Structural distortion of biogenic aragonite in Ranella Olerea mollusc shell layers

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1- INTRODUCTION

Mollusc shells are fascinating organic-mineral biocomposites with high mechanical performances. With only 1–5 wt% of organic material, a nanometer-sized growth-control is achieved which increases the shell toughness and mechanical properties considerably compared to the non-biogenic mineral. Both hardness and low crack propagation in nacre are attributed to strong structural bounds between organic macromolecules and inorganic crystals [1,2]. Mollusc shells are mainly made of calcite and aragonite crystalline polymorphs. The major part of organic materials is intercrystalline, and in a minor way intracrystalline. Recently, using high resolution synchrotron radiation, Pokroy et al. [3,4]showed that the biogenic unit-cells are deformed anisotropically compared to the non-biogenic crystals. The distortions observed are due to intracrystalline organic molecules. However, all the previous X-ray and neutron studies were done on powderised samples, with the samples being composed of several shell layers and eventually several phases.

In this work we made use of the so-called ''Combined Analysis'' [5] to determine simultaneously the structure (unit-cell distortions and atomic positions) and the preferred orientations (texture) of constituting aragonite crystallites of several shell layers. This approach was chosen because it allows working on real samples, without grinding operation or specific preparation.

2- PREPARATION AND CHARACTERISATION METHOD

Great Ranelle , *Ranella olearium* (Linnaeus , 1758) is a mollusc of the Cymatiidae family . This family is well represented in all warm seas. It includes large species, including *Charonia lampas lampas* (Linnaeus , 1758) of the Ranellidés family (which belongs to the triton and trumpets shells) (Beu, 1987) .With its length of 30 cm, it is one of the largest gastropods of north-east of the Atlantic and the Mediterranean sea. Great Ranelle has smaller dimensions , but its shell can reach 20 cm high and 10 cm wide; which places it in terms of size in the second position just after the triton.

We collected a shell of 21 cm (Figure 1a) on the coast of Bejaia situated in the north- east of Algeria (North Africa). The inorganic portion of the shell is composed only of aragonite . SEM image at low magnification of the shell (Figure 1b) shows a fractured section of the shell where we can distinguish three layers: the outer, intermediate and inner layers , shown on the image respectively from top to bottom.
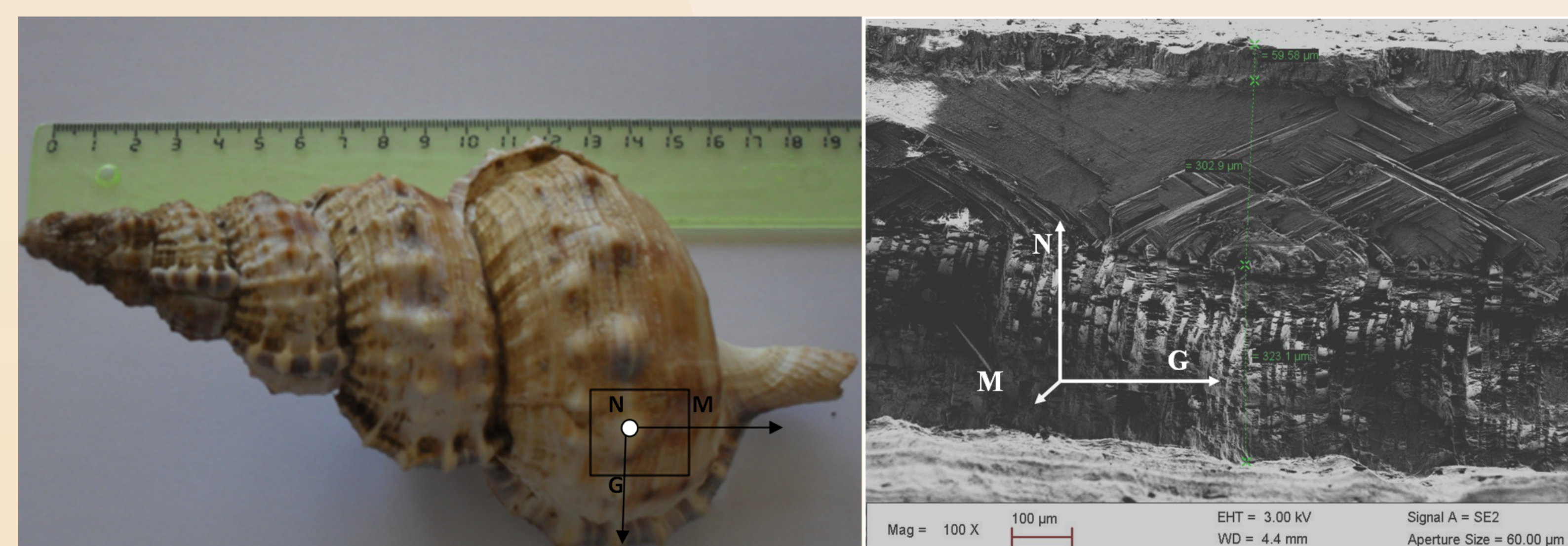

Figure1: The *Ranella olearea* shell(**a)** and Cross-section SEM image of the fractured shell at the location indicated in **G**, **M** and **N** indicate the Growth, Margin and Normal directions, respectively (b).

3-COMBINED TEXTURE–STRUCTURE X-RAY ANALYSIS

Diffraction measurements were done on a 4-circle diffractometer equipped with a curved position sensitive detector (CPS 120, Inel, figure 3) using copper K_a radiation (Morales, 2005). The use of a 1D detector covering an enough large 2θ range is essential for a reasonable total acquisition time of the necessary diagrams.

We used a 2.5°x 2.5° grid in tilt (χ) and azimuth (ϕ) and covered the pole figures up to 60° in χ (figure 4) , resulting in 3744 diagrams per each layer. Such an acquisition gives access to the possibility of a refining texture, cell parameters, atomic positions, etc. Pole figures are normalised in distribution density units (or m.r.d. for multiple of a random distribution), a unit depending only on orientation. In such units, a specimen without any preferred orientation exhibits 1 m.r.d. homogeneous level on all pole figures, while a textured specimen shows the minima and maxima of the densities. Instrumental resolution was calibrated using the NIST LaB6 (SRM 660b) standard (Ouhenia et al., 2008). Data treatment is done through the ''Combined Analysis'' formalism (Chateigner, 2011) implemented in the MAUD software (Lutterotti et al., 1999) [6] .

After measuring the first outer layer of the shell, we removed this layer with a dilute HCl solution to analyze the intermediate layer, and then, we repeated the same operation with the intermediate layer to measure the inner layer under the same conditions .
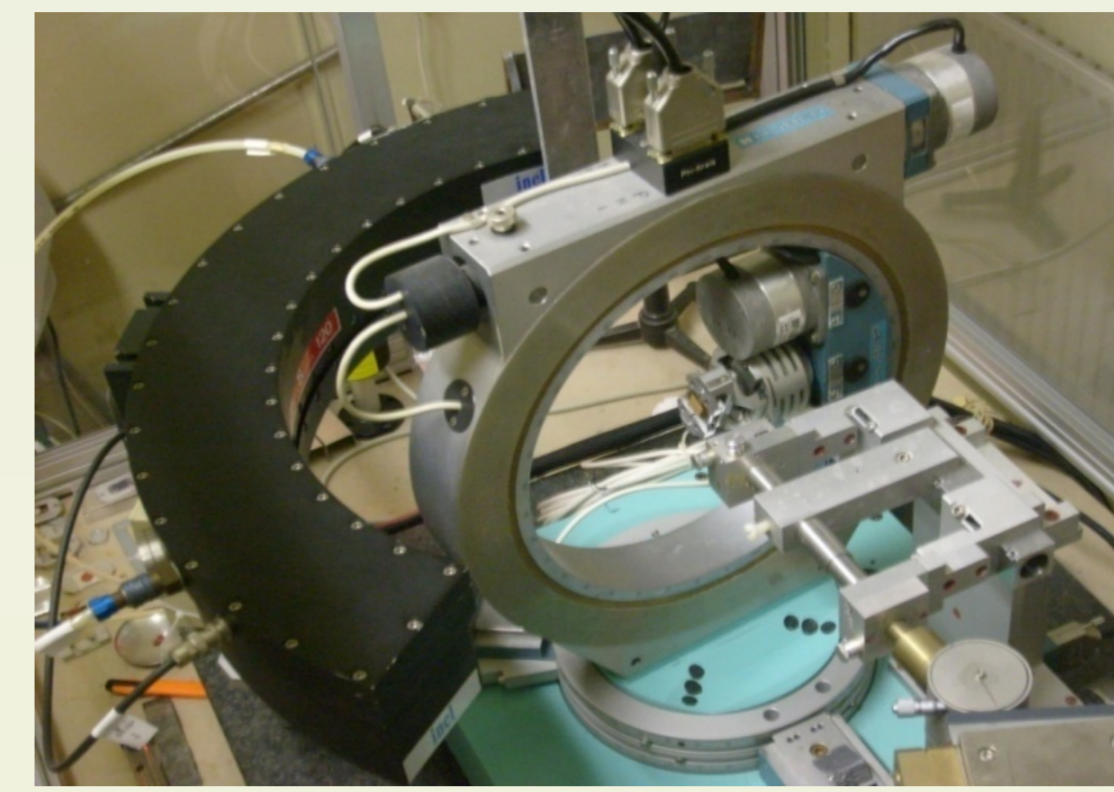

Figure 3 : 4-circle diffractometer equipped with curve position sensitive detector (CPS 120, Inel) covering an angle of 120°.
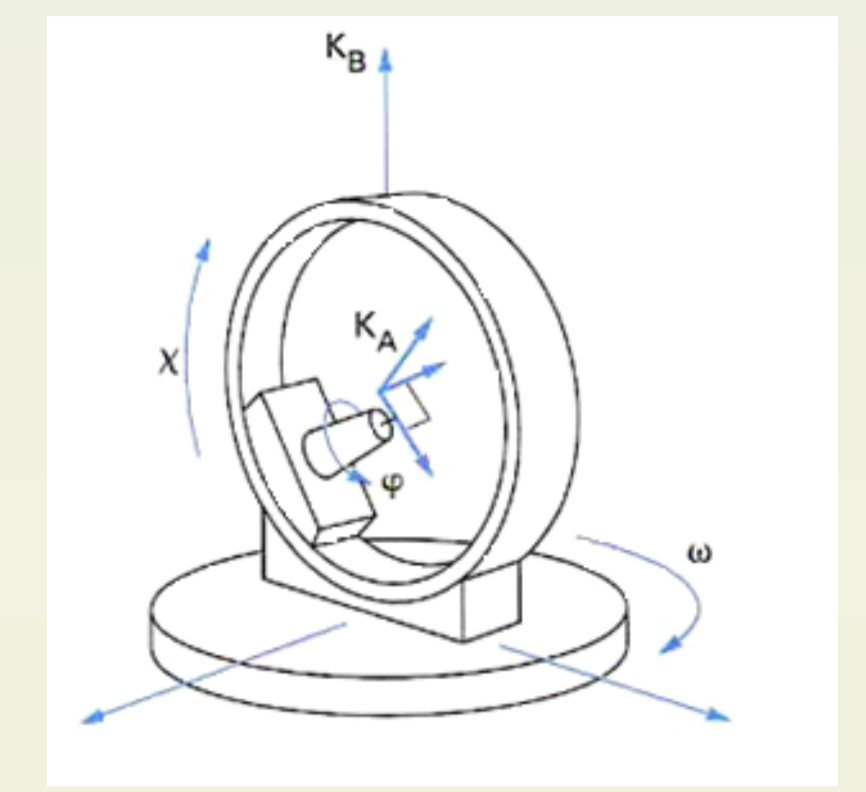

Figure 3 : 4-circle goniometer and Euler angle

4- Structural distortion in biogenetic aragonite shell

The results of the cell refinement are summarized in Table 2 for each layers (index parameter of the refinement are indicated in table 1 and figure 4 show Randomly selected diagrams showing the good reproducibility of the experimental after the last refinement cycle for the three layers). An anisotropic unit-cell distortion is quantified for the three layers without necessity of grinding them. There are sensitive deviations from the usual geological aragonite for all lattice parameters *a*, *b* and *c* of the three layers. The obtained lattice distortions are highly anisotropic, being of positive sign (cell expansion) for the a- , b- and c-axes for the outer layer . While for the intermediate layer the distortion parameters a- and c-axes are of negative sign (cell contraction) . For the inner layer, the distortion of lattice parameters are negative for a- and b-axes but positive for the c-axes (cell contraction). the effect of inter-crystalline portion is visible as a distortion of the cell combined with the influence of intra-crystalline organic molecules. Inter-crystalline macromolecules have been associated with the selection of the polymorph of calcium carbonate, but also with the particular organization of crystals in the shells of molluscs.

Table 1: Resulting parameters of combined analysis of the external, intermediate and inner layers of Ranella Olerium. GoF: Goodness of Fit.

layer		ORCL	ICCL	ICL
Reliability factor of the OD	R_w (%)	25	24	25,2
	R_b (%)	24.8	23	24,3
	GoF (%)	1,7	1,06	1,07
Rietveld Reliability factors	R_{wp} (%)	28.08	27.05	28.06
	R_b (%)	32.80	28.03	28.14

Table 2

Cell parameters, refined atomic positions and DZ values for *Ranella olerea* layers, our non-biogenic reference and the Strombus species of Pokroy et al. (2007)

|	Geological reference	Ranella Outer	Ranella Intermediate	Ranella Inner	Strombus decorus
a (Å)	4.9623(3)	4.98563(7)	4.94021(4)	4.94844 (1)	4.9694(3)
b (Å)	7.968(1)	8.0103(1)	7.94906(6)	7.96077 (3)	7.9591(4)
c (Å)	5.7439(3)	5.74626(3)	5.74222(1)	5.74963 (2)	5.7528(1)
Δa/a	0	0.0047	-0.0044	-0.0028	0.0014
Δb/b	0	0.0053	0.0023	-0.0024	-0.0011
Δc/c	0	0.0004	-0.00029	0.001	0.00154
ΔV/V	0	1.06%	-0.69%	-0.38%	0.18%
Ca					
y	0.415	0.41529 (4)	0.41372(4)	0.41560 (4)	0.4135(7)
z	0.7597	0.75930 (3)	0.75612(3)	0.75967 (3)	0.7601(8)
C					
y	0.7622	0.7631(2)	0.7625(2)	0.7639 (2)	0.7607(4)
z	-0.086	-0.0768 (1)	-0.0889(9)	-0.08437 (8)	-0.0851(7)
O1					
y	0.9225	0.9382 (1)	0.9454(1)	0.9157 (1)	0.9228(4)
z	-0.096	-0.10112(6)	-0.10861(7)	-0.09254 (6)	-0.0905(9)
O2					
x	0.4736	0.4679(1)	0.4652(1)	0.4687 (1)	0.4763(6)
y	0.681	0.6839 (1)	0.69015(9)	0.6803 (1)	0.6833(3)
z	-0.086	-0.08760(5)	-0.08516(5)	-0.08718 (4)	-0.0863(7)
DZC–O1 (Å)	0.05744	0.139	0.113	0.047	0.031
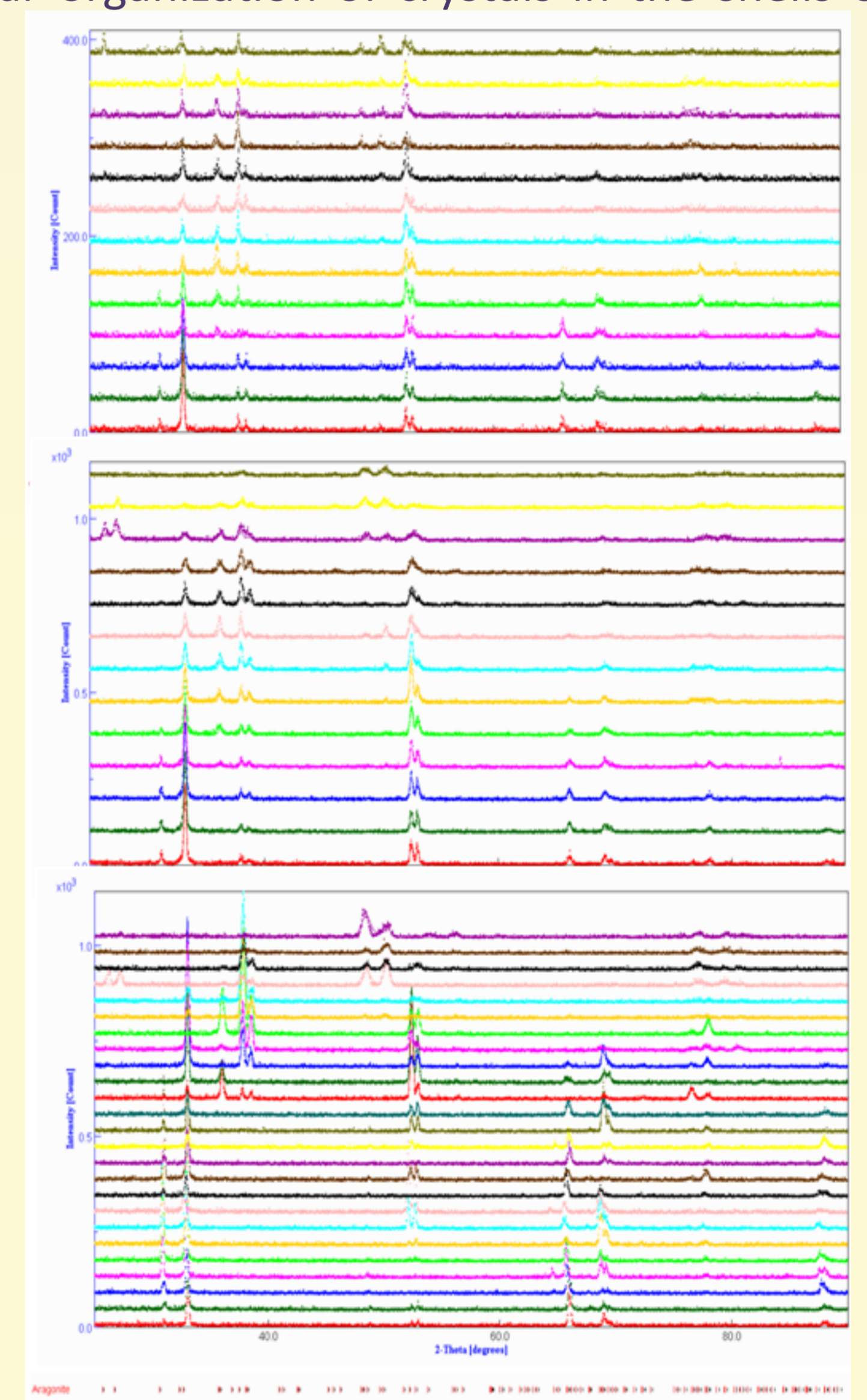
Figure 5: Randomly selected diagrams showing the good reproducibility of the experimental (dots) patterns from the combined analysis refinement (lines) after the last refinement cycle for the (a) outer (b) intermediate and (c) inner layers.

5- CONCLUSION

Two techniques of characterisation have been used to investigate a *Ranella olerea* shell. the scanning electron observation investigations reveal that this species is composed of three distinct layers ; the inner layer composed of Radial Lamellar (IRL), the intermediate layer which is comarginal crossed lamellar (ICCL) and outer layer composed of crossed lamellar (OCCL). Using the X-ray diffraction and the powerful combined analysis approach, we refined quantitatively the structure of the three layers and their respective aragonite unit-cell distortions. An anisotropic unit-cell distortion is quantified for the three layers without necessity of grinding them. These distortions are attributed to the combined effects of inter- and intracrystalline macromolecules, by comparison with other authors' works.

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