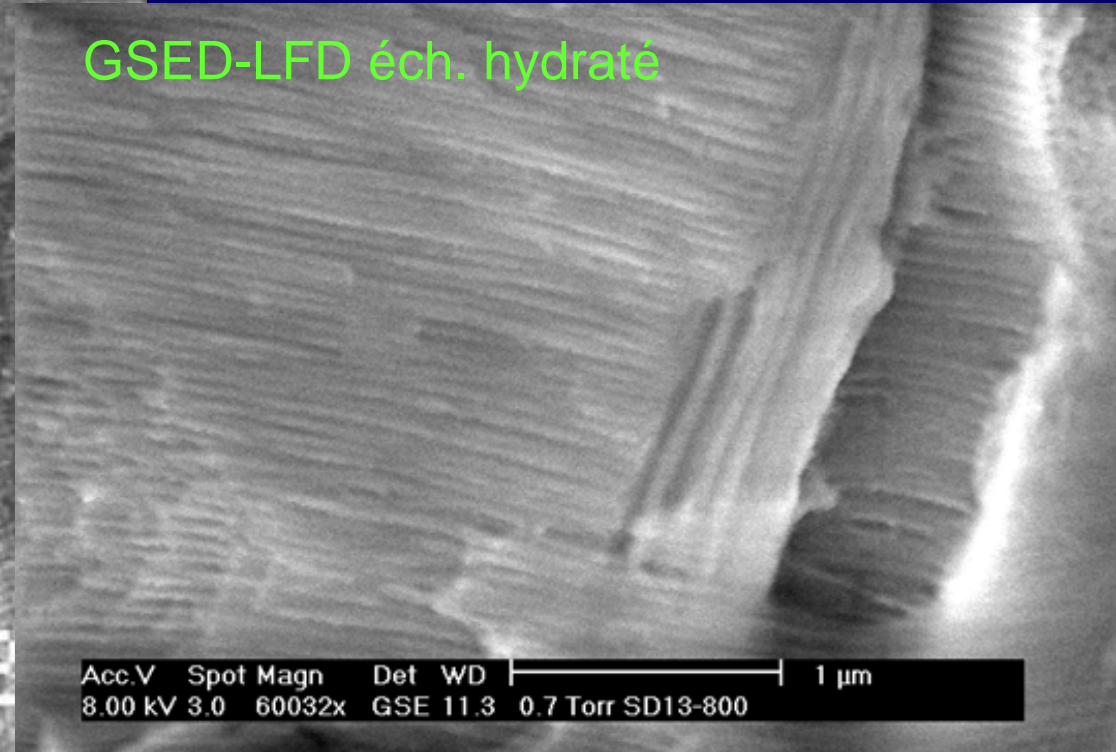
3. Le MEB à pression contrôlée (LV-SEM, ESEM)

Exemple: détail des fibres
(50 -100 nm)
dans la carapace d'un crabe

MEB à haut vide éch. métallisé

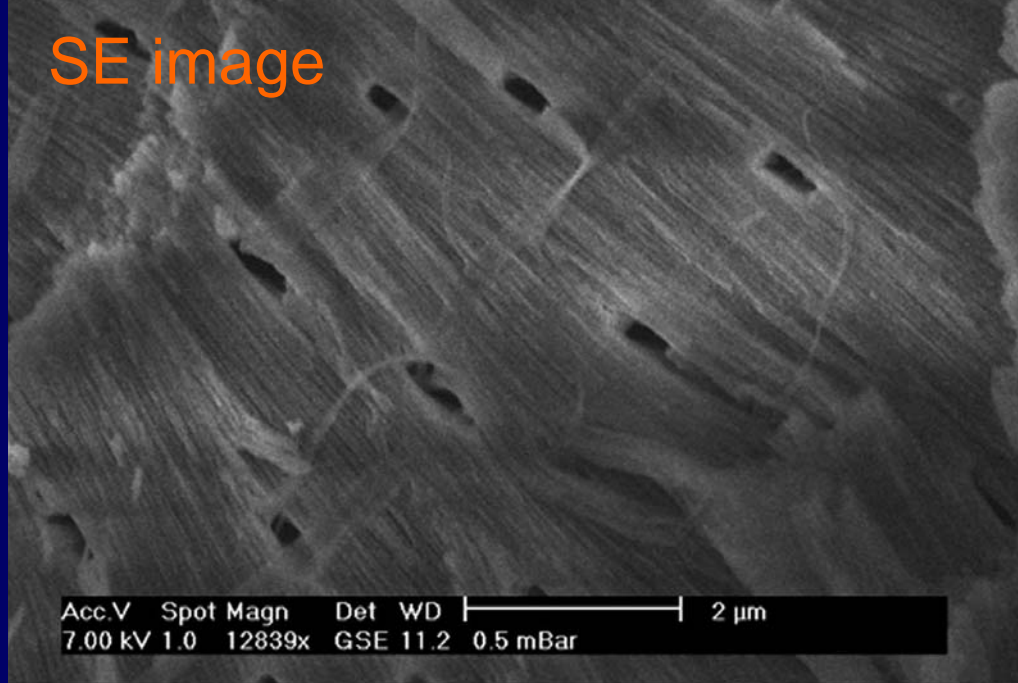


GSED-LFD éch. hydraté



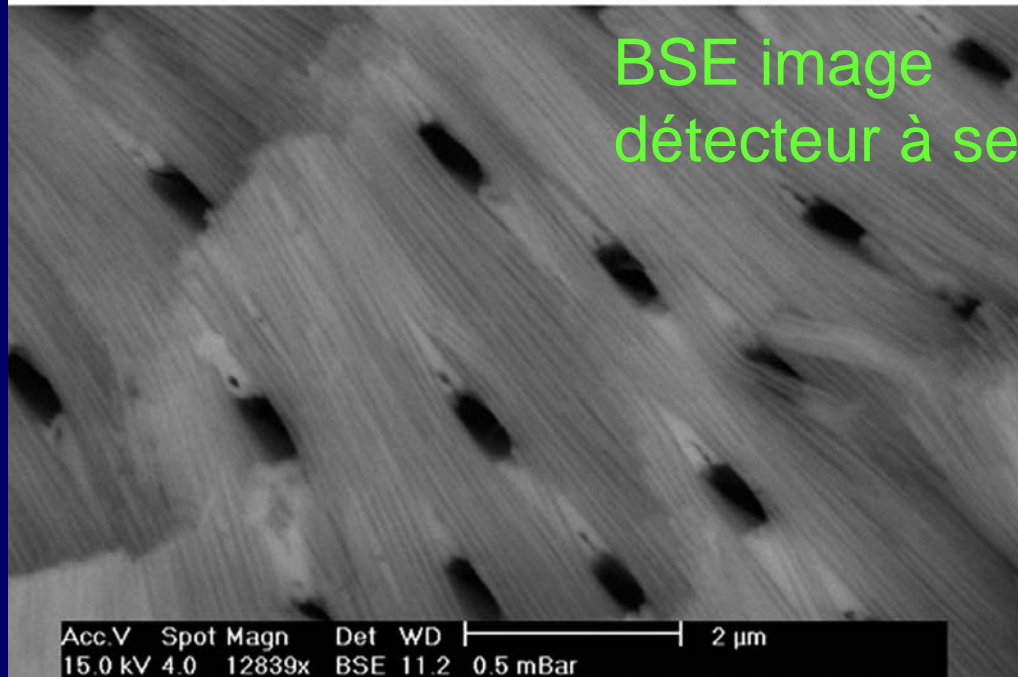
GSED LFD image

SE image



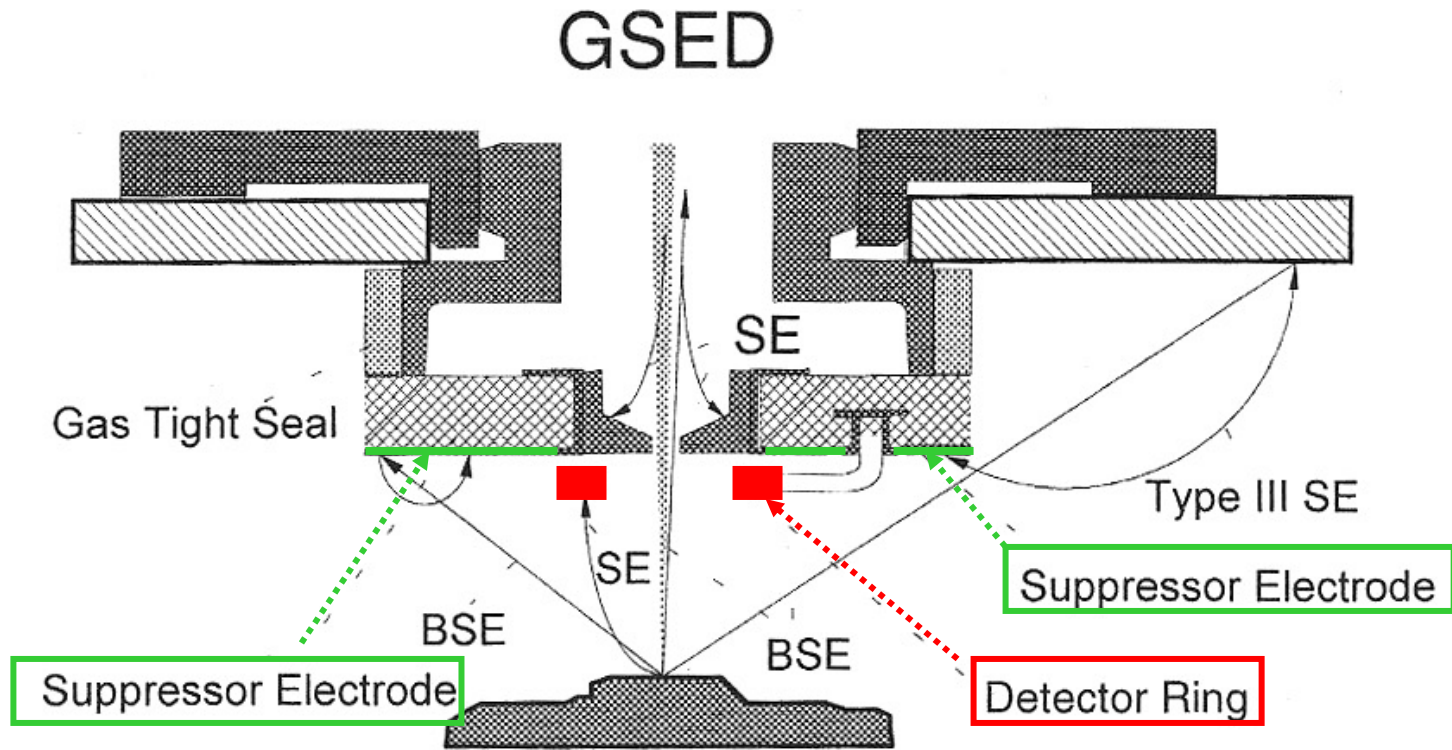
Fibres
50-100 nm
de diamètre

BSE image
détecteur à semiconducteur



CaCO₃ entre
les fibres

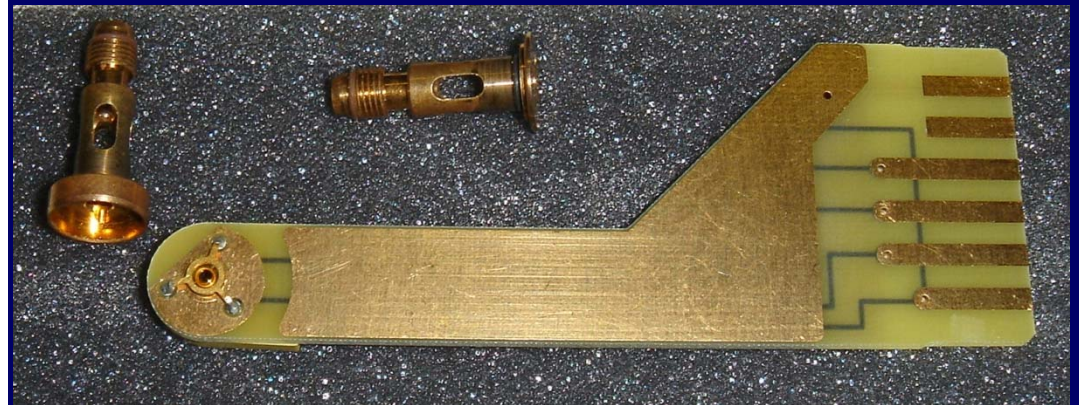
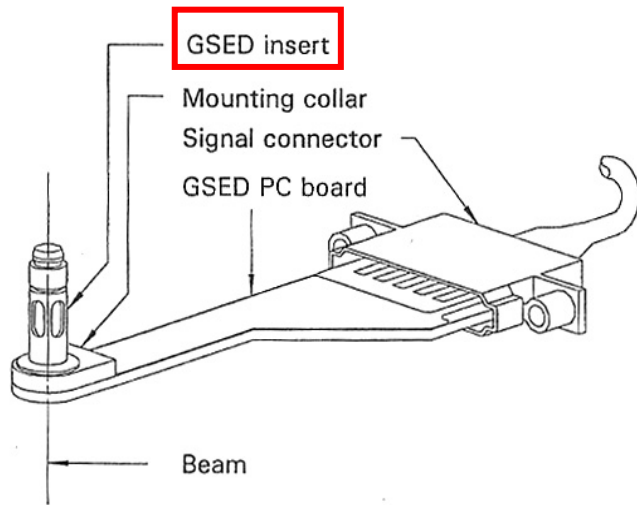
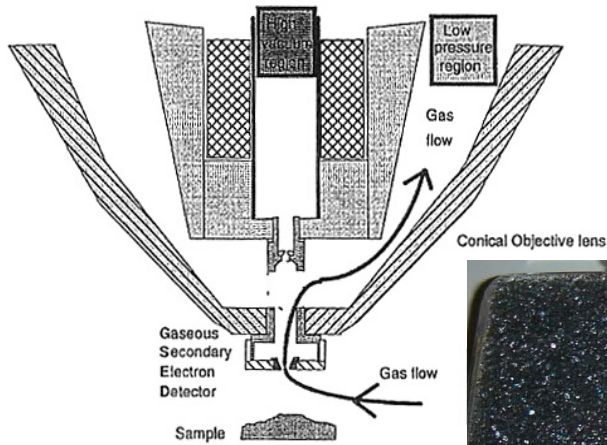
GSED = « Gaseous secondary electron detector »



Améliore la détection des SE et les distingue des BSE (anneau de détection plus petit) et des SE-III (créés par les BSE) grâce à un suppresseur

GSED

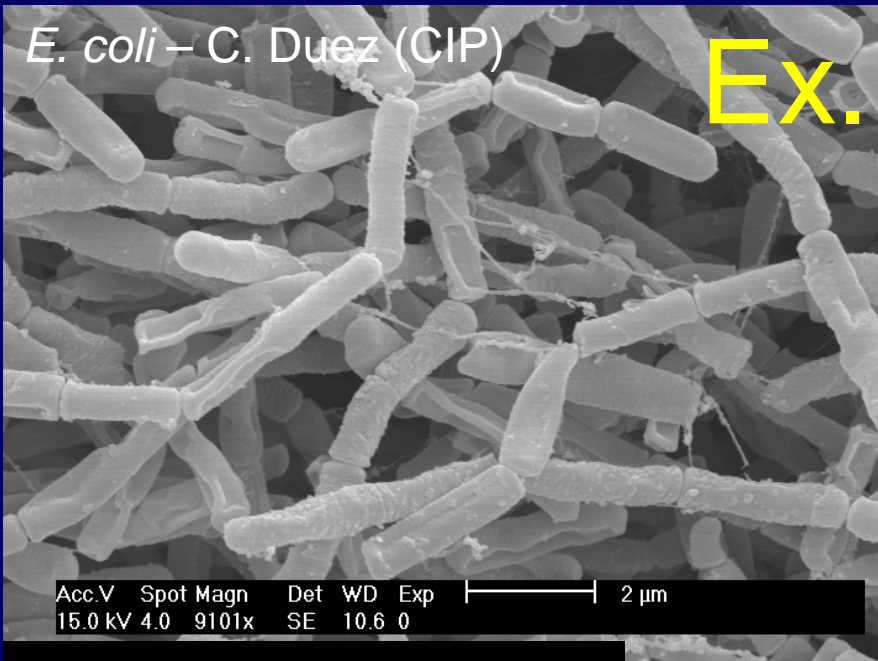
Face supérieure



Face inférieure

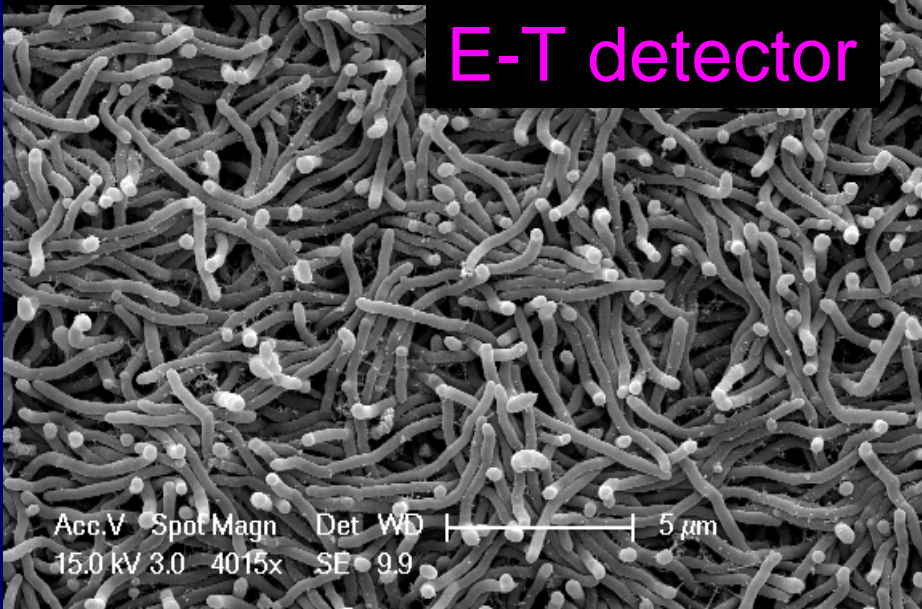
Ex.: Bacterial biofilms

E. coli – C. Duez (CIP)



High vacuum mode

E-T detector



GSED

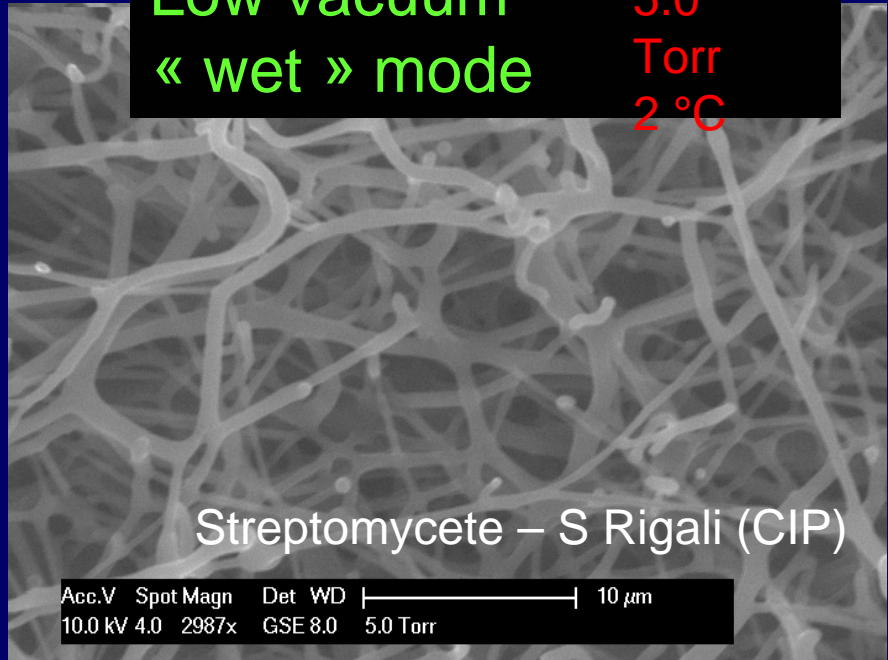
Low vacuum

« wet » mode

5.0

Torr

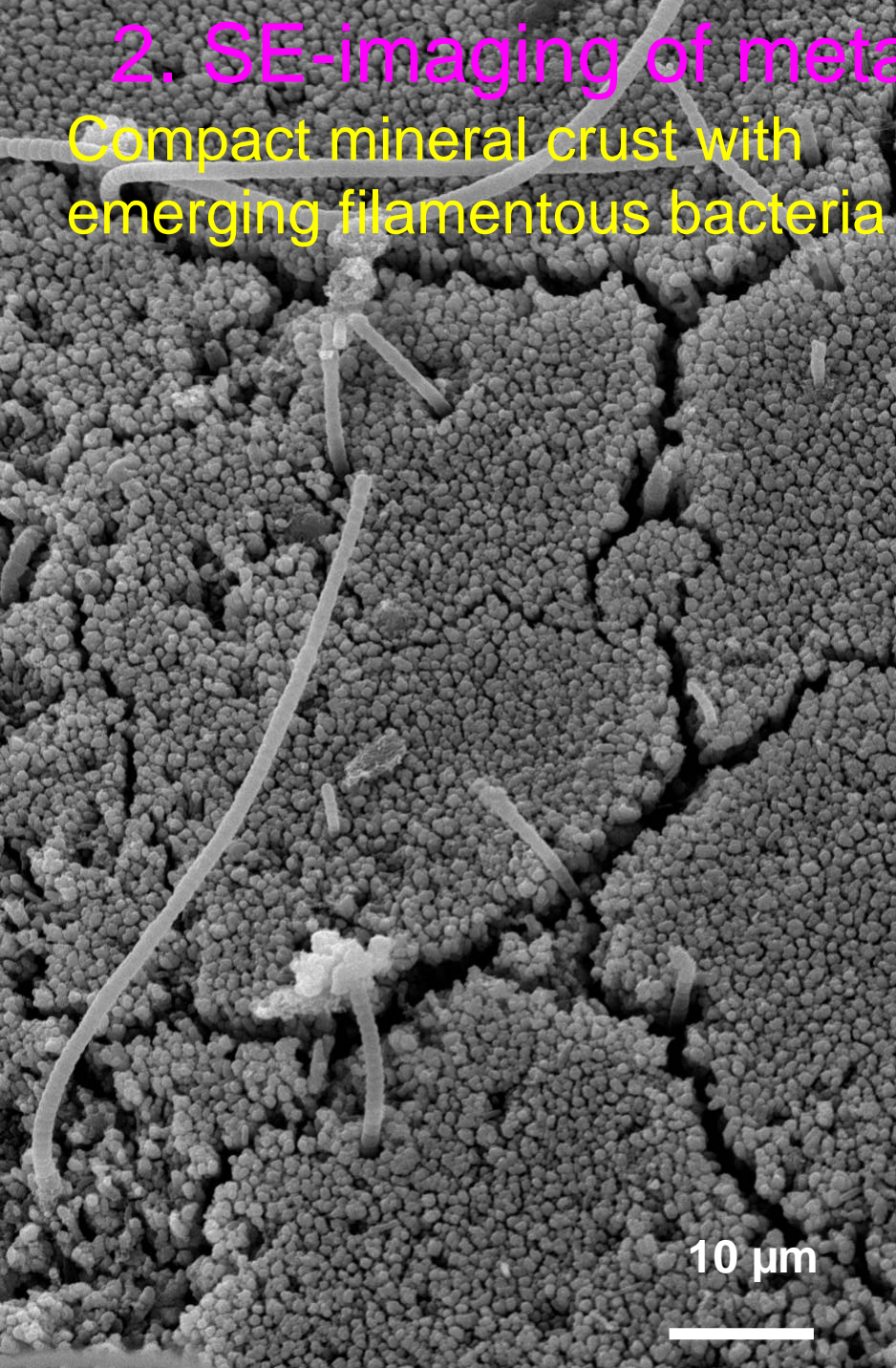
2 °C



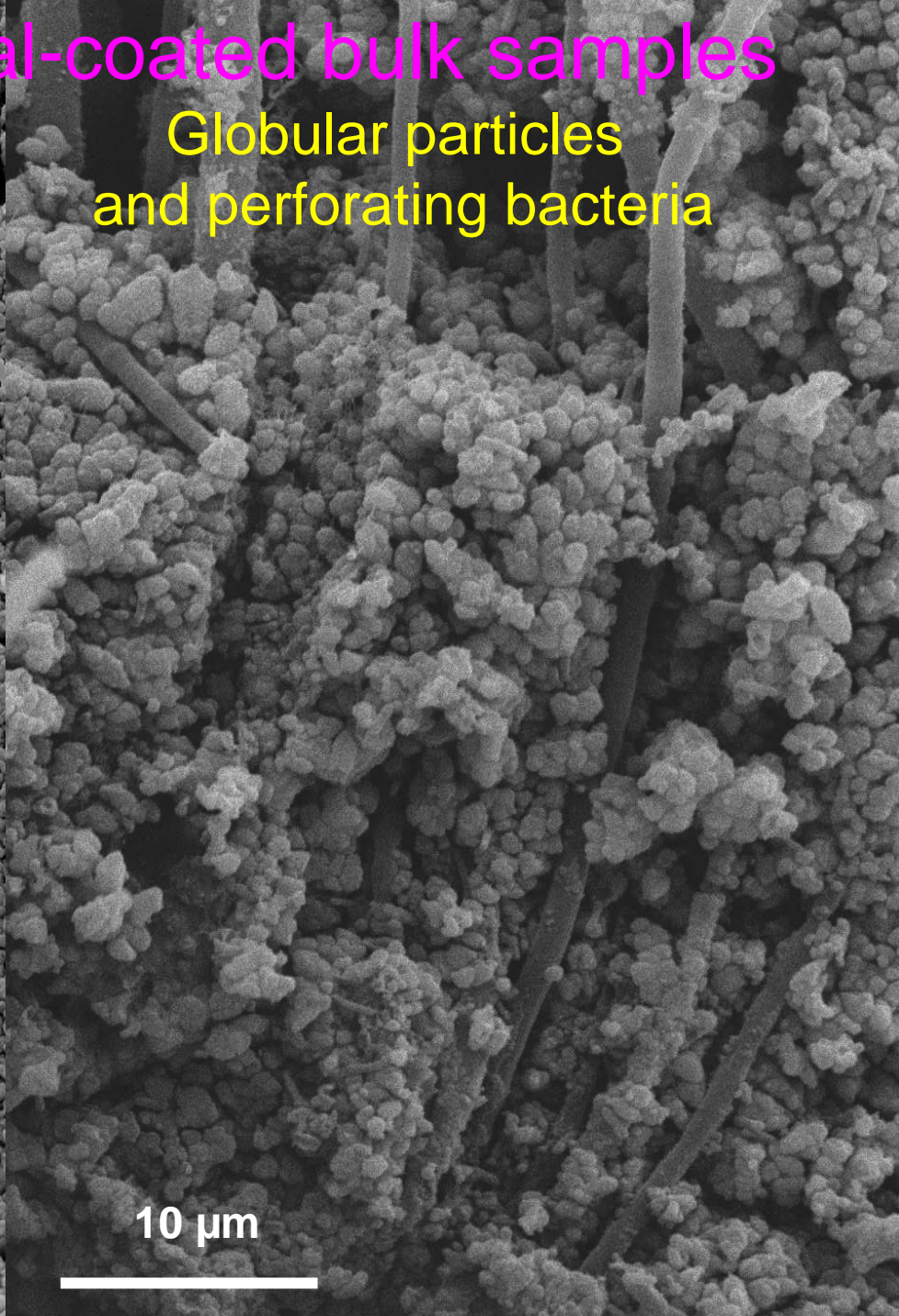
Streptomycete – *S Rigali* (CIP)

2. SE-imaging of metal-coated bulk samples

Compact mineral crust with emerging filamentous bacteria



Globular particles and perforating bacteria



Bactéries filamenteuses and bâtonnets

Epsilon-proteobacteria (Polz & Cavanaugh, 1995)
Chimiosynthétique (Wirsen et al., 1993)
Sulfo-oxidantes (Polz & Cavanaugh, 1995)

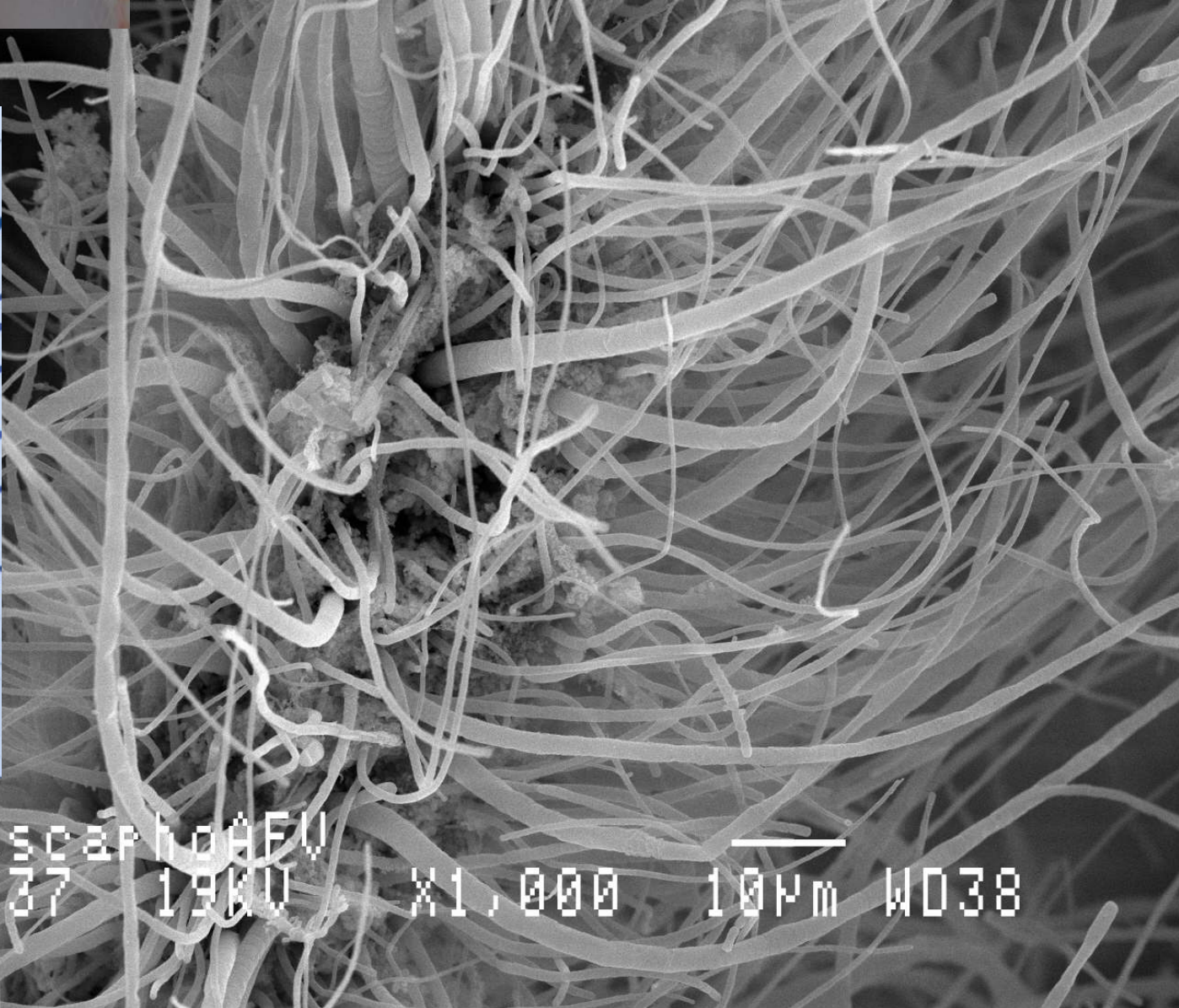
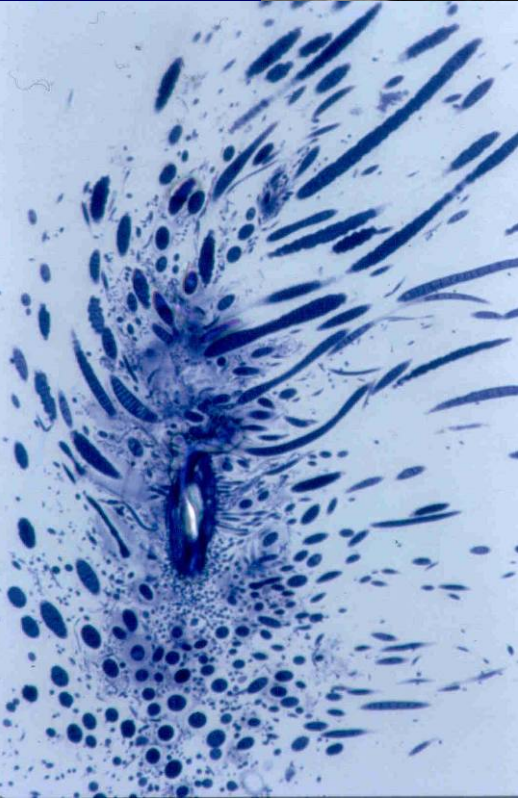
Surface interne
de la paroi de la
cavité branchiale
(branchiostegite)

Dépendance trophique ($\delta^{13}\text{C}$, acides gras) de la crevette
(Gebruk et al. 1993, 2000; Rieley et al. 1999; Pond et al. 1997, 2000)
et transferts nutritionnels bactéries-hôte (radiotraceurs)
(Ponsard et al. 2011, en préparation)

10 μm

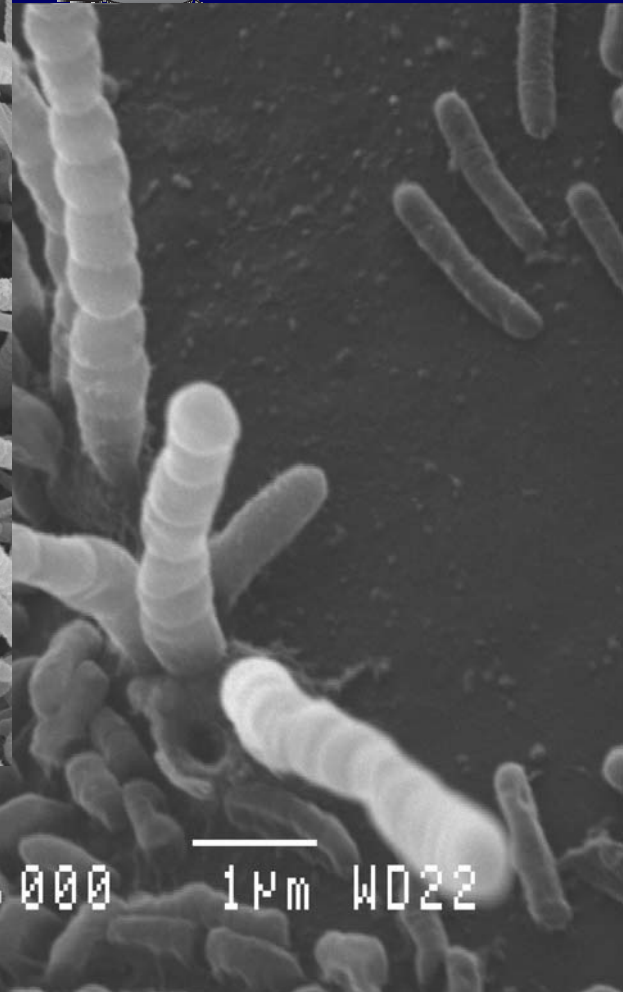
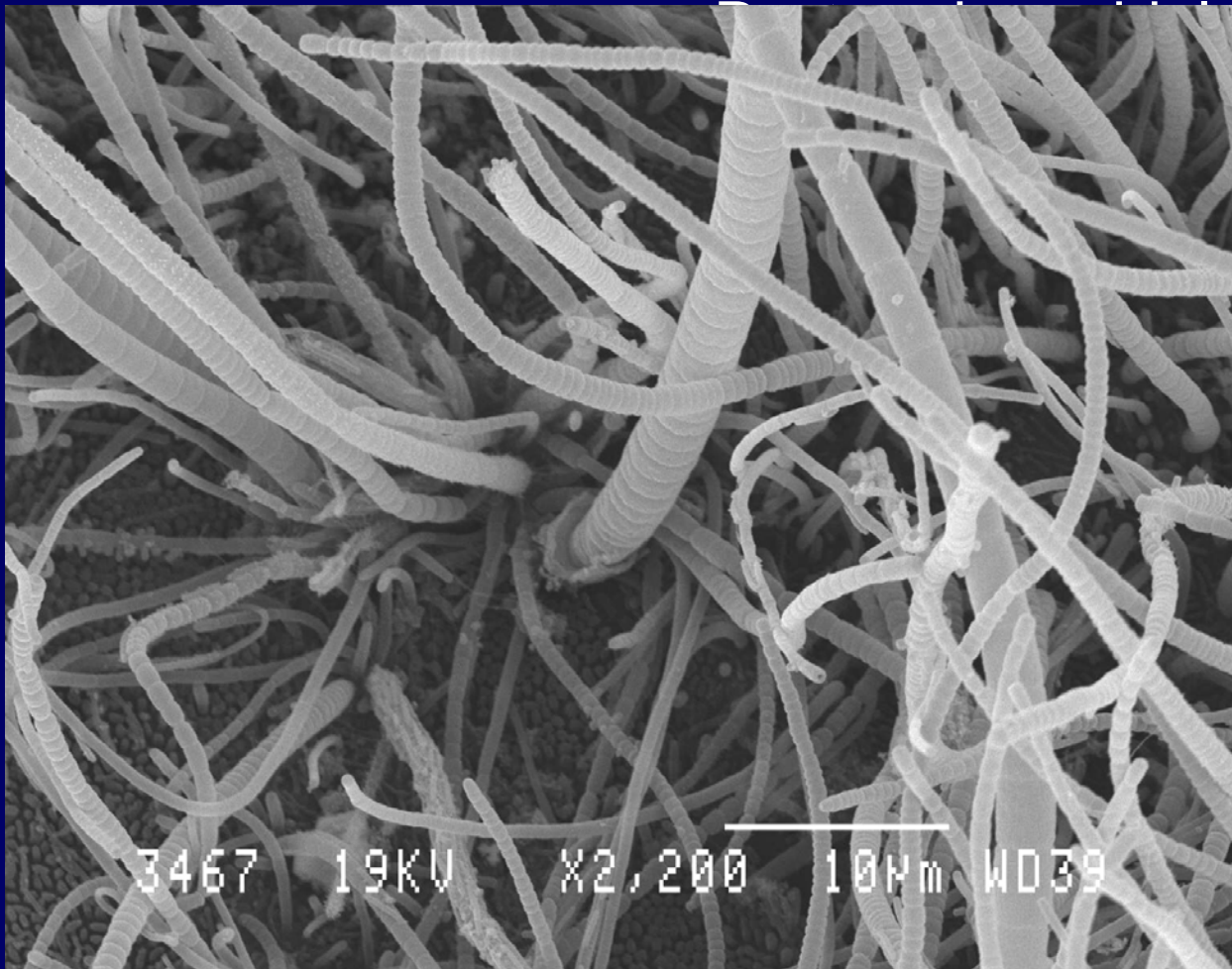
X1,900 10 μm WD39

Soies bactériophores des scaphognatites



R10scaphoAEU
7137 19KV X1,000 10µm WD38

Distribution de la communauté bactérienne

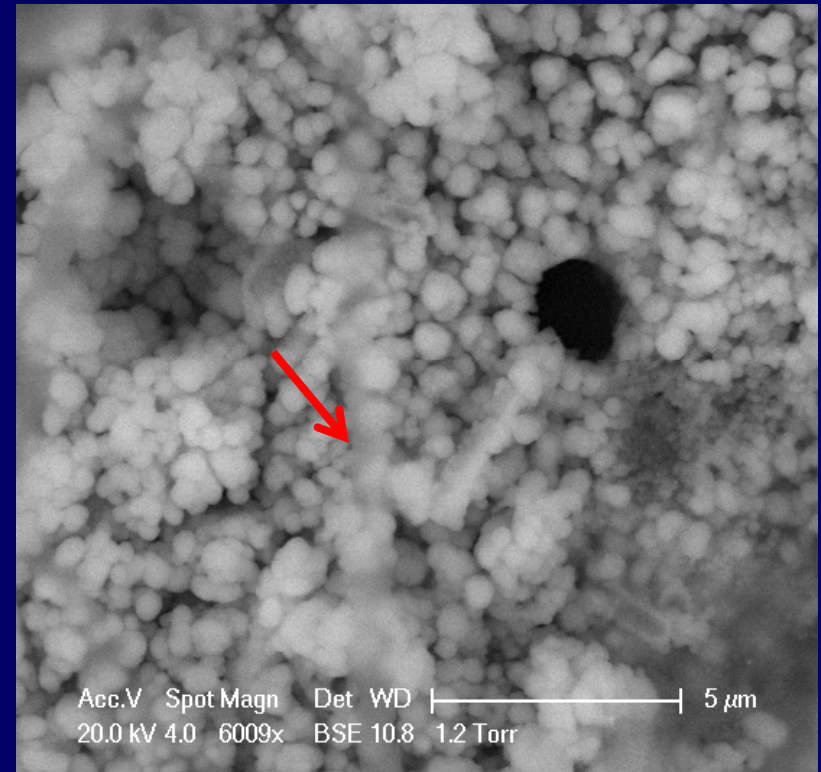
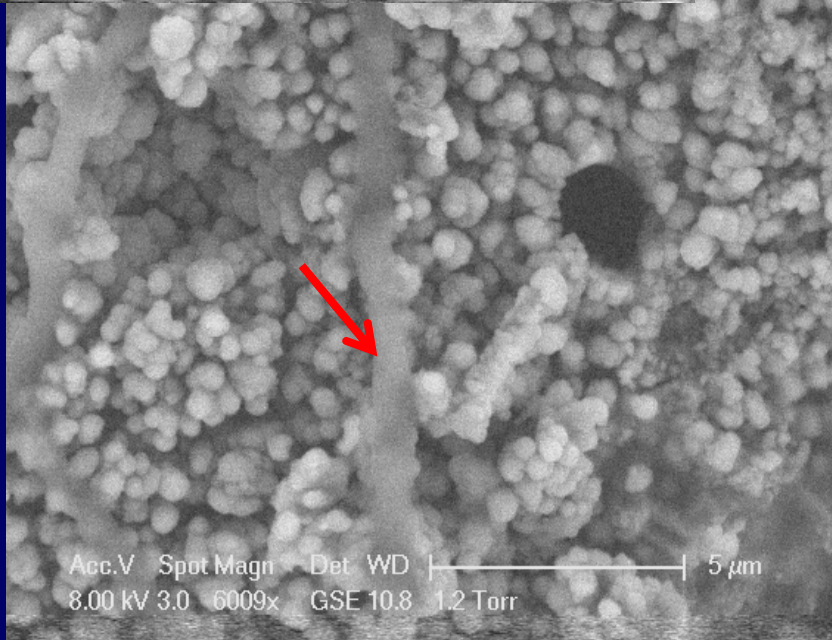
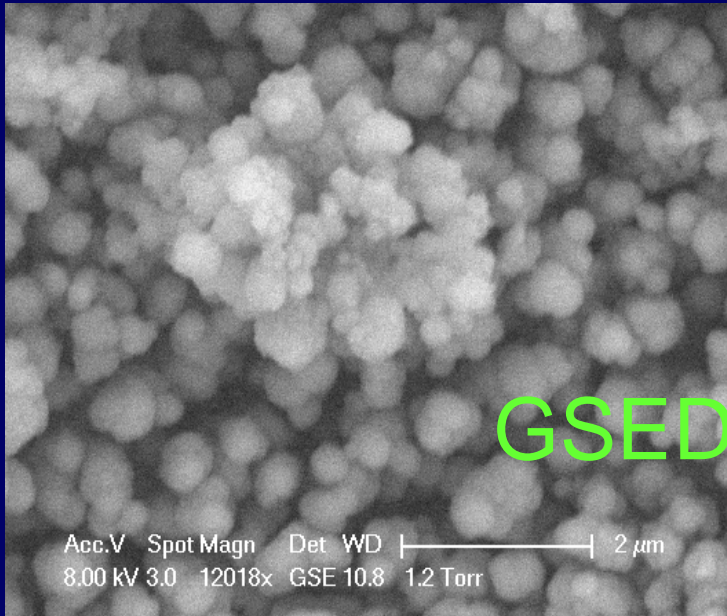


Various
morphotypes

3. MEB à bas vide LV-SEM

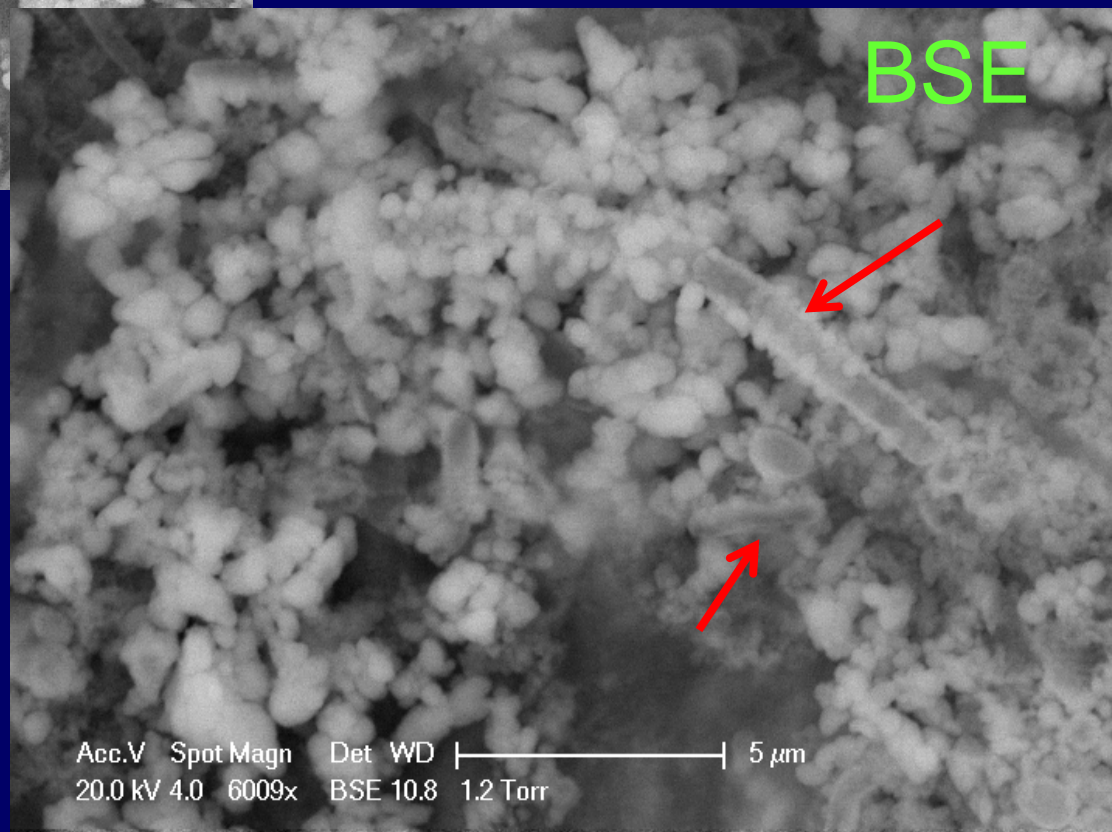
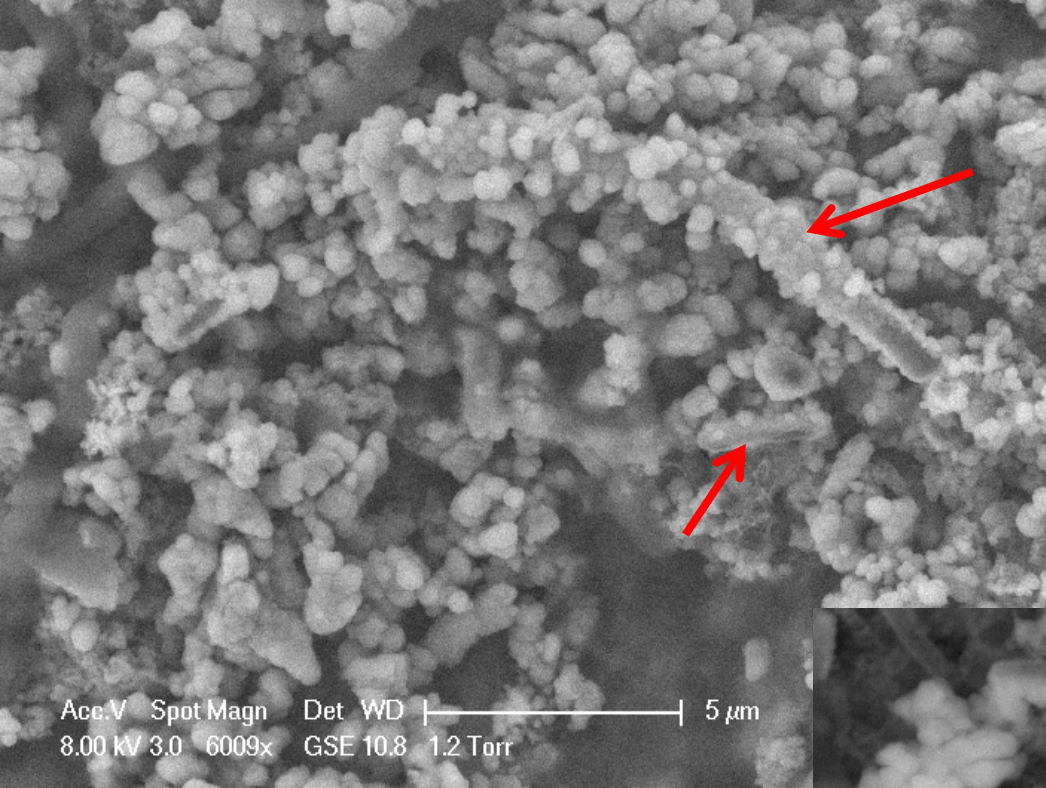
GSED-LFD

BSE



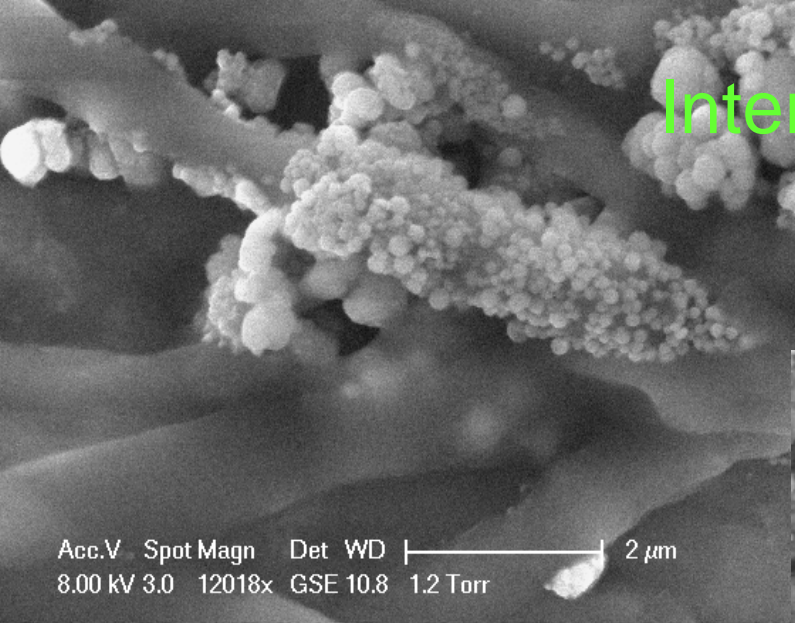
LV-SEM

Bactéries filamenteuses et bâtonnets avec des minéraux fixés sur leurs parois



GSED-LFD

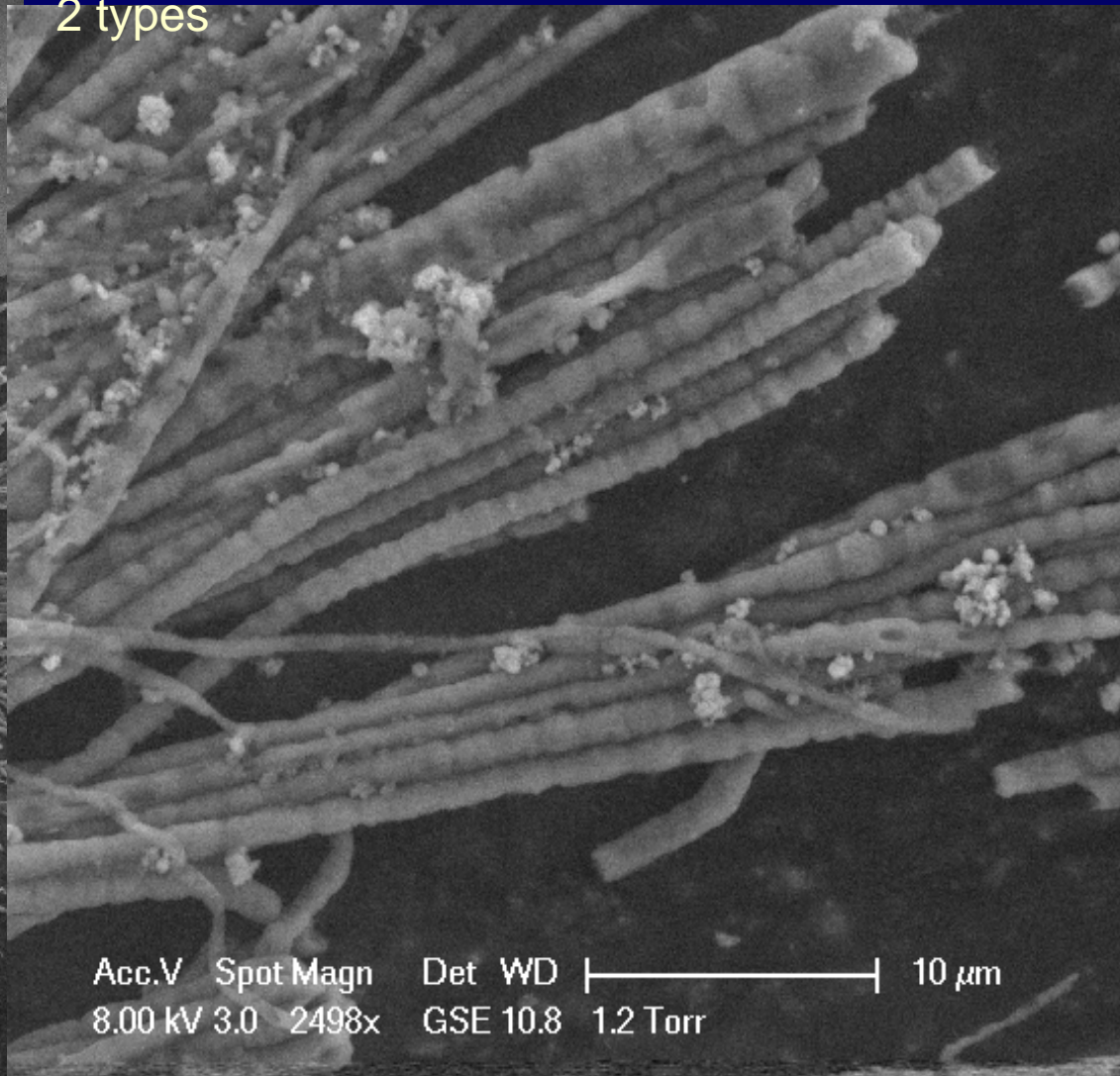
Interactions bactéries-oxydes de fer



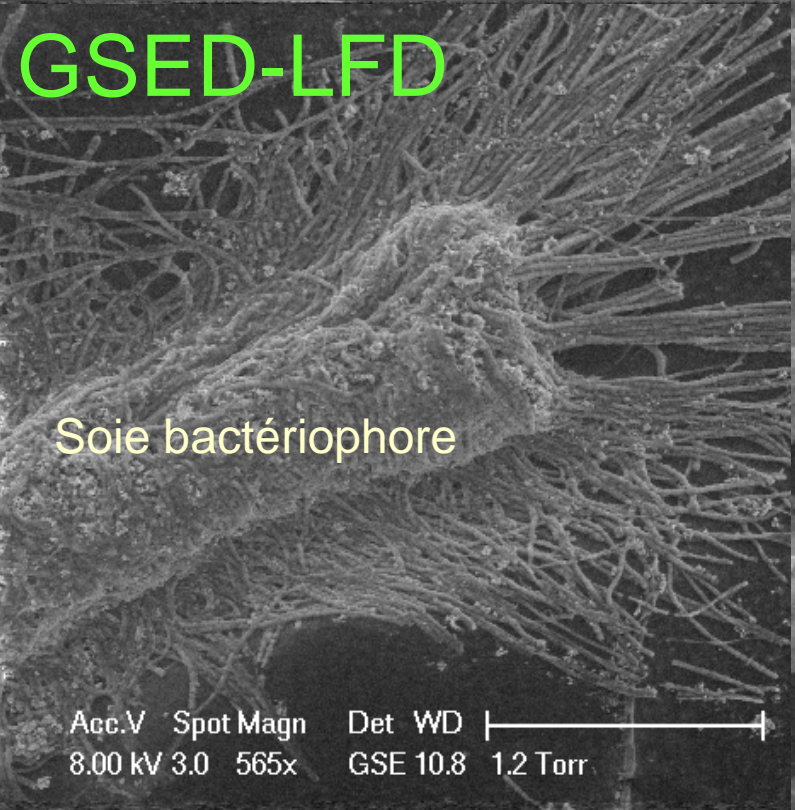
Acc.V Spot Magn Det WD |-----| 2 μ m
8.00 kV 3.0 12018x GSE 10.8 1.2 Torr

Bactéries filamenteuses
2 types

LV-SEM



Acc.V Spot Magn Det WD |-----| 10 μ m
8.00 kV 3.0 2498x GSE 10.8 1.2 Torr

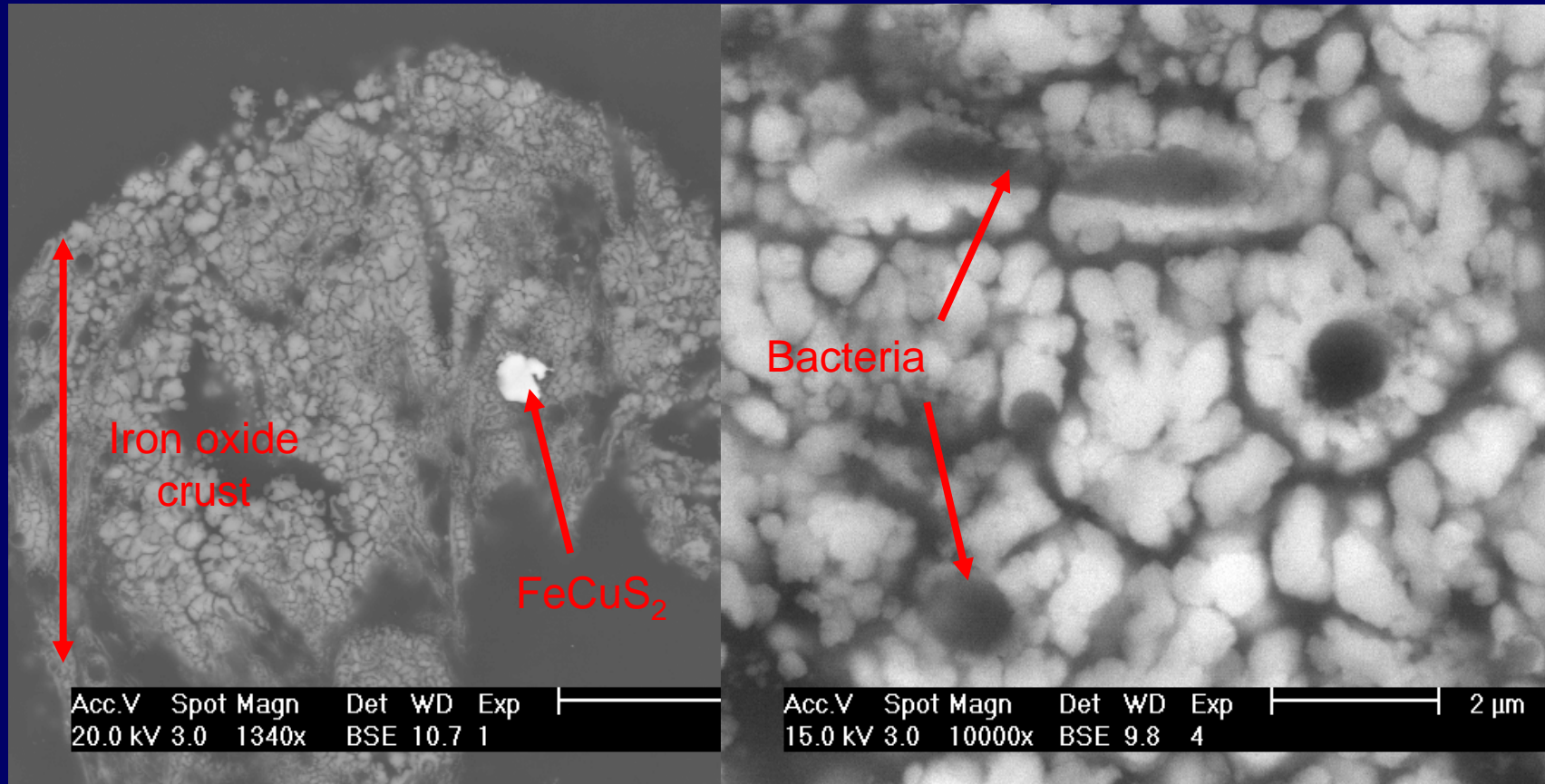


GSED-LFD

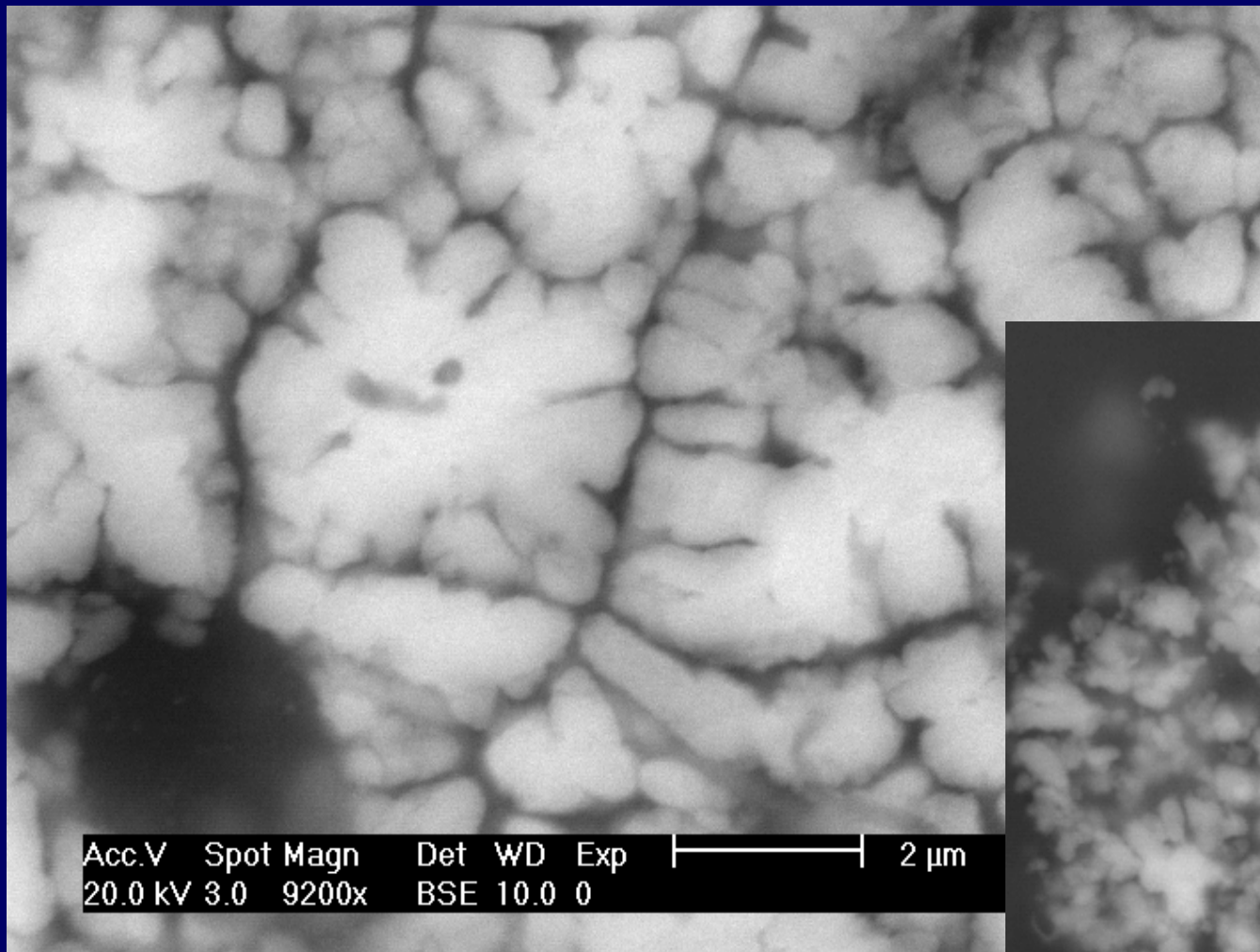
Soie bactériophage

Acc.V Spot Magn Det WD |-----|
8.00 kV 3.0 565x GSE 10.8 1.2 Torr

4. BSE-imaging of polished thin slices

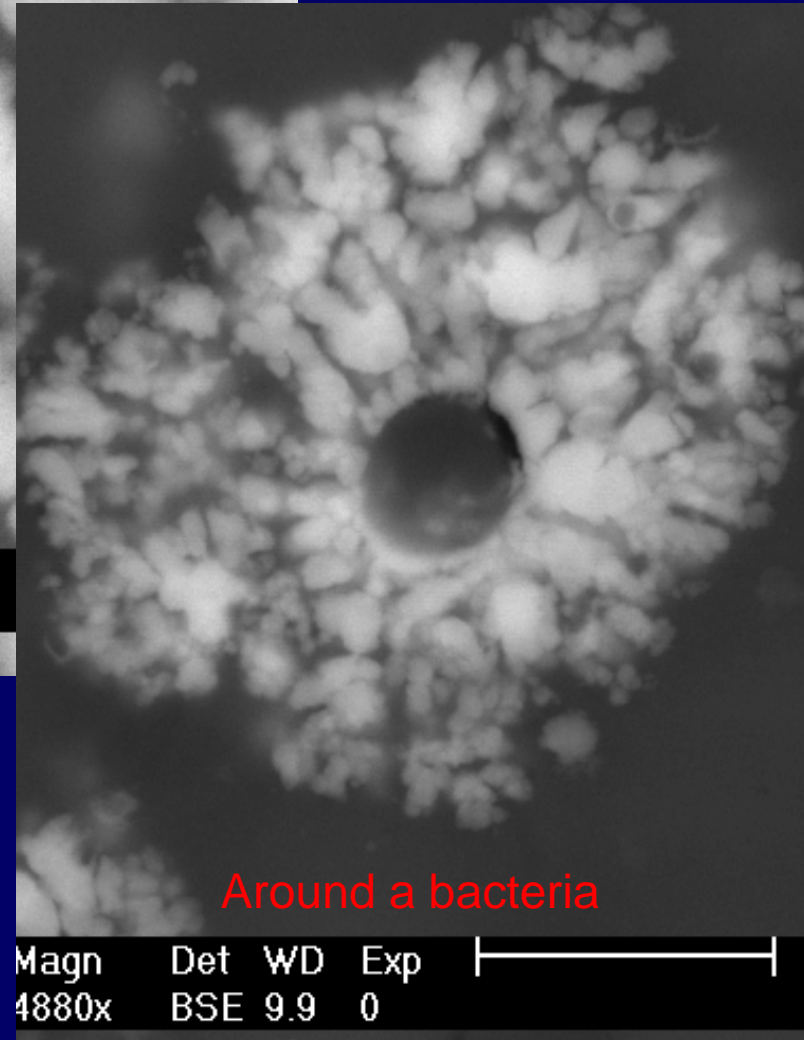


Petal-like section shapes in the (multi)globular particles sheathing bacteria



BSE-images

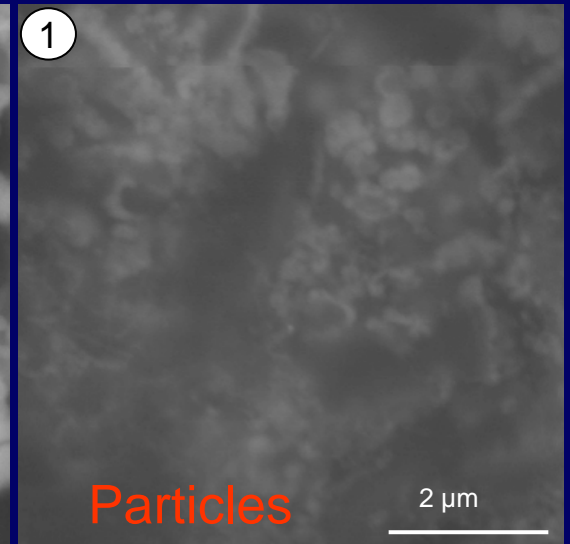
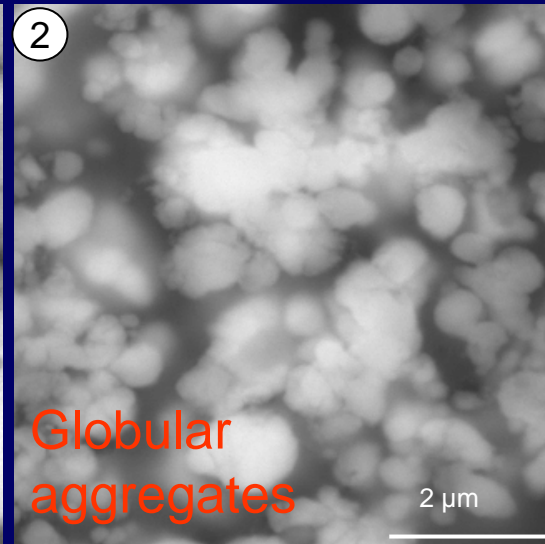
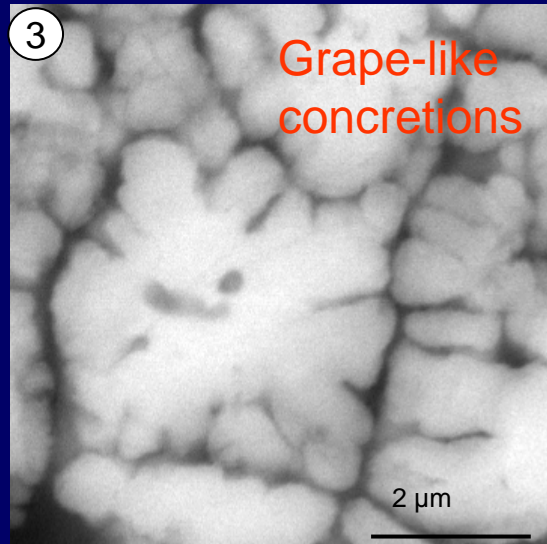
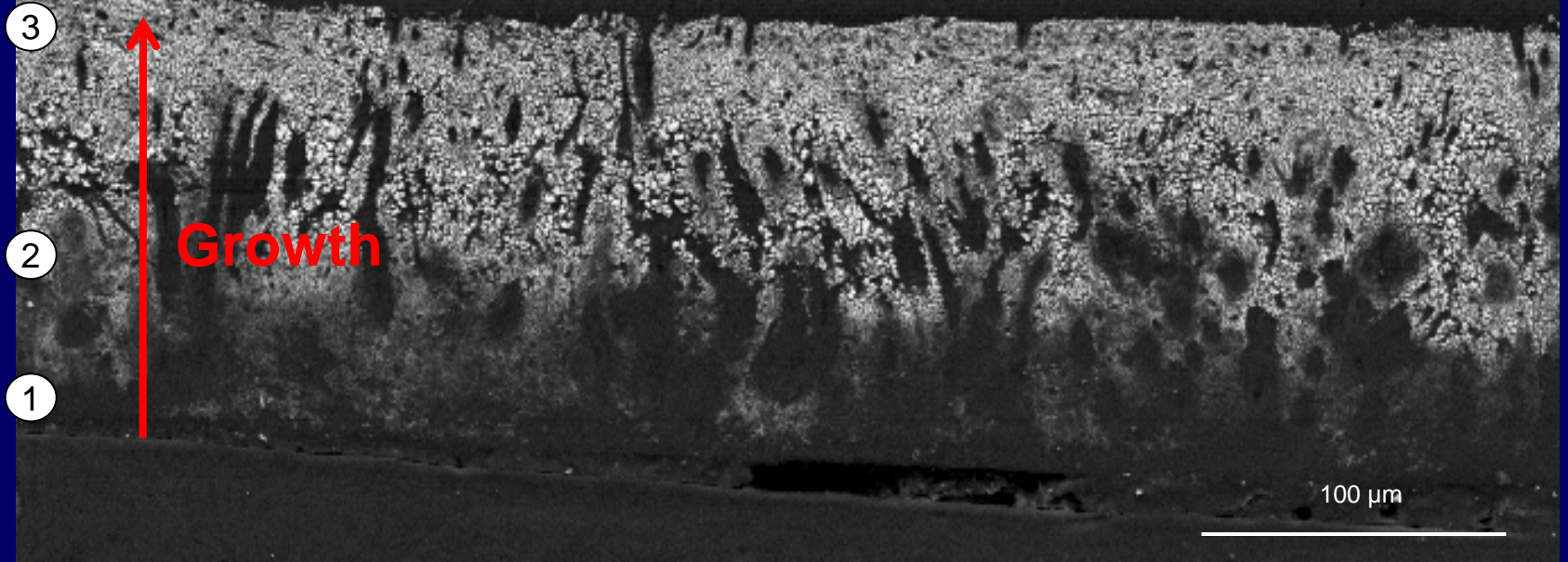
Details of joining and isolated
petal-like particles



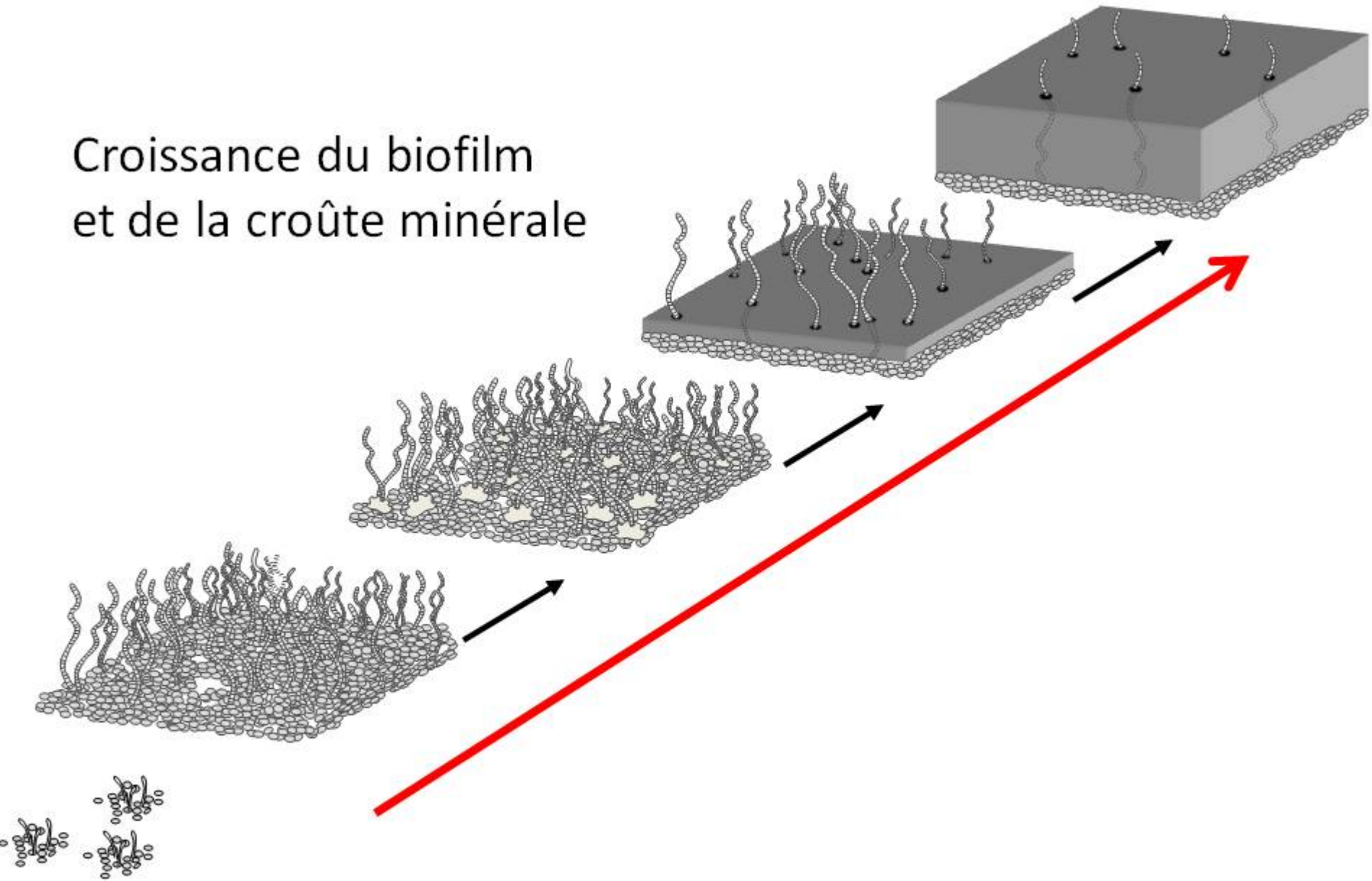
Around a bacteria

Three layered iron oxide crust

BSE



Croissance du biofilm et de la croûte minérale

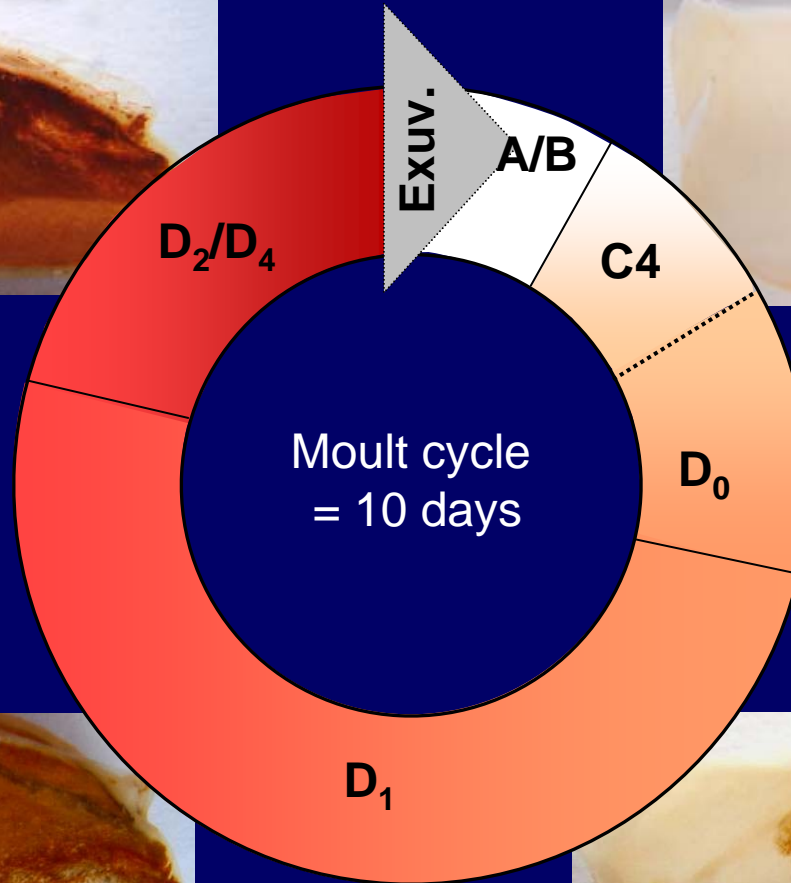




Premoult shrimp with heavy Iron oxide deposits



Newly moulted cuticle free of epibionts and mineral deposits

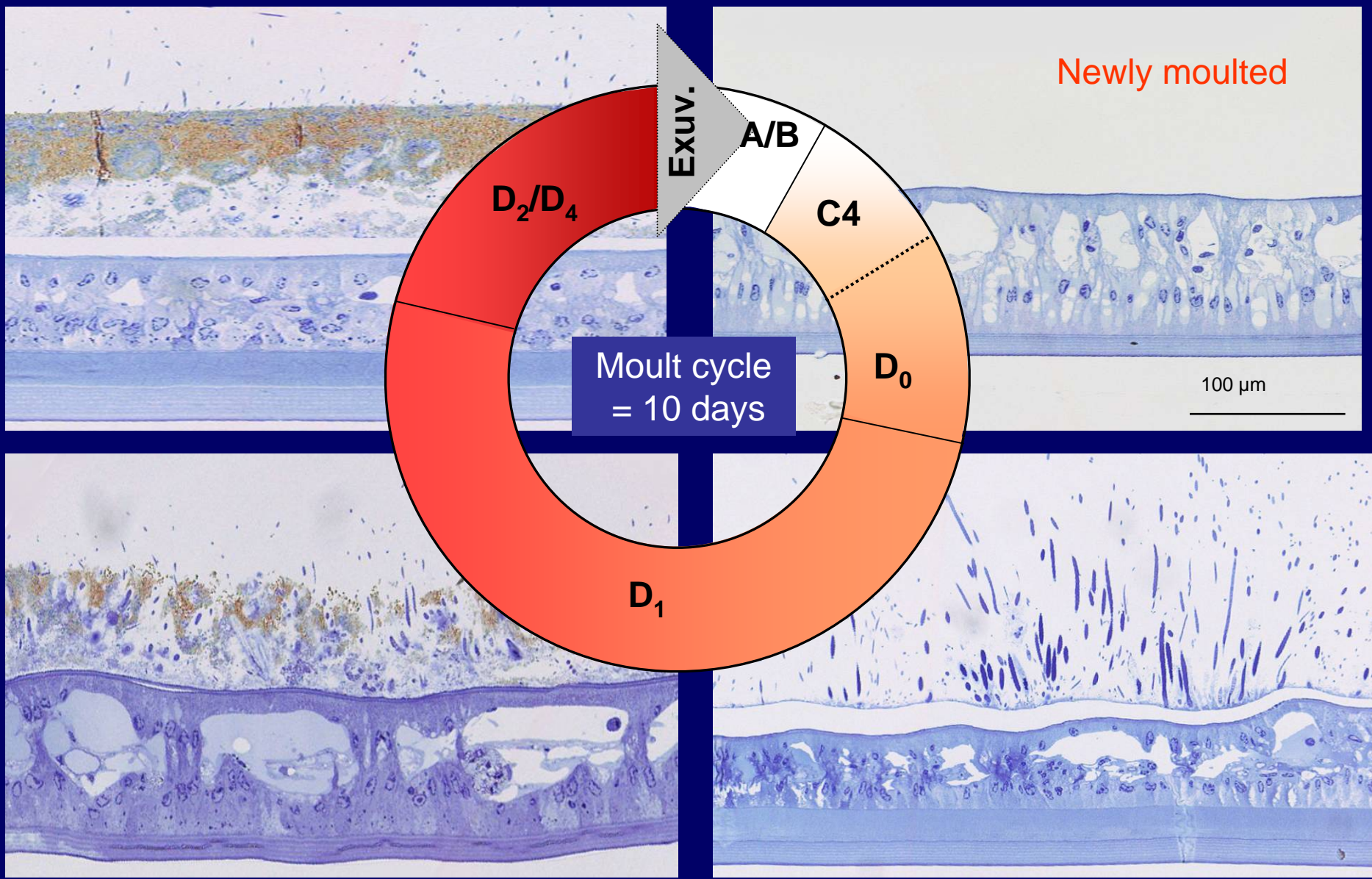


Developing bacterial community

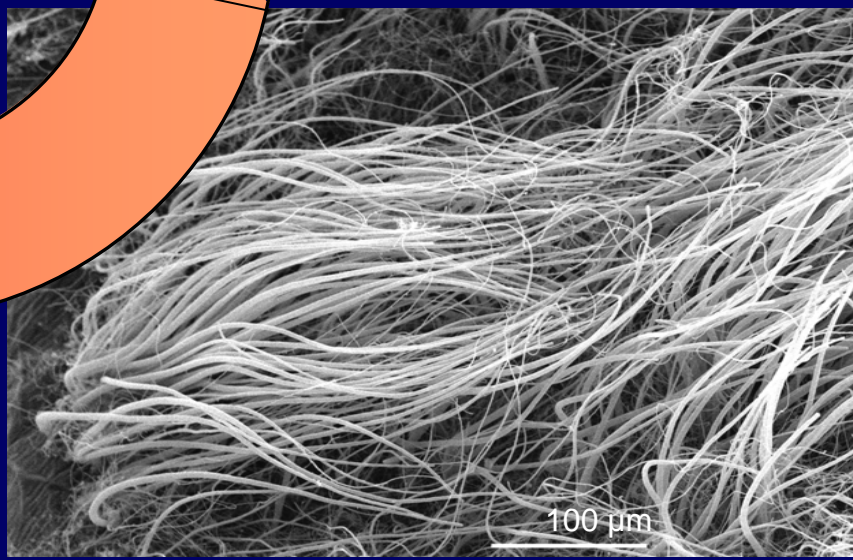
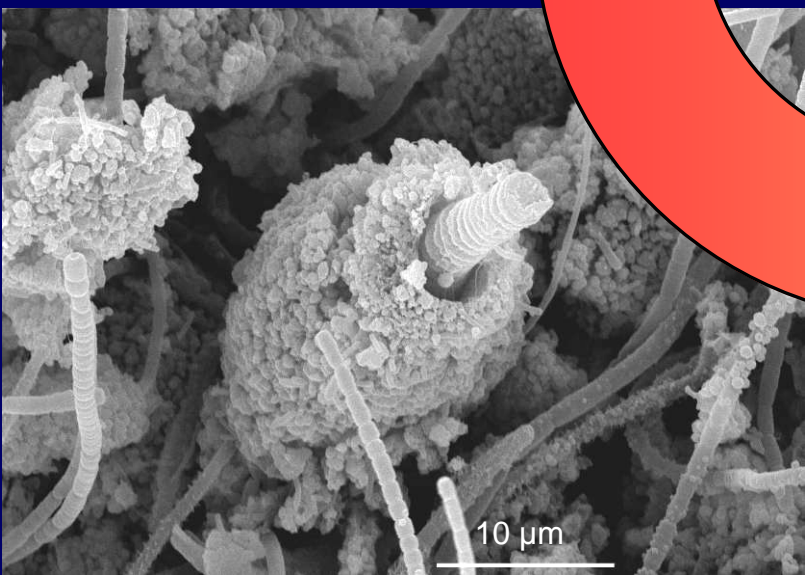
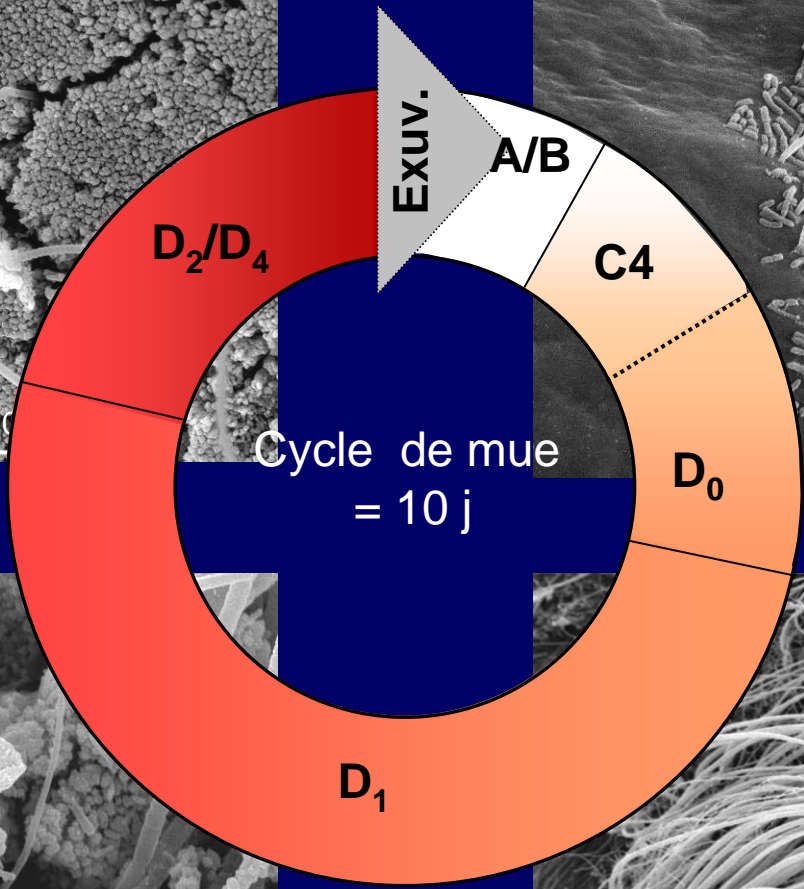
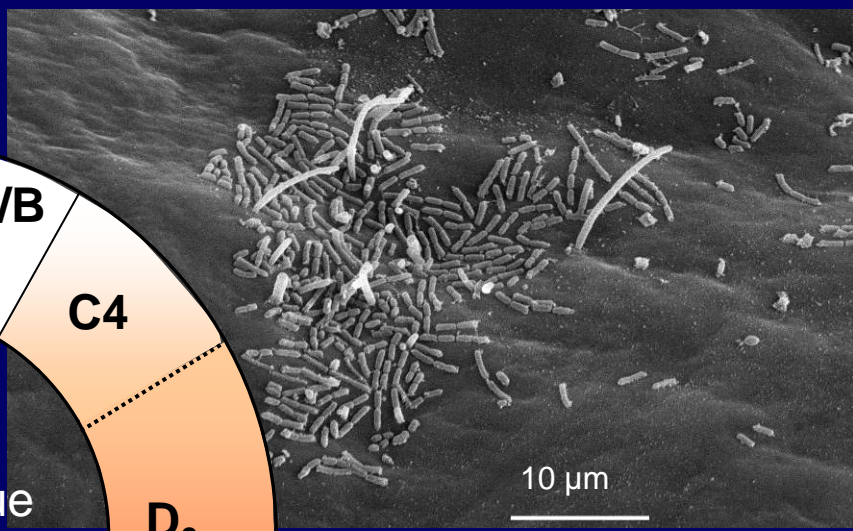
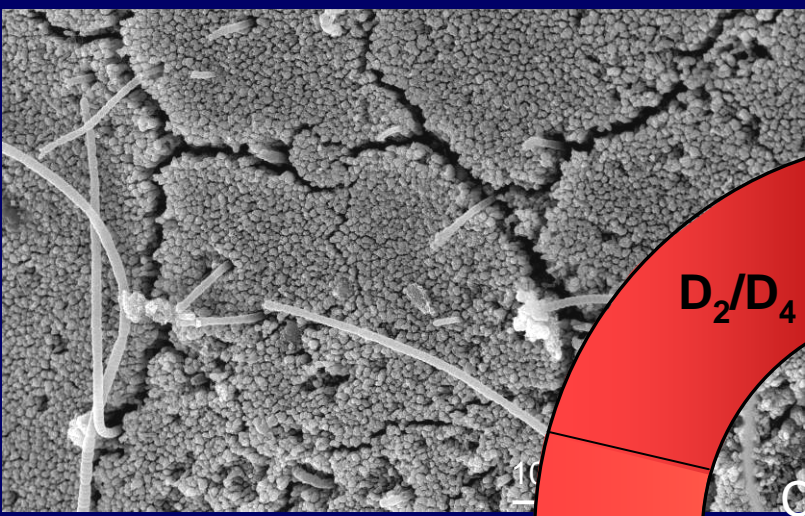


- Very rapid
- Atypical with predominant preecdysial stages

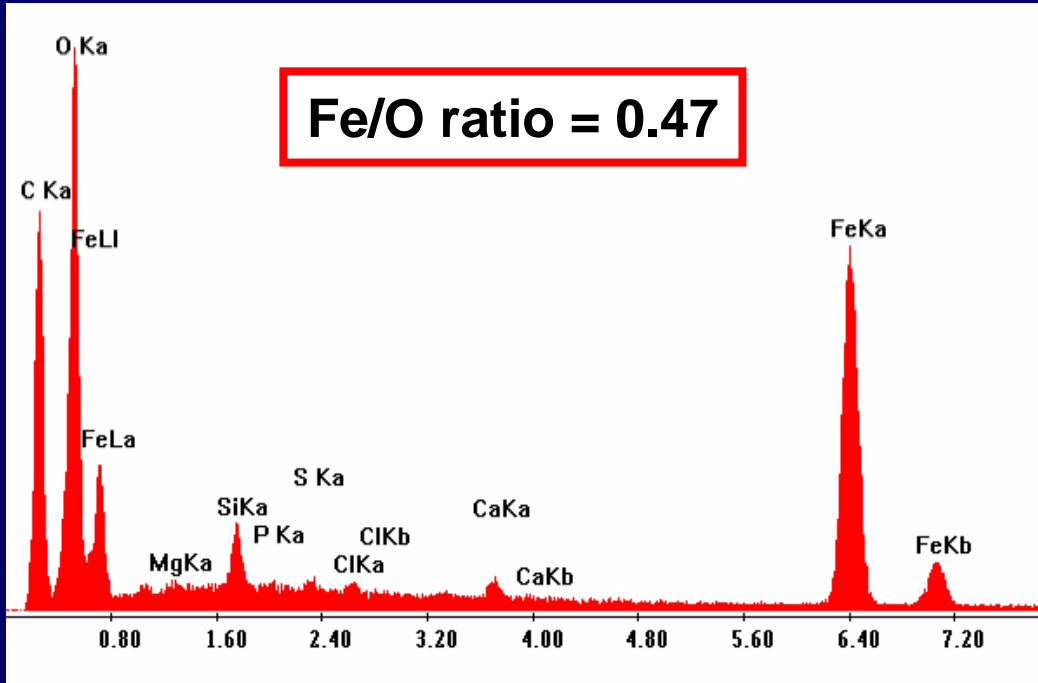
Moult cycle



Moult cycle



Quantitative X-ray microanalysis (EDAX)

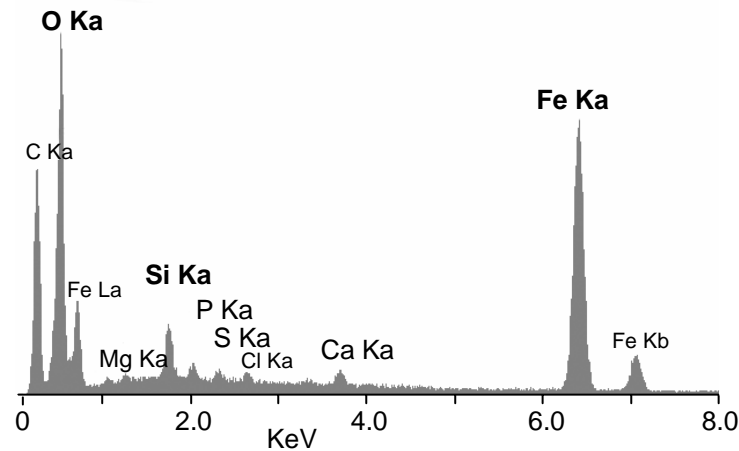
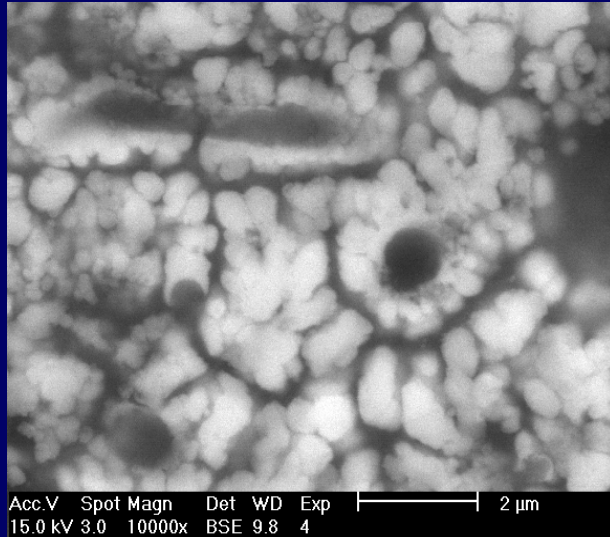


Elements	Wt %	At %
C K	37.5	57.3
O K	25.5	29.2
MgK	0.4	0.3
SiK	1.7	1.1
P K	0.6	0.3
S K	0.3	0.2
ClK	0.3	0.2
CaK	0.6	0.3
FeK	32.7	10.8

Elements	O	Fe	Si	S	P	Ca	Mg
Mean	67.8 ± 1.4		2.6 ± 0.4	0.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.5 ± 0.3
Available O	-		5.3 ± 0.8	2.1 ± 0.4	2.0 ± 0.9	-	-
Anionic groups				SO_4^{2-}	PO_4^{3-}		
		Ferrihydrite		2.6 ± 0.5	2.5 ± 1.2		(n=14)
Mineral compounds		$\text{Fe}_x\text{O}_y(\text{OH})_z + v\text{H}_2\text{O}$	SiO_2	$(\text{Ca},\text{Mg})\text{SO}_4$	$(\text{Ca},\text{Mg})_3(\text{PO}_4)_2$		Total
			7.9 ± 1.2	3.1 ± 0.6	3.2 ± 1.5		99.9 ± 0.2

Ligands

Microanalyse aux rayons X



Element	Wt %	At %
C K	37.5	57.3
O K	25.5	29.2
MgK	0.4	0.3
SiK	1.7	1.1
P K	0.6	0.3
S K	0.3	0.2
ClK	0.3	0.2
CaK	0.6	0.3
FeK	32.7	10.8

Mössbauer spectroscopy

2L-Ferrihydrite with $\text{Fe}^{3+} > 90\%$
nano-particles = 2-5 nm
Aggregated in
multiglobular concretions

Quantitative X-ray microanalysis

2L-Fh 85.8 %

Minor mineral ligands

$\text{Si}(\text{O}_2)$ 7.9 %

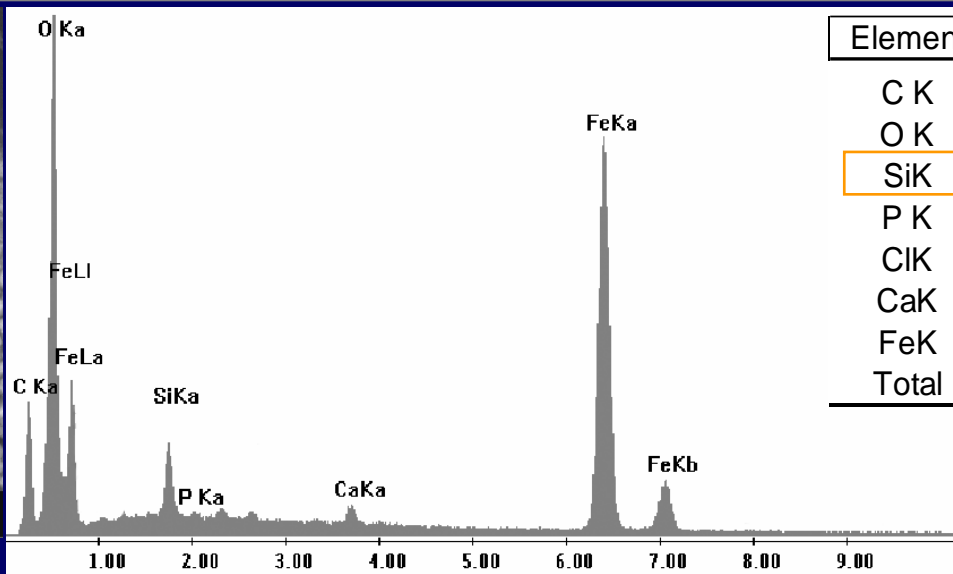
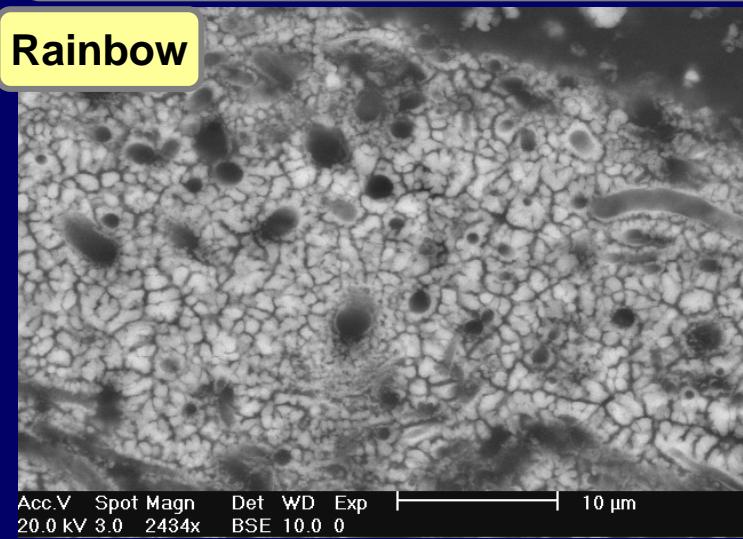
(Ca,Mg) 6.3 %

$-\text{PO}_4^{3-}$ $-\text{SO}_4^{2-}$

Microanalyse aux rayons X

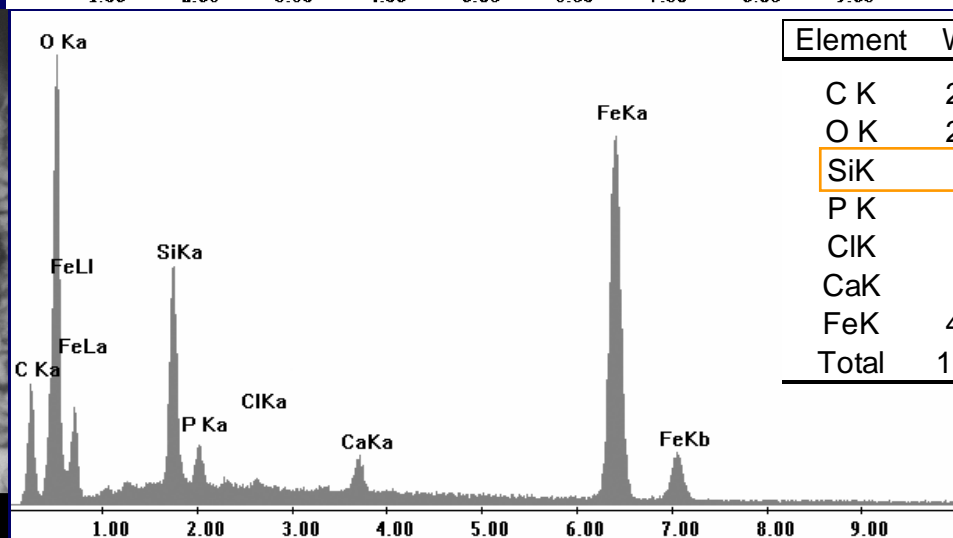
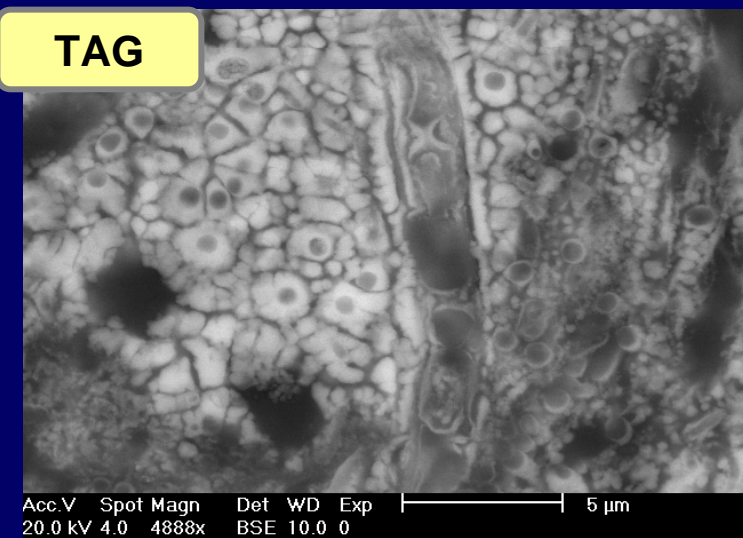
Composition élémentaire : oxydes de fer en rapport avec celle du fluide hydrothermal

Rainbow



Element	Wt %	At %
C K	23,0	41,4
O K	28,6	38,6
Si K	2,2	1,7
P K	0,2	0,2
Cl K	0,3	0,2
Ca K	0,7	0,4
Fe K	44,4	17,2
Total	100,0	100,0

TAG



Element	Wt %	At %
C K	23,2	42,0
O K	24,6	33,4
Si K	6,6	5,1
P K	1,5	1,1
Cl K	0,4	0,2
Ca K	1,3	0,7
Fe K	40,3	15,7
Total	100,0	100,0

KeV

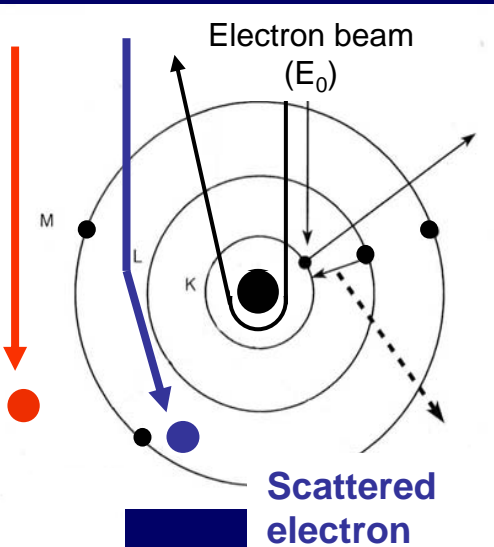
KeV

BF-TEM and HAADF-STEM imaging modes

in the **Tecnai G2 Twin STEM**

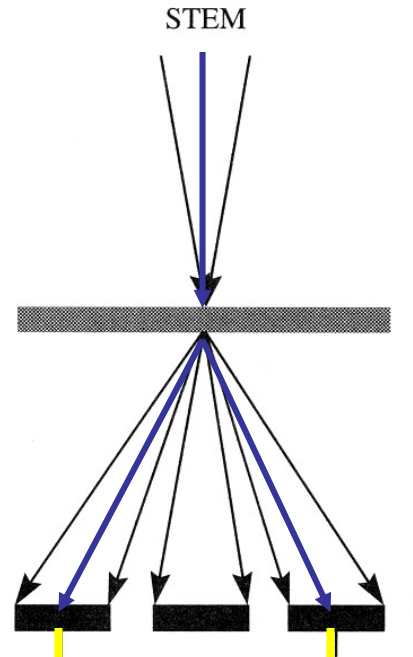
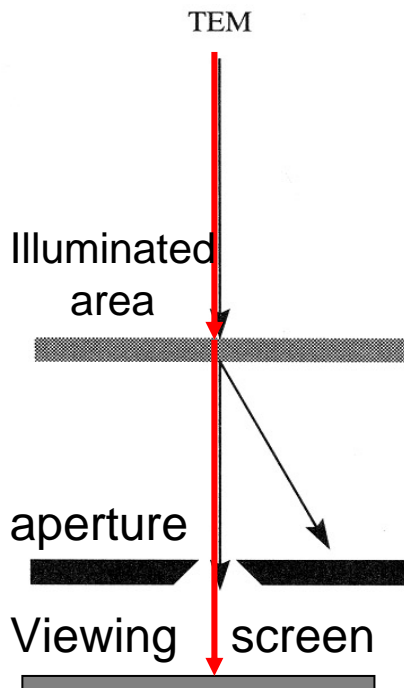
Transmitted electron
($E = E_0$)

Parallel beam



Convergent scanning electron beam

**TEM-
Bright field
Mass
-thickness
contrast**



**STEM
Dark field Z-contrast**

**High angle annular
dark field detector**

5. Microscopie électronique à transmission - Champs clair

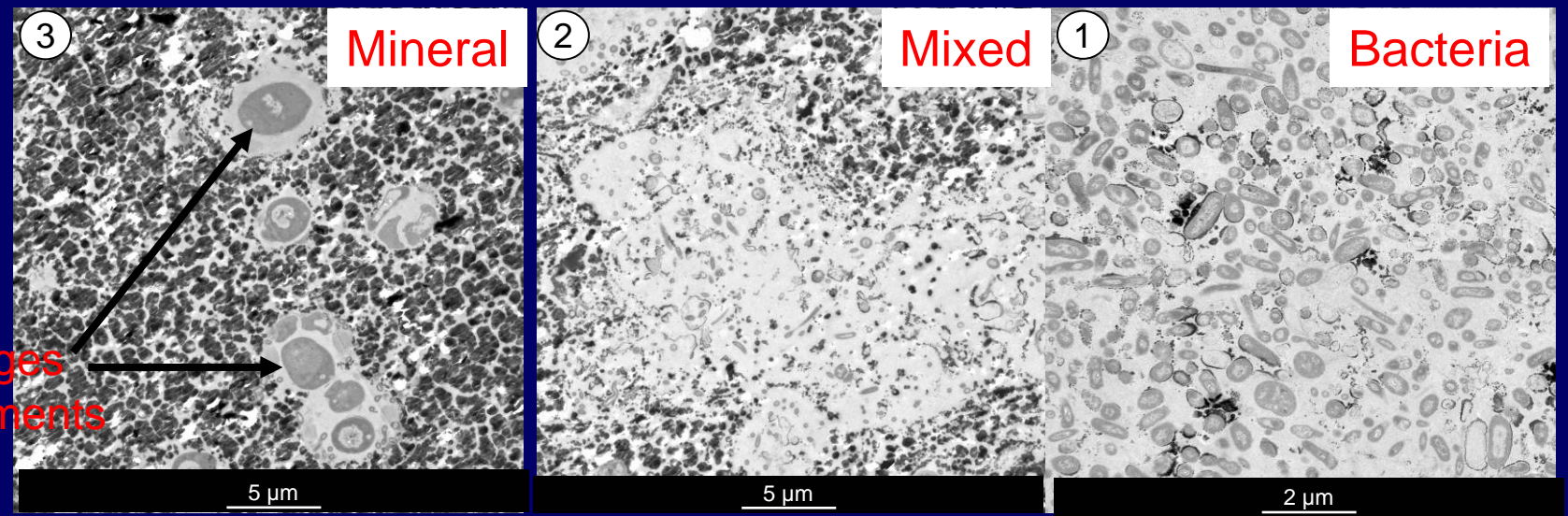
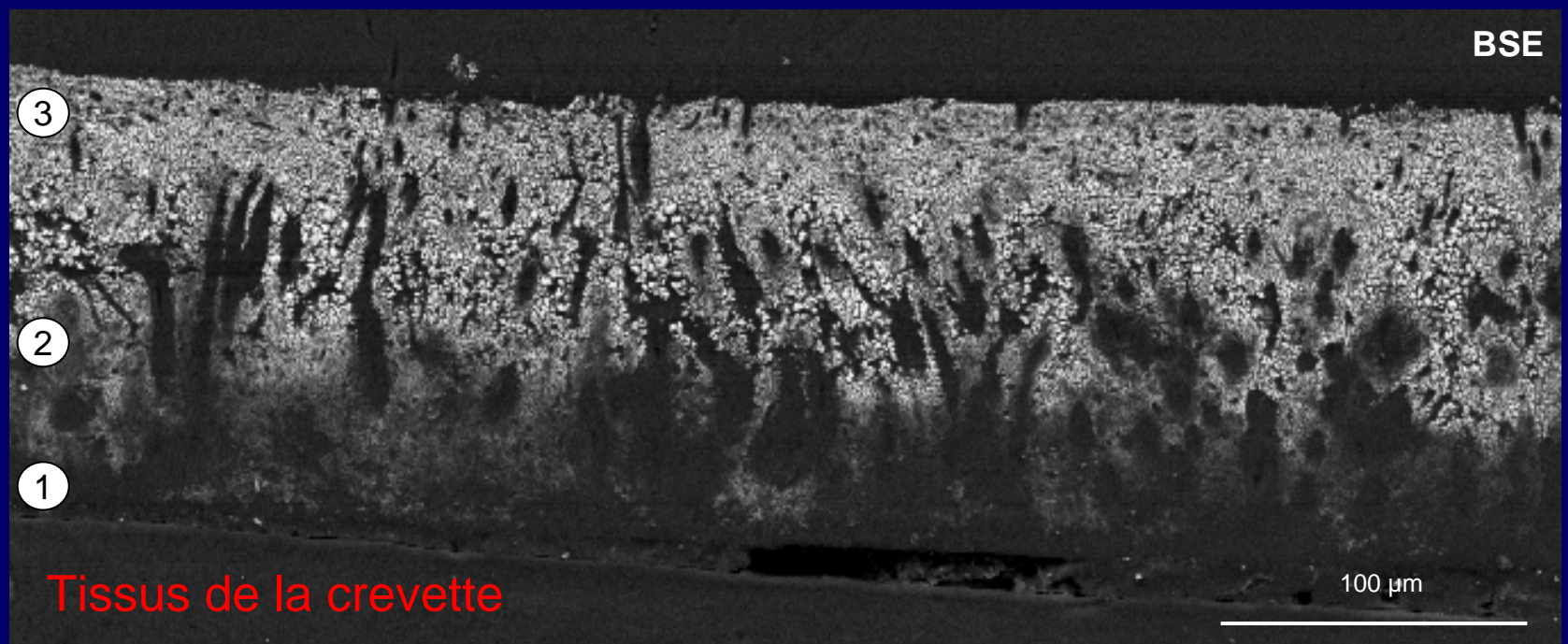


Différents morphotypes, métabolismes et gènes caractéristiques de bactéries (sulfo-,méthane- et Fe-oxydant)

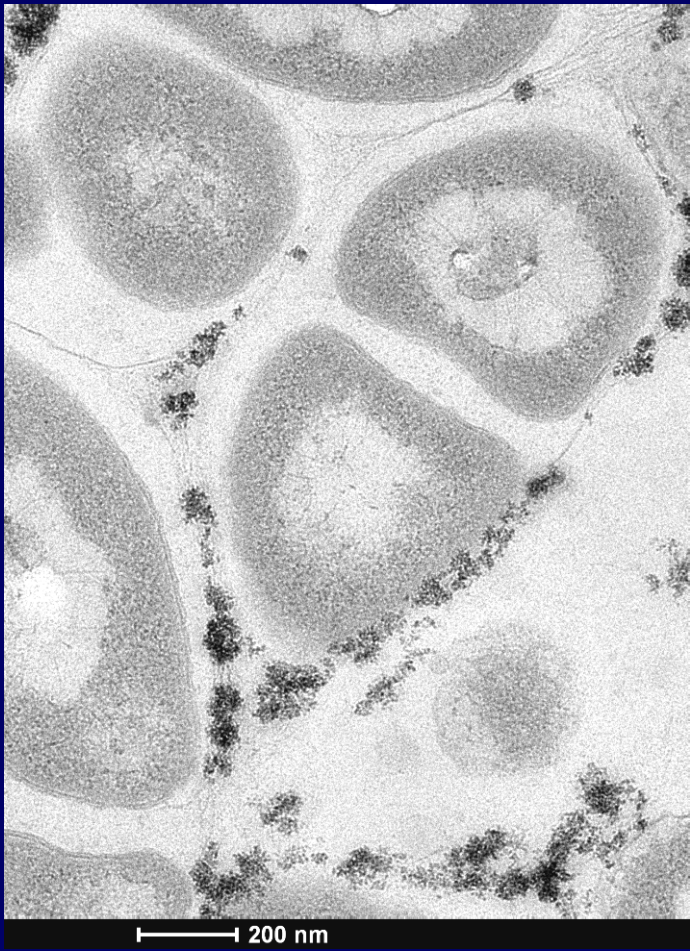
Zbinden et al. 2008; Corbari et al. 2008, Petersen et al., 2010)



5. MET



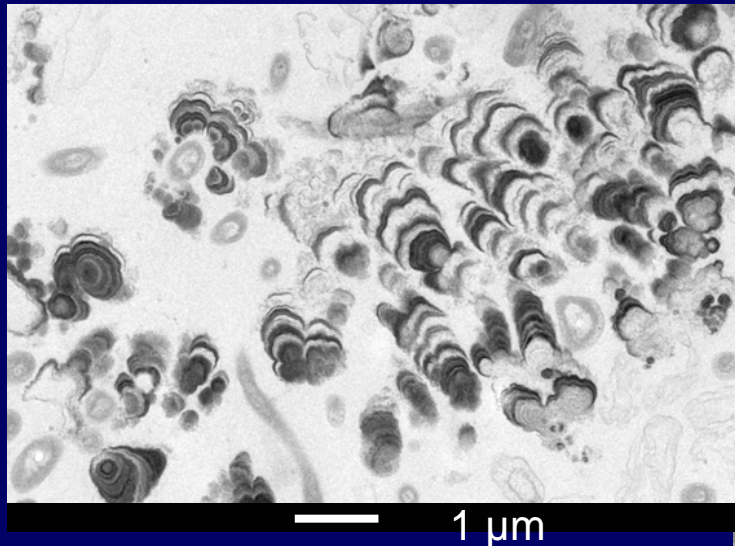
5. MET – Interactions bactéries -minéraux



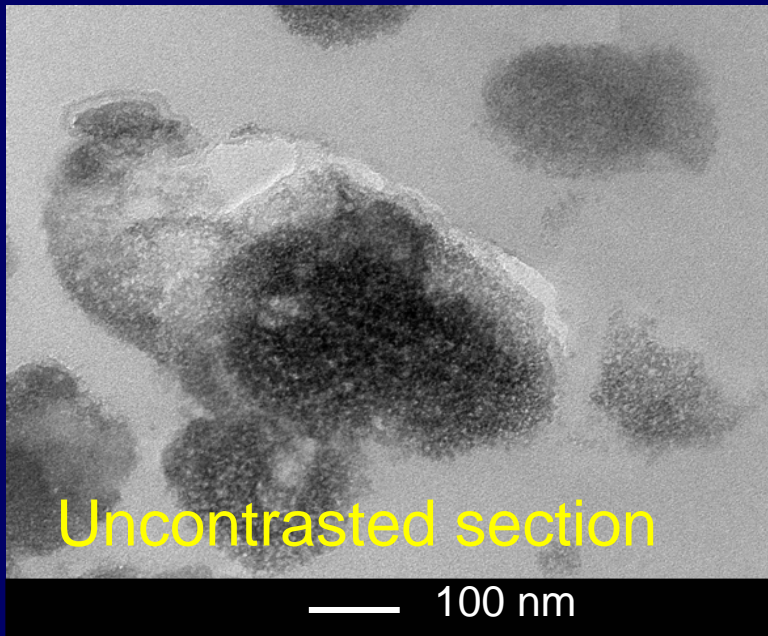
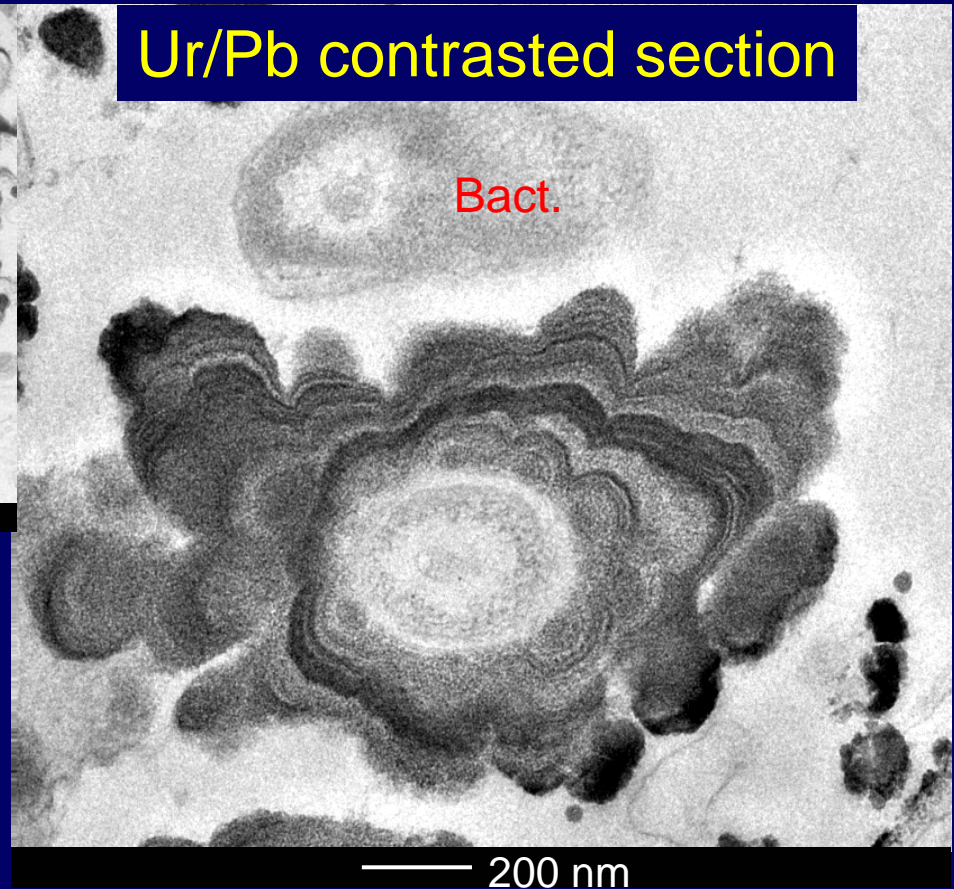
Biogenic iron oxides initiated by bacterial cell walls or secretions
Biotic origin = byproducts of the bacterial Fe^{2+} -oxidising metabolism
Abiotic precipitation

50 nm

5. Conventional BF-TEM



Ur/Pb contrasted section

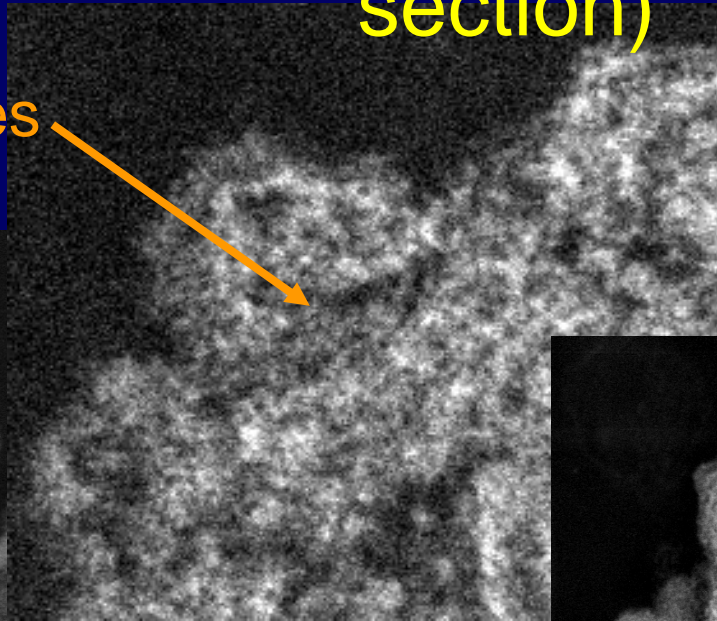


Uncontrasted section

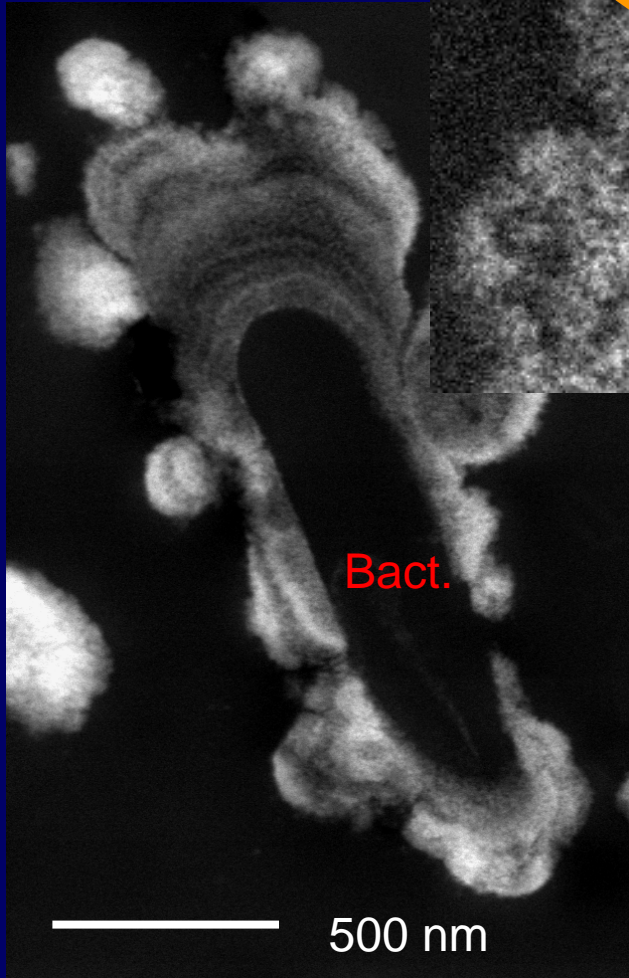
Nanometric banding (nanostrata)
due to section contrast or changes
in mineral composition ?

6. HAADF-STEM views (uncontrasted section)

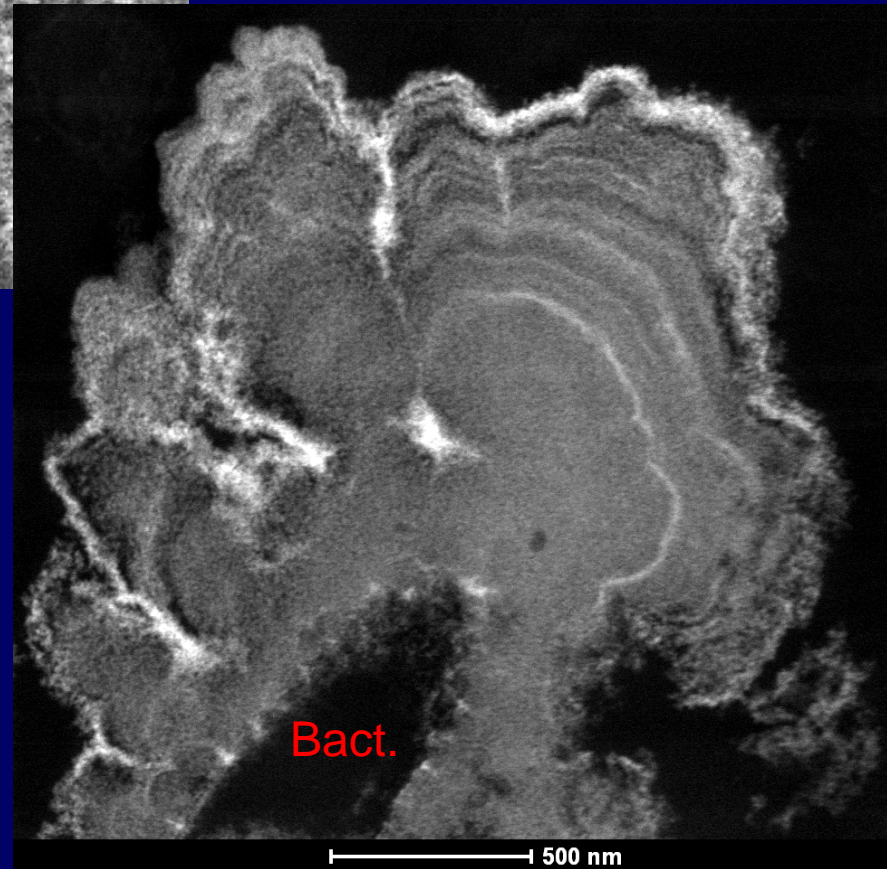
Nanoparticles
(2-3 nm)



Dark Field
Z-contrast images

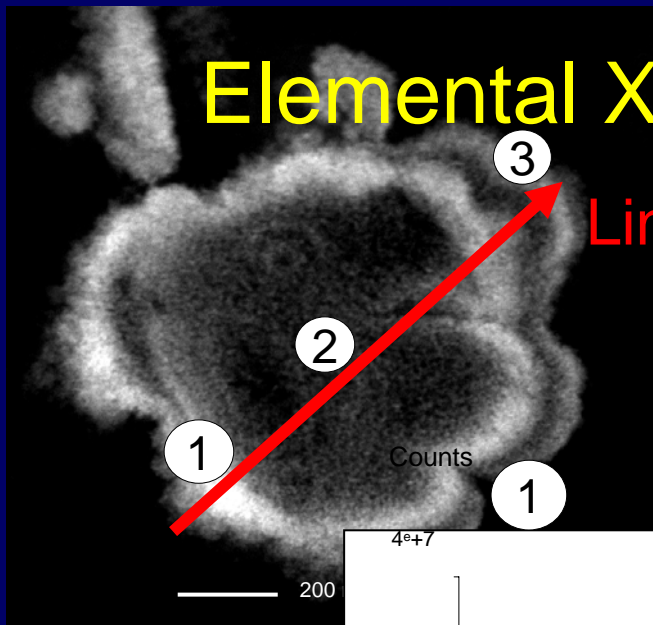


Nanostrata



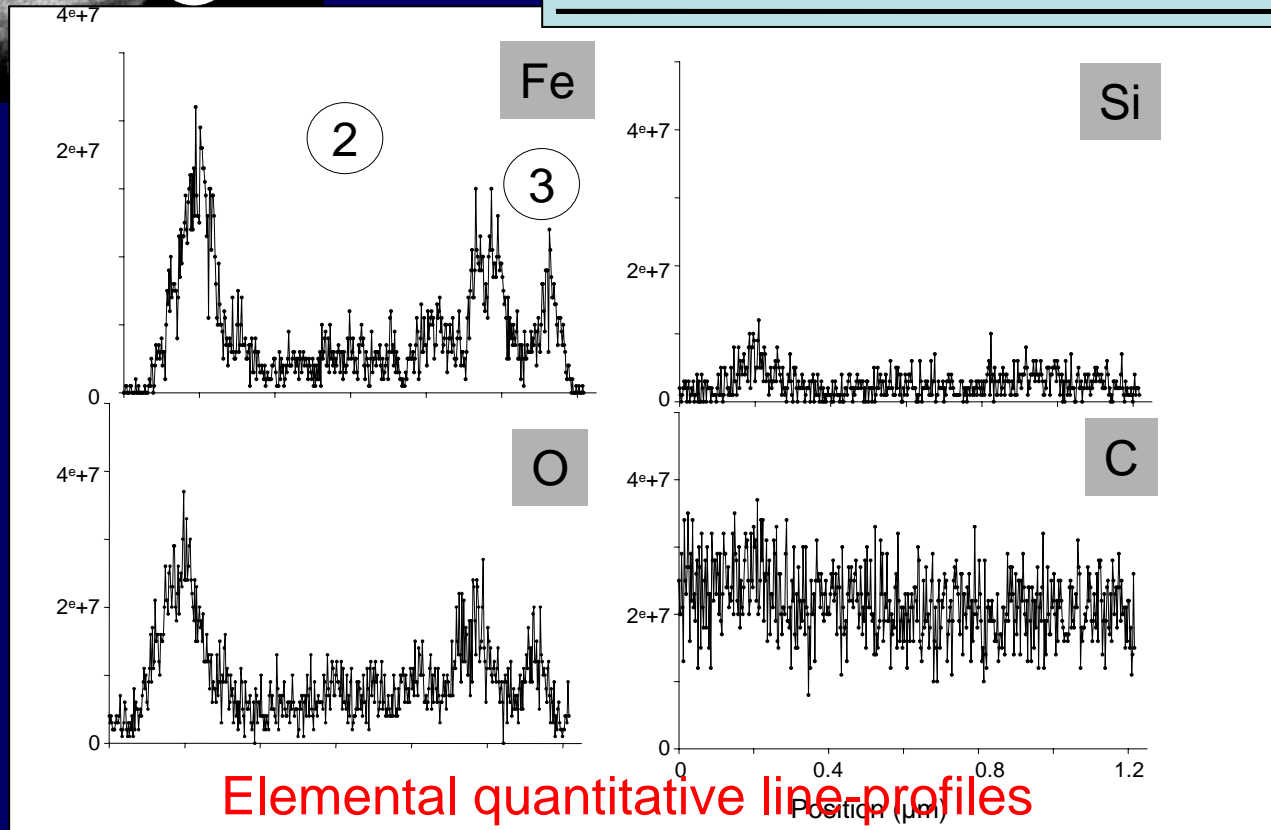
Elemental X-ray microanalysis (EDAX)

Quantitative results



Line-profile

% At	Fe(K)	O(K)	Si(K)	C(K)
1	4.62	10.95	0.55	81.22
2	1.07	4.57	0.34	94.02
3	1.99	6.54	0.36	90.29

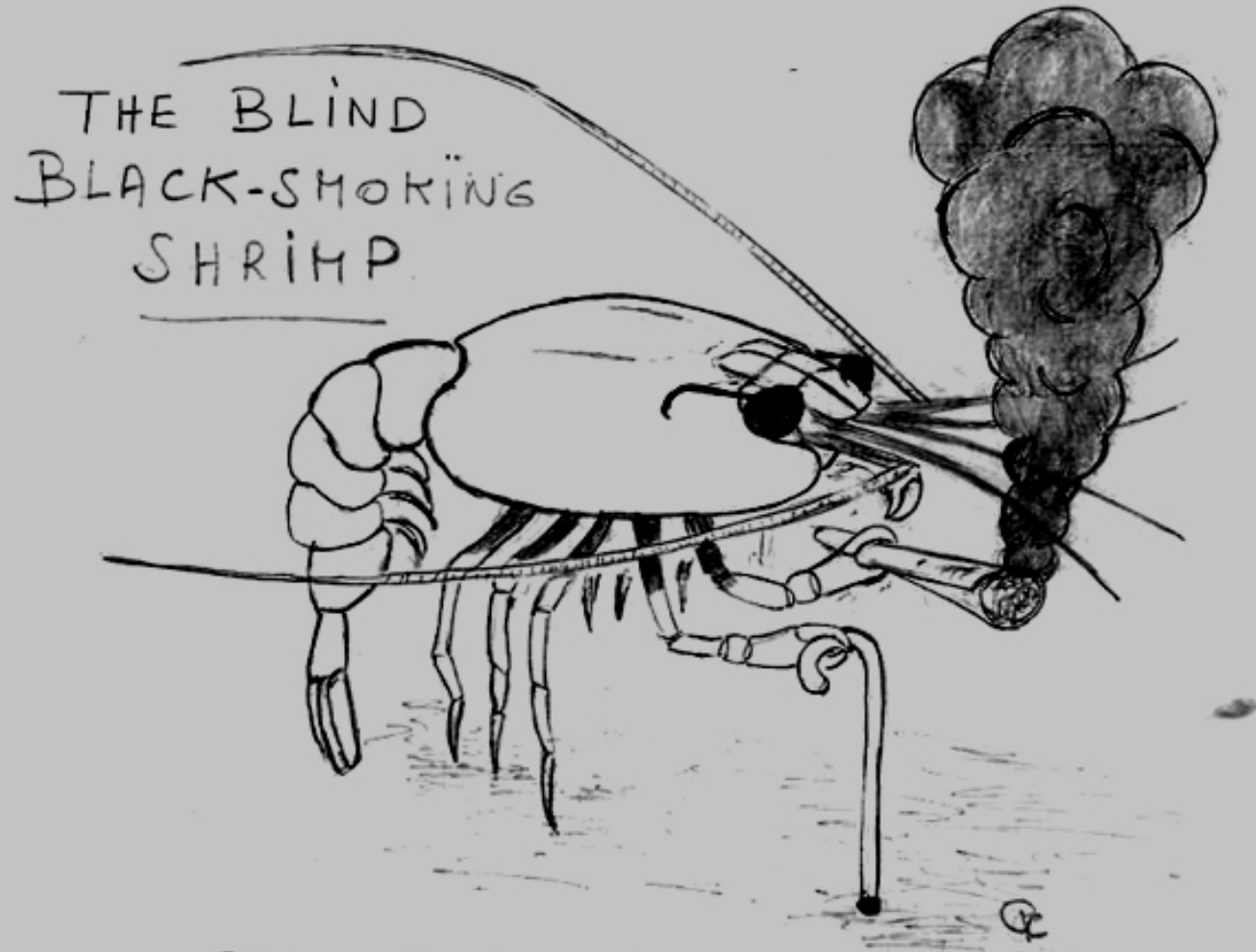


Conclusions

- 1. Microscopie optique – Organisation de la croûte minérale
- 2. MEB-SE (HV) - Forme des minéraux et des bactéries
- 3. MEB (bas vide ou environnemental) – GSE -BSE
Forme des particules et contacts avec les bactéries
- 4. MEB (HV) – Lames mince polies
– BSE (contraste de Z) + EDAX
Croissance et composition chimique (ferrihydrite)
- 5. MET conventionnel - Initiation de la croissance des minéraux
- 6. STEM-HAADF + EDAX
Nanostrates de densité de nanoparticules de ferrihydrite et co-precipitation avec des ligands

Arguments d'une origine biotique des oxydes de fer associés aux bactéries (biominéraux)

Quand on ne les regarde pas !!!!



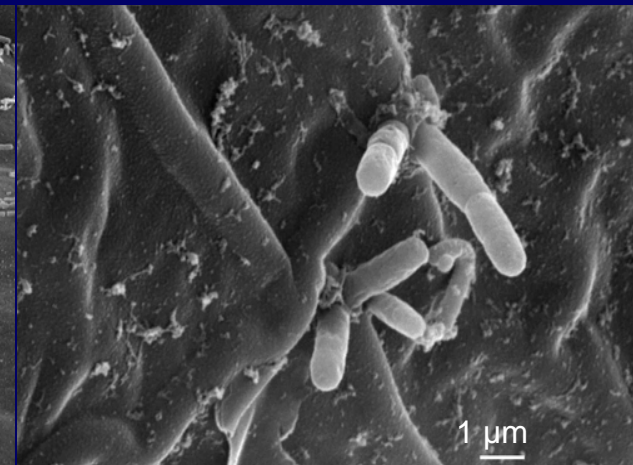
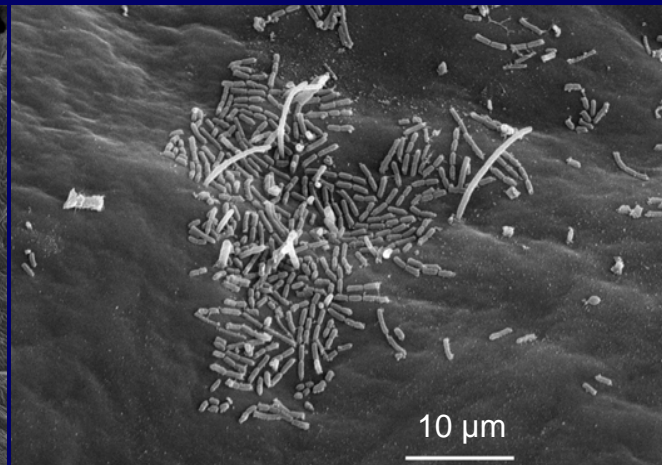
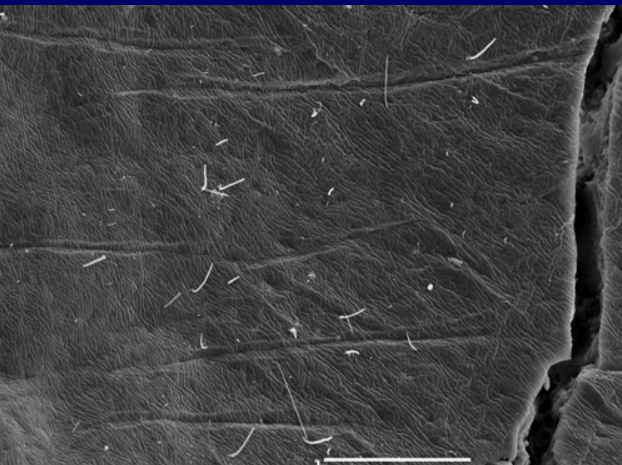
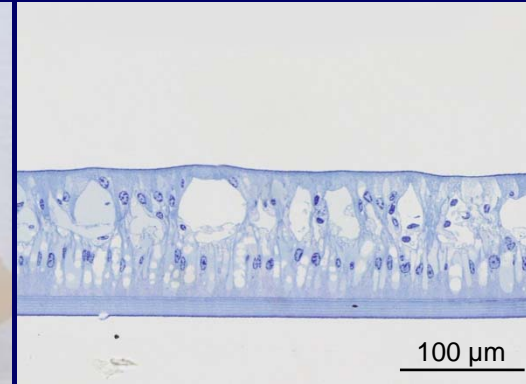
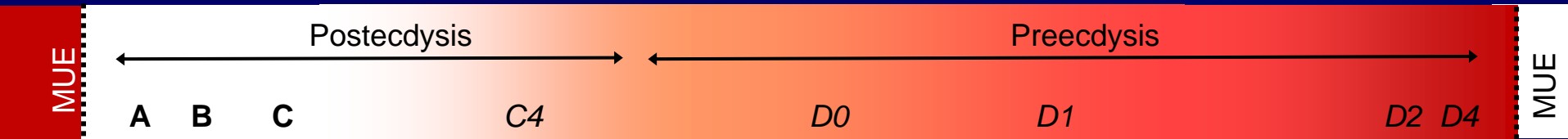
Merci de votre attention !



JE NE FAIS
QUE DES
PHOTOS
SANS
INTÉRÊT

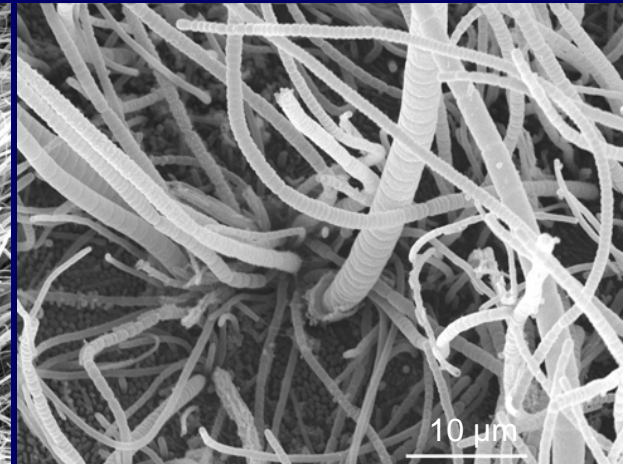
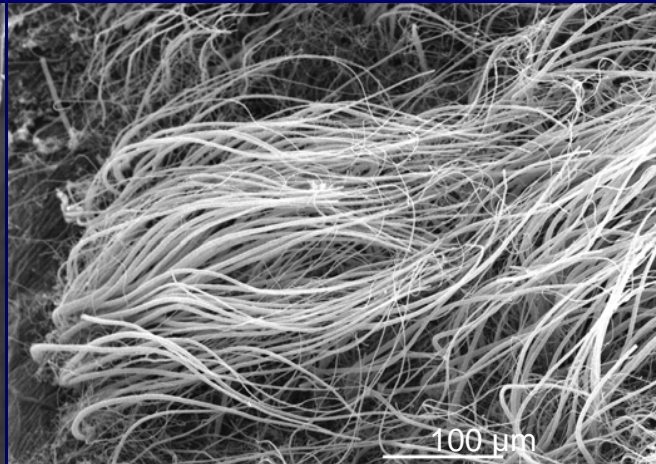
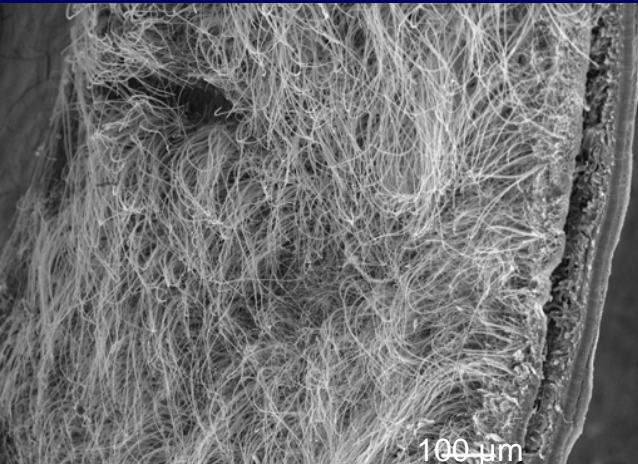
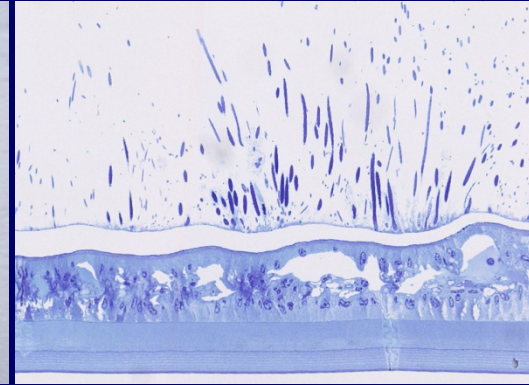
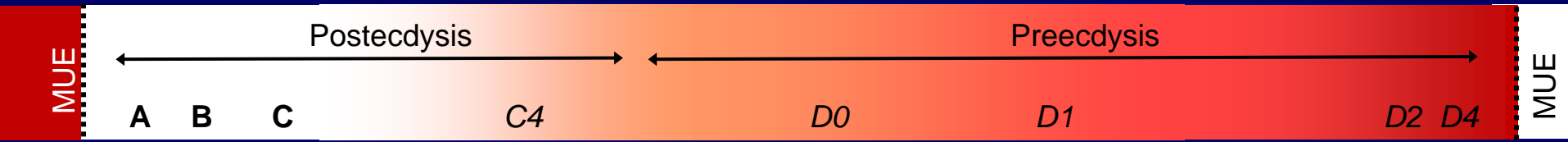
L'AVANTAGE,
C'EST QUE QUAND
ELLES SONT
RATÉES..
JE M'EN
FOUS

Rimicaris exoculata



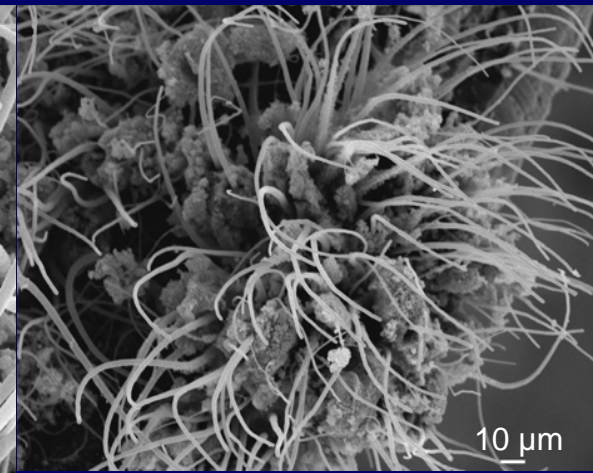
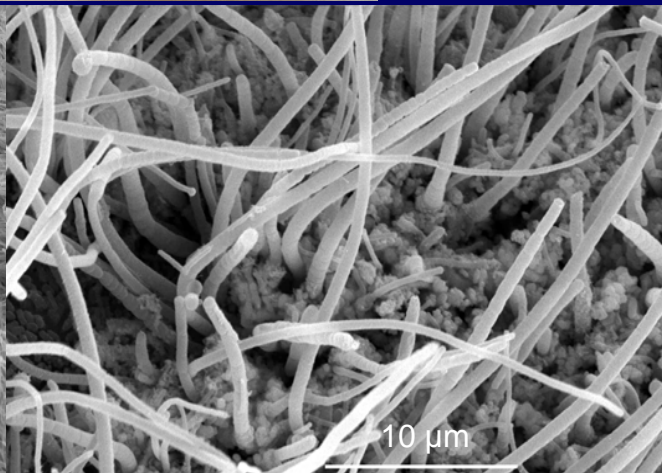
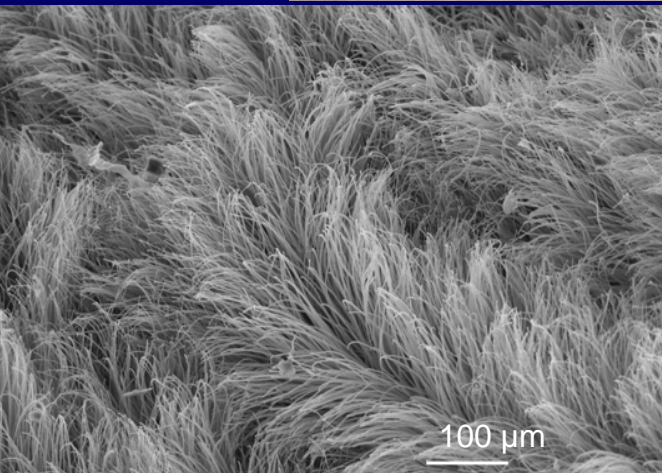
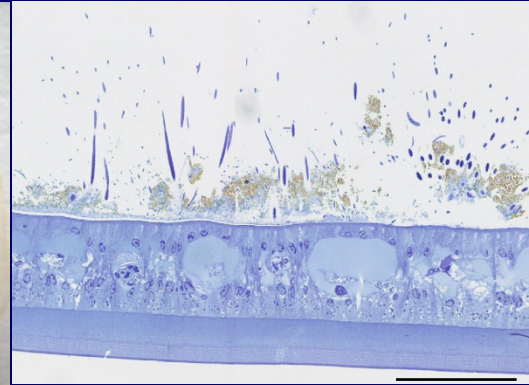
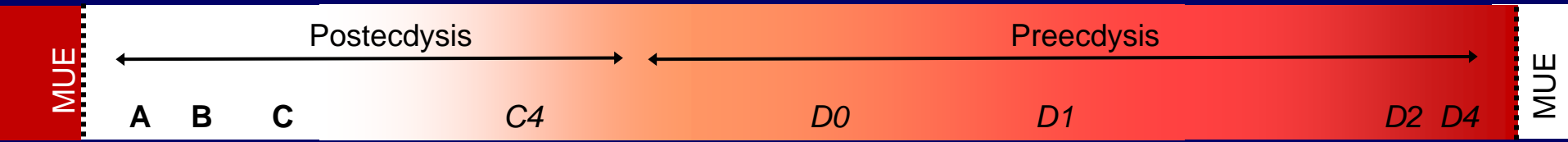
1- Premières bactéries en patches

Rimicaris exoculata



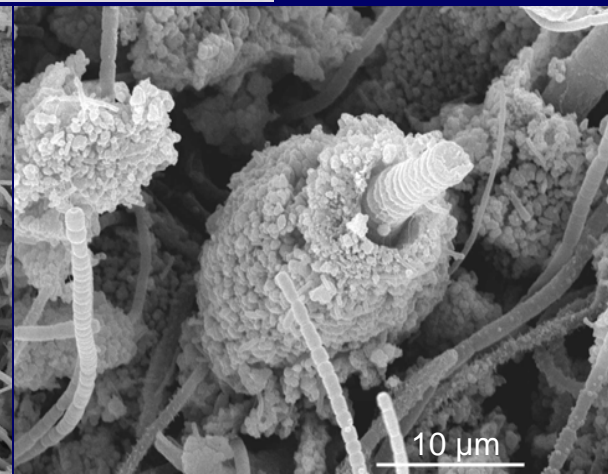
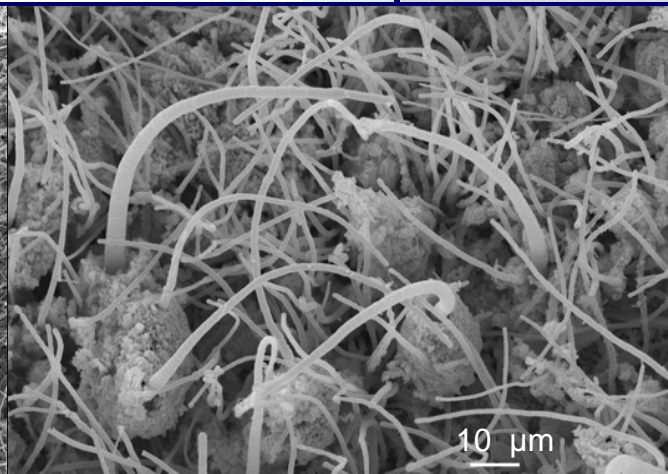
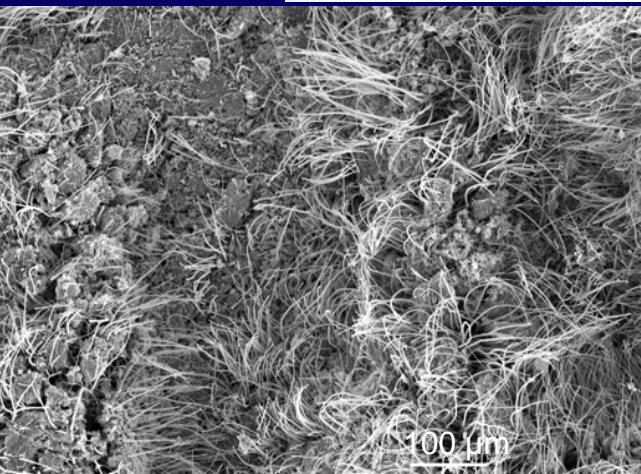
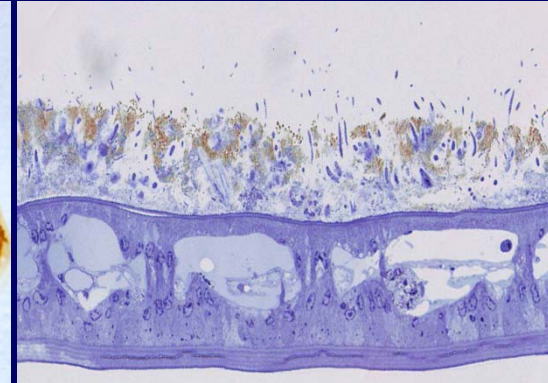
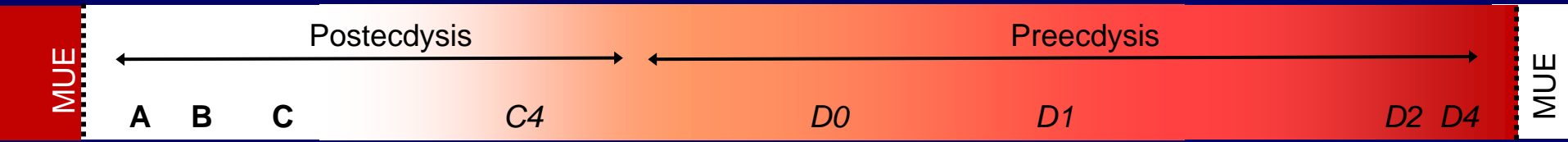
2- Développement max. bactéries (bat. & Fil.)

Rimicaris exoculata



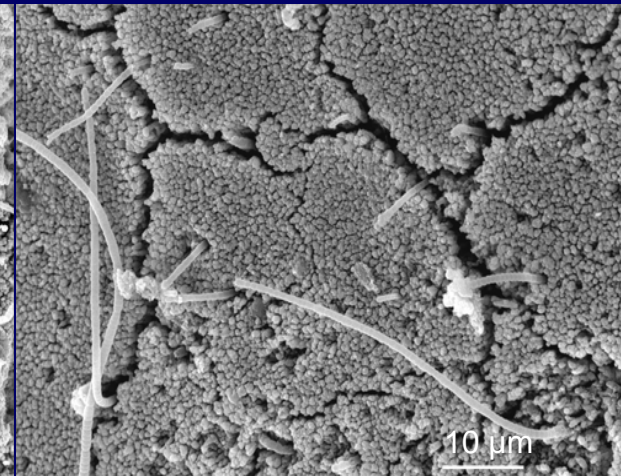
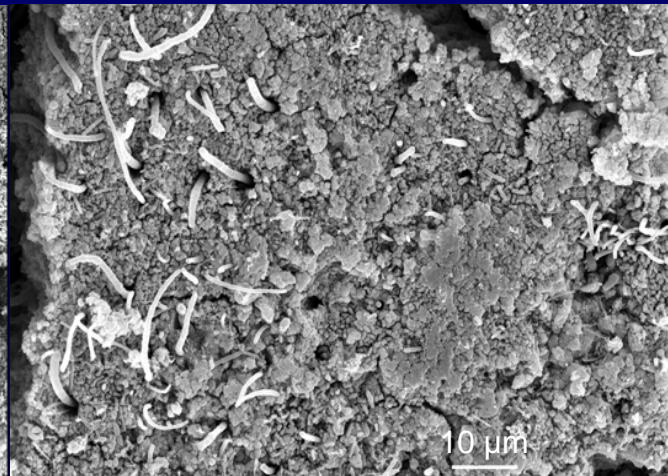
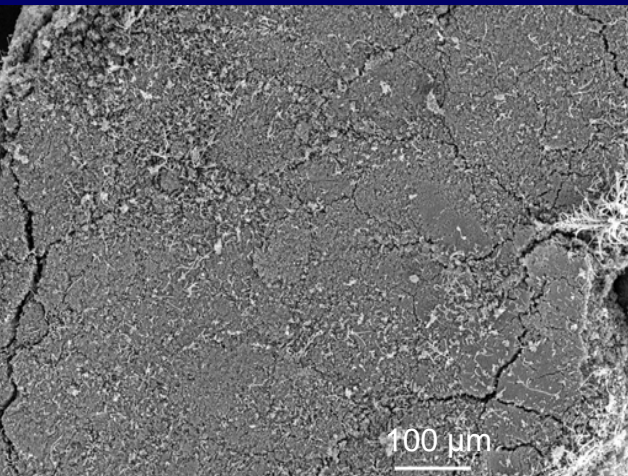
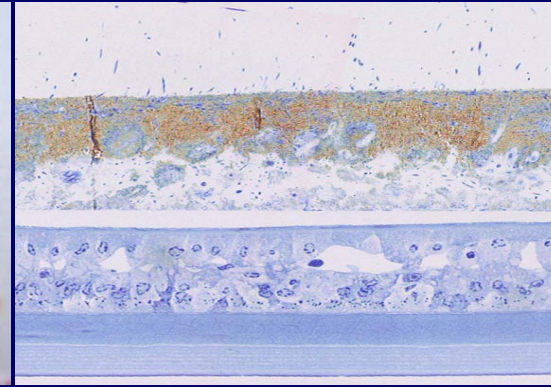
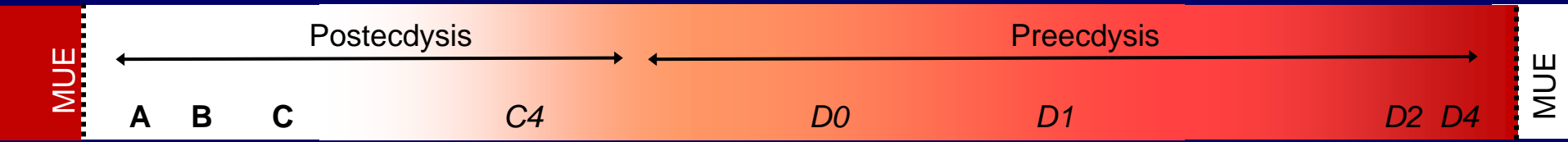
3- Détection des premières particules minérales

Rimicaris exoculata



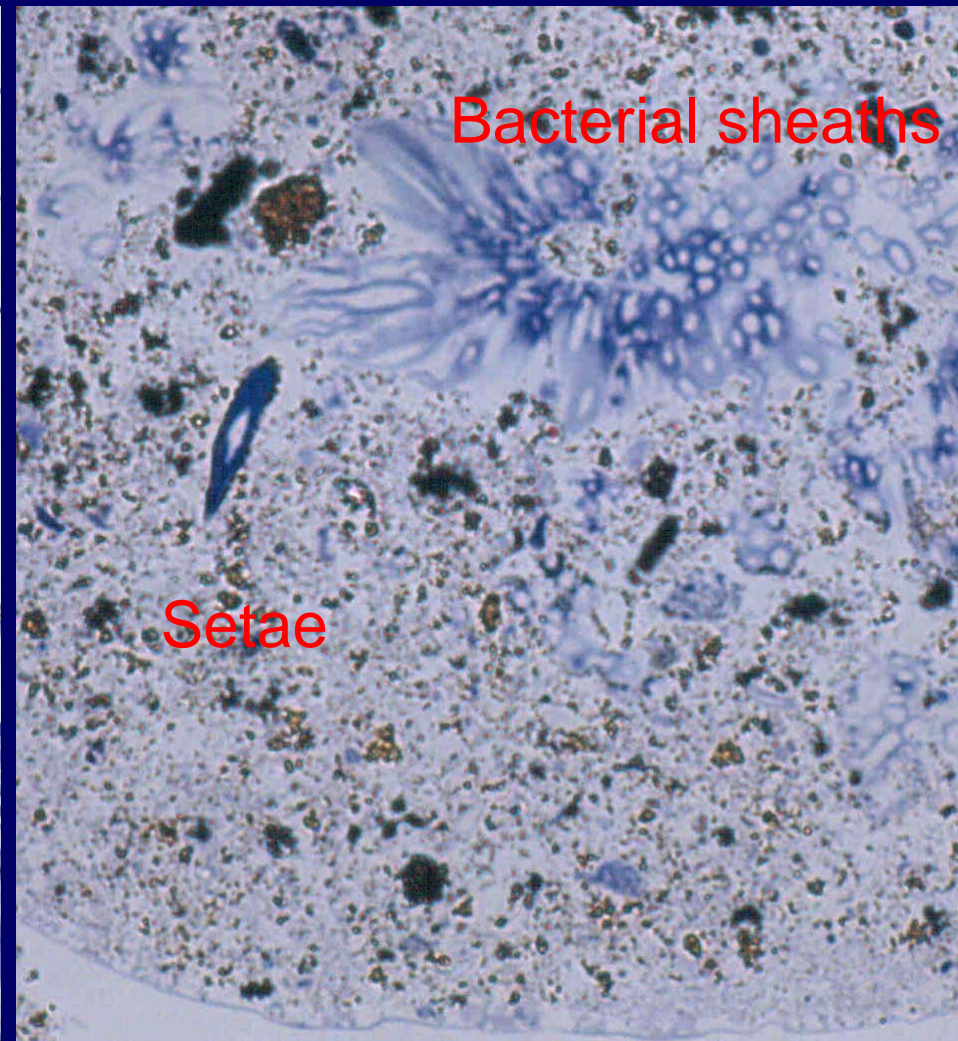
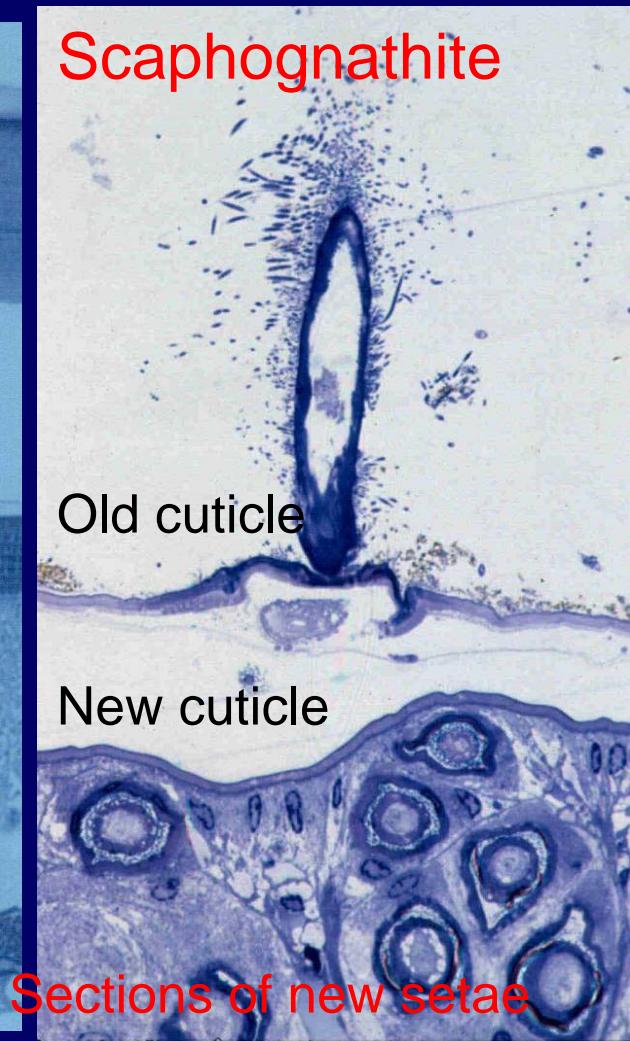
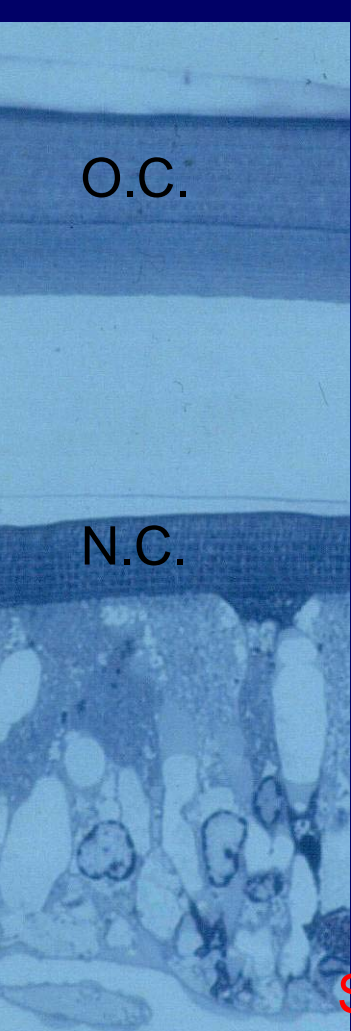
4- Accroissement des dépôts minéraux

Rimicaris exoculata



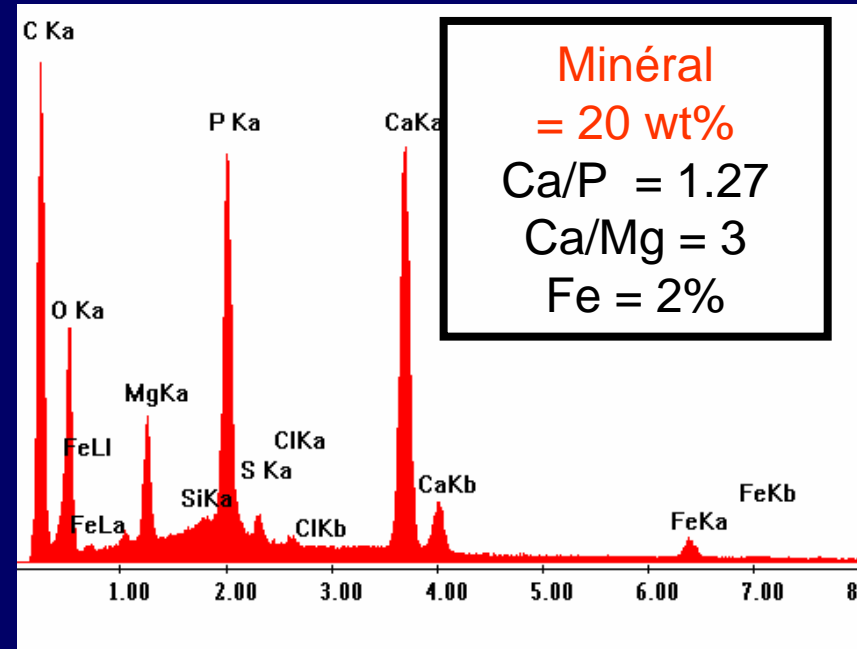
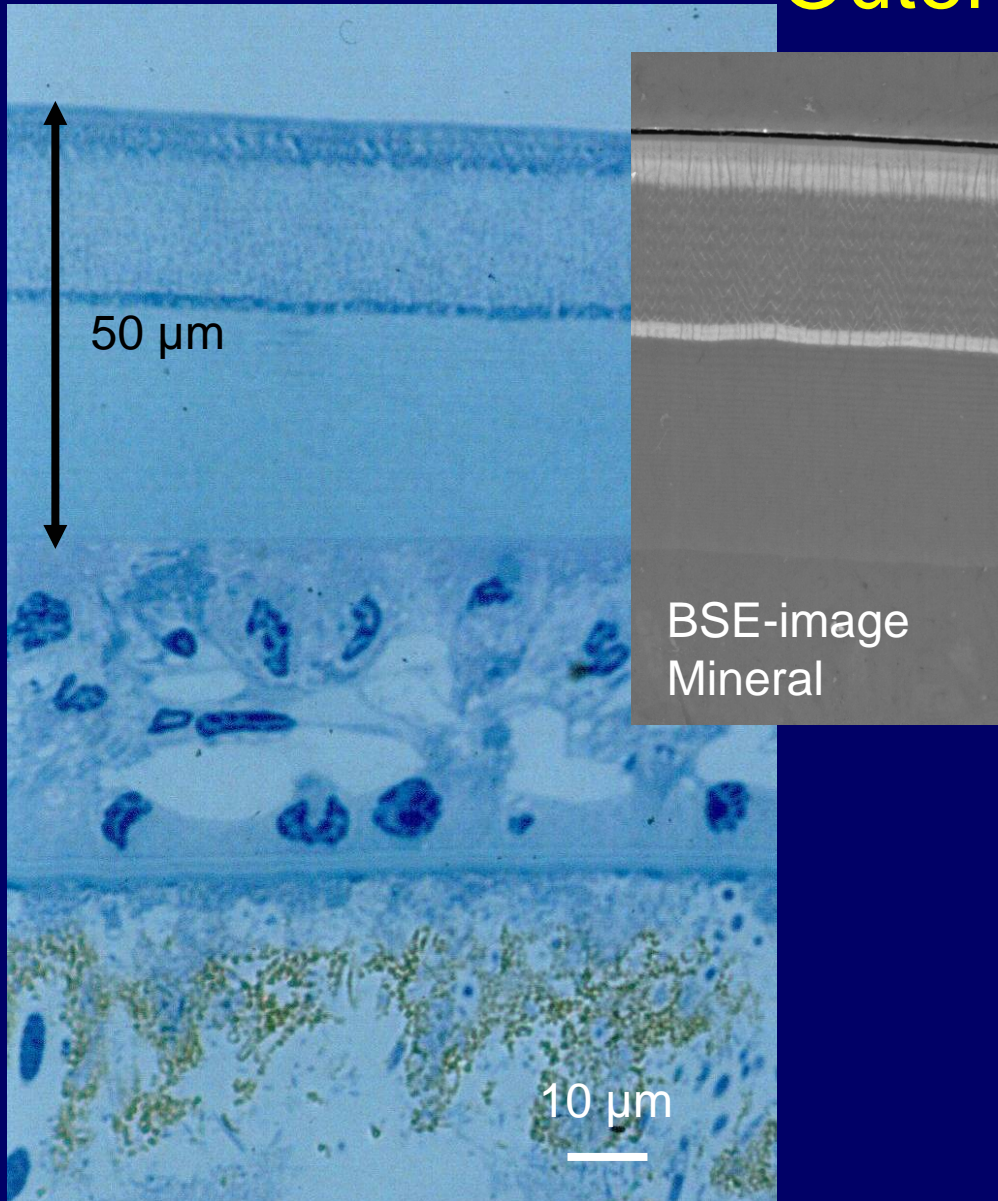
5- Croûte minérale importante

Eating exuviae other individuals with their bacteria



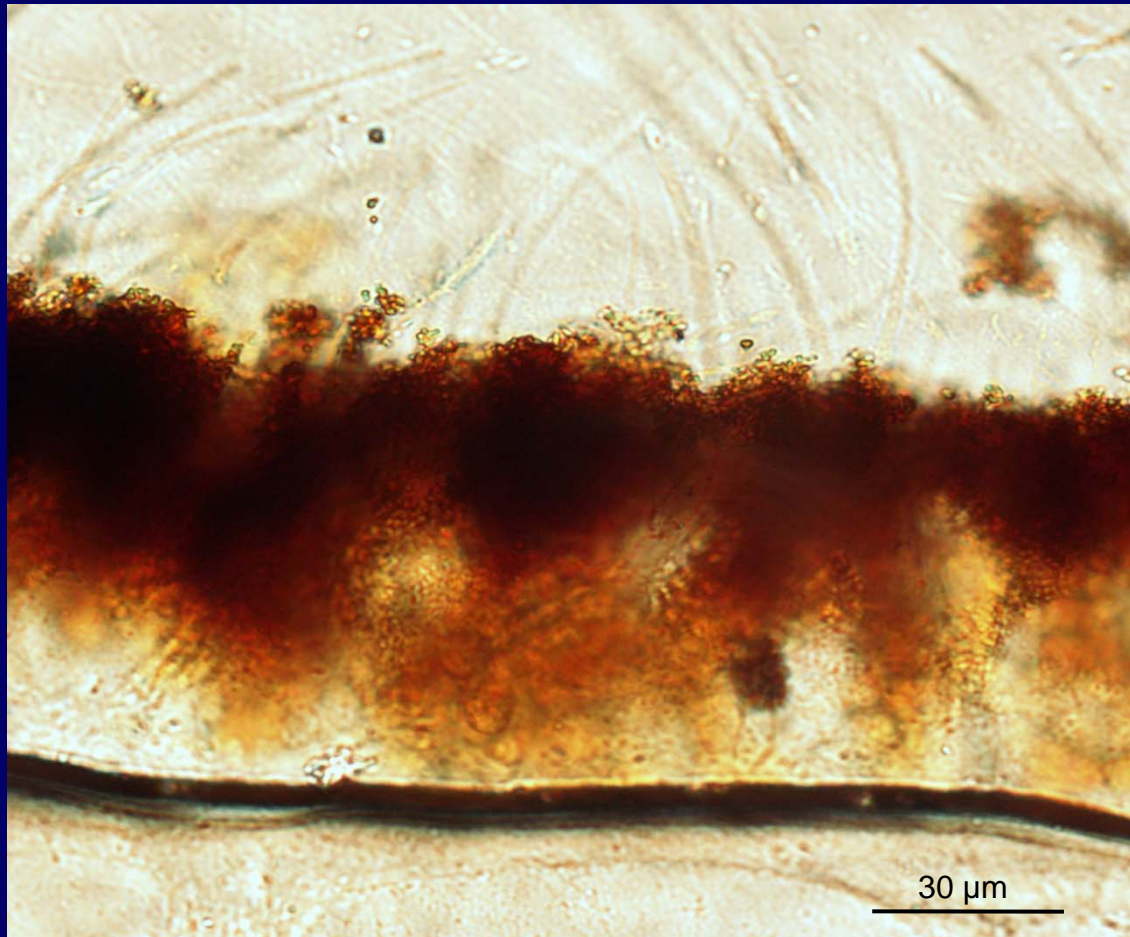
Outer branchiostegite cuticle

Amorphous calcium phosphate and carbonate



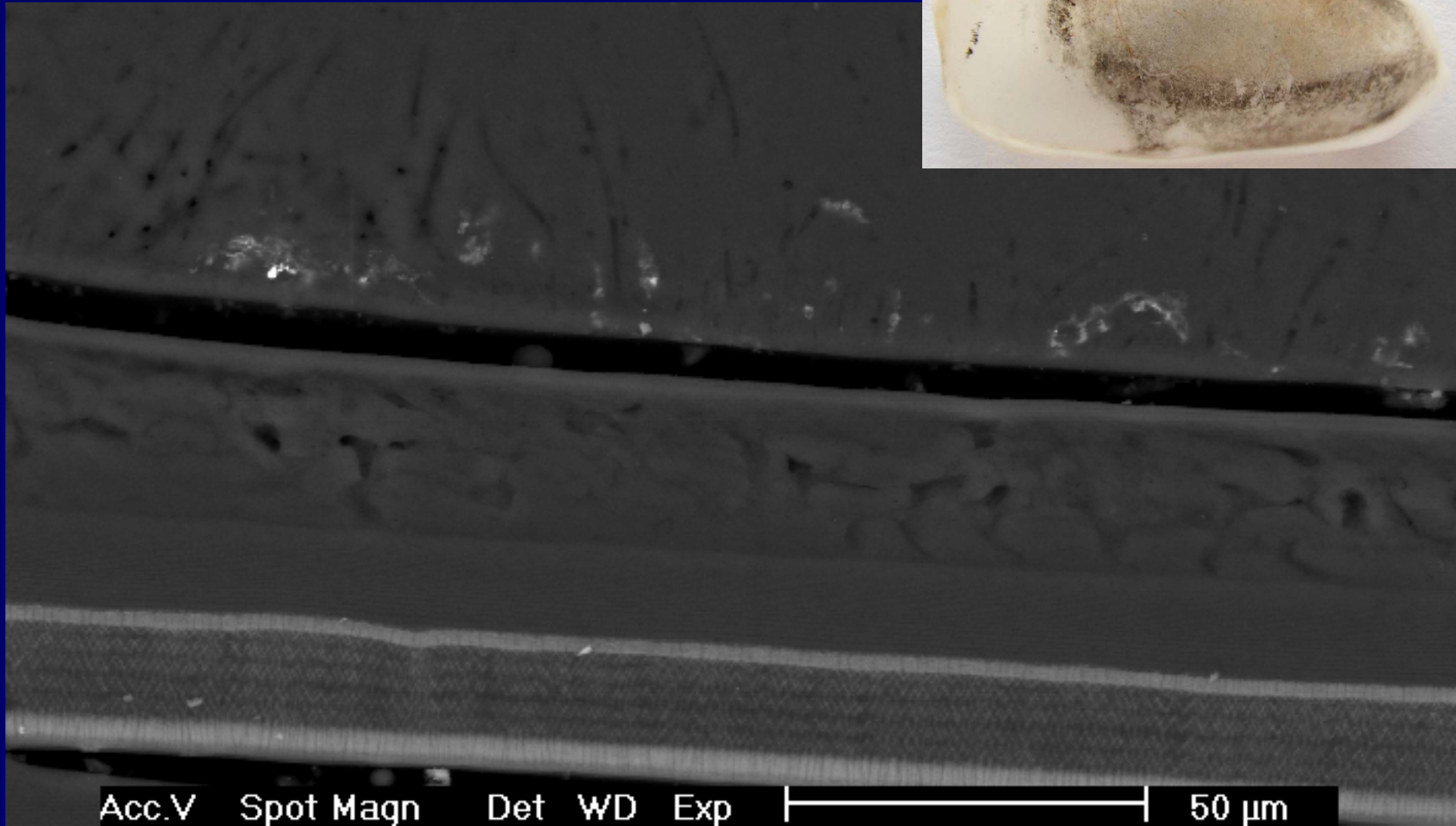
X-ray microanalysis

Composition des minéraux : oxydes de fer



Biominéraux

Structure : minéraux diffus - amorphes?



Acc.V

Spot Magn

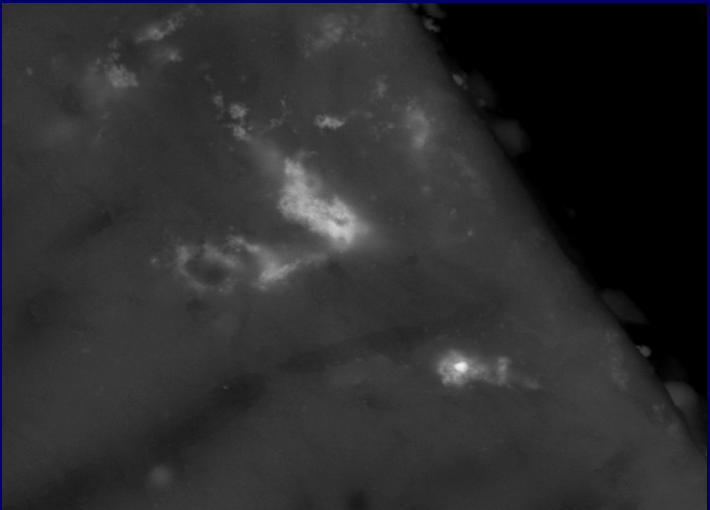
Det

WD

Exp

50 μm

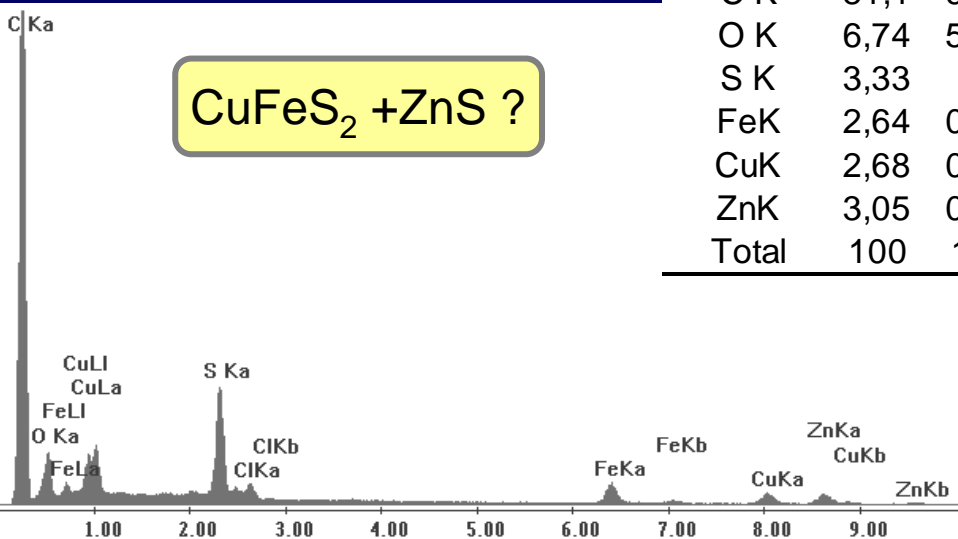
Composition élémentaire : minéraux diffus



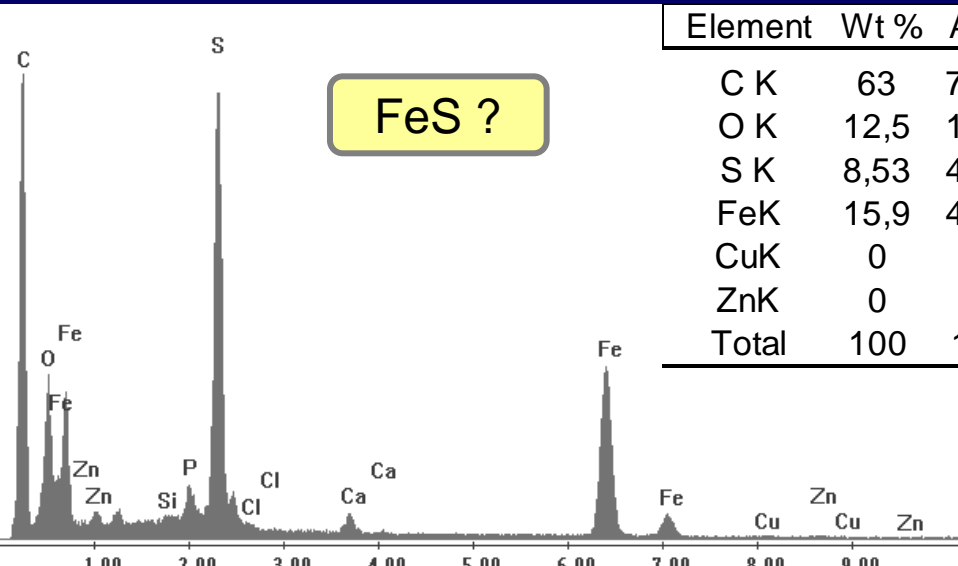
Acc.V Spot Magn Det WD Exp |-----| 5 µm
 20.0 kV 3.0 4843x BSE 10.0 0



Acc.V Spot Magn Det WD Exp |-----| 5 µm
 20.0 kV 4.0 6000x BSE 9.8 1



Element	Wt %	At %
C K	81,1	90,9
O K	6,74	5,67
S K	3,33	1,4
Fe K	2,64	0,64
Cu K	2,68	0,57
Zn K	3,05	0,63
Total	100	100



Element	Wt %	At %
C K	63	79,7
O K	12,5	11,9
S K	8,53	4,04
Fe K	15,9	4,33
Cu K	0	0
Zn K	0	0
Total	100	100



Exemple

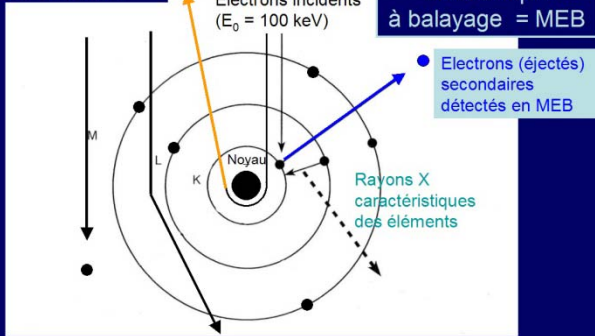
Crevette frappeuse



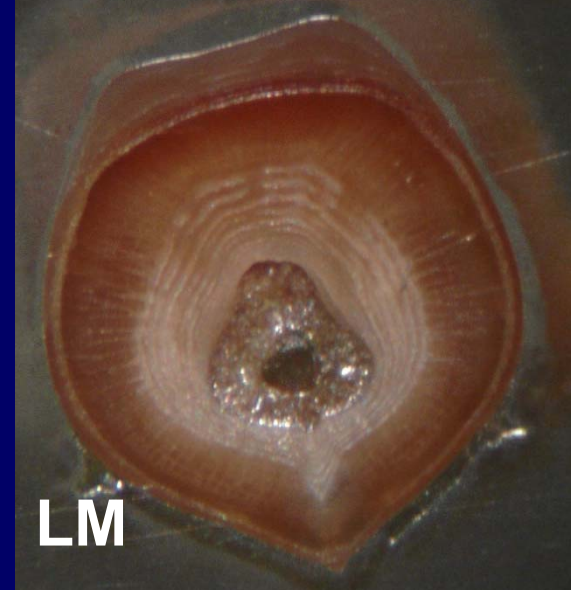
Gonodactylus sp.

Electrons rétrodiffusés déviés par le noyau) détectés en MEB

Microscopie électronique à balayage = MEB

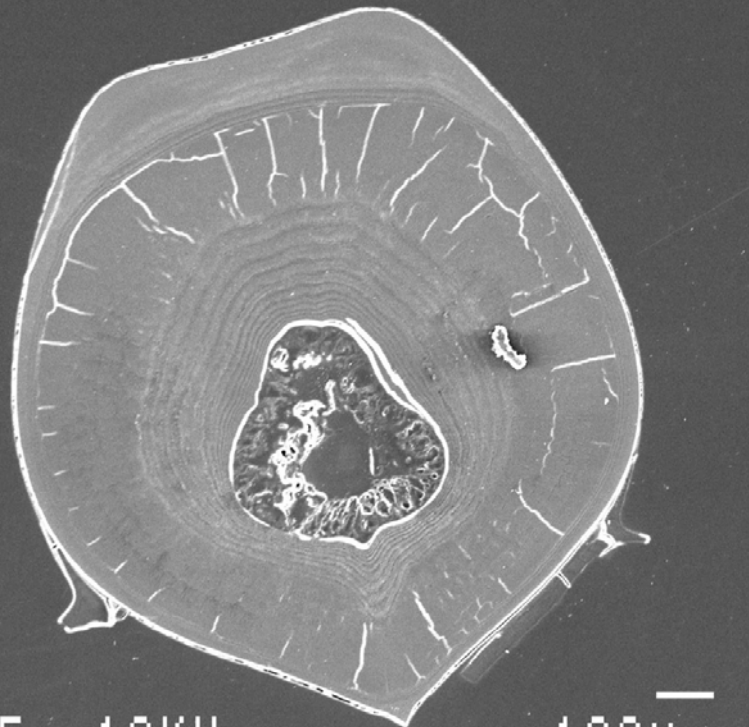


Section transversale de la patte frappeuse



LM

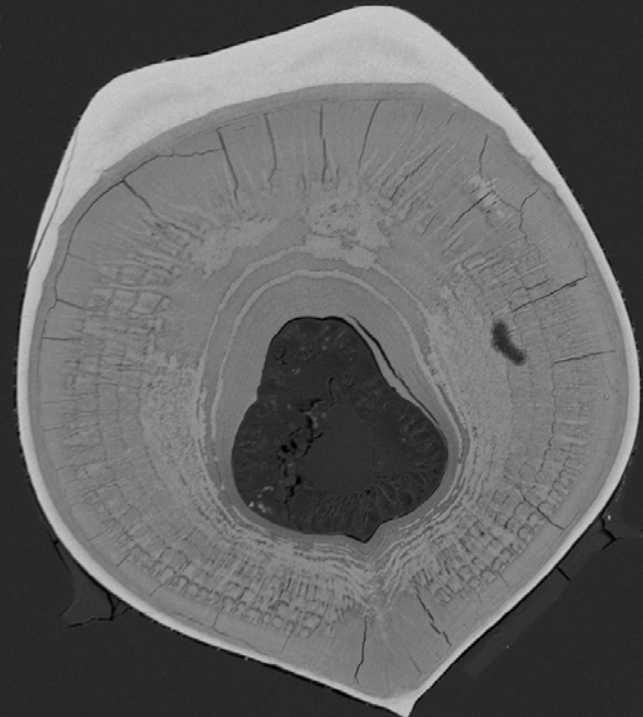
SE



3865 19KV

100µm

BSE



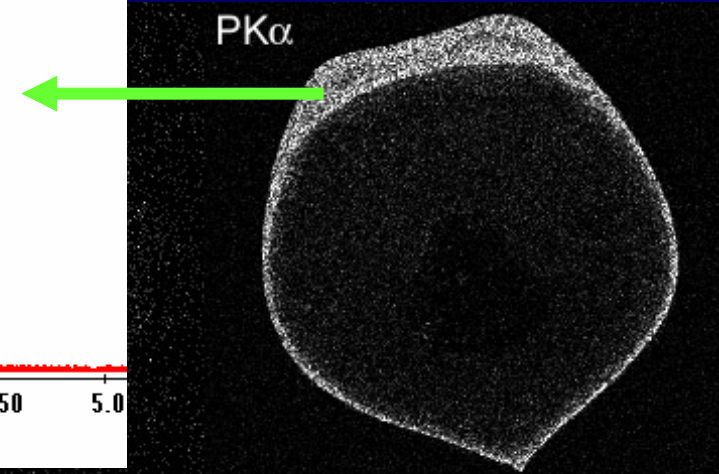
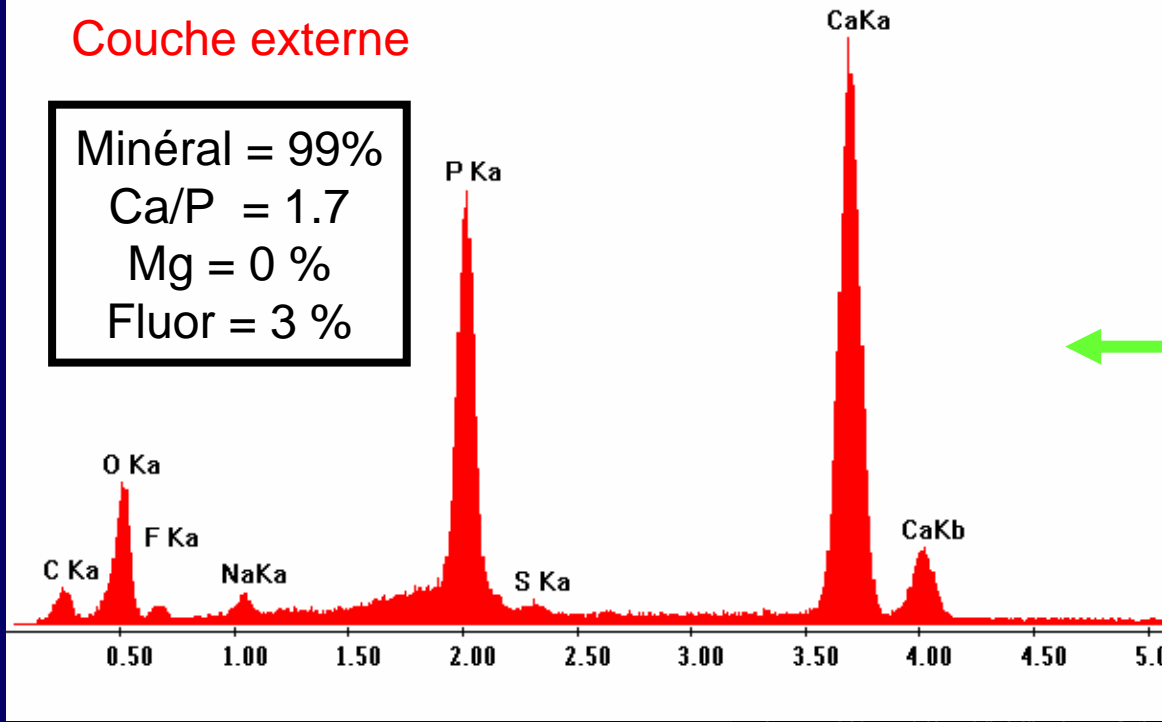
3867 19KV

100µm

Gonodactylus Patte frappeuse

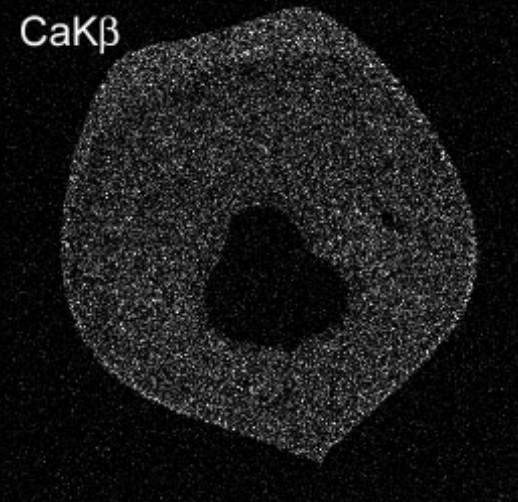
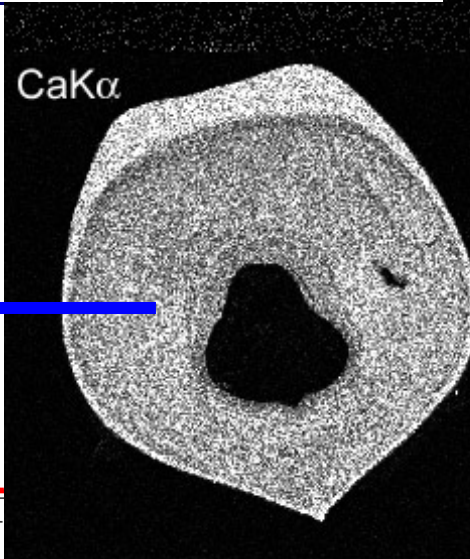
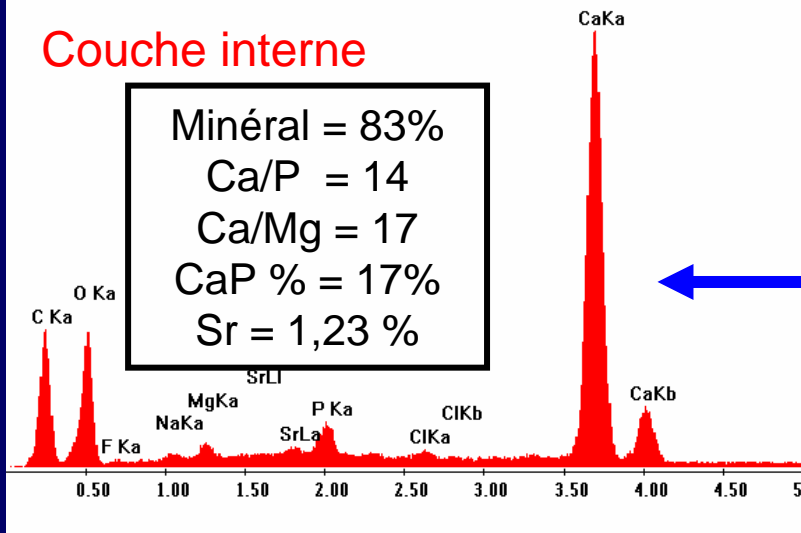
Couche externe

Minéral = 99%
Ca/P = 1.7
Mg = 0 %
Fluor = 3 %

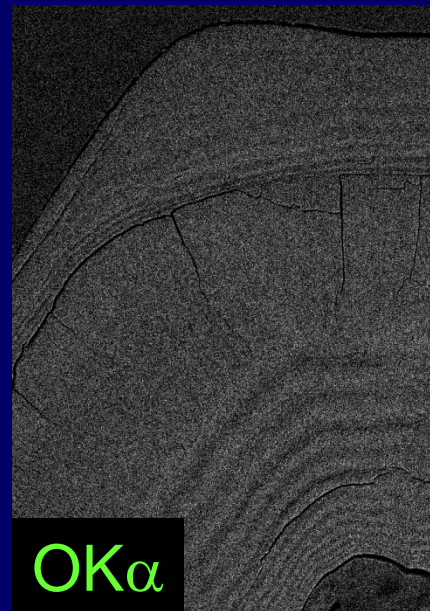
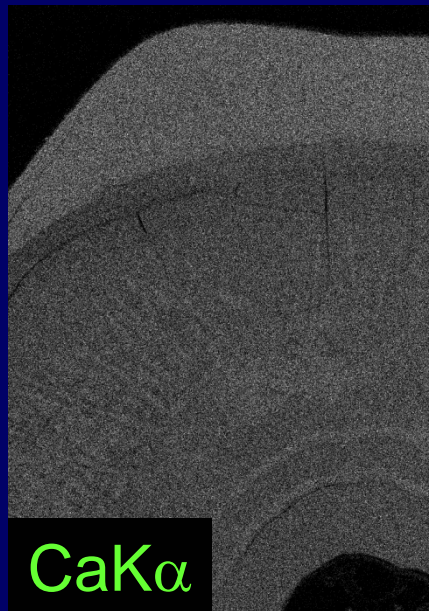
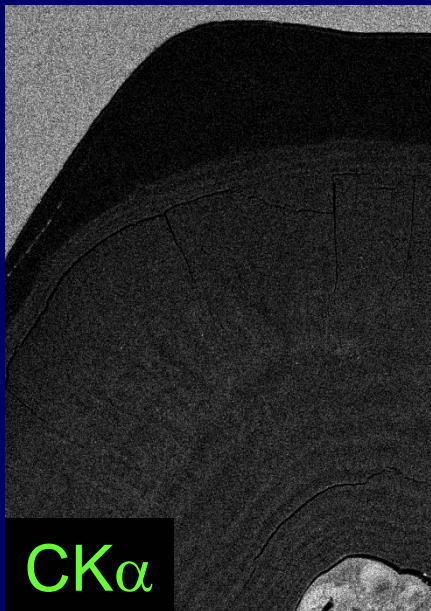
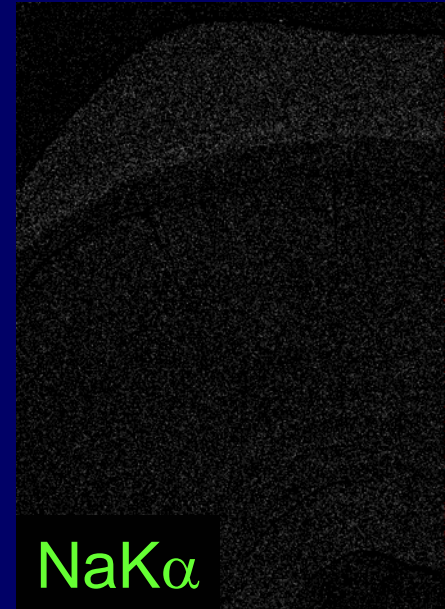
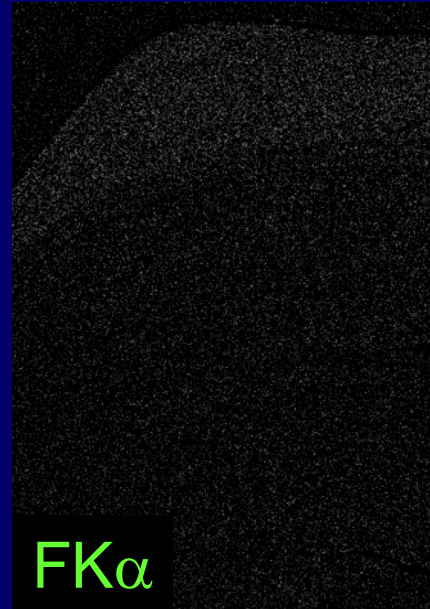
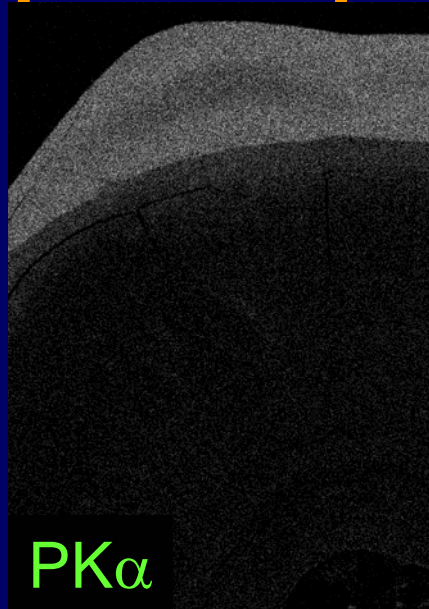


Couche interne

Minéral = 83%
Ca/P = 14
Ca/Mg = 17
CaP % = 17%
Sr = 1,23 %

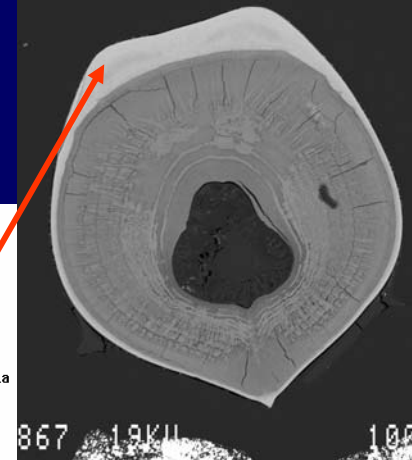


Cartographies à plus haute résolution

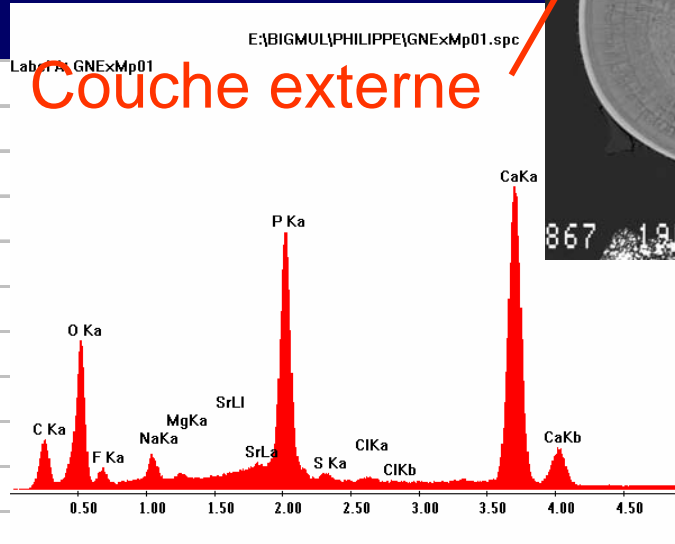


Exemple d'analyse quantitative

Patte frappeuse de *Gonodactylus*



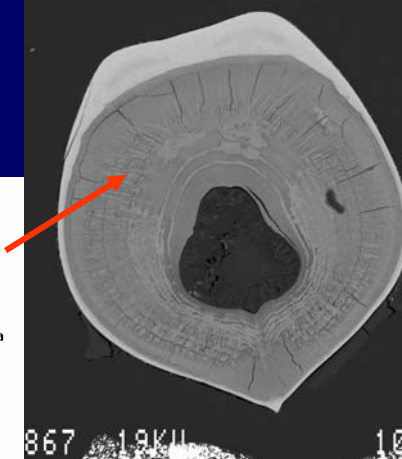
E:\BIGMUL\PHILIPPE\GNExMp01.spc			
Acquisition Time	Date: 8-Nov-2007		
kV:15.00	Tilt: 0.00	Take-off:34.6	Tc:35.0
Detector Type	Resolution :1	Lsec :100	
EDAX ZAF C Standardless			
Element Normalized			
SEC Table : Default			



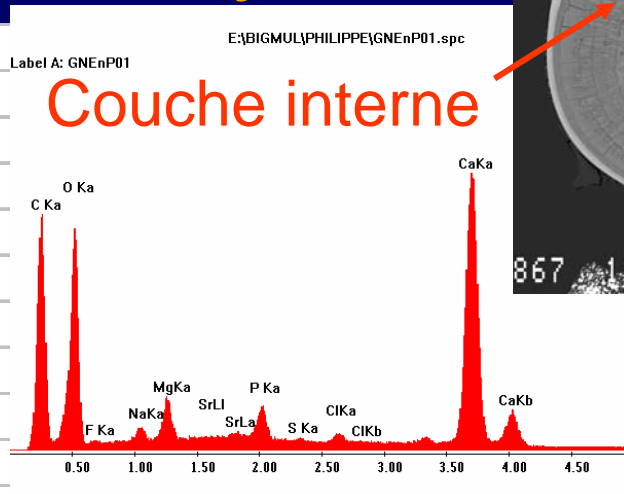
Element	Wt %	At %	K-Ratio	Z	A	F
C K	16,08	29,11	0,0371	1,0657	0,2166	1,0005
O K	25,24	34,31	0,0468	1,0454	0,1772	1,0002
F K	3,19	3,66	0,0065	0,9814	0,2076	1,0004
NaK	2,05	1,94	0,0095	0,9752	0,4769	1,0016
MgK	0,77	0,69	0,0048	0,9988	0,6168	1,0032
SrL	2,98	0,74	0,0234	0,7754	1,0002	1,0117
P K	15,4	10,82	0,1312	0,9597	0,8814	1,0072
S K	0,76	0,51	0,0063	0,9832	0,8455	1,0105
ClK	0,49	0,3	0,0042	0,9399	0,894	1,018
CaK	33,04	17,93	0,3095	0,9629	0,973	1
Total	100	100				

Exemple d'analyse quantitative

Patte frappeuse de *Gonodactylus*



E:\BIGMUL\PHILIPPE\GNEnP01.spc			
Acquisition Time	Date: 8-Nov-2007		
kV:15.00	Tilt: 0.00	Take-off:34.6	Tc:35.0
Detector Type	Resolution :1	Lsec :100	
EDAX ZAF C	Standardless		
Element Normalized			
SEC Table : Default			



Element	Wt %	At %	K-Ratio	Z	A	F
C K	33,81	49,09	0,1512	1,0382	0,4305	1,0005
O K	31,75	34,61	0,0614	1,0186	0,1898	1,0001
F K	0,72	0,66	0,0014	0,9563	0,2025	1,0002
NaK	0,87	0,66	0,004	0,9505	0,4849	1,0008
MgK	1,99	1,43	0,0123	0,9736	0,6352	1,0012
SrL	0,67	0,13	0,0051	0,7528	1,0076	1,0056
P K	1,74	0,98	0,0148	0,9323	0,9052	1,0077
S K	0,11	0,06	0,001	0,956	0,9383	1,0141
ClK	0,5	0,25	0,0045	0,9144	0,9682	1,0247
K K	0,61	0,27	0,006	0,9173	0,9976	1,0709
CaK	27,24	11,85	0,2559	0,9367	1,0032	1
Total	100	100				