Raman microscopy in sclerochronology

a powerful non-impact tool to render hidden information visible in fossil bio-archives

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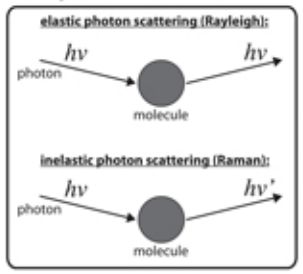
WHY & HOW ?

Throughout the last decades the anatomical and/or geochemical properties of marine biogenic hard-parts became a valuable source for palaeo-environmental information. However, standard methods of growth pattern visualization may fail in fossil bio-archives owing to alterations of the organic compounds within the biogenic materials with time. We demonstrate that confocal Raman microscopy (CRM) can identify and visualize growth patterns of mollusc shells from different geological ages with high spatial resolution (300 nm). In contrast to standard staining techniques (e.g., Mutvei´s solution) CRM has been applied successfully to samples in which the organic components are altered.

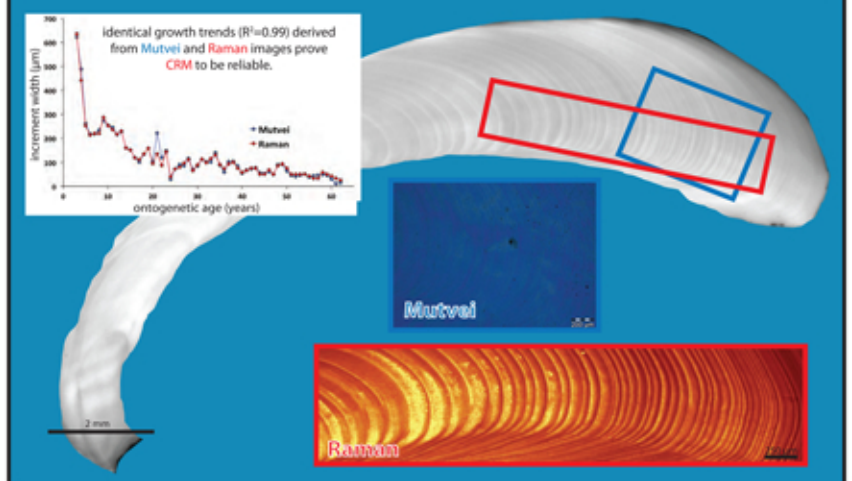


- Monochromatic light (laser) is needed; but a non-destructive method.
- Very small part of the light is scattered inelastically --> Raman effect !
- Difference in frequency between irradiated and scattered light (Stokes shift) can be used to identify analysed material.

CALIBRATION - PROOF IT WORKS

Infobox modern *Arctica islandica* from Norway

Modern specimen from Tromsø, Norway (69°39'N 18°57'E), collected in 2006 by Salma Begum (AWI Bremerhaven). Specimen ID: TT-N2-R. Mutvei´s solution immersion time: 23 minutes.
Identical growth increments visible in Mutvei and Raman images, resulting growth trends in very good agreement (R^2=0.99). **Therefore, CRM is a reliable method to visualize growth structures.**



TECHNICAL DETAILS

- Raman mapping: performed on a WITec alpha 300 R (diode laser with an excitation wavelength of 488 nm vv; 20x objective (Zeiss EC Epiplan)) using WITecProject software (version 2.10); high spatial resolution (300 nm); can be applied to fluids, gases, solids.
- Mutvei´s solution staining according to Schöne et al., 2005. Immersion times varied from 23 (in modern specimen) to more than 60 minutes (in fossil specimens). Mutvei images were taken using an Olympus SZX12 stereo microscope equipped with an Olympus U-CMAD CCD camera and analySIS docu software (Olympus, version 5.1).
- Increment measurements in Mutvei and Raman images were conducted using AxioVision software (Zeiss, release 4.8.1).

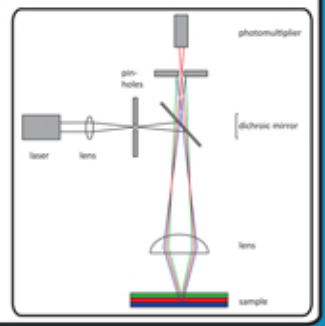
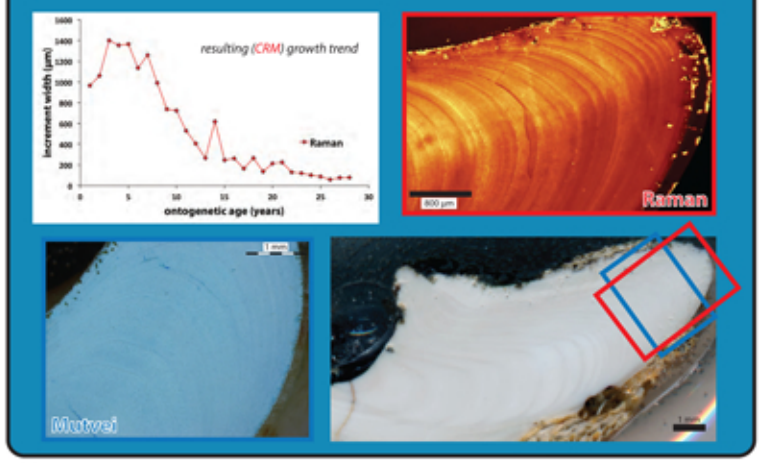


Figure: Schematic illustration of a Confocal Raman microscope. Hereby, confocal means that the focal plane can not only be chosen on the sample surface but also in depth or above the sample. This enables the reconstruction of three-dimensional structures.

FOSSIL EXAMPLES: RAMAN SUCCEEDS WHERE MUTVEI FAILS

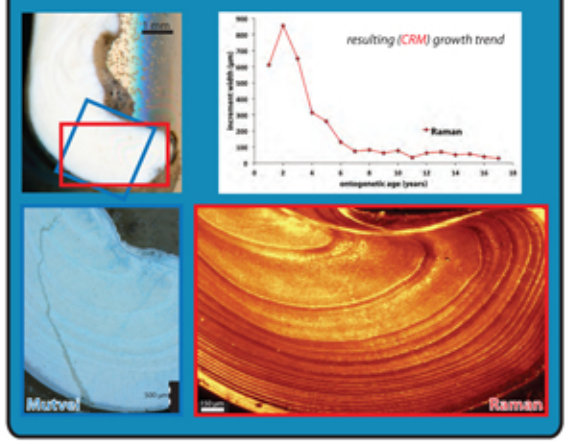
Infobox fossil *Arctica islandica* from the Coralline Crag, UK

From the Coralline Crag formation, UK (52°6'N 1°03'E). Pliocene (Ramsholt Member) specimen collected in 1974. Specimen ID: AI-CoCr-01. Mutvei´s solution immersion time: 60 minutes (stepwise). Due to a strong bleeching effect within the specimen and the relatively low amount of organics, the umbonal growth increments are only visible through Raman microscopy. Consequently, an entire growth trend can only be measured when this technique is applied, allowing a palaeoenvironmental investigation even in extremely bleeched specimens.

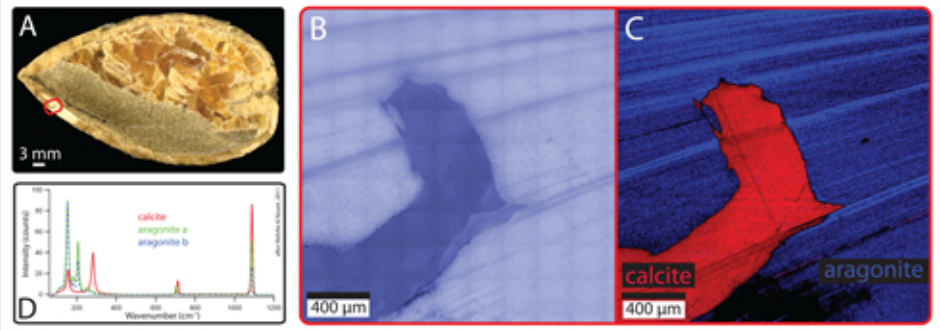


Infobox fossil *Pygocardia rustica* from Belgium

Pliocene (probably Piacenzian) specimen from Antwerp, Belgium. Collected in 1958. Specimen ID: RGM609.096. Mutvei´s solution immersion time: 46 minutes. *P. rustica* is a close relative to *A. islandica* but became extinct during the Pleistocene. Due to preservation and the decay of organics, Mutvei´s solution failed to visualize growth increments. By applying the Raman mapping technique, even intra-annual growth increments in the umbo become apparent.



IN ADDITION: CHECK FOR RECRYSTALLIZATION



Figures: Fossil *A. islandica* (Pliocene, Tjörnes Beds, Iceland) **A**, image of shell thick section. Both valves are preserved. **B**, stitched reflected-light micros-copy image of indicated shell portion in A. **C**, high-resolution Raman image of indicated shell portion in A. Original, aragonitic parts in blue and recrystallised, calcitic parts in red. **D**, Stokes lines can be used to distinguish between two polymorphs of calcium carbonate (aragonite and calcite) shell parts.

CRM is ideal to identify mineral (and organic) phases and potential taphonomic alterations (e.g., recrystallization from aragonite to calcite) in marine biogenic carbonates. Checking for such alterations should be a mandatory step prior to any kind of biogeochemical analysis (e.g., stable isotopes or trace elemental ratios) of fossil samples. Therefore, CRM can play an crucial role in the quality control of biogenic carbonate studies.

ACKNOWLEDGEMENTS & REFERENCES

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