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Interactive comment on “Identification of two organic bands showing different chemical composition within the skeleton of *Porites lutea*: a confocal Raman microscopy study” by M. Wall and G. Nehrke

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Received and published: 3 August 2012

This paper exemplifies the high analytical potential reached by the optical microscope when, in addition to its classical use based on visible light, laser produced lights are focused onto the polished sample surface and the resulting Raman radiations submitted to spectral analysis associated with digital mapping methods. The two-century old instrument is then transformed into a powerful device allowing multiple characterizations to be made. The paper focuses on *Porites*, a representative of this Scleractinia coral genus whose calcareous skeletons are the most used as source of information

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concerning evolution of sea-surface temperatures in tropical areas. Climate change investigations require continuous analytical improvements in accuracy and reliability of the numerical values integrated into predictive models. With respect to accuracy, SIMS instruments dedicated to chemical or isotopic measurements now allow spatial resolutions in the micrometer range, but regarding reliability analysts are now facing a new type of problem. As pointed out by Nothdurft and Webb (1) in conclusion of a remarkable microstructural study of several coral species: “Although finer temporal scales have become possible with high resolution, high precision microsampling techniques, this sampling must take the exact temporal relationships between adjacent elements of microstructure into account. Even a slight temporal divergence may invalidate time series data”. Conclusively “a greater understanding of microstructure [i.e. the three-dimensional arrangement of the skeletal components] is required if coral skeletogenesis is to be understood adequately for coral skeleton to serve as repository of temporally constrained geochemical data”.

In the current practice, establishing growth patterns for any coral sample with such a resolution requires observation at the SEM (as did Nothdurft and Webb). A preparative process comprising chemical etching and metal coating of the sample is needed. As a result, when microstructural arrangement of skeleton components is well known the sample cannot be longer used for chemical or isotopic measurements. Removing the coating by new polishing (coarser to finest grains) obviously modifies the surface microstructures, making useless the first observational step. This is quite different when observing the polished (but non etched and not coated surfaces) through Laser Confocal Raman Microscopy, as Wall and Nehrke are reporting in this paper. Not only images of fine skeletal patterns are easily obtained: they can be compared to polarizing microscope views (as seen in their Fig. 1) but in addition, Raman imaging allows obtaining information about organic compounds that have driven skeletal growth. From a single and uncoated section a wealth of structural and spectral information contributes to reinforce interpretations concerning skeleton growth patterns at a given place. Without a doubt, experienced analysts will appreciate that without any additional preparative

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steps, the sample now well known with respect to major temporal growth steps and fine scale microstructural patterns can be immediately submitted to high resolution analytical instruments (that now operate at a comparable dimensional level).

From both points of view, originality of the used method and potential contribution to the on-going evolution of practice in the field of high resolution environmental studies, this report deserves careful attention.

Structure and Content of the paper

This 32-page paper (including 11 figures, scheme and color plates) follows the usual organization. Part 1: Introduction As the paper focuses on microscopic evidences revealing fine scale structures, its introduction summarizes the historical sequence of observations which has progressively improved our representation of coral skeleton structures and mode of growth. With respect to adequacy of references and sometimes historical exactitude of facts, it appears that authors should be asked to somewhat modify their first version of this introduction.

Examples. (p. 8273, line 17-18): "... an intricate and complex skeleton, which represents a chronological layered archive (e.g., Lough and Barns, 2000; Cohen and McConnaughey, 2003)." This is a typical example of inadequate reference. In 1980, i.e. long before the cited papers, the first book in the series "Topics in Geobiology" was published under the title: Skeletal Growth of Aquatic organisms: Biological Records of Environmental Change (Plenum Press, D.C. Rhoads and R.A. Lutz Editors). In this book, tens of examples concerning most of the calcifying organisms are carefully studied with respect to their potential as "chronological layered archive." If some reference has to be cited there (reviewer doesn't think so), this should be this book (and more precisely the Dodge & Vaisnys' contribution dealing with corals). Authors will find that the first report on growth patterns as biological archive in coral skeletons was made by Whitfield 1896 !! As a general remark, when dealing with occurrence of a new concept, citation of the relevant authors is simply a matter of equity regarding the efforts of our

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predecessors.

Even worst is the next citation (p 8275, line 5). After an excellent sentence (“The morphology of skeletal structures of corals represents the foundation of this investigation..”) the cited reference is J.E. Sorauf 1972!! No doubt that J.E. Sorauf, whose SEM contribution to understanding fiber growth is well recognized, should be surprised to be considered as a reference for discovery of the basic morphological organization and overall diversity of the corallites as well as for general relationships between skeletons and soft tissues in corals. Owing to his deep knowledge of coral literature, he would surely suggest paying some attention to much older investigations and figures like the following ones (Fig. 1), which exemplify what was published as early as 1848 (yes, eighteen forty-eight) about corallite morphology.

Fig. 1: Examples of figures from H. Milne-Edwards and J. Haime 1848-49: *Recherches sur les polypiers*, Mémoire n° 4-7, *Annales des Sciences Naturelles*, 3^e sér. Zoologie, t. X, 16 pl. a,b,c : Skeleton of the *Porites* (these are eye-lens observations and hand-made drawings!).

In parallel to a selection of justified references, readers may expect that modern imaging techniques allow authors to provide data substantially better than those produced in the midst of 19th century. Concerning the W. & N. paper, readers not familiar with *Porites* microstructure cannot make any clear relationship between spatial arrangement of fibrous tissue in this species and the series of polished sections brightly illustrating the experimental results in the following parts of the paper (e.g. to fully appreciate Fig. 7, which provides good examples of this correspondence, Fig. 1 of the manuscript must be largely improved). Presented alone, the annual banding (Fig. 1d) is irrelevant with respect to the scale of further observations and authors' objective. This picture must be completed by morphology of skeletal growth layer seen at the micrometer scale. Conclusively, figure 1 in its present status is missing its essential function: introducing readers to three-dimensional arrangements of skeleton fibers in *Porites*, allowing him to immediately appreciate the microstructural significance of the

Raman results.

The “building block” question p. 8274, lines 18 to 20, a sentence summarizes the general organization of the skeletons in corals: “Both macro- and micro-morphological elements are composed of two building blocks (microstructural elements) the centers of calcification (COC) and fibers, first described by Ogilvie (1896)”. Historically this is not fully correct. The Ogilvie’s paper entitled: Microscopic and Systematic Study of the Madreporarian Types of Corals is a milestone among coral studies because, after the first description of fibers in the Scleractinia skeletons (made by Pratz, 1882), the spatial arrangement of these fibers was, for the first time, used as a taxonomic criteria by Ogilvie. She noted the very frequent radial arrangement of fibers: therefore she used the term “centres of calcification” as the points from which fibers diverge. But, it was simply a geometrical note: she cannot think that centers of calcification could be “building blocks” of the skeletons owing to her conception of corallite mineralization. Historically, a virulent controversy was raging at that time between two different concepts of calcification in corals: intracellular calcification, which was advocated by von Heider (1886) opposed to the von Koch’s point of view, defending an extracellular calcification (same year). Unfortunately Ogilvie supported the erroneous view. Thus she wrote (1896, p. 102):”I find however that Koch, Fowler and Bourne are wrong in their conception of the calicoblasts. . . [name of the cells forming the basal layer of the Scleractinia polyp in the histologist language]. The calicoblasts build up successive layers of calcified cells, which hang together at first by their cell wall, and ultimately, as crystalline change continue, form the individual laminae of the skeleton”. To understand the origin of Ogilvie’s opinion we have to consider her very remarkable observations exemplified by figure 2. A comparison is made between Ogilvie’s drawings of thin sections she made in coral septa (a, b, c) and what can be obtained when looking at a fibrous fan-system after etching of a polished surface.

Fig. 2: Comparison between Ogilvie’s figures of fiber growth in septal structure (a-d) and recent observations. e: SEM view of a fibrous fan-system; f: thin section in

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polarized light (note that growth layers are weakly visible); SEM view of an etched corresponding surface.

It is fascinating to see that Ogilvie actually described the layered growth of the coral fibers, but the layers she draw were interpreted by her as layers of calcified cells. Owing to her opinion about the cellular structure of the whole septum, her “centres of calcification” were not specific structures (not at all “building blocks”), but simply the starting point of cell layer superposition. As a result, even after general acceptance of the extracellular calcification in corals, centers of calcification were considered as doubtful structures. For instance Wells (in the RC Moore Treatise on Scleractinia) always used quotation marks when he wrote about “centers of calcification”. It is only when further investigations provided evidence for specific structural characteristics and chemical composition of the COCs that their actual role as “building blocks” was recognized in models of coral mineralization.

Comment on the introductory chapter: Topic of this paper does not require a full presentation of the historical steps by which the descriptive terms now in use have progressively acquired their present meanings. Historical details are simply given to avoid oversimplification in writing of a new introduction. Considering the general content of the paper, introduction in its present status seems inadequately oriented as it focuses on the input of Raman method as a contribution to understanding of the biomineralization process in corals. By so doing, authors are led to explain present concepts in this research field, leading to an excessively long introduction (which even comprises some disputable statements). Much of this introduction is not at all necessary to make readers receptive to the forthcoming Raman structural pictures. Suggestion is made to write an introductory chapter largely simplified by limiting information to what is needed for a clear understanding of author’s technical approach and methodologic objective. Most of information and comment regarding biomineralization should be more useful in the discussion chapter (possibly) and -from reviewer view-point- should be simply removed from this paper. A next one, in which focus will be placed on compositional

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information obtained from Raman spectral analysis, should be of major interest and in-depth discussion about biomineralization would be at a right place there.

Clearly, to ensure a wider audience to this paper its introduction should focus on what is the primary advantage of Raman mapping in description of coral microstructure and growth patterns. The title itself should be reworked in such a view.

Part 2: Material and method Observations were carried out on sections from a single *Porites* specimen which had been stained in situ during 16 hours by alizarin red, three months before collection at Andaman Islands (Tailand). Authors assume that daily growth rate can be calculated by dividing the distance between alizarine marking and the sample surface by the number of days (p 8282, line 5). To be valid this simple calculation requires assumption that growth was continuous and regular during the three months after marking. Figure 10, for instance, showing the three distinct growth steps (the two lower ending with strongly fluorescent surfaces), suggests that growth is not so regular. This seems a common growth pattern in *Porites*, as Nothdurft and Webb in the above cited paper, have evidenced some equivalent mode of growth (see Fig. 3). Some information must be given to make this point clear. Note that simply by itself this point fully justifies the use of Raman observation as a first rank practical tool to ensure reliability of environmental measurements by a strict correspondence with growth patterns, as pointed out by Nothdurft and Webb. Figure 3 makes this obvious.

Fig. 3: SEM view and scheme of skeletal microstructure shown by Nothdurft and Webb (a-b) and Raman picture of a comparable section shown by Wall and Nehrke.

The purely technical information concerning widely used methods (2.3 and 2.4) should be reduced to a minimum, whereas part 2.2 Confocal Raman microscopy should be somewhat extended, taking care of using a “simplified mode”. Please consider that a paper like this one, which should exert a methodological influence, must be a stand-alone text. If basic explanation concerning Raman method and potential results were already given elsewhere, they must be adapted to this case study and repeated, al-

lowing non Raman-familiar readers to immediately capture the deep sense of the displayed pictures. Accordingly, comments related to biomineralization mechanism (part 2.5) should be made in the relevant part of the discussion (3).

Part 3: Results and Discussion This part is essentially formed by 8 composite figures (Fig. 4 to 11) showing the potential of the Laser Raman Confocal observation as microstructural and analytical tool.

The first one (Fig. 4) is excellent. Comparison of 4b and 4c (thin section of the same area in cross nicols) leaves no doubts about ability of the method to clearly reveal the fibrous arrangements through variation in proportions of the main peaks produced by interaction with laser light and mineral lattices. Reviewer draw attention on the importance of having the pictures printed as large as possible. Therefore editorial compromise has to be made concerning paper length. Perhaps Fig. 5 and 5 should be merged, with suppression of most of the “mixed” pictures (numbered 3): their input is limited excepted for 6 A3 and B3 which clearly show the position of fiber bundles with respect to the major growth limits.

Fig. 7, and specifically 7B is also excellent, as it beautifully reveals the specific composition of the limit between superposed growth layers. This is a significant contribution of Raman investigation to improve our representation of the biomineralization cycle. Ideally, chemical information obtained from EMZ circular areas and the thin limits of the growth layers should be the objective of a next investigation

Fig. 8 illustrates the magic of Confocal microscopy combined to Laser Raman. Not only sample surfaces can be seen in their structural aspects but below the surface, the same sample can be examined without losing imaging resolution and structural characterization. From a practical view point, three-dimensional reconstruction of the calicinal rod (trabecule) by imaging software should allow free observation from any point of view: an ideal situation to select areas to be investigated.

Fig. 9-10. In order to gain space for publishing the pictures as large as possible,

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repetition of the A and B columns in fig. 10 should be avoided (similar to fig. 8). Alteration of the mineralizing process at the major growth limit is well illustrated by the exact correspondence between Raman and microprobe mapping.

Fig. 11 In addition to Fig. 9, this last figure shows that LCRM may also contribute to improvement of our understanding of macro and microscopic growth patterns through biochemical information.

Comments Author's interpretation of these remarkable pictures comprises additional examples of inadequacy in cited references: referring to Cuif and Dauphin 2005 when speaking of fan-like arrangement of fibers is not serious. This pattern is one of the oldest identified in coral skeleton: it was already visible in Pratz' figures (1882) and so well drawn by Ogilvie (notwithstanding her interpretation of fibers themselves) that Wells 1951 was still using her drawings (see 2 p. F377, Fig. 231 and others).

However, author's comments on their data are constructive. Slight reworking should make them a bit shorter, with a more clear focus on the distinct information provided by this new type of microscope and the main difference between the two wavelength domains simultaneously available. Between the 200 to 1200 cm⁻¹ the main peaks allow structural mapping, opening the way to detailed spatial reconstructions of skeletal structures, whereas minor peaks (in the 600–1850 cm⁻¹ and 2800–3600 cm⁻¹) are produced by intra-skeletal organic components. A huge difference in respective intensities causes the major problem concerning the latter: overall fluorescence is a strongly limiting factor to mapping of organic compounds. This is also why this paper must concentrate on the spectacular progress in structural analysis made possible by this method. Once more, considerations regarding biomineralization should be reduced. The growth cycle model (Fig. 3) should be completed by clearly drawing fibers (instead of simply writing "fibers". This single word does not draw attention on the essential aspect of fiber's growth mode: the continuity of the growth layers between adjacent fibers, clearly disproving the concept of fibers as independently growing crystals (here, in contrast to the above citation of "fibrous fan-systems", is the right place

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to mentioning the Cuif and Dauphin 2005 BG paper). Avoiding repetition of reference (e.g. in part 3.5 may also contribute to shortening the manuscript (e.g. p. 8287: lines 11-13 and 29-30-33).

However, information regarding the potential of the Raman method to get localized compositional information should be maintained. Therefore figs. 9 - 11 are in right place to be commented as illustration of a possible contribution of the Raman method to our knowledge of the coral biomineralization process, implicitly announcing a future paper on this topic.

Recommendation

Reviewer strongly recommends publishing the paper after refocusing it in order to gain interest of a wider audience: the environmental geologists looking for a convenient method ensuring reliability of measurements made in close correlation with major growth steps. This is the only approach allowing accurate relationships to be established between numerical time-series and environmental oscillations. Accordingly important reworking of introduction, better selection of citations and comments and even relevant change in the paper title itself should to be considered.

1 Nothdurft L. D. and Webb, G. E.: Microstructure of common reef-building coral genera *Acropora*, *Pocillopora*, *Goniastrea* and *Porites*: constraints on spatial resolution in geochemical sampling, *Facies*, 53, 1–26, 2007.

2 Wells J. W., 1956. *Scleractinia*. – [In:] MOORE, R.C. [ed.] *Treatise on Invertebrate Paleontology, Part F, Scleractinia: F328-F444*, New York and Lawrence/Kansas (Geological Society of America and University of Kansas Press).

Interactive comment on Biogeosciences Discuss., 9, 8273, 2012.

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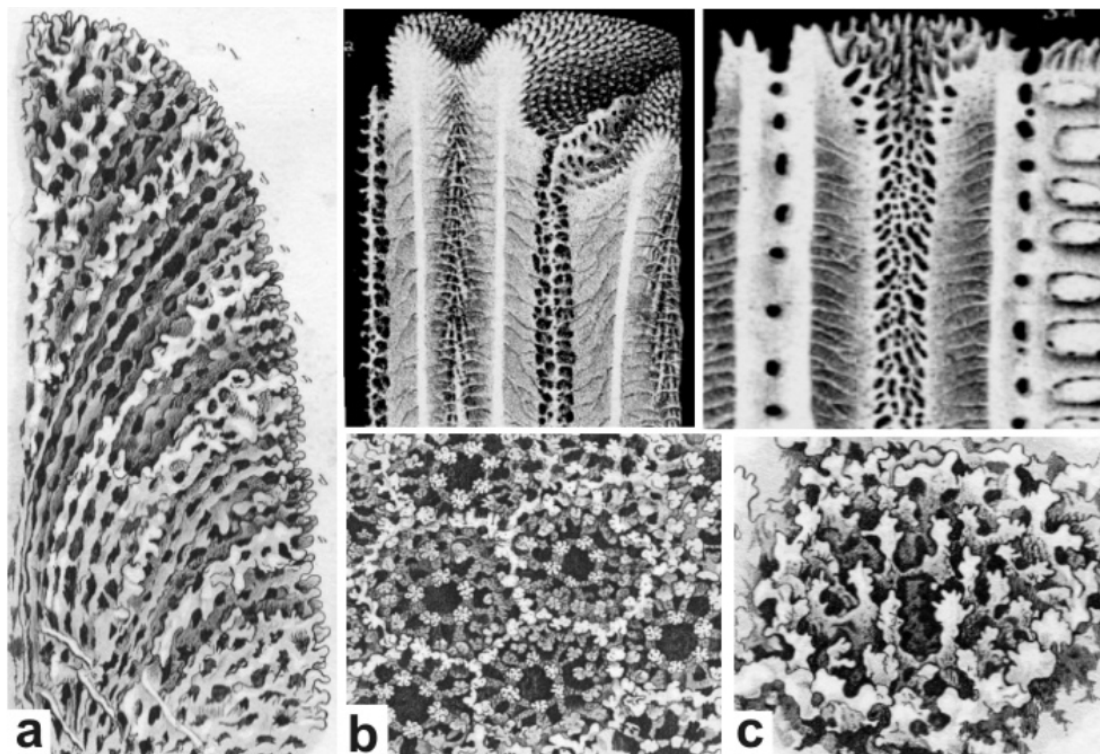


Fig. 1.

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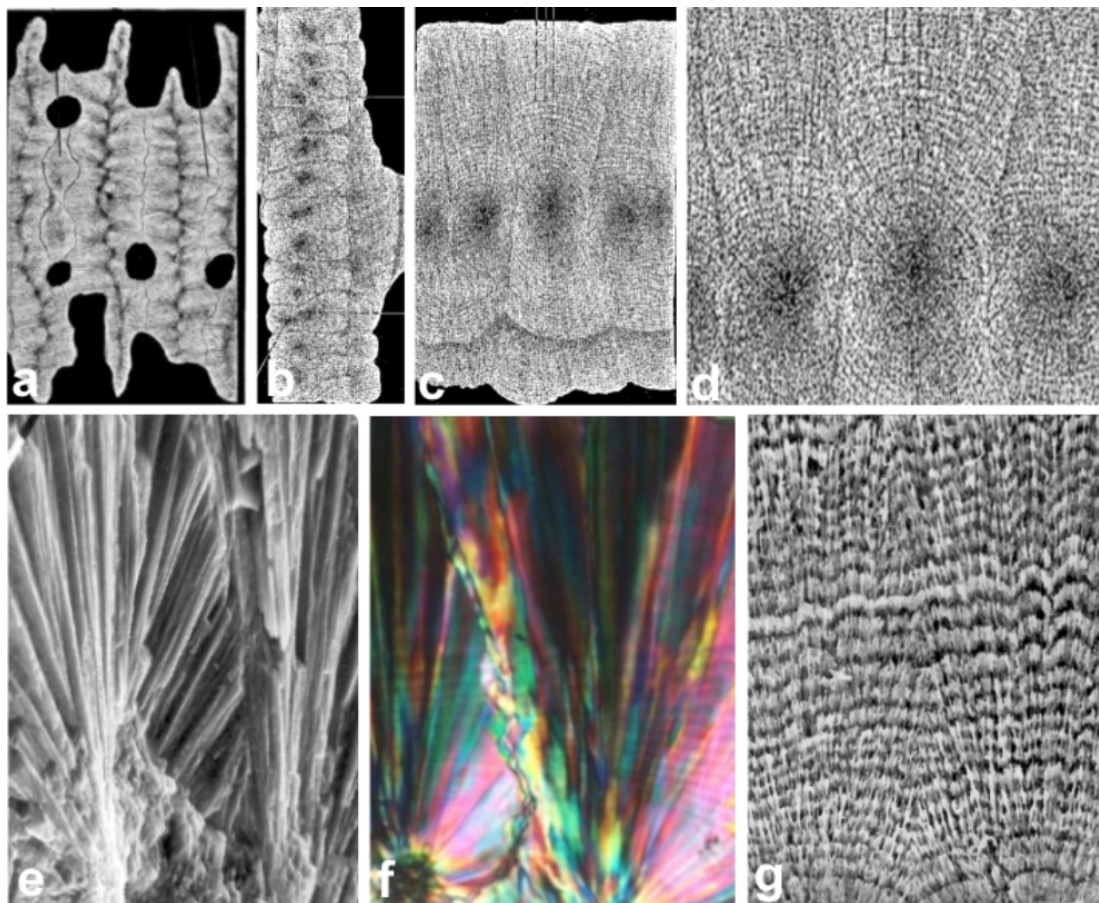


Fig. 2.

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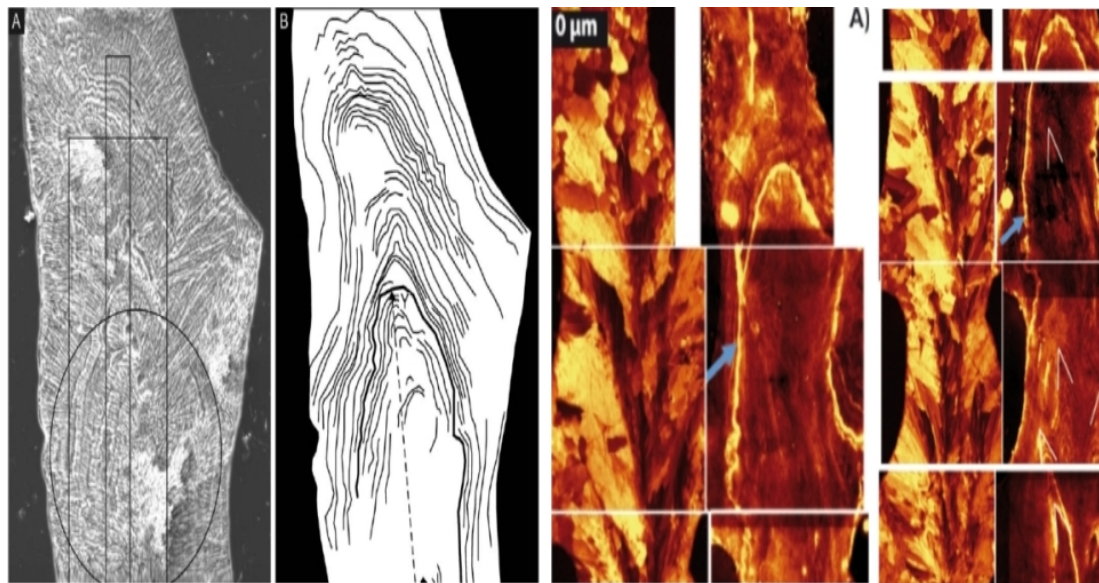
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Nothdurft & Webb, 2007, Fig. 17 (part)

Wall & Nehrke, Fig. 8 (part)

Fig. 3.

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