MARIE SKLODOWSKA-CURIE ACTIONS

Co-funding of regional, national and international programmes (COFUND)

DOC2AMU PROJECT 2017 CALL FOR APPLICATIONS

In vivo CARS microscopy for biomineralization study of *Foraminifera*

1. DESCRIPTION OF THE PHD THESIS PROJECT

1.1 OBJECTIVES OF THE PROJECT BASED ON THE CURRENT STATE OF THE ART

Many marine organisms build complex $CaCO_3$ shells, whose fossils archive past climates in their trace element chemistry. Organic–mineral interactions are a crucial, poorly understood aspect of shell formation, which may alter shell composition and bias climate records. The PhD proposal aims at deciphering the in vivo *biomineralization processes in foraminifera, a major marine group, using an advanced microscopy technique based on coherent Raman scattering. Crystal and organic maps of shells will offer a detailed view of the chemical interactions at an organic mineral template, and will allow to determine the fundamental biomineralization processes.*

Biomineralization is the fascinating ability exhibited by many living organisms to tailor minerals into highly regulated complex shapes in order to build hard tissues, fully adapted to specific structural and/or biological functions. Only considering calcium carbonate polymorphs in molluscs, corals, sponges, foraminifera or coccoliths, a variety of (sub-)millimetric shapes, crystalline polymorphs, micrometric structure and architectures are observed, often related to outstanding mechanical properties. Moreover, the sensitivity of the biomineralization process to physical and chemical environmental conditions results in geochemical changes recorded in the shells of foraminifera and exploited for paleoceanographic and paleoclimatic reconstructions. This context thoroughly justifies the efforts pursued by the scientific community in order to decipher the mechanisms underlying their biomineralization. While biomineralization has been fairly extensively studied in marine organisms such as molluscs and corals, studies of foraminiferal biomineralization are still relatively rare, due undoubtedly to the complexity of their biomineral structures at the relevant sub-micrometric scale.

The hierarchical multi-scale structures of calcareous biominerals are never seen in inorganic mineralogy: the morphologies displayed by foraminiferal tests (the calcified parts of the organism) constitute striking examples of the highly complex 3D shapes that can be produced by biomineralization (Fig. 1). While it is known that biomineralization is under the influence of organic molecules (which control crystallographic orientations, crystal size and texture), to date a detailed understanding of biomineralization is still lacking. This is due to the fact that to date, observations of calcification are either made before calcite crystallization through fluorescent labeling [e.g. Nehrke, 2013] or after the death of the organism through elemental mapping (NanoSIMS) [Branson, 2016] but not during the life of the foraminifera.

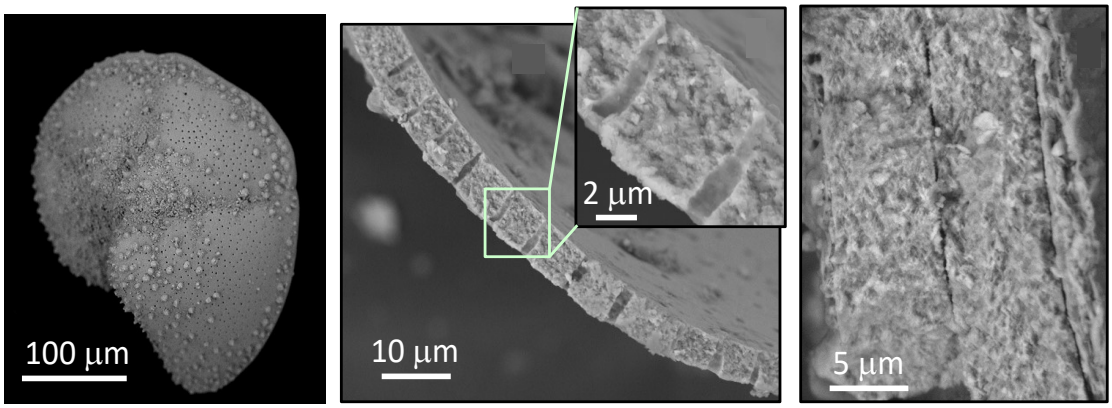

Figure 1: Scanning electron microscopy views of the living planktonic foraminifera *Globorotalia truncatulinoides* (collected in the Bay of Marseille)

Addressing this question would ideally require *in vivo* analysis of mineralizing organisms, a highly challenging program that relies on our capability to simultaneously target biomineralization sites within the living organism, the relevant length scales *and* the relevant chemical and/or physical parameters. **However, the current state of the art of imaging techniques does not allow *in vivo* observation, associated to chemical sensitivity, label-free and highly resolved. An important demand exists for more quantitative approaches that should overcome the lack of chemical and structural information during the development of organism in real time.**

Coherent Raman scattering (CRS) technique has proved to be powerful due to its label-free, three-dimensional, chemical selective and real-time imaging capability. In coherent anti-stokes Raman scattering (CARS), two beams of different frequencies interact within the sample to excite a vibrational resonance [1]. A probe beam is used to probe the vibrational excitation by generating a new anti-Stokes frequency shifted beam. The anti-Stokes frequency is specific to a given chemical bond and the associated beam intensity is quadratically dependent on the molecular concentration. In the microscopy-scanning mode, 3D density distribution images are thereby obtained with lateral and axial resolutions of about 350 nm and 2.5 µm, respectively. In the vicinity of the CO_3^- resonance, CARS allows distinction between crystalline and amorphous calcium carbonate, making it ideally

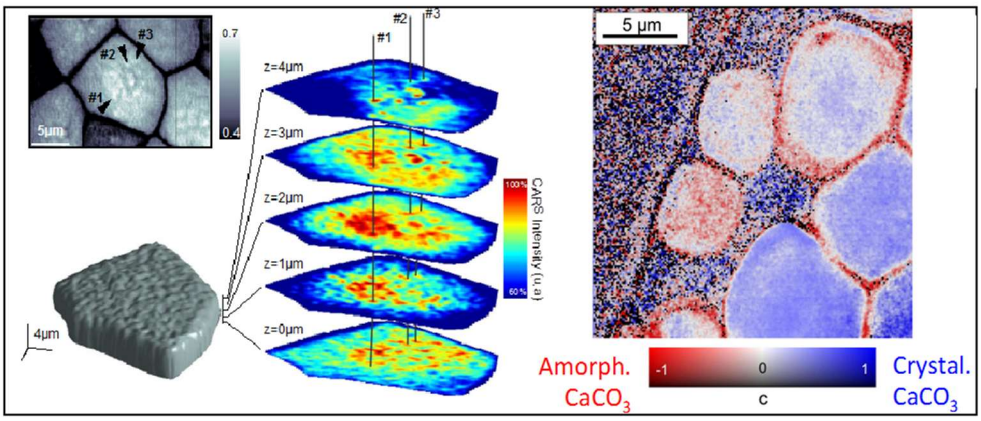

Figure 2: Carbonate distribution measured by CARS optical microscopy on *Pinctada margaritifera* pearl oyster, preliminary result from J Duboisset Fresnel Institute.

suited for the investigation of the proposed biominerals. This is timely illustrated in Fig. 2, where the calcareous crystalline-to-amorphous ratio is imaged in an extremely thin (0.5-4 µm) prismatic unit of a molluscs shell. **It has then become apparent that the Raman spectrum is the very signature of the shell construction process and that coherent Raman spectroscopy is able to give information unavailable until now.**

1.2 METHODOLOGY

Methodology: The goal of the PhD thesis is to bring original structural information on the morphology, chemistry and crystallinity of one major marine calcifier, with the ultimate aim of paving the way for an *in vivo* analysis of biomineralization. The development of the thesis, designed to reach this goal, is based on three sequences: (1) one for methodological developments of the microscopy CARS, in order to provide the throughput level, sensitivity and resolution needed for investigation of the chosen biominerals, the imaging parameter on test samples and foraminifera fixed samples, (2) one for the development of foraminifera cultures under controlled conditions that will be used for (3) the *in vivo* imaging during organism life.

To succeed along these lines, the PhD student will share his/her time between Fresnel Institute and CEREGE laboratory. The CARS microscope is present at Fresnel Institute and will be developed in order to focus on dead shells of foraminifera. Once this objective reached, the PhD student will develop the culture of living planktonic foraminifera at CEREGE. His/her formation will be acquired with several visits in Santa Catalina Marine station (USA) and Eilat marine station (Israel), where foraminifera cultures are done yearly. Furthermore the PhD student will participate to cruises on the RV Antedon (INSU / OSU Pytheas) in the Mediterranean Sea in order to collect living foraminifera. Lastly, the PhD student will develop at Fresnel Institute a dedicated controlled sea water incubator in order to study live organisms with a CARS microscope.

Feasibility in 3 years: The technology used in the PhD work has been already validated. CARS imaging is mastered at the Fresnel Institute (see publication) and have already demonstrated on similar sample, like oysters (see figure 2). The PhD will start from these preliminary achievements.

Originality: There is no reported work on CARS microscopy that enable to study *in vivo* sea organism. The combination between one world leader in advanced imaging technique and one expert in paleoclimatology, would bridge the gap between physic-chemists and oceanographers, and yield unique live insights on calcification processes.

Impact: With respect to industrial developments, biominerals constitute an amazing source of inspiration for the design of novel materials. The production of nanostructured materials that resemble the hierarchical structures of natural hard tissues like bones and teeth remains a primary objective of medical material engineering. As a consequence, one looks at developing schemes for assessing the quality of man-made, bio-inspired materials and their resemblance, at the nanoscale, to the original biominerals. More generally, mimicking biogenic mineralization processes opens an avenue for the bottom-up synthesis of innovative hierarchical materials, compatible with ecologically-friendly production environments.

1.3 WORK PLAN

The goal of the PhD thesis is to bring original structural information on the morphology, chemistry and crystallinity of one major marine calcifier, with the ultimate aim of paving the way for an *in vivo* analysis of biomineralization. The PhD student will share his/her time between Fresnel Institute (IF), Cerege (C) and Parc National des Calanques (PNC).

<table>
<tr><td></td><td>0 – 6 month</td><td>6 - 12</td><td>12 - 18</td><td>18- 24</td><td>24 - 32</td><td>32 -36</td></tr>
<tr><td>IF</td><td colspan="2">Microscopy development</td><td>Ex vivo imaging</td><td></td><td>In vivo imaging</td><td rowspan="3">Writing thesis</td></tr>
<tr><td>PNC</td><td>1w</td><td>1w</td><td colspan="3">2w</td></tr>
<tr><td>C</td><td></td><td></td><td colspan="3">Foraminifera culture</td></tr>
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1.4 SUPERVISORS AND RESEARCH GROUPS DESCRIPTION

1- Fresnel Institute carry out research on four themes: electromagnetism and metamaterials, nanophotonics and optical components, data processing and random waves and, finally, advanced and living imaging. Our two strong lines of research can be identified around the notions of image and optical components. **Biomineralization at Fresnel Institute:** Biomineralization processes received recently a great attention. An ERC Consolidator grant starts in 2017 around biomineralization process using X-Ray microscopy lead by Dr Chamard. The skills develop at Fresnel Institute will ensure an ideal environment for the PhD student. **Mosaic group** is an interdisciplinary research group aiming at raveling life science problems using advanced photonic tools. Mosaic principal investigators are physicists and biologists working together at the cross roads between advanced optical imaging, and biology.

Julien Duboisset (maître de conferences) will supervise the PhD student at the Fresnel Institute under the supervision of **Hervé Rigneault** (CNRS, HDR, head director of Mosaic Group) until Dr Duboisset has the right to supervise officially a PhD thesis (during 2017).

2- **CEREGE** has a recognized expertise in paleoclimates and geochemistry. The **group Bioindicateurs et traceurs des paleoenvironements** takes advantage of microfossils to document past environmental changes, based on a two pronged approach combining extensive fieldwork - warranting direct access to scientifically robust samples and a recognized knowledge of the ecology and taxonomy of different planktonic groups. **Thibault de Garidel-Thoron** is a CNRS researcher working on planktonic foraminifera. He is head of the scientific theme « **Variabilité Climatique and Impact sur les Ecosystèmes** » and has published articles in Nature (2), Science and PNAS. He will co-supervise the PhD at CEREGE. He has also developed a long time interest in outreach (coordination of Fete de la Science de l'Arbois, largest environmental festival in France; former RealClimate blog contributor).

2. 3I DIMENSIONS AND OTHER ASPECTS OF THE PROJECT

2.1 INTERDISCIPLINARY DIMENSION

The proposed PhD connects the sectors of physics, with the development of label-free microscopy technique, and paleoclimatology with the study of one of the most widely used paleoceanographic indicator. Ultimately it is expected that the methodological framework develop will apply to translational research as label free microscopy technology will have the ability to observe biomineralization process by looking at the chemical and crystal structure. It will then make possible to define microscopy protocol for other organisms where biomineralization is involved.

2.2 INTERSECTORAL DIMENSION:

The PhD project will include a critical outreach dimension with the **Parc National des Calanques.** The National Park of Calanques is located in France, in the heart of the metropolis of Aix-Marseille-Provence. It is one of the ten national parks in France. Environmental education is at the heart of the missions of the Calanques National Park. For this, the Park has a field staff who shares its passion and passes on its knowledge to the public. It also works within the framework of the partnership Educalanques with 5 partner associations that contribute to this mission. We have the formal agreement of one of this partner (Petits Débrouillards PACA) which will help the PhD student in developing outreach products. This association already collaborates with the PNC through the "Sciencetour".

The doctoral candidate will participate to the development of environmental education actions by a set of 4 of weekly stays at the HQ of PNC. The knowledge acquired during his/her PhD thesis, and more specifically the unique 3D images of planktonic organisms retrieved within the PNC, will be integrated within the series of event planned by the PNC: exposition on the role of deep sea canyons (MedSeaCam), planned in 2017-2018; Camargo Foundation art-science residence (2017-2018); and an exposition on interdependence of biocenoses (dates to be determined).

This project will directly respond to the regional SRI-S3 objective "Tourisme - Industries culturelles et contenus numériques". The project will help to develop the educational role and the tourism attractiveness of the Parc National des Calanques. We foresee to provide through intriguing images and 3D models of marine plankton an unexpected view of the Mediterranean Sea biodiversity. This content might be later used for the Manifesta 2020 event to be held in the Marseille-Provence metropolis.

2.3 INTERNATIONAL DIMENSION:

The PhD trainee will be included in a network of young scientists working on paleoclimates and foraminifera. As the PhD student will be a physics student, he will have to train into paleosciences. The PhD student will attend the yearly International Urbino Summer School on Paleoclimatology which gathers scientists and students from all over the globe. The acquisition of culture techniques for foraminifera will be done in collaboration with UC Davies (Dr. H. Spero, CA - USA), which is the pioneer of planktonic foraminifera cultures, and JAMSTEC (Dr. Kimoto, Japan) series of field trips to the main marine stations where planktonic foraminifera are grown (Catalina Is., Puerto Rico and Eilat). Those collaborations will be critical to acquire the know-how for foraminifera cultures.

3. RECENT PUBLICATIONS

[1] Carsten Cleff, Alicja Gasecka, Patrick Ferrand, Hervé Rigneault, Sophie Brasselet, Julien Duboisset, Nature Commun **7** 11562 (2016)

[2] Regoli, F., de Garidel-Thoron, T., Tachikawa, K., Jian, Z., Ye, L., Droxler, A. W., et al. (2015). Progressive shoaling of the equatorial Pacific thermocline over the last eight glacial periods. *Paleoceanography*, *30*(5), 439–455. http://doi.org/10.1002/2014PA002696

[3] Morard, R. et al. (2015). PFR 2: a curated database of planktonic foraminifera 18S ribosomal DNA as a resource for studies of plankton ecology, biogeography and evolution. *Molecular Ecology Resources*, *15*(6), 1472–1485. http://doi.org/10.1111/1755-0998.12410

4. EXPECTED PROFILE OF THE CANDIDATE

To apply to this PhD proposal, the candidates must hold an internationally-recognized Master equivalent degree in physics, engineering, bio-engineering or medical-engineering.

Experience in experimental research in optics, biophotonics will be highly appreciated, but is not mandatory. Interest in biology is desirable.

The selected PhD student will work under close supervision of a senior researcher and benefit from direct mentorship. PhD students will also benefit from several courses specially developed within the European Erasmus Mundus Doctorate program Europhotonics and POESI running at the AMU Fresnel Institute.

A PhD degree from the University of Aix-Marseille will be granted after successful completion of the PhD research.

PhD speaking language will be English and/or French (English speaking is mandatory as being the language of the Mosaic group).

5. SUPERVISORS' PROFILES

Hervé Rigneault (DR1 CNRS – PhD director – 151 publications – >3000 citations – h-index 30)) is leading the Mosaic group at the Fresnel Institute (http://www.fresnel.fr/mosaic) and has been involved for almost a decade in developing dedicated optical instrument for biology. The group is presently involved in the national infrastructure programs France Bio Imaging and France Life Imaging for its expertise in optical microscopy. Since 2002, H. Rigneault has been developing coherent Raman microscopy and was the first in France to build a CARS microscope (http://www.fresnel.fr/spip/spip.php?article570 - see CARS image gallery). **Julien Duboisset** (Maître de Conférences – 25 publications >500 citations – h-index 14) reach the Mosaic team in 2010. He develops CARS microscopy and will supervise the PhD student when he will obtain the "Habilitation à diriger des recherches" (during 2017).

Past PhD students in the field of nonlinear coherent Raman imaging (H Rigneault PhD Director, *supervised by Julien Duboisset):

N. Djaker, 'Microscopie par diffusion cohérente Raman CARS : Application à l'imagerie des milieux biologiques', Sept 2003 - Oct 2006, Marseille, 4 publications – Now Assistant Professor Univ Paris 13

D. Gachet, ’Microscopie CARS (Coherent anti-StokesRaman scattering). Génération du signal au voisinage d'interfaces et à l'intérieur d'une cavité Fabry-Perot.’, Sept 2004 - Nov 2007, Marseille, 15 publications – 2 patents – Now chief engineer ATTOLIGHT (Switzerland)
F. Munhoz, ‘Polarization resolved coherent anti-Stokes Raman scattering (CARS) microscopy’, Sept 2007 - Sept 2010, Marseille, (prix de thèse PACA 2011) – 4 publications – Now chief engineer at L’OREAL
Pascal Berto, ‘Microscopie et spectroscopie de phase. Développements en diffusion Raman cohérente (CRS) et en thermo-plasmonique’, Sept 2010- Janv 2013, 12 publications, 1 patent - Now assistant professor at Paris V
Sarah Saint-Jalm, ‘Sources optiques fibrées solitoniques pour la spectroscopie et la microscopie nonlinéaires’, Sept 2011 - Nov 2014, 3 publications, Now in postdoctoral study at Exeter Univ – UK (S. Saint Jalm will interact with the PhD student as developing Raman imaging at Exeter Univ)
Xueqin Chen, ‘Développements en spectroscopie et microscopie non linéaire pour l'étude morphologique et fonctionnelle de la peau humaine’, Sept 2011 - Dec 2014, 2 publications, Now engineer at L’Oreal Recherche.
Fatma-Zhora Bioud*, ‘Microscopie de mélange à quatre ondes résolue en polarisation pour sonder l’ordre moléculaire dans les milieux biologiques’ sept 2010-Dec 2013, 3 publications, now postDoc Univ St Andrews

PhD supervised (100%) in Oct 2017:
Xavier Audier – Sept 2015 – Sept 2018
Camille Scotte – Oct 2016 – Oct 2019

Thibault de Garidel-Thoron (CR1 CNRS, 1270 citations, 22 papers, h-index 15) is co-leading the Thème Variabilité Climatique et Impact sur les Ecosystèmes at CEREGE. He has a recognized expertise in paleoclimatology based on the diversity, ecology and geochemistry of planktonic foraminifera. He is the PI of the ongoing ANR project FIRST (Foraminifera Imaging and Recognition Sorting Tool), and is a member of different scientific panels.

Former PhD students :

Aurore André : ‘Taxonomies moléculaire et morphologique chez les foraminifères planctoniques : élaboration d'un référentiel et cas particuliers de Globigerinoides sacculifer et Neogloboquadrina pachyderm’a (sept 2010-nov. 2013) – 3 publications, now assistant professor at Université de Reims.
Fabienne Régoli : ‘Diversité cryptique du zooplancton carbonaté et réponse aux changements globaux du pléistocène à l'anthropocène’ (oct. 2009-juil. 2014) – 1 publication, now school teacher in Marseille.

No ongoing PhD student