Calcium carbonate microstructures in mollusc sea-shells: a voyage with the x-ray shuttle

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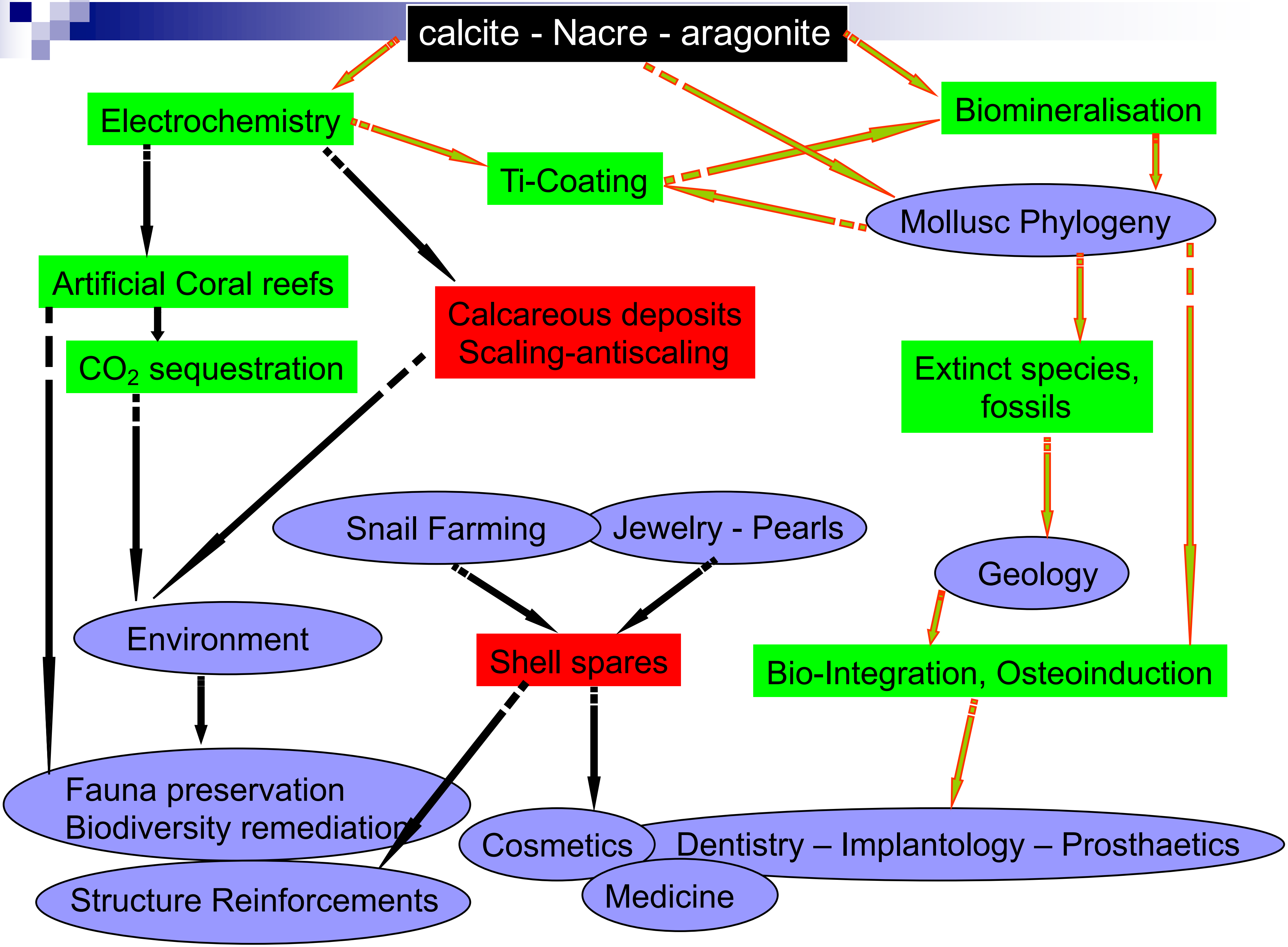
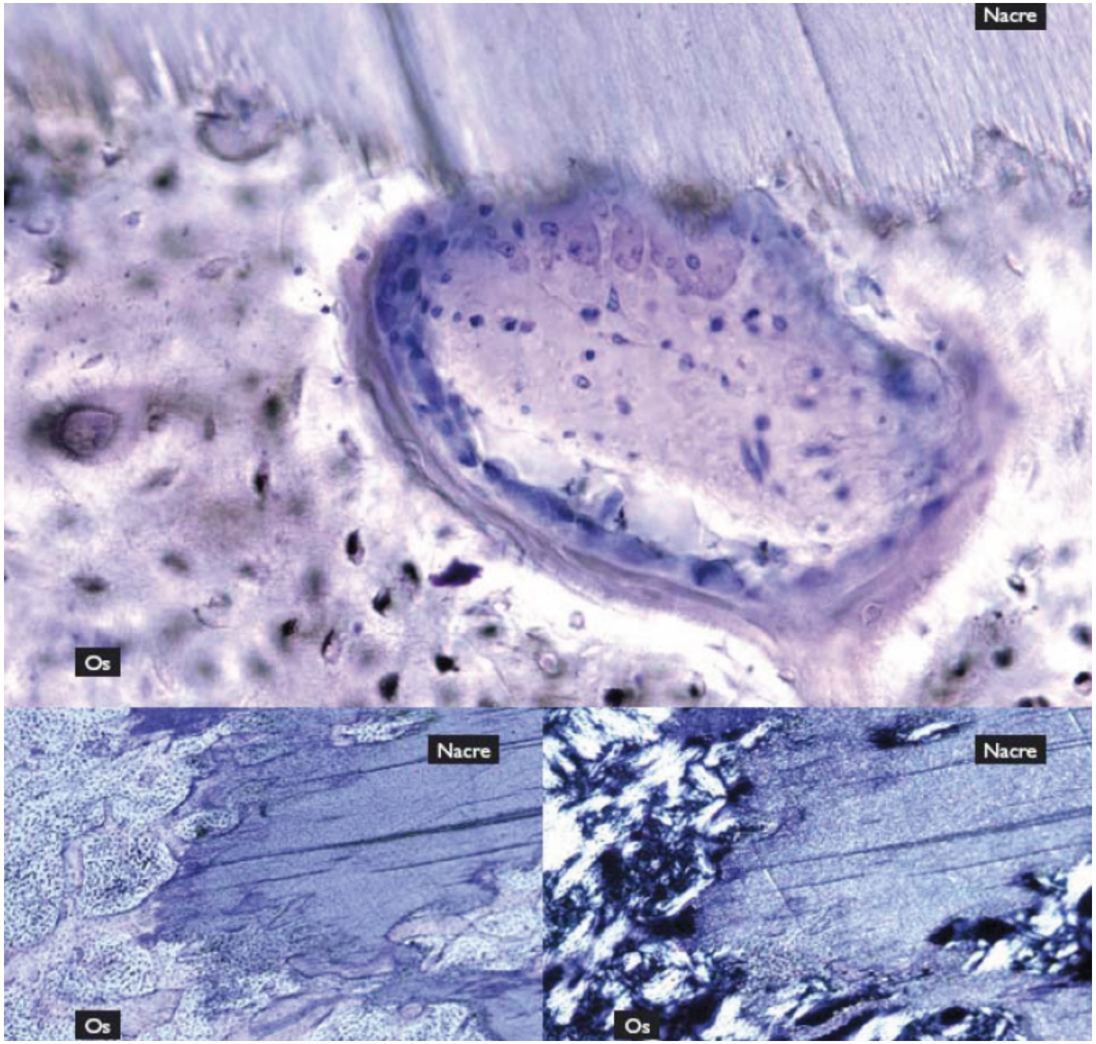

calcite - Nacre - aragonite
Electrochemistry
Biomineralisation
Ti-Coating
Mollusc Phylogeny
Artificial Coral reefs
Calcareous deposits
Scaling-antiscaling
CO_2 sequestration
Extinct species,
fossils
Snail Farming
Jewelry - Pearls
Geology
Environment
Shell spares
Bio-Integration, Osteoinduction
Fauna preservation
Biodiversity remediation
Cosmetics
Dentistry – Implantology – Prosthaetics
Medicine
Structure Reinforcements

4000 BC maya cranes, Honduras

Amadéo Bobbio (1972) *Bull. Historical Dentology*

Evelyne Lopez, MNHN, Paris



Bone-cells stimulation at the nacre/bone interface

Penetration of neo-bone inside nacre

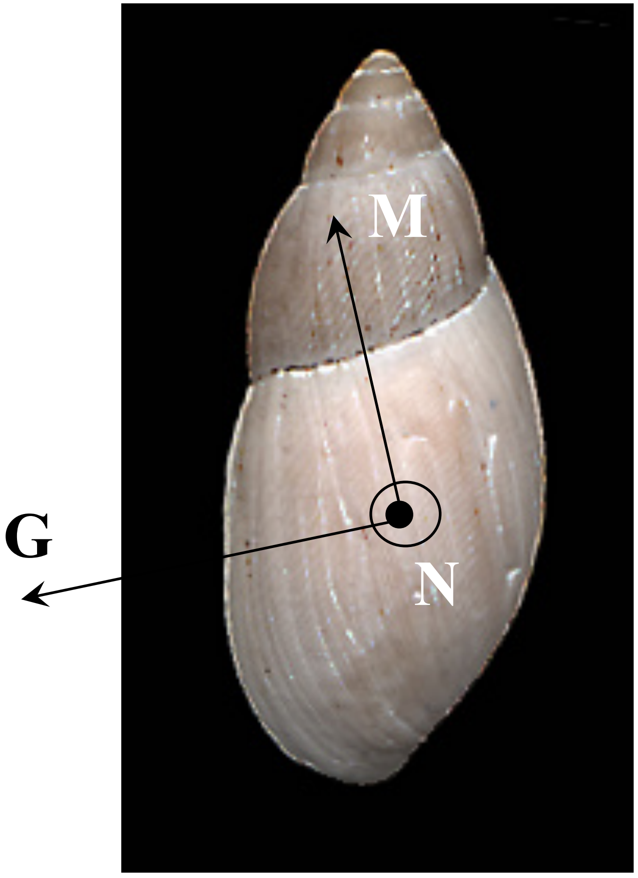
Evelyne Lopez *et al*. (1992) *Tissue & Cell*

Why rays (X, n, e^-) diffraction ?

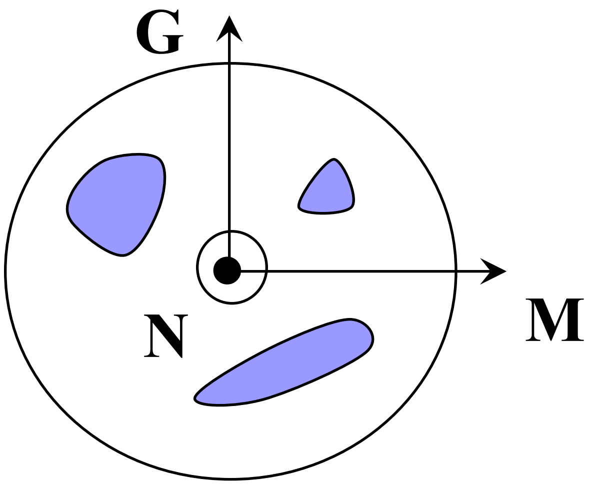
- Microstructure versus texture
- Mollusc Phylogeny (Texture …)
- A link to mollusc ancestors
- Cell distortions in biogenic crystals
- Synthetic nacre-like biocrystals
- In corals

Reference frame

Euglandina rosea: a land snail, carnivorous mollusc introduced in Pacific and Indian oceans, to regulate *Achatina fulica*

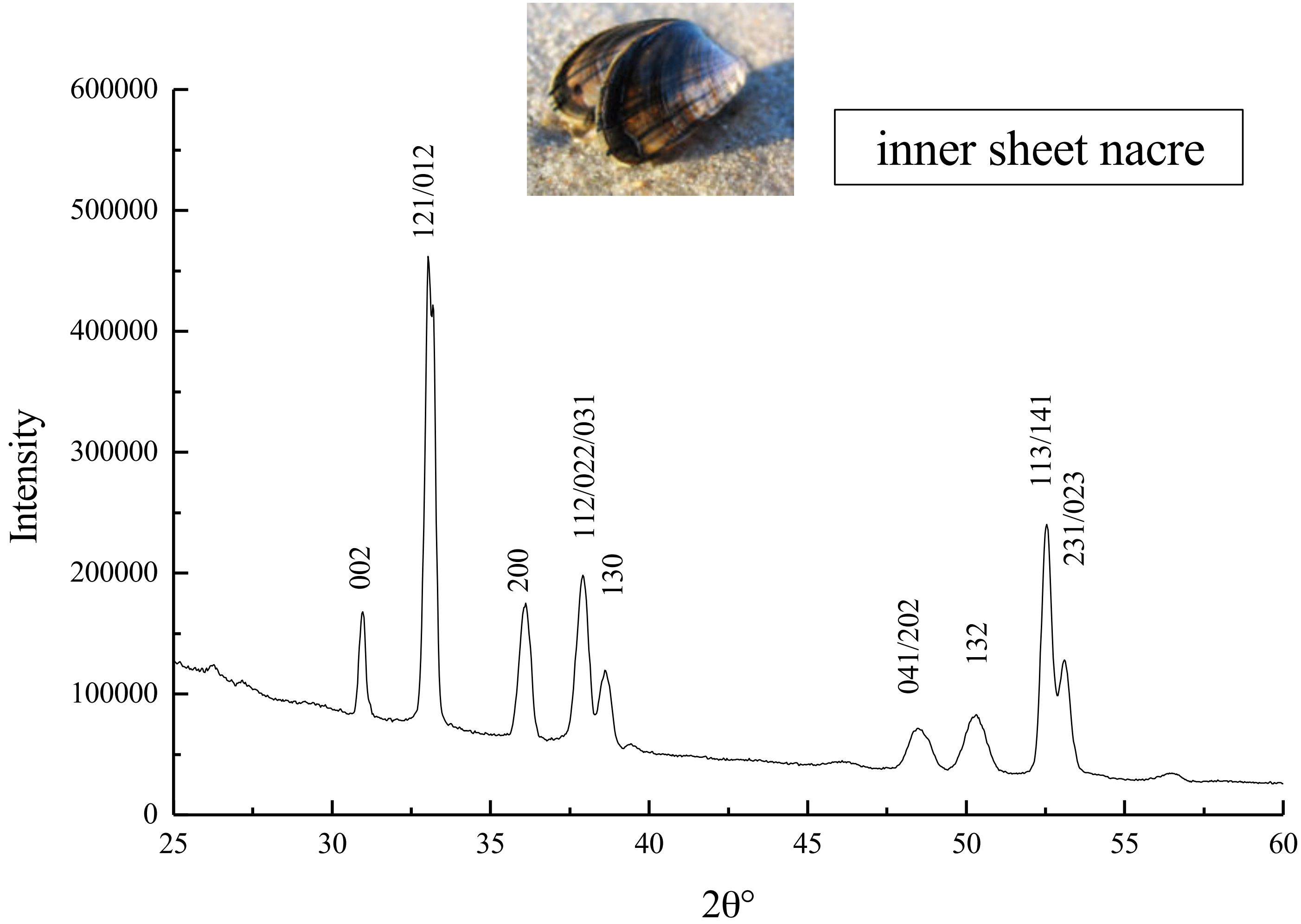


- Crystal: $CaCO_3$, aragonite (Pmcn) or calcite ($R\bar{3}c$)
- Sample: triclinic

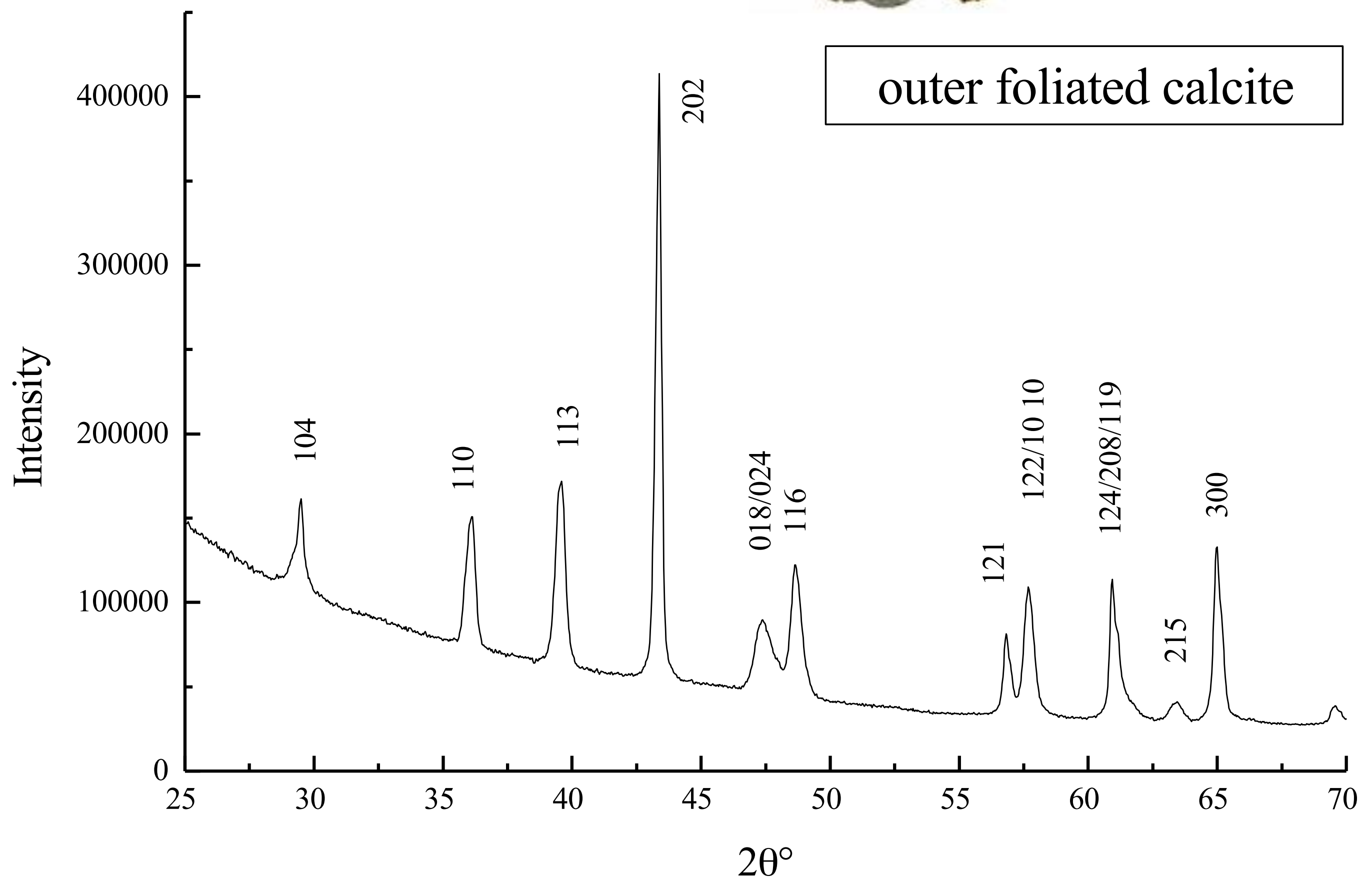


Typical patterns: using the CPS120-INEL

Mytilus edulis (common mussel): sum diagrams

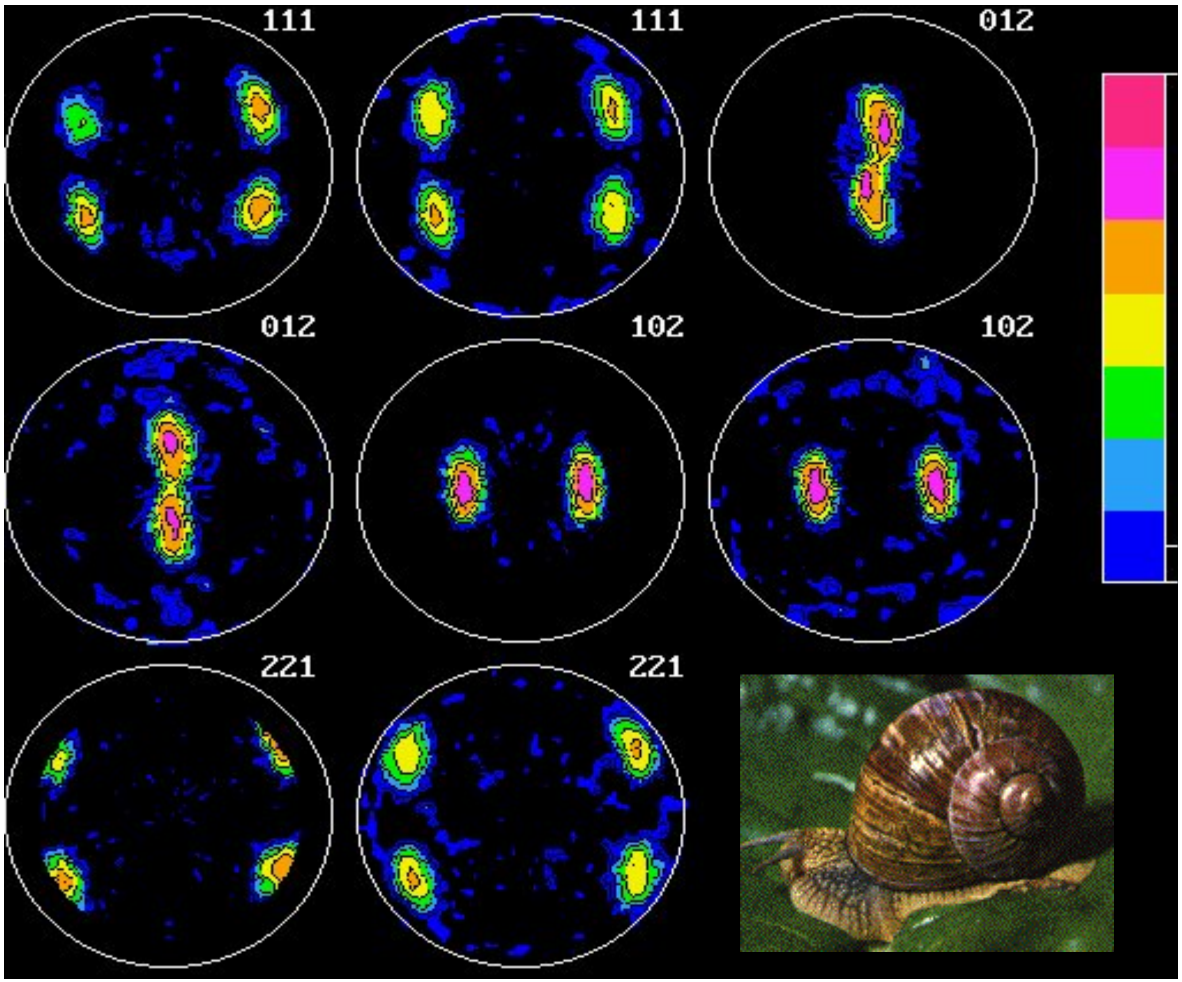


Crassostrea gigas (common oyster)



Measured for around 1000 sample orientations, using x-rays, neutrons or electrons, depending on the desired probed volume

OD-reliability: *Helix pomatia* (Burgundy land snail: Outer com. crossed lamellar)



22.7

Lin. scale

Eq. area

1 m.r.d.

$RP_{0.05} = 67\%$

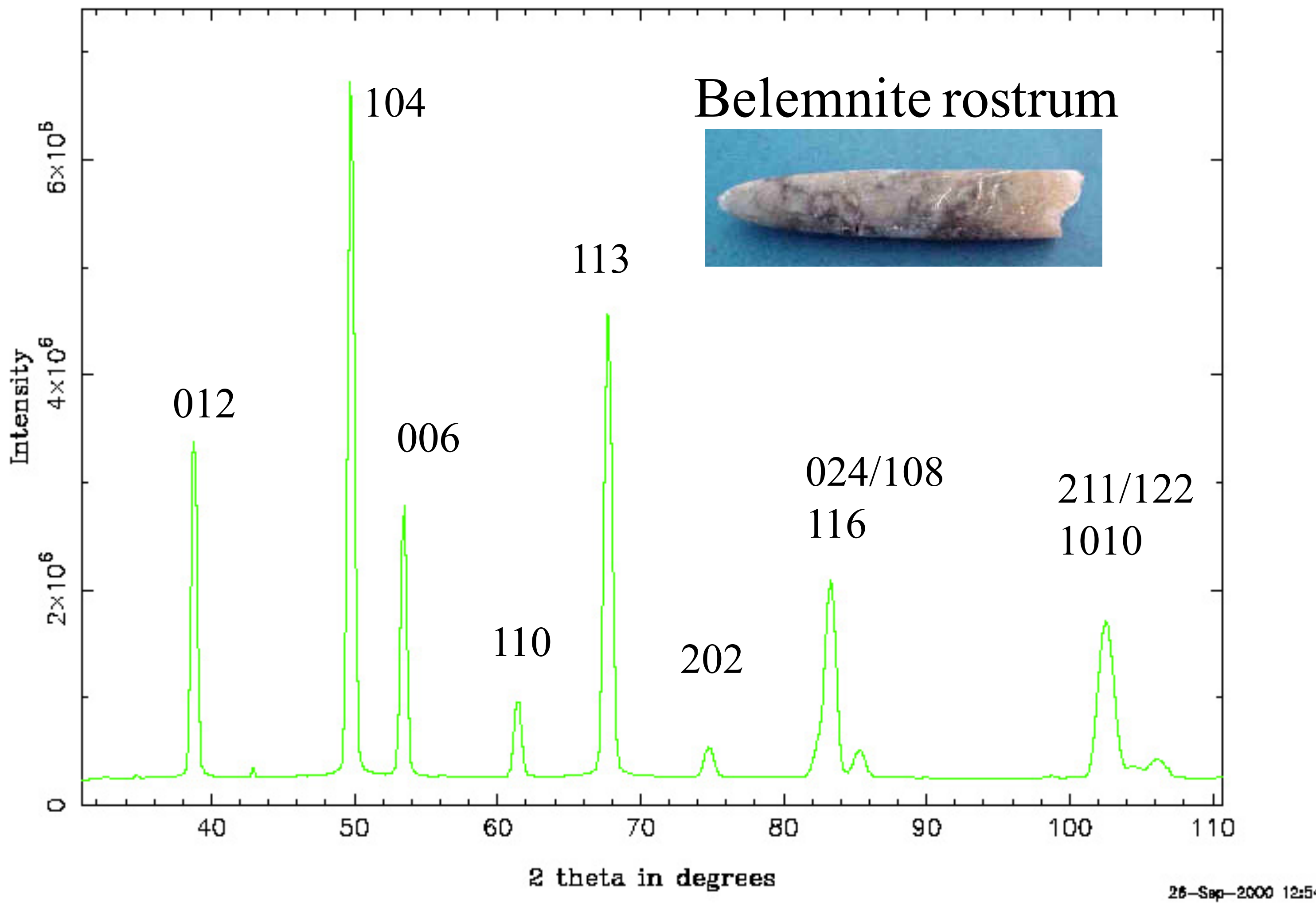
$RP_1 = 40\%$

$S = -4.1$

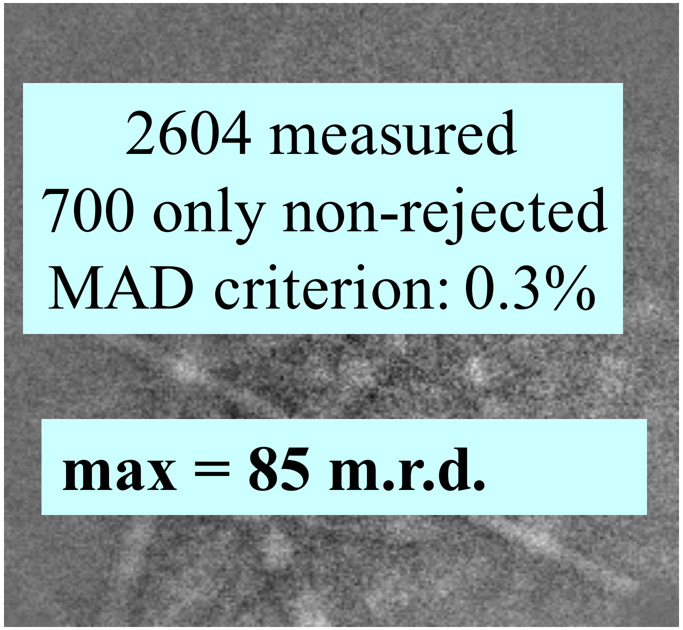
$F^2 = 106$ m.r.d.^{2}

$OD_{max} = 444$ m.r.d.

D1B-ILL experiments

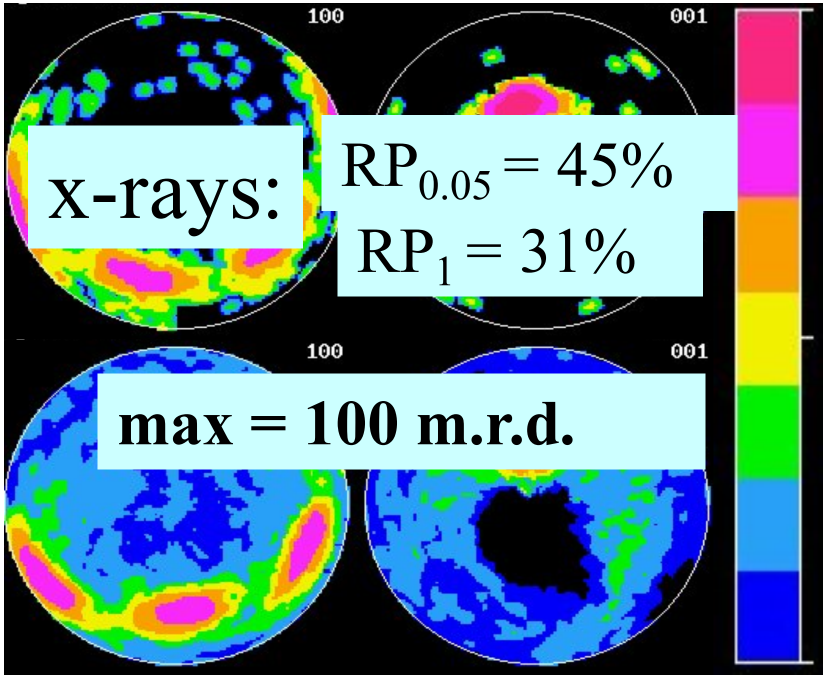


Crassostrea gigas (Inner foliated calcite)



Kikuchi diagrams

Electrons

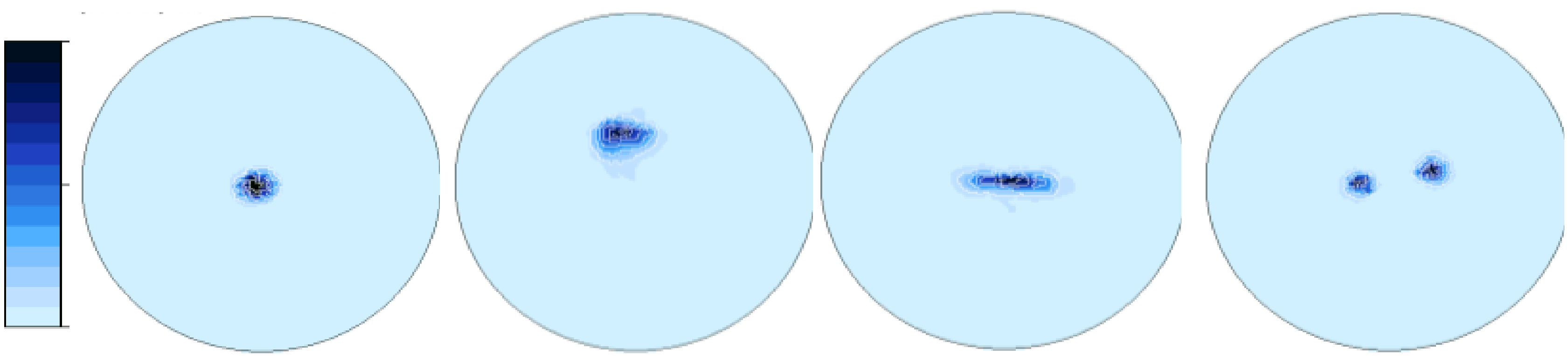


x-rays

Global analysis is coherent with local ones like synchrotron microfocus x-rays *(Aizenberg et al. (1996) Connective Tissue Research* ***34*** *255)*

c-axes texture patterns

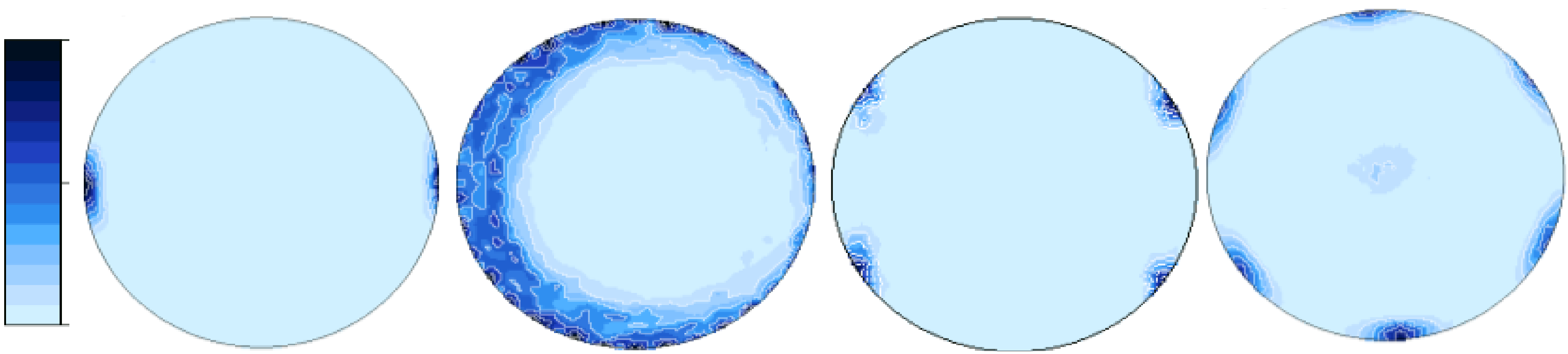
Pinctada maxima	*Nerita polita*	*Fragum fragum*	*Cypraea testudinaria*
ISN	ICCL	ICCL	ICCL
"gold pearl oyster"	"polished nerite"	"cockle"	"turtle cowry"



⊥ ∠ ∀ ∨

a-axes texture patterns

Helix pomatia	*Tectus niloticus*	*Conus leopardus*	*Nautilus pompilius*
OCCL	ICN	ICCL	ICN
"burgundy land snail"	"commercial top shell"	"leopard cone"	"new caledonia nautilus"

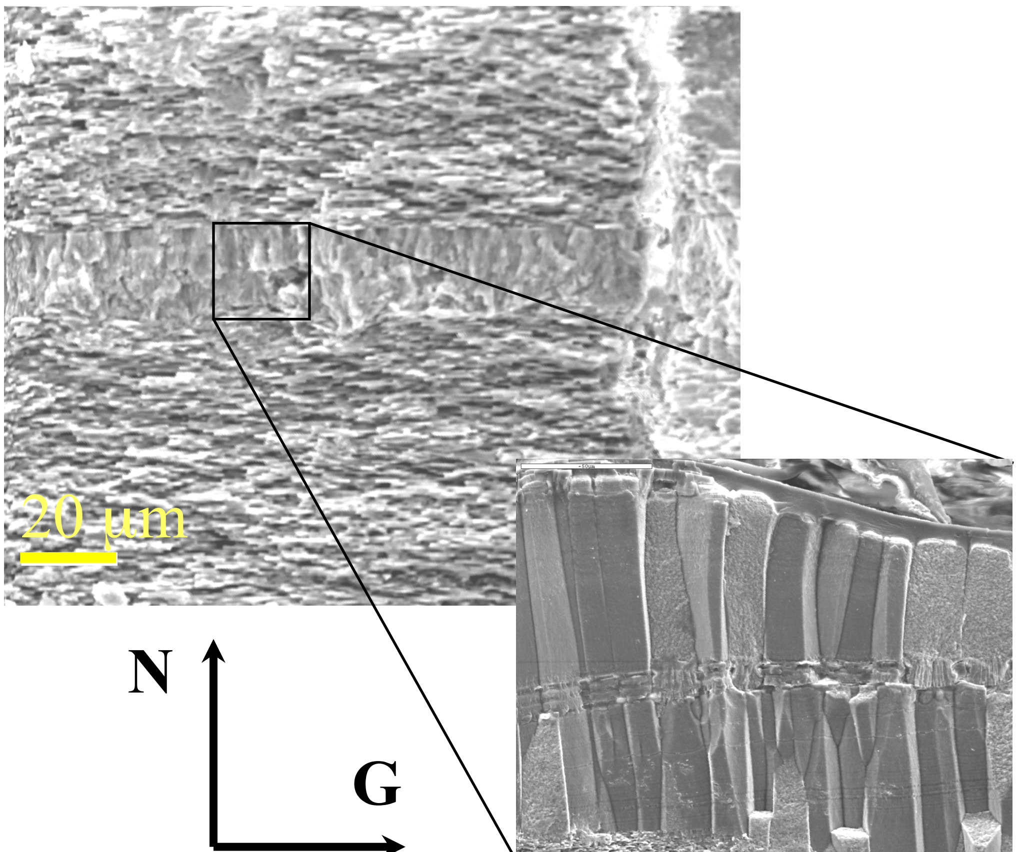


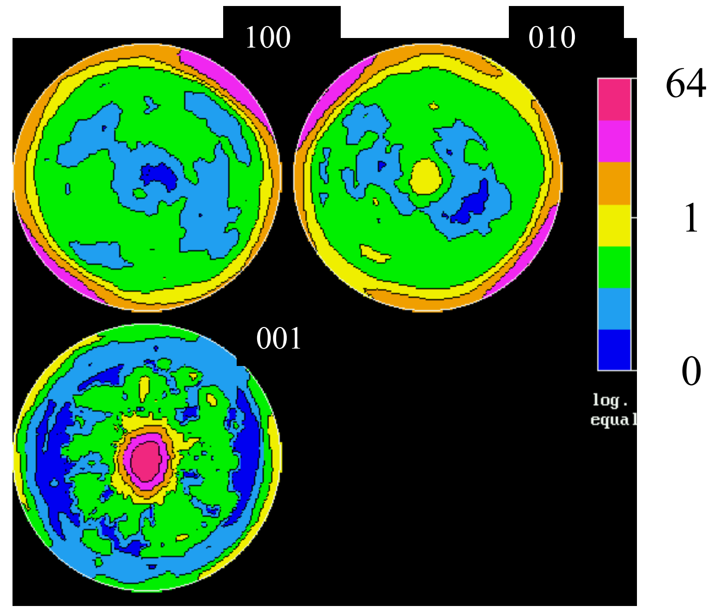
|	⭘	×	✱

Chateigner, Hedegaard, Wenk, J. Struct. Geol. ***22*** *(2000) 1723*

Microstructure versus texture

Inner sheet nacre of *Anodonta cygnea* (freshwater swan mussel)

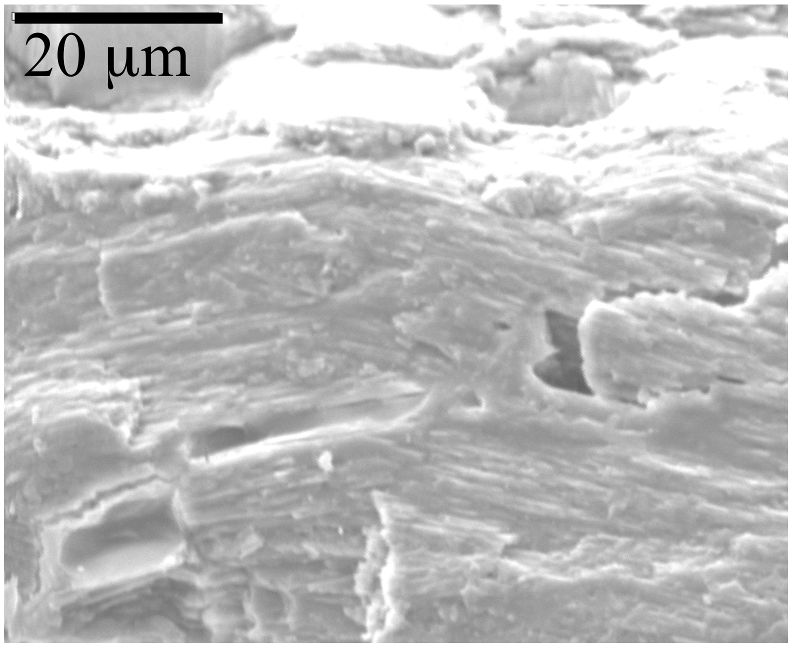


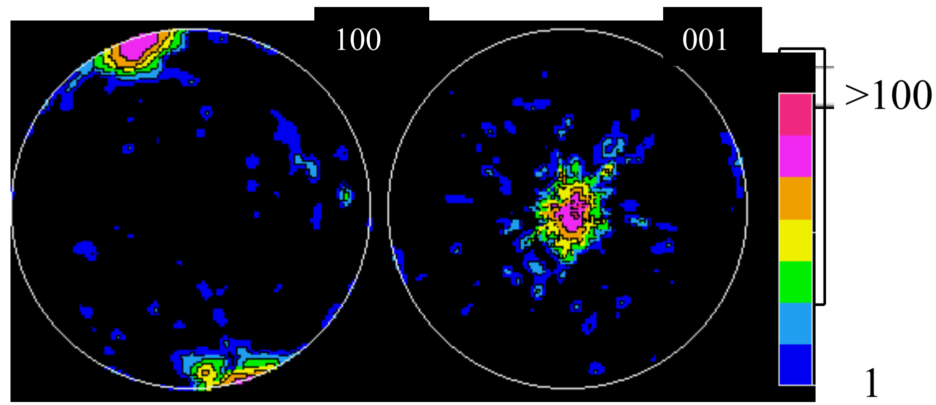


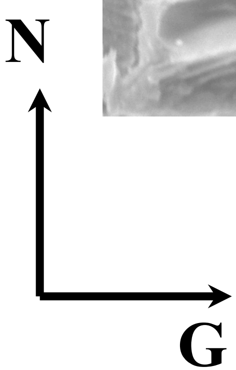
$$\left\langle \perp \middle| \mathrm{ISN} \middle| *^{a,\,-45}_{25} \right\rangle$$

Microstructure versus texture

Cyclophorus woodianus: different crystal orientations look like single crystal from diffraction !







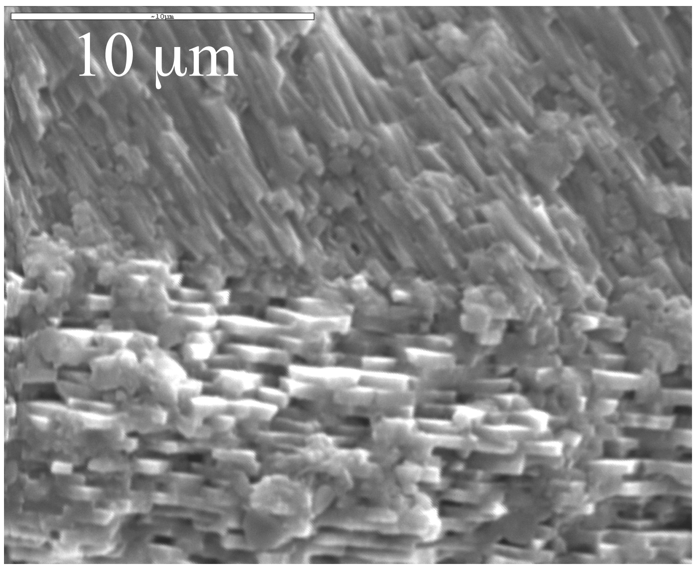
$$\left\langle \perp \middle| \mathrm{IRCL} \middle|^{a,\,20} \right\rangle$$

Texture parameters may deserve phylogenic analysis

Microstructure versus texture

Bathymodiolus thermophilus (-2400m deep event mussel)

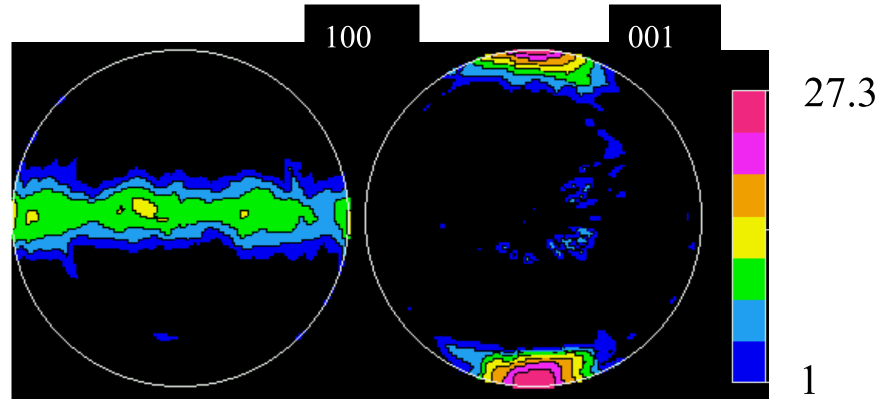
$\left\langle \angle,90 \middle| \mathrm{OFC} \middle| \mathrm{I}^{c,\,0} \right\rangle$

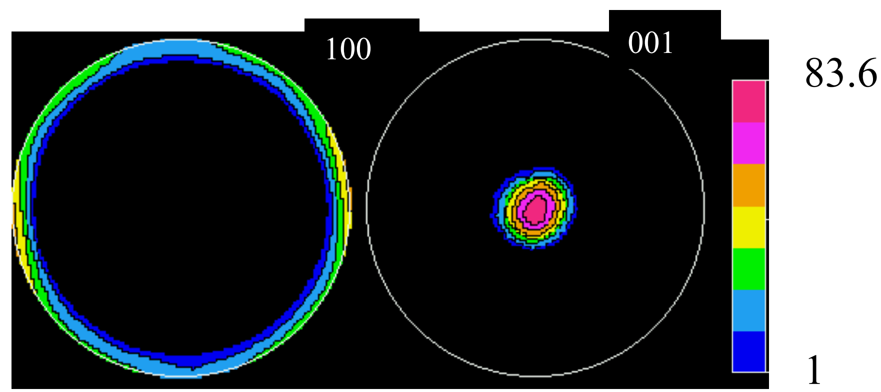


N

G

$\left\langle \perp \middle| \mathrm{ISN} \middle| *^{a,\,90}_{38} \right\rangle$

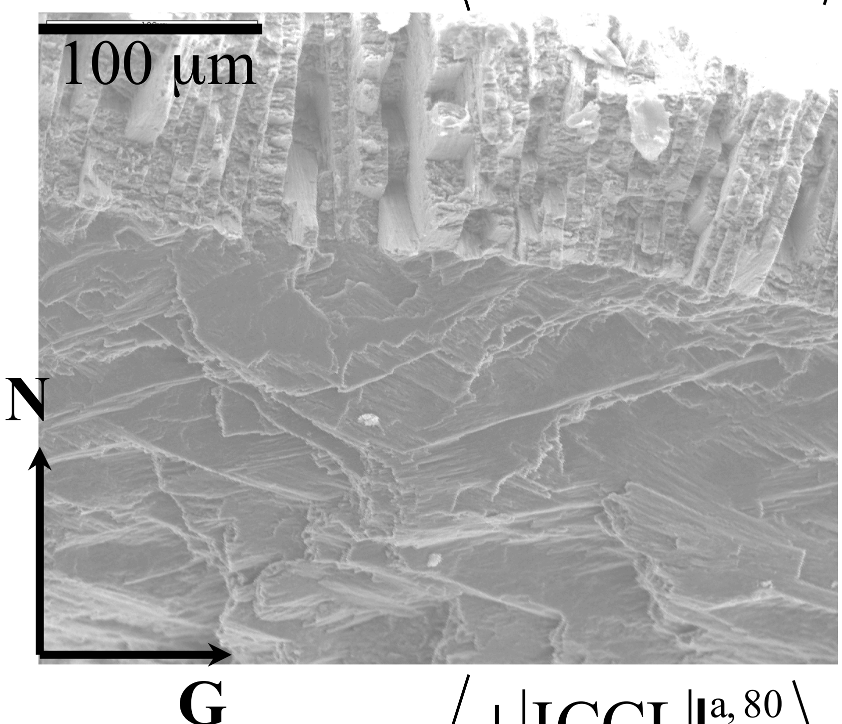




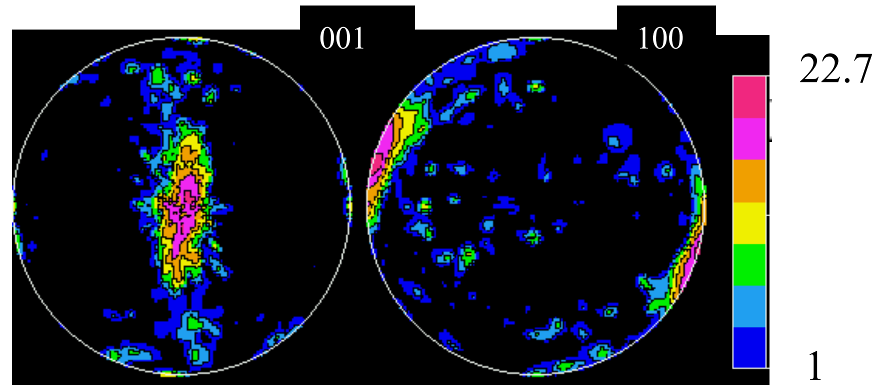
Microstructure versus texture

Euglandina rosea different crystallite shapes, close orientations !

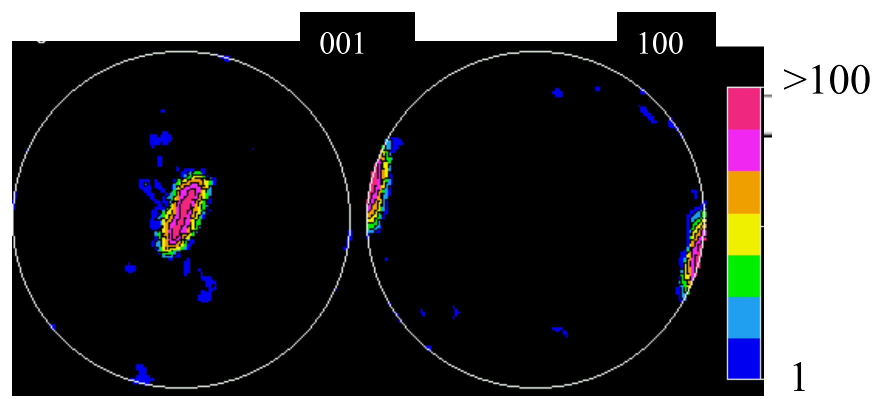
$$\langle \perp | ORCL |^{a,75} \rangle$$



$$\langle \perp | ICCL |^{a,80} \rangle$$



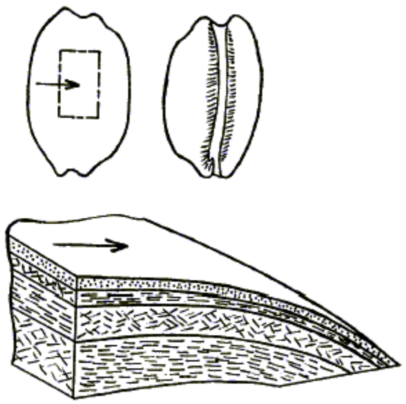
no inter-mineral epitaxy

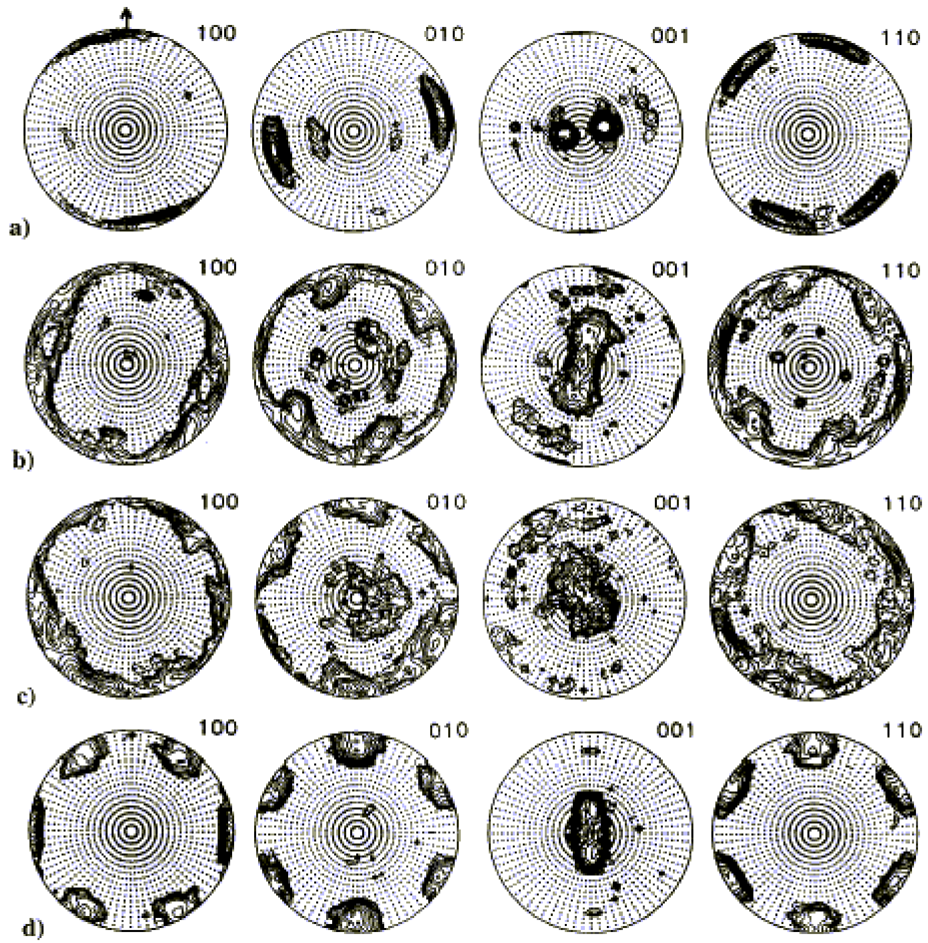


Microstructure versus texture

Inner sheet nacre of *Cypraea testudinaria* (cowry):

no inter-layer epitaxy



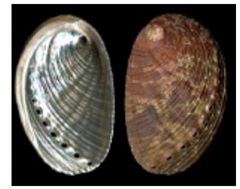


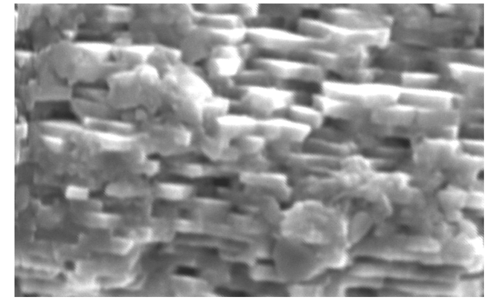
Organically driven growth

Dealing with nacre

Gastropods *Columnar Nacre*

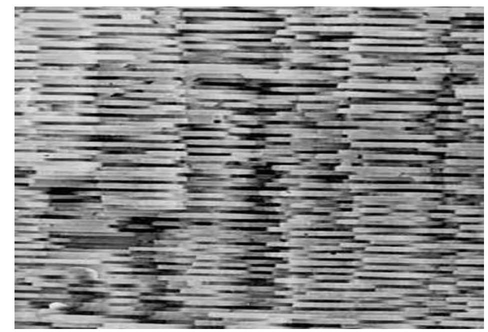
Haliotis tuberculata (common abalone)



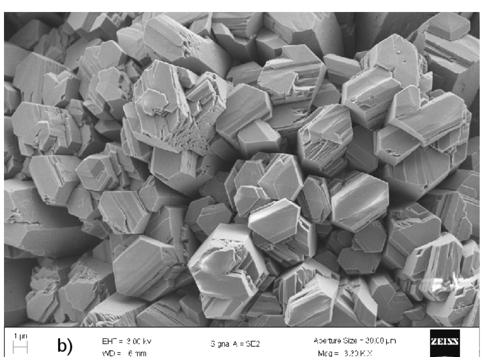


Bivalves *Sheet Nacre*

Pinctada maxima (Mother of pearl oyster)



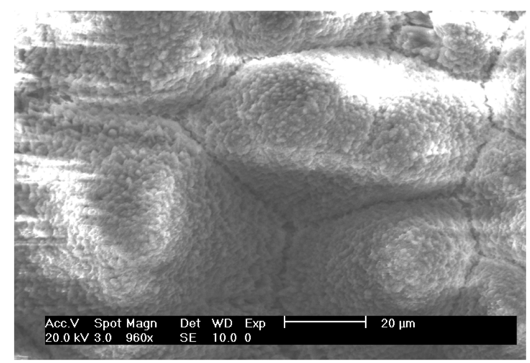
Electrodeposited $CaCO_3$/Ti-Al-V coatings



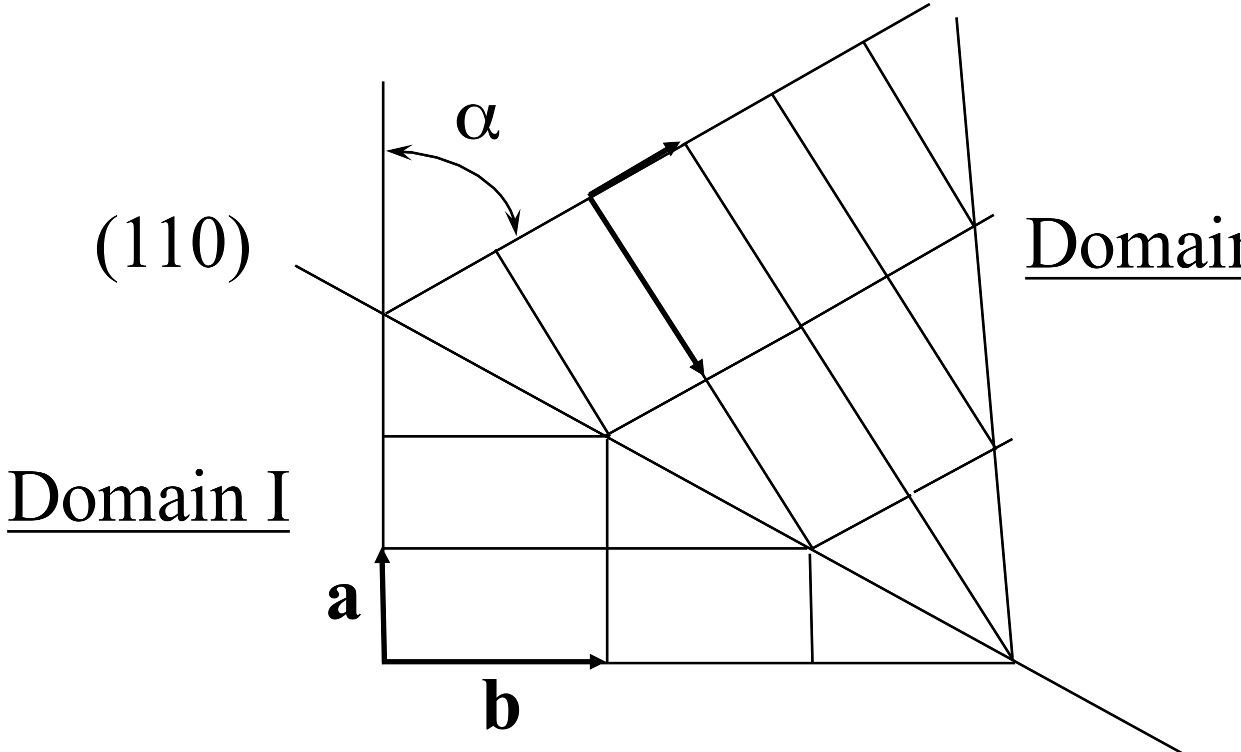
Inorganic

non-polar extract

Pinctada maxima

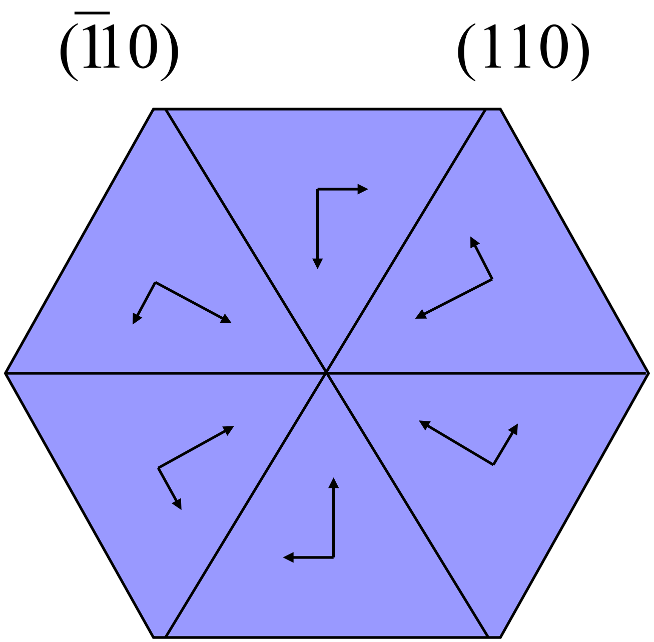


Twinning in aragonite ...

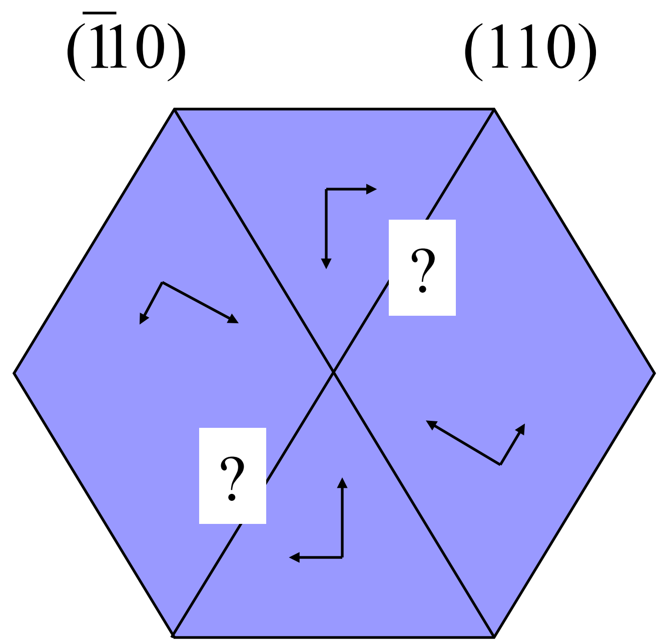


$\alpha = 2\arctan(a/b) = 63.8°$

… forms nacre platelets ...

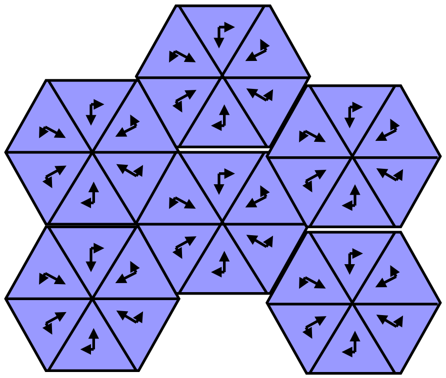


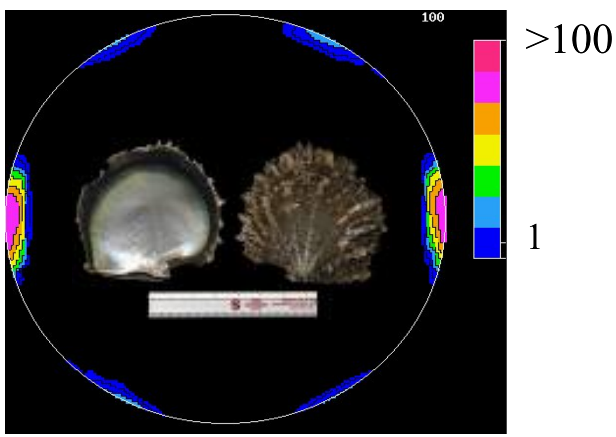
Bragg, 1937



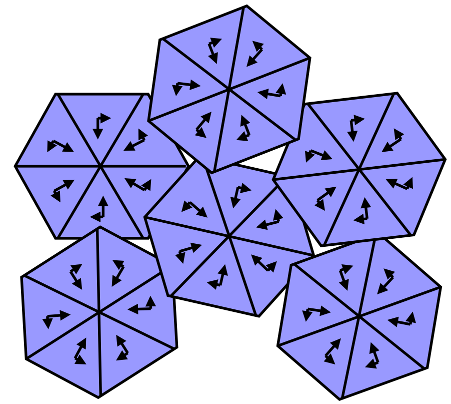
Mutvei, 1980

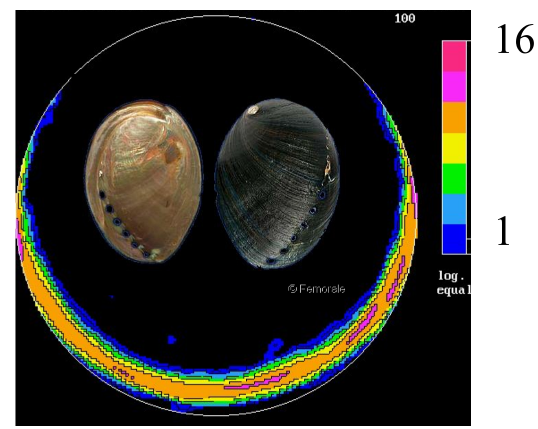
… that rearrange ...





Pinctada margaritifera
(black pearl oyster)



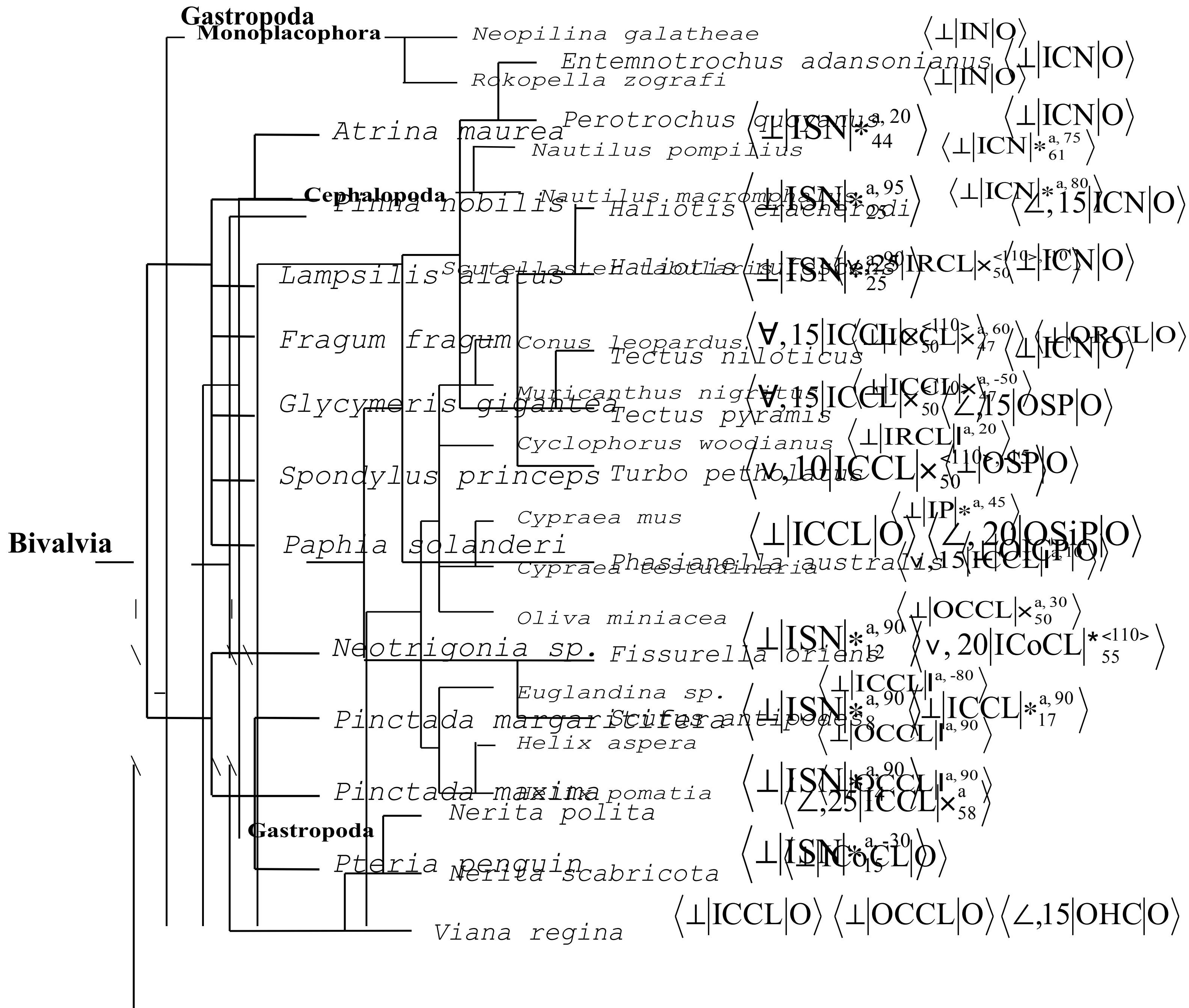


Haliotis cracherodi
(black abalone)

QTA and Mollusc Phylogeny

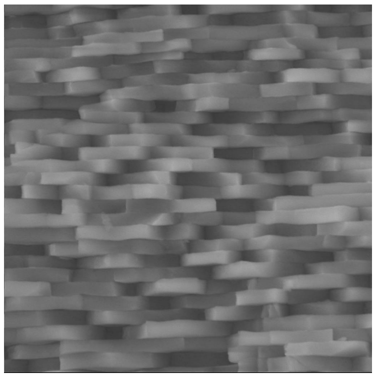
Around 80 mollusc species (gastropods, bivalves, monoplacophoras and cephalopods), around 150 layers studied, incl. fossils

Closely related species, close textural characters, but significant variations: textural parameters can serve character analysis

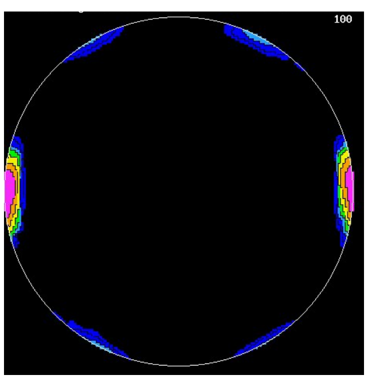


Gastropoda
Monoplacophora
Neopilina galatheae
Entemnotrochus adansonianus
Rokopella zografi
Atrina maurea
Perotrochus quoyanus
Nautilus pompilius
Cephalopoda
Pinna nobilis
Nautilus macromphalus
Haliotis discus
Lampsilis alatus
Fragum fragum
Conus leopardus
Tectus niloticus
Glycymeris gigantea
Muricanthus nigritus
Tectus pyramis
Cyclophorus woodianus
Spondylus princeps
Turbo petholatus
Cypraea mus
Bivalvia
Paphia solanderi
Phasianella australis
Cypraea testudinaria
Oliva miniacea
Neotrigonia sp.
Fissurella oriens
Euglandina sp.
Pinctada margaritifera
Helix aspera
Pinctada maxima
Helix pomatia
Nerita polita
Gastropoda
Pteria penguin
Nerita scabricota
Viana regina

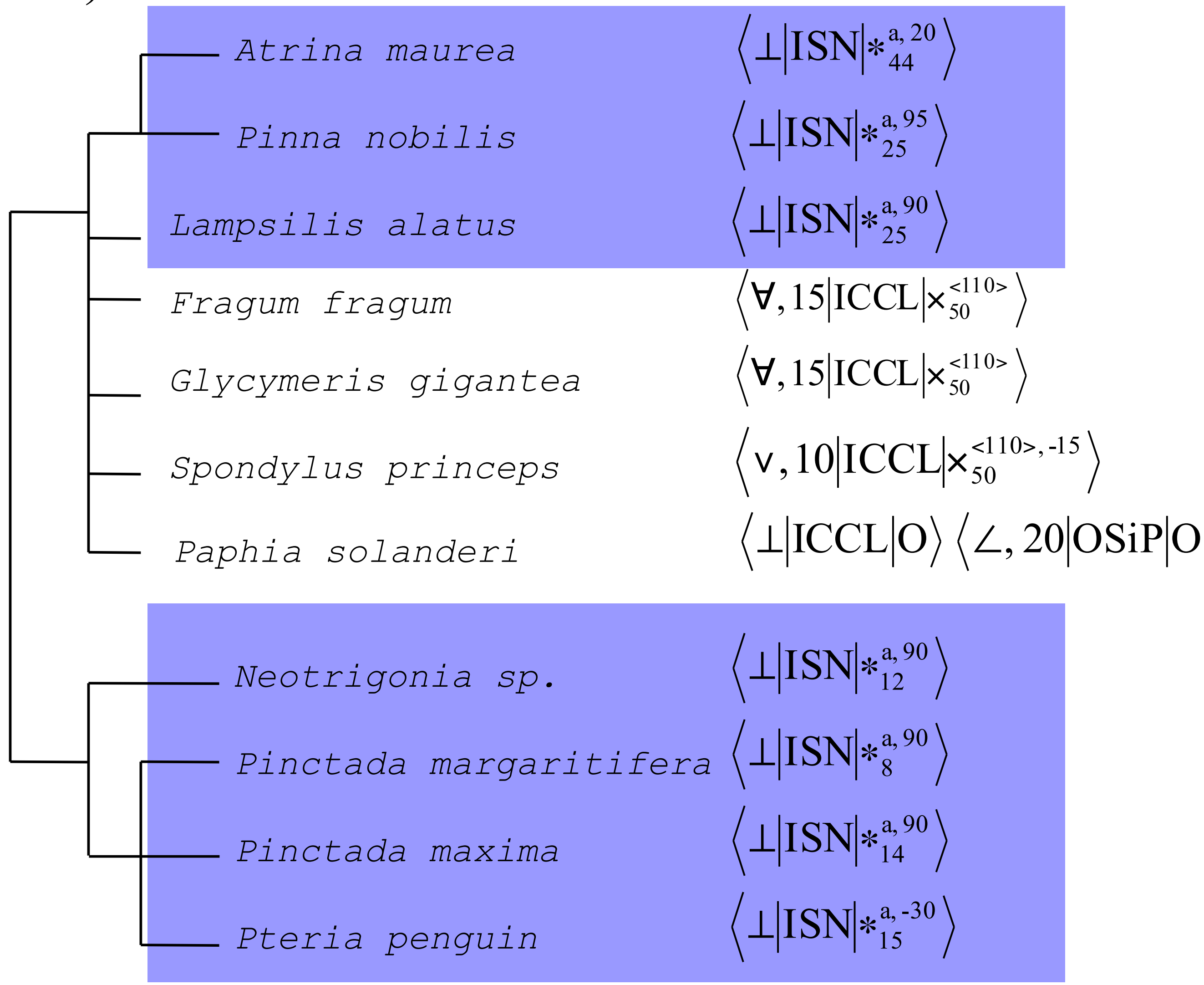
Pinctada margaritifera, P. maxima and *Pinna nobilis* nacres: Bio-compatible and osteo-inductive for human osteoblasts (E. Lopez (MNHN, Paris)



Bivalvia

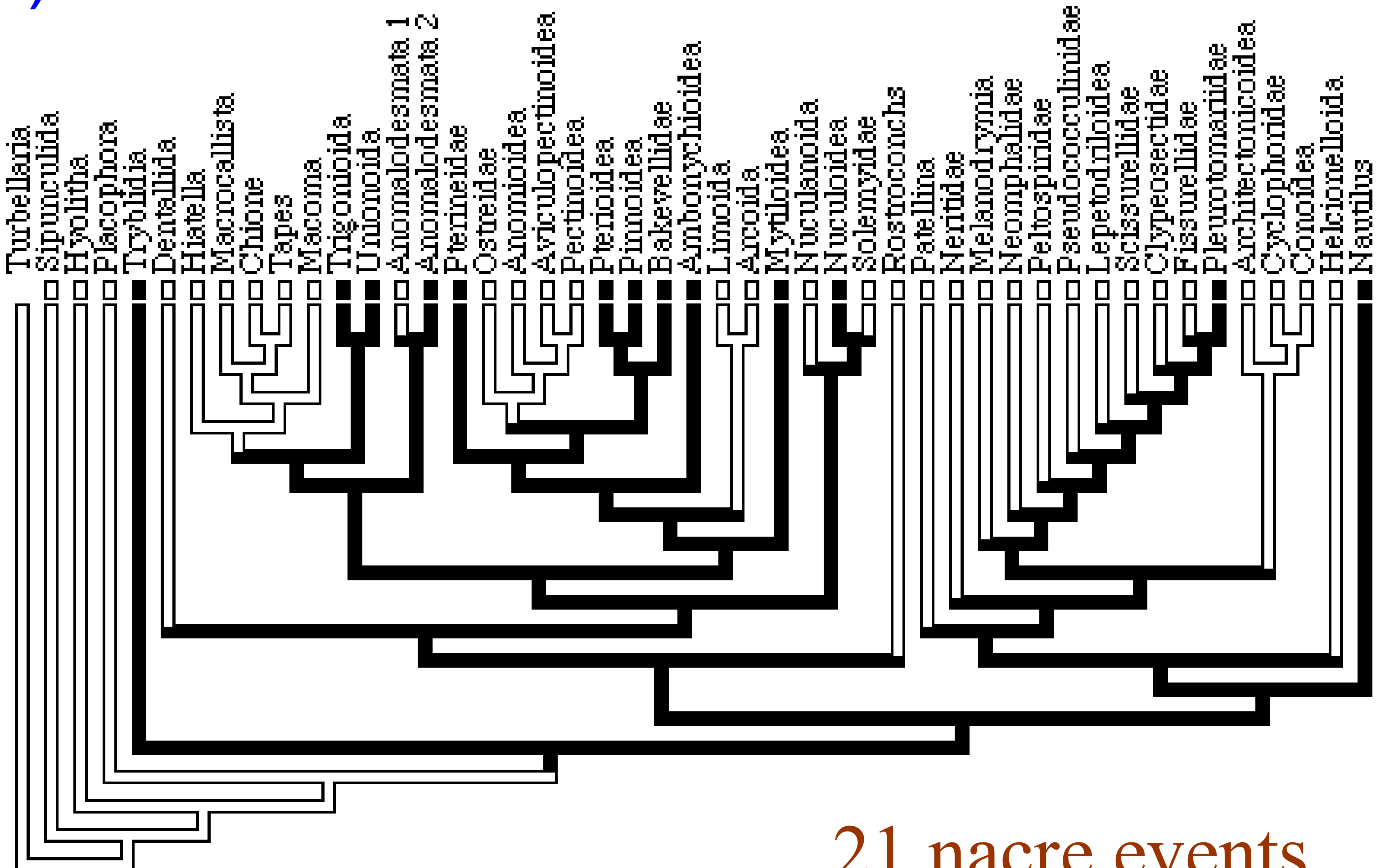


P. Margaritifera



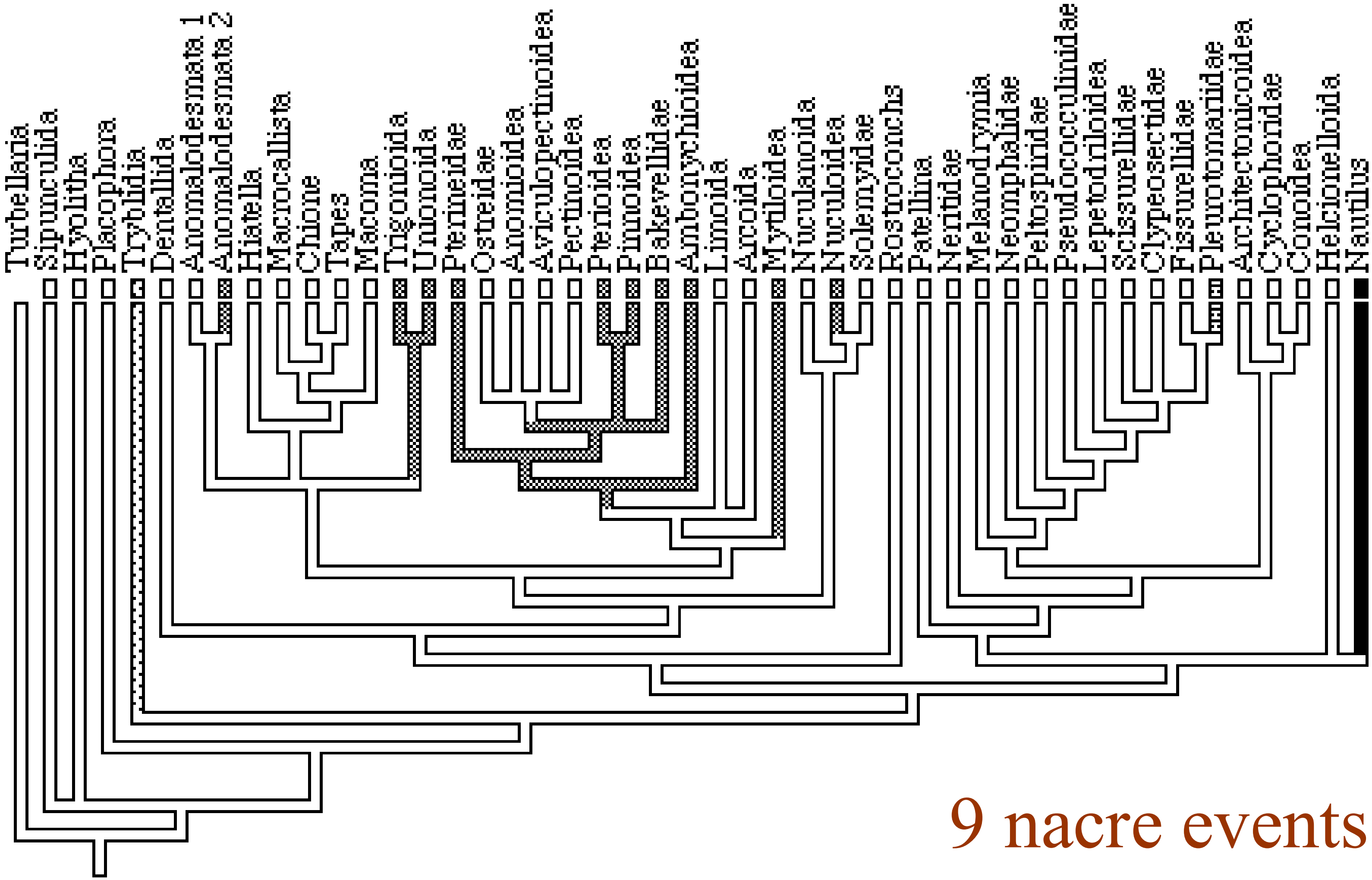
Monoplacophora	*Neopilina galatheae*	Nacre: c: ⊥ a: **O**
	Rokopella zographi	
	Tryblidium sp.	
Bivalvia	*Neotrigonia sp.*	
	Pinctada margaritifera	Osteoinductive
	Pinctada maxima	Sheet nacre
	Pinna nobilis	c: ⊥ a: *
	Pteria penguin	Different twin levels
	Lampsili alatus	
	Atrina maurea	
	Acila castrensis	
	Mytilus edulis	
	Mytilus californianus	
	Bathymodiolus thermophilus	
	Anodonta cygnea	
Cephalopoda	*Nautilus pompilius*	Columnar nacre: c: ⊥ a: *
	Nautilus macromphalus	
	Baculities sp.	
Gastropoda	*Entemnotrochus adansonianus*	
	Perotrochus quoyanus	Columnar nacre: c: ⊥ a: **O**
	Haliotis cracherodi	
	Haliotis rufescens	
	Haliotis tuberculata	
	Tectus niloticus	

cladistics: nacre = ancestral (Carter & Clarck, 1985)



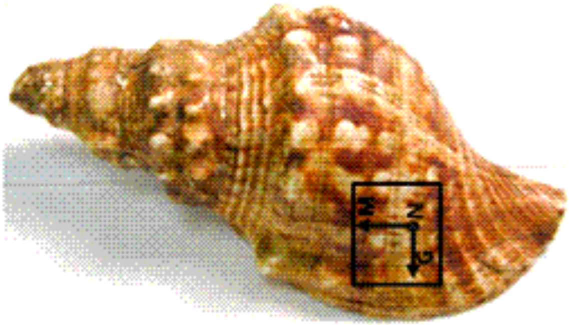
21 nacre events

nacre not ancestral: more parsimonious



9 nacre events

Mediterranean sea and Eastern Atlantic carnivorous gastropod, protected (Bern conference)

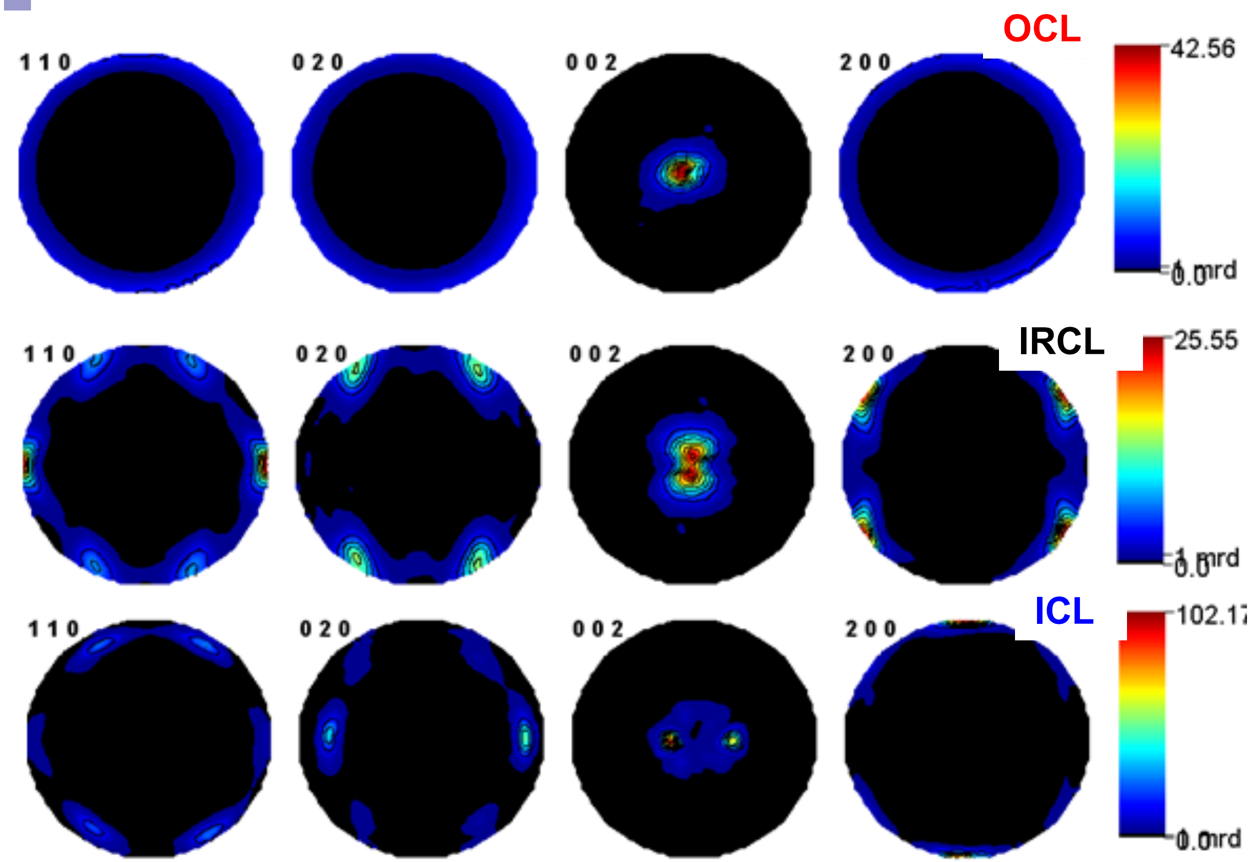


Charonia lampas lampas

OCL : Outer Comarginal Crossed Lamellae : lamellae plane // M

IRCL : Intermediate Radial Crossed Lamellae : lamellae plane ⊥ M

ICCL : Inner Irregular Complex Crossed Lamellae



Fiber texture: $\vec{c}$ // N

Split of $\vec{c}$ axes around N + two contributions // (G,N) plane.

Split of $\vec{c}$ axes from N + two contributions // (M,N) plane.

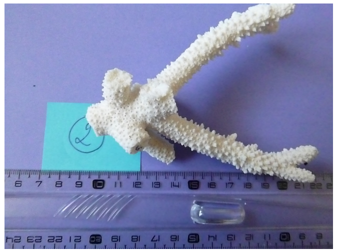
Texture information coherent with usually admitted gastropods phylogeny for this taxon

Ouhenia et al., *J. Struct. Biol.* **163** (2008)

Elastic stiffnesses

Single crystal	160	37.3	1.7			
		87.2	15.7			
			84.8			
				41.2		
					25.6	
						42.7
ICCL	96.5	31.6	13.7			
		139	9.5			
			87.8			
				29.8		
					36.6	
						40.2
RCL	130.1	32.6	10.3			
		103.3	14.1			
			84.5			
				36.3		
					31.1	
						40.5
OCL	111.1	32.9	13.2			
		119	11.8			
			84.8			
				32.8		
					34.6	
						40.9

In corals (Cnidaria) ?



Seriatopora

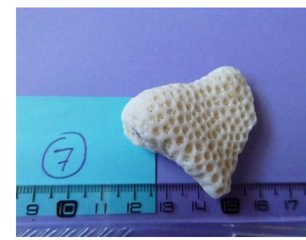
Millepora

Acropora

Phaceloida

Invariably aragonite
Except fossils !

Fossilized sp.



Stylocoeniella

Turbinaria

Fungia

Lobophyllia

Cerioda

Conclusions

- ⟹ Intracrystalline molecules distort cell and structures
- ⟹ Structures change through shell thickness
- ⟹ Intercrystalline molecules modify crystal sizes
- ⟹ QTA + Structural analysis deserve character analysis

But not in corals !

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E. Lopez, MNHN Paris
X. Bourrat, BRGM Orléans
C. Hedegaard †2009
D. Desbruyeres, MARVEL expedition (1997)
F. Lallier, HOPE expedition (1999)

EC: SOLSA
ANR: Ecocorail
Région Centre: SMAM

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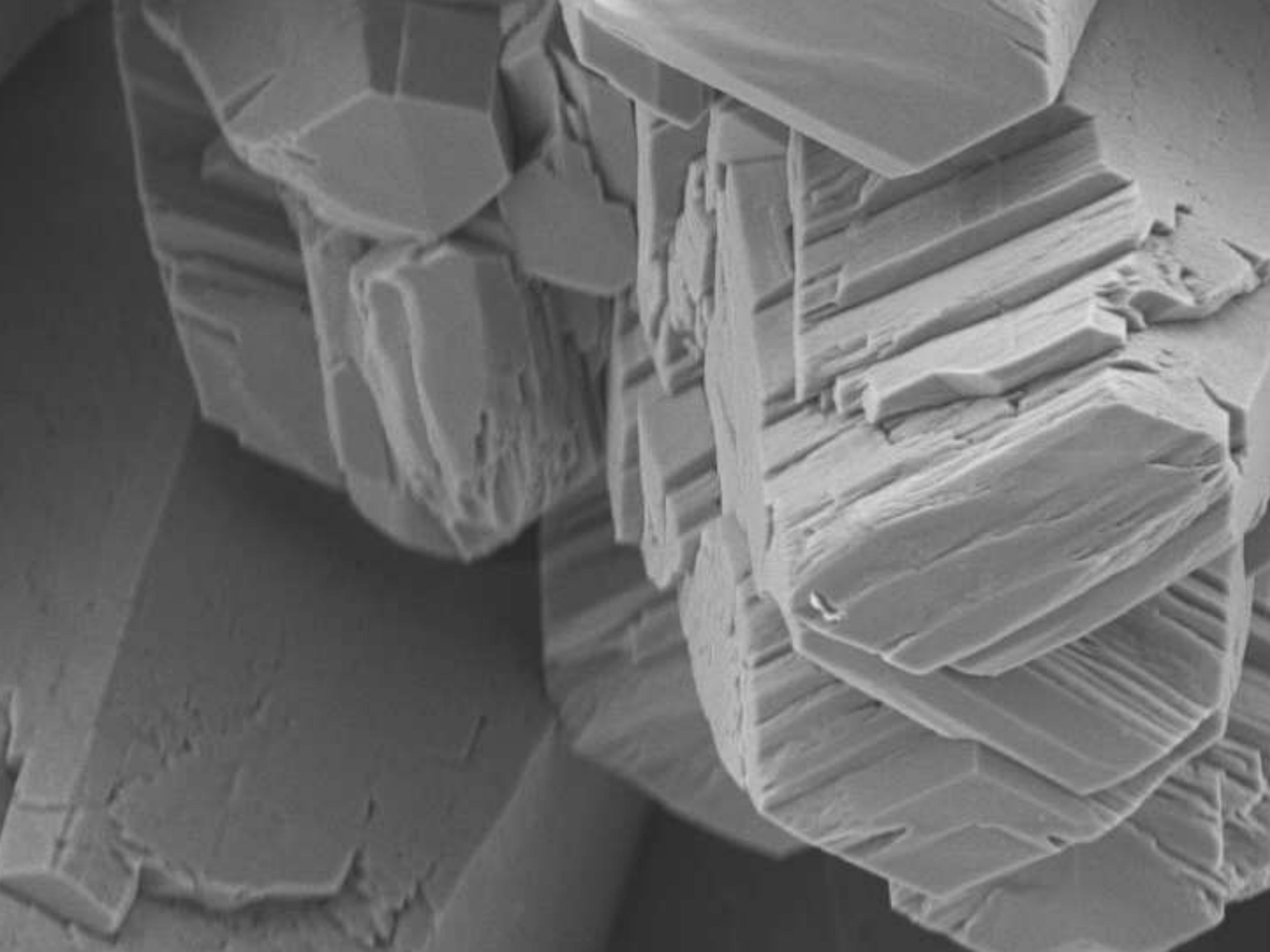
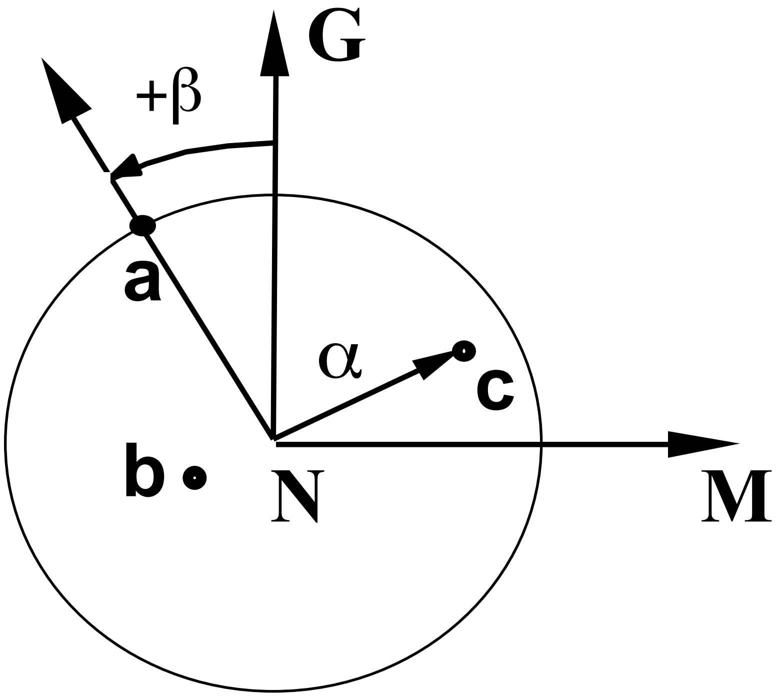
H.-R. Wenk, DEPS Berkeley
M. Morales, CIMAP Caen
L. Lutterotti, Trento Univ
E. Lopez, MNHN Paris

Special dedicace to Claus Hedegaard, Aarhus Univ (1963-2009)



Texture terms



$$\left\langle \mathbf{c}^{\alpha} \middle| \mathbf{L} \middle| \mathbf{a}_{\mathrm{T}}^{\langle hkl \rangle,\beta} \right\rangle$$

c: ● , ∀, ∨, ∠, ⊥

a: ● , **○**, ✱, ×, |

L: ISN, ICN, ICCL

T: % twinned volume

<hkl>: direction in (**G**,**M**)

A link to mollusc ancestors

Calcitic fossils: trichites

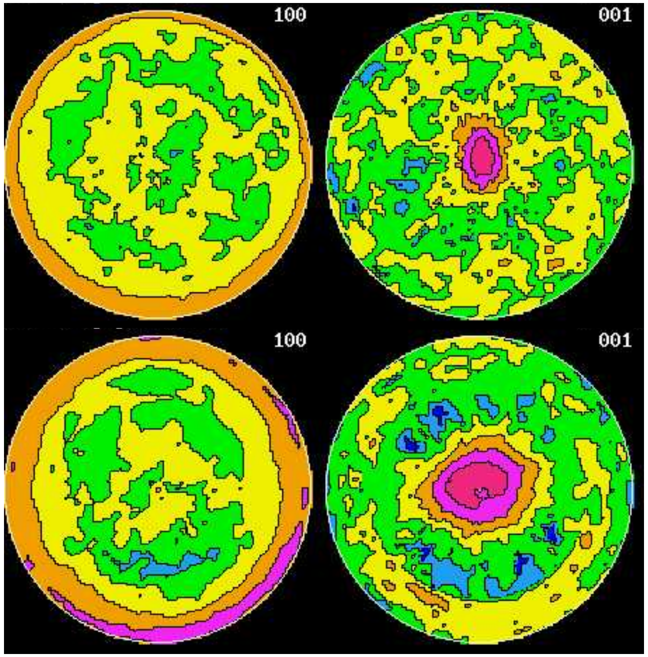
- Fragments of the large bivalve *Trichites* relatively abundant in shallow marine sediments from the Middle to Upper Jurassic of Europe, Asia and Africa

- Entire individuals are rare and the palaeobiology of the genus is poorly understood because of this

- Studied specimens are thick, some fragments up to 3 cm in thickness, composed of a coarse simple prismatic calcite

- Taxonomic position of Trichites remains problematic: pinnoids ?

Pinnoid and Pterioid prismatic layers



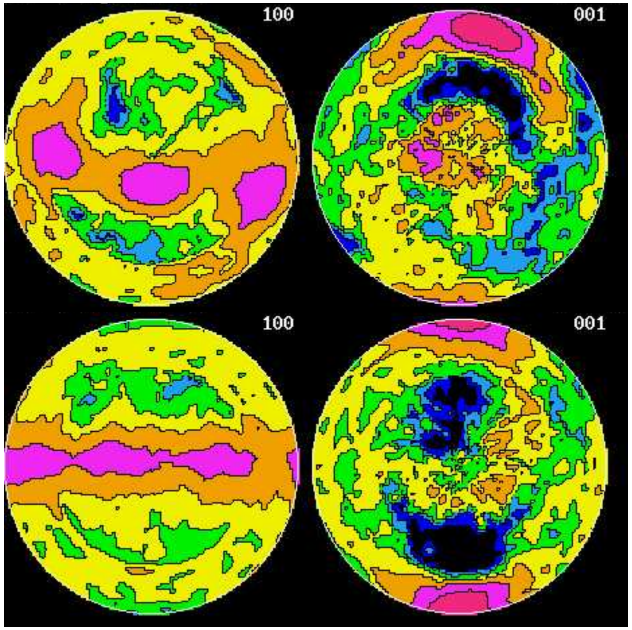
Pinna nobilis

c-axes // **N**

a-axes at random

Pteria penguin

Mussels prismatic layers



Mytilus edulis

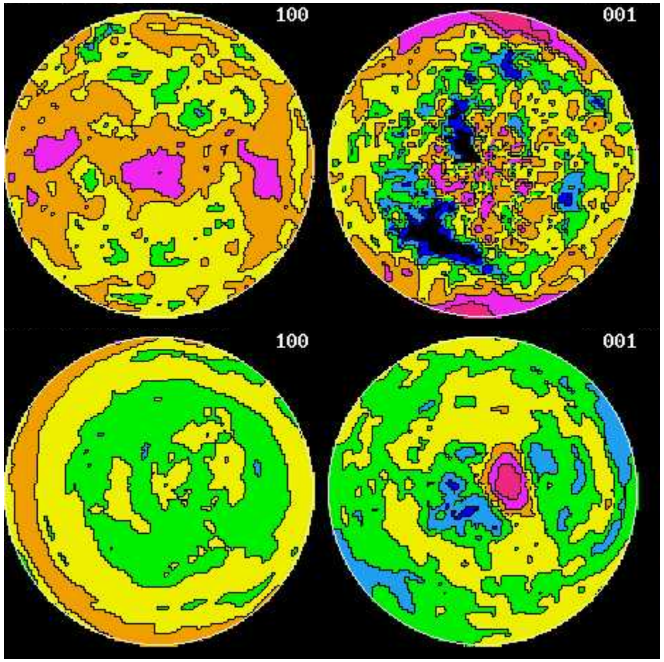
c-axes ∠ **N**

a-axes single-crystal like

c-axes ⊥ **N**, // **G**

Bathymodiolus thermophilus

Scallop and trichite prismatic layers



Amussium parpiraceum (scallop)

c-axes ⊥ **N**, // **G**

a-axes single-crystal like

Trichites (fossil)

c-axes ∠ **N**

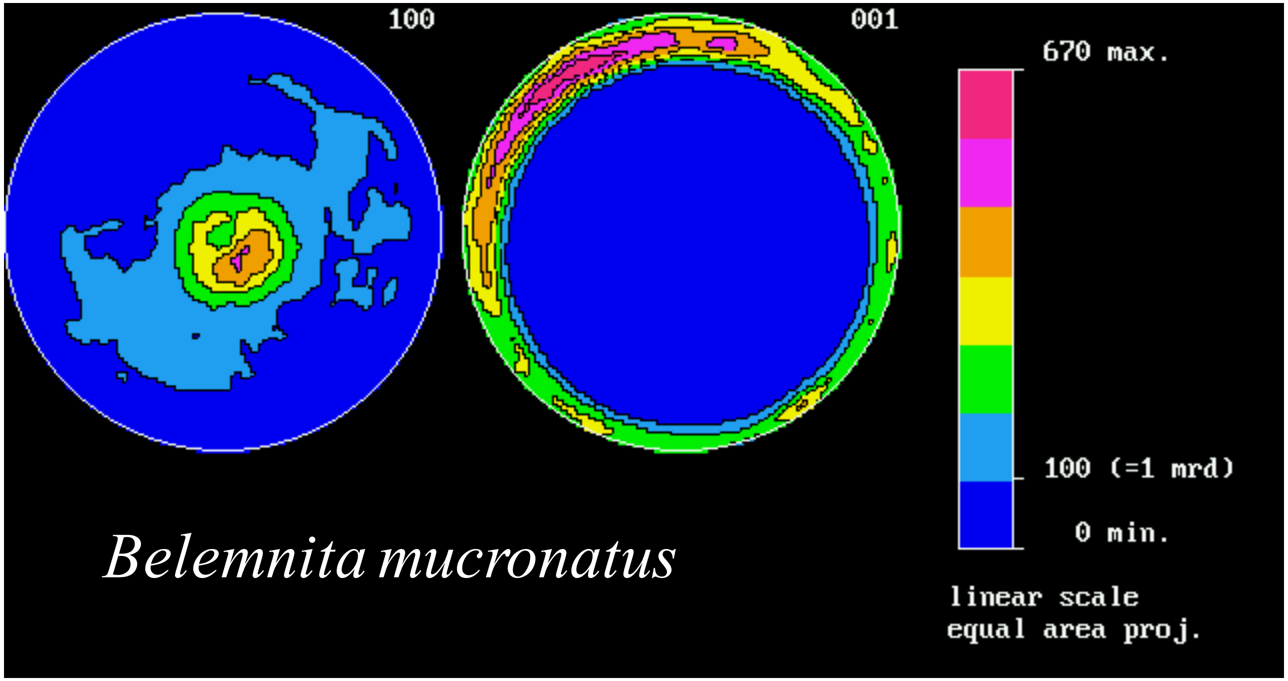
a-axes random

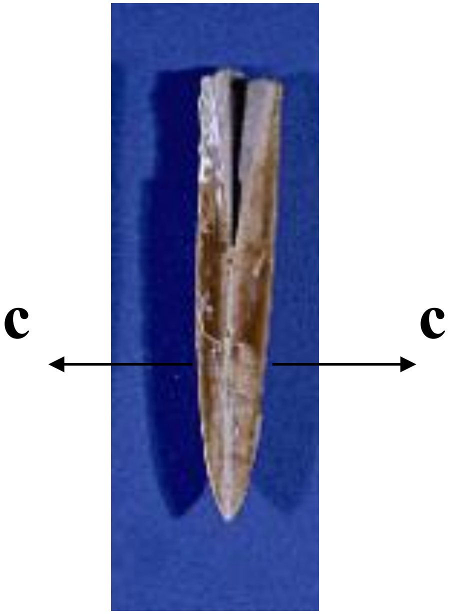
	Layer type	ODF Max (mrd)	ODF min (mrd)	RP0 (%)	RP1 (%)	c-axis	a-axis	{001} Max (mrd)	F^2 (mrd^2)	- S
Pinna nobilis	OP	303	0	50	29	// N	random	68	29	2.3
Pteria penguin	OP	84	0	29	15	// N	random	31	13	1.9
Amussium parpiraceum	OP	330	0	53	33	// G	<110> // M	20	31	2.6
Bathymodiolus thermophilus	OP	63	0	25	18	// G	// M	27	13	1.9
Mytilus edulis	OP	207	0	41	25	75° from N	<110> // M	23	21	2.2
Trichites	P	390	0	52	28	15° from N	random	56	41	2.2
Crassostrea gigas	IF	908	0	45	31	35° from N	// M	>100	329	5.1

No DNA is available on fossils like Trichites, but Trichite's textural parameters are close to the ones of *pinnoids* or *pterioids*: interesting for the classification of extinct species

Chateigner, Morales, Harper (2002), *Mat. Science Forum* ***408*** *1687*

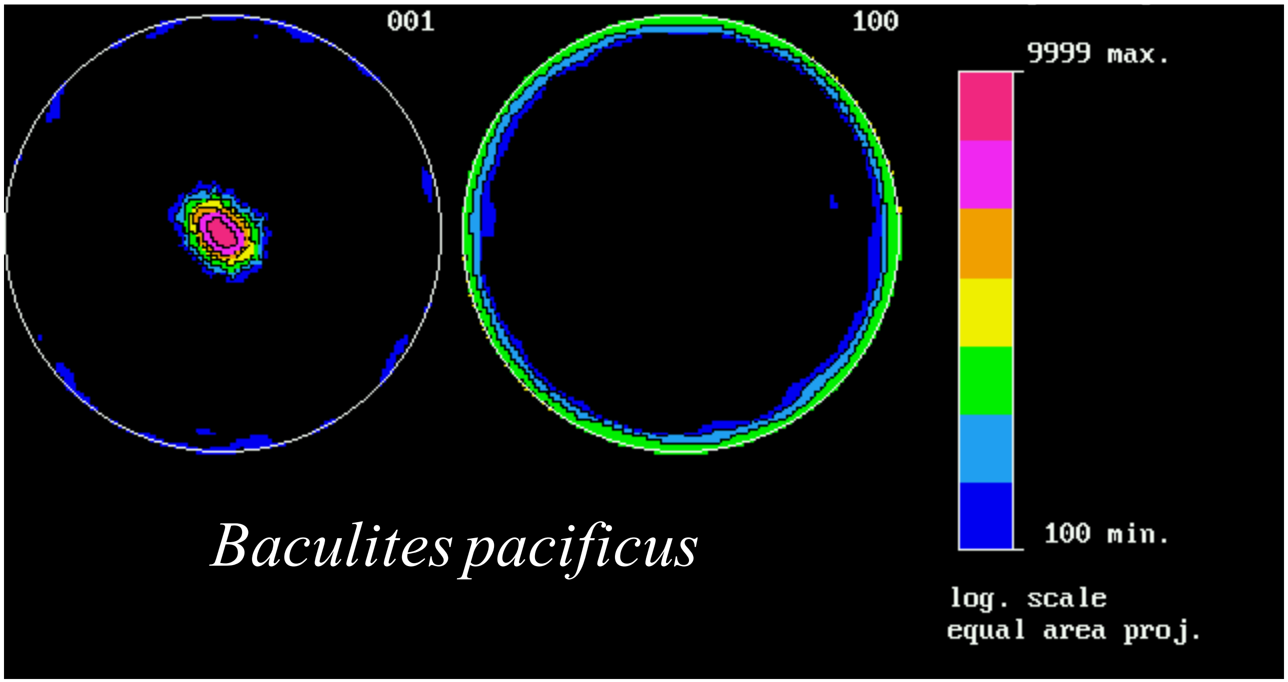
Calcitic fossils: *Belemnites*: Belemnoidea

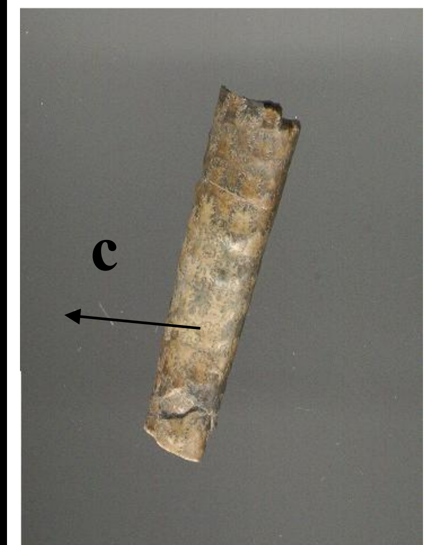




c-axes perp. to the shell: as in other cephalopods
No significant phylogenetic differences between Cretaceous (145-65 Mya) and Jurassic (200-145 Mya) species

Aragonite fossils: *Baculites sp.*: Amonoidea, late Cretaceous



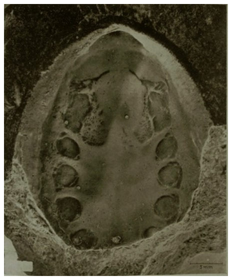


c-axes perp. to the shell: as in other cephalopods, strong **c**-calcite to **c**-aragonite fossils interaction

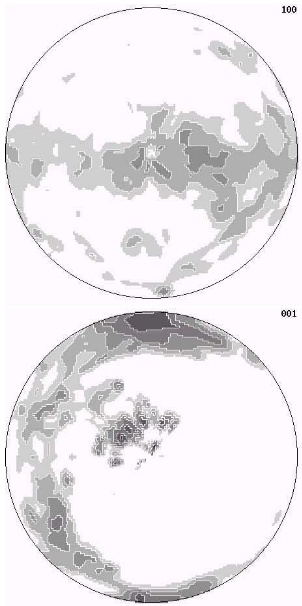
Is nacre the ancestor form ?

Recrystallized Aragonite ? *Pilina unguis*: Tryblidiidae Monoplacophora, Paleozoic (550-250 Mya)

{100}



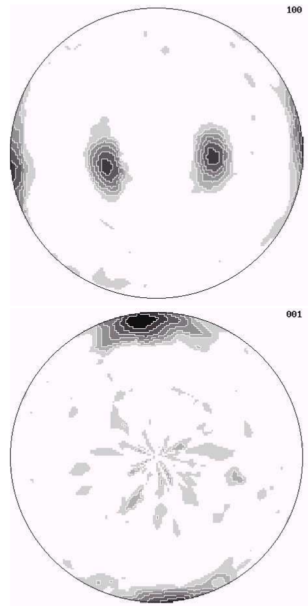
{001}



$\left\langle \angle,90 \middle| \mathrm{IFC} \middle| *^{<100>} \right\rangle$

Pilina unguis

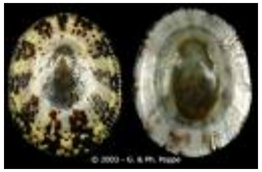
Recrystallised aragonite ? (Erben 1968)

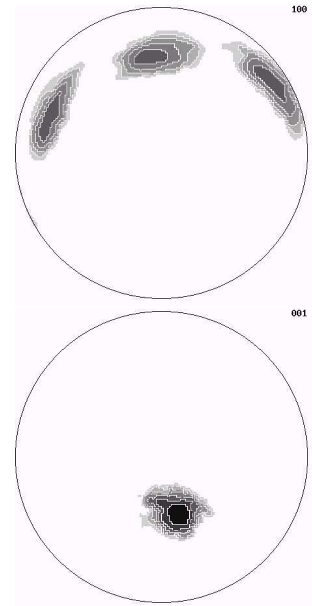


$\left\langle \angle\, 90 \middle| \mathrm{IRFC} \middle| *^{<110>,\,90}_{50} \right\rangle$

Cellana testudinaria

Rather original foliated calcite ?





$\left\langle \angle\, 30 \middle| \mathrm{IRFC} \middle| *^{<110>,\,90}_{50} \right\rangle$

Crassostrea gigas

Nacre ancestor ?

Structural distortions from x-rays

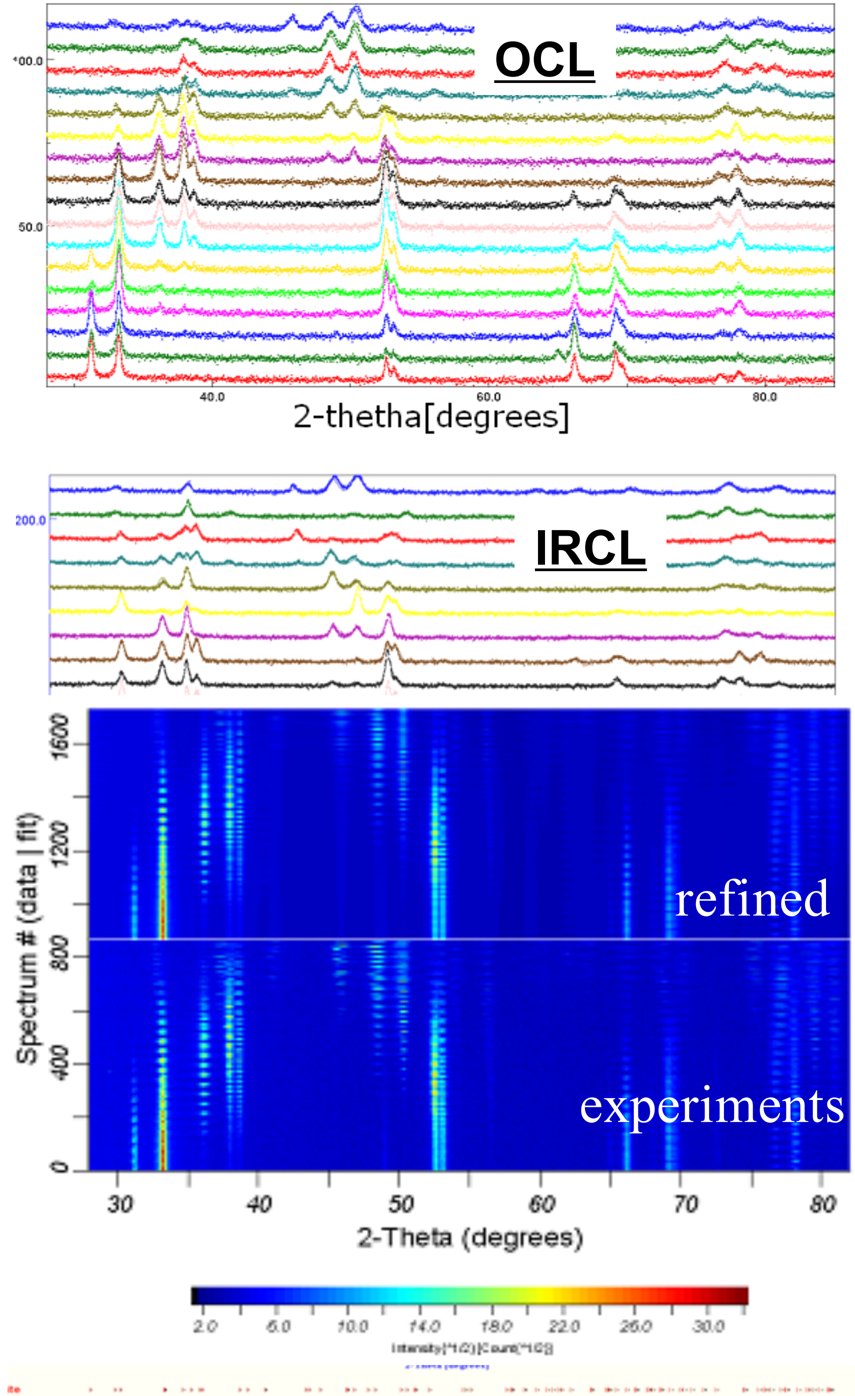
Aplanarity of carbonate groups in $CaCO_3$

$\Delta Z_{C\text{-}O1} = c(z_C - z_{O1})$

Calcite	*Biogenic aragonite*	Mineral aragonite
0 Å	*Intermediate, more distorted ?*	*0.05744 Å*

How to probe this ?

Synchrotron (Pokroy & Zolotoyabko), but also Lab XRD, in the Combined Analysis frame



layer		OCL	IRCL	ICCL
a (Å) b (Å) c (Å)		4.98563(7) 8.0103(1) 5.74626(3)	4.97538(4) 7.98848(8) 5.74961(2)	4.9813(1) 7.9679(1) 5.76261(5)
ΔV/V		1.05 %	0.62 %	0.71 %
OD maximum (m.r.d.) OD minimum (m.r.d.)		299 0	196 0	2816 0
Texture index (m.r.d.^{2})		42.6	47	721
OD reliability factors	R_w (%)	14.3	11.2	32.5
	R_B (%)	15.6	12.7	47.8
Rietveld reliability factors	GoF (%)	1.72	1.72	3.05
	R_w (%)	29.2	28.0	57.3
	R_B (%)	22.9	21.7	47.2
	R_{exp}(%)	22.2	21.3	32.8

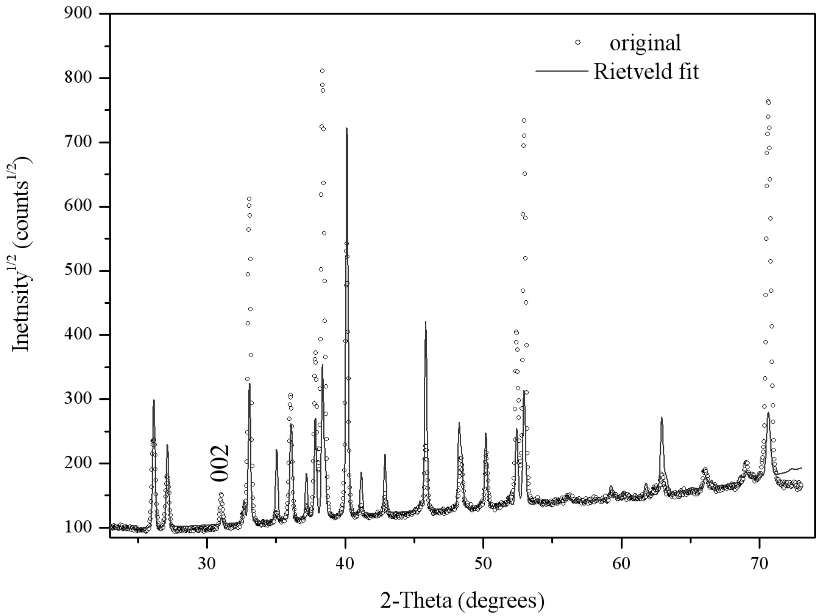
Largest crystallite organisation closer to the animal

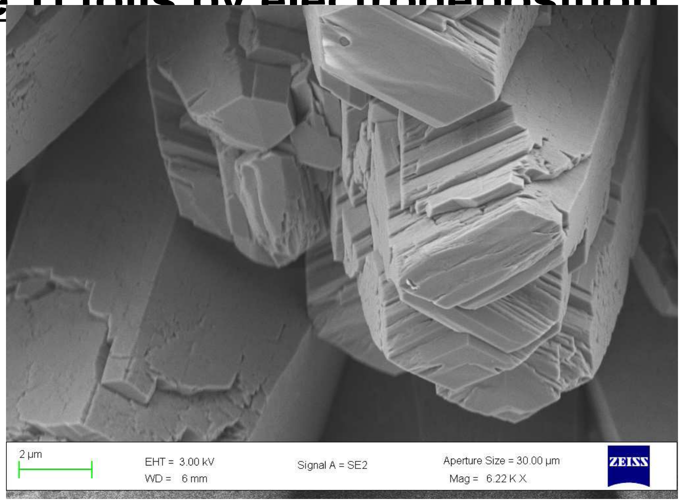
		Geological reference	*Charonia lampas* OCL	*Charonia lampas* IRCL	*Charonia lampas* ICCL
a (Å) b (Å) c (Å)		4.9623(3) 7.968(1) 5.7439(3)	4.98563(7) 8.0103(1) 5.74626(3)	4.97538(4) 7.98848(8) 5.74961(2)	4.9813(1) 7.9679(1) 5.76261(5)
Ca	y z	0.41500 0.75970	0.41418(5) 0.75939(3)	0.414071(4) 0.76057(2)	0.41276(9) 0.75818(8)
C	y z	0.76220 -0.08620	0.7628(2) -0.0920(1)	0.76341(2) -0.08702(9)	0.7356(4) -0.0833(2)
O1	y z	0.92250 -0.09620	0.9115(2) -0.09205(8)	0.9238(1) -0.09456(6)	0.8957(3) -0.1018(2)
O2	x y z	0.47360 0.68100 -0.08620	0.4768(1) 0.6826(1) -0.08368(6)	0.4754(1) 0.68332(9) -0.08473(5)	0.4864(3) 0.6834(2) -0.0926(1)
ΔZ_{C-O1} (Å)		0.05744	0.00029	0.04335	0.1066

ΔZ_{C-O1} ↗ from outer to inner layer correlated to the organic macromolecules presence + coherent with the ↘ of texture strength → control loss from macromolecules on aragonite stabilization farther from animal!

Anisotropic cell distorsions yet observed in biogenic aragonite powderised layers

l grade Ti foils by electrodeposition

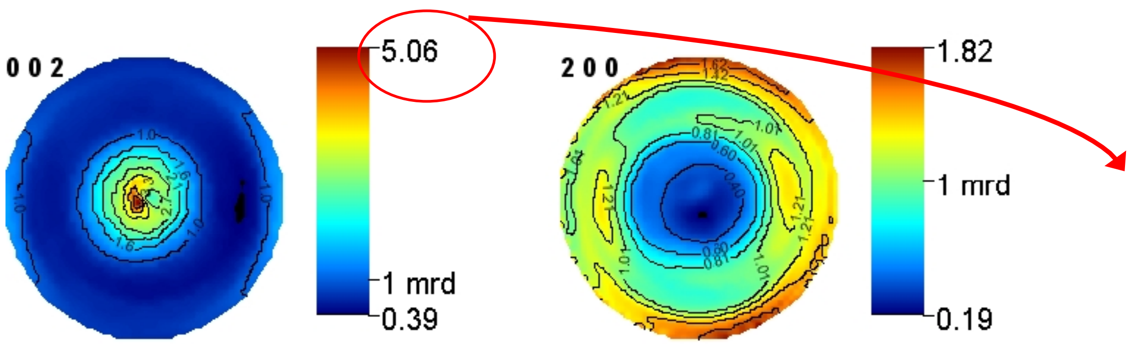




Nonoptimized deposited films: cauliflower-shaped aragonite + calcite + vaterite

Corresponding X-ray diagram: only aragonite is evidenced with a pronounced (00l) texture

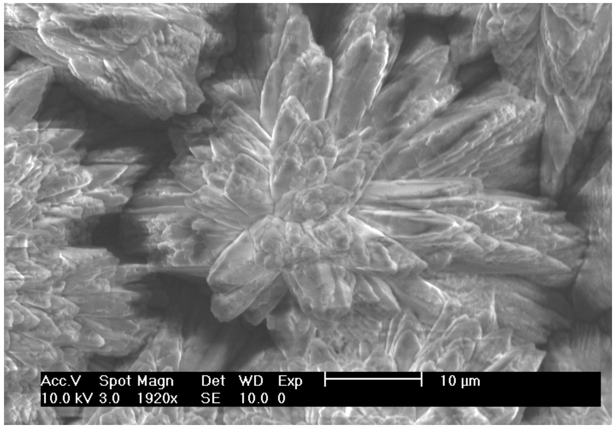
Optimized deposited films with nacre like pseudo hexagonal shaped crystals



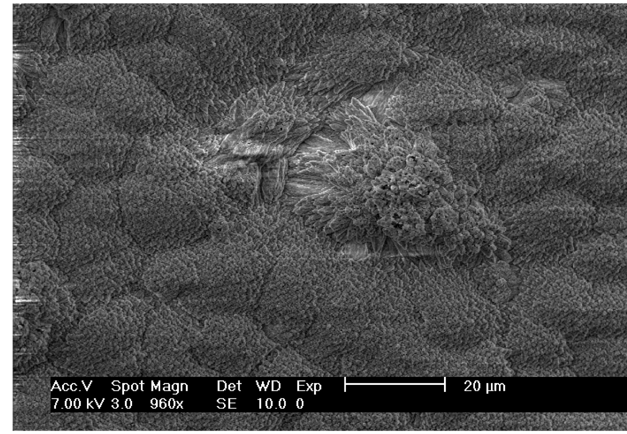
Texture strength far from natural nacre → differences can be associated to organic driven processes

Recalculated pole figure : <00l> fiber like texture

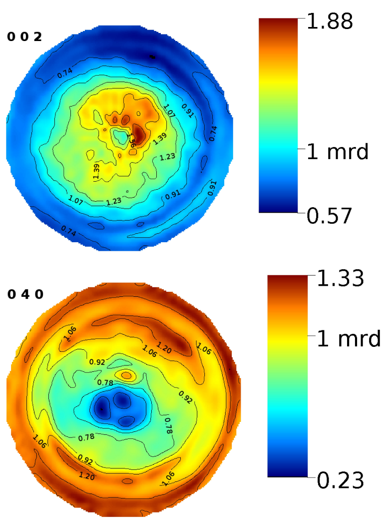
Krauss et al., *Cryst. Growth & Design* **8** (2008)

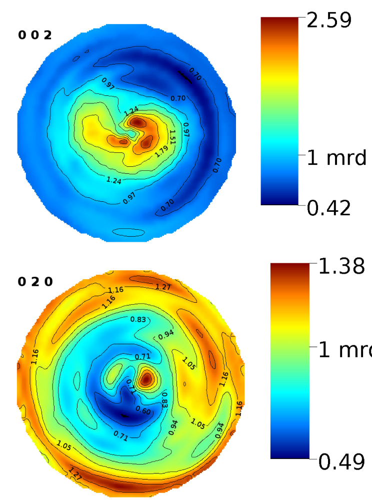


<u>Apolar Ethanol extracted molecules:</u> **cauliflower-shaped aragonite**



***<u>Polar Water extraction</u>*: compact cauliflower-shaped aragonite**

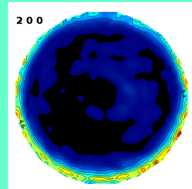
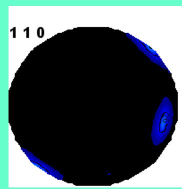




reduction of the <00l> texture

Structural distortions ?

$\Delta Z_{C\text{-}O1}$ (Å) Geological reference **0.05744**

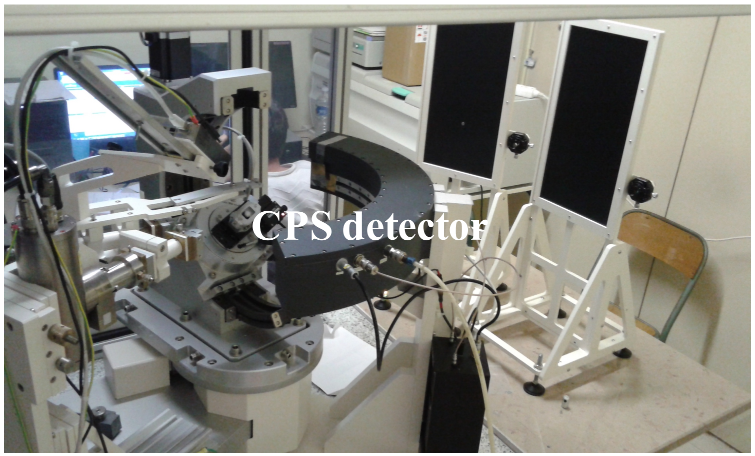
Gastropods	*Haliotis tuberculata* ICN		*Charonia lampas* ICCL	*Charonia lampas* IRCL	*Charonia lampas* OCL	*Strombus decorus* All layers
	0.089		**0.107**	**0.043**	**0.0003**	**0.031**
Bivalves	*Pinctada maxima* ISN		*Mercenaria mercenaria* IP	*Mercenaria mercenaria* IntP	*Mercenaria mercenaria* OP	
	0,054		**0.069**	**0.092**	**0.11**	

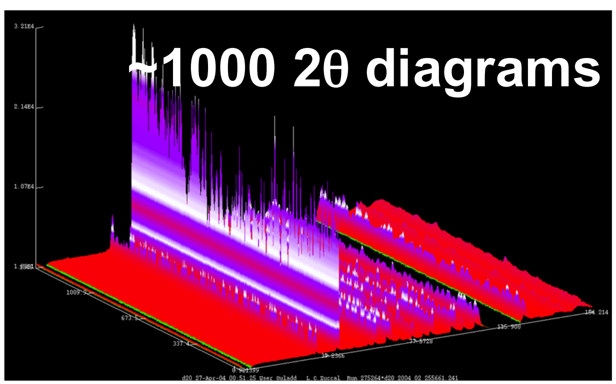
Synthetic layers **Crystallite size**	Inorganic **890Å**	Chitosan **1272Å**	Non-polar Extraction 10 mg/l **1211Å**	20 mg/l **1126Å**	Polar Extraction 10 mg/l **1284Å**	20 mg/l **1150Å**
$CaCO_3$ / Ti	**0,087**	**0.04**	**0.173**	**0.086**	**0.134**	**0.081**

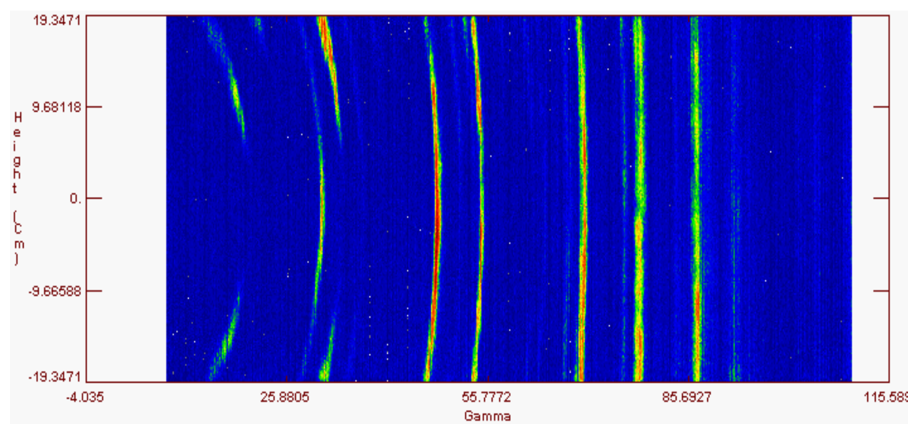
[illegible]
[illegible]
In *Pinctada*: ΔZ=0.05, both inter- and intramolecules act

Minimum experimental requirements:

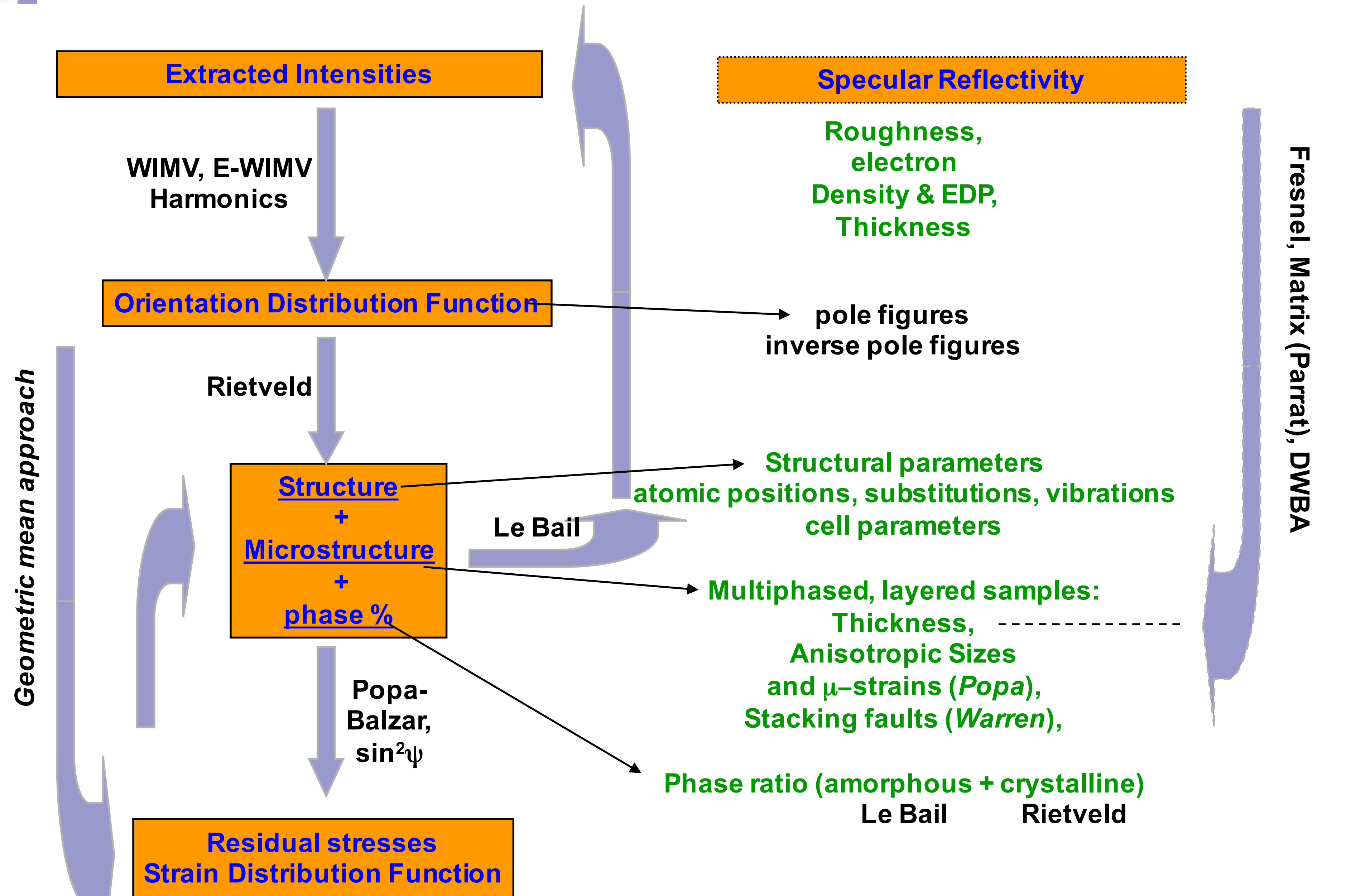
1D or 2D Detector + 4-circle diffractometer (X-rays and neutrons)







~200 2θ diagrams



Combined analysis approach

Rietveld enlarged: Structure – Texture – Stress – Phase – Microstructure – Layering analyses - Reflectivity

$$y_{ic}(\mathbf{y}) = y_{ib}(\mathbf{y}) + \sum_{\Phi=1}^{N_\Phi} S_\Phi \sum_{k=K_1}^{K} j_{\Phi k} Lp_{\Phi k} P_{\Phi k}(\mathbf{y}) \left|F_{\Phi k}\right|^2 \Omega_{i\Phi k} A_{i\Phi}(\mathbf{y})$$

$$P_{\mathbf{k}}(\mathbf{y}) = \int_{\varphi} f(g,\tilde{\varphi})\, d\tilde{\varphi}$$

Tensor homogeneisation, geometric mean …