

● High-resolution climate reconstruction using MRMS MALDI Imaging

Reconstruction of past climate conditions, e.g. sea surface temperature, with organic marker molecules archived in marine sediments is a well-established technique in paleoclimate research.

Abstract

Conventionally, biomarkers indicative of past climate conditions such as C_{37} -alkenones from haptophyte algae are chromatographically analyzed from lipid extracts generated from centimeter- and gram-sized sediment samples.

Consequently, the temporal resolution of the results is rather coarse and abrupt events or small-scale climate changes are averaged into a smoothed signal. Processes operating on these timescales are, however, the ones impacting modern ecosystems. Insights into these

paleoenvironments are crucial to assess their potential response to future climate change. This study shows how molecular imaging of these target molecules with MRMS MALDI Imaging enables paleoclimate research at unprecedented resolution.

Keywords:
Imaging, solarIX,
flexImaging,
climate proxies

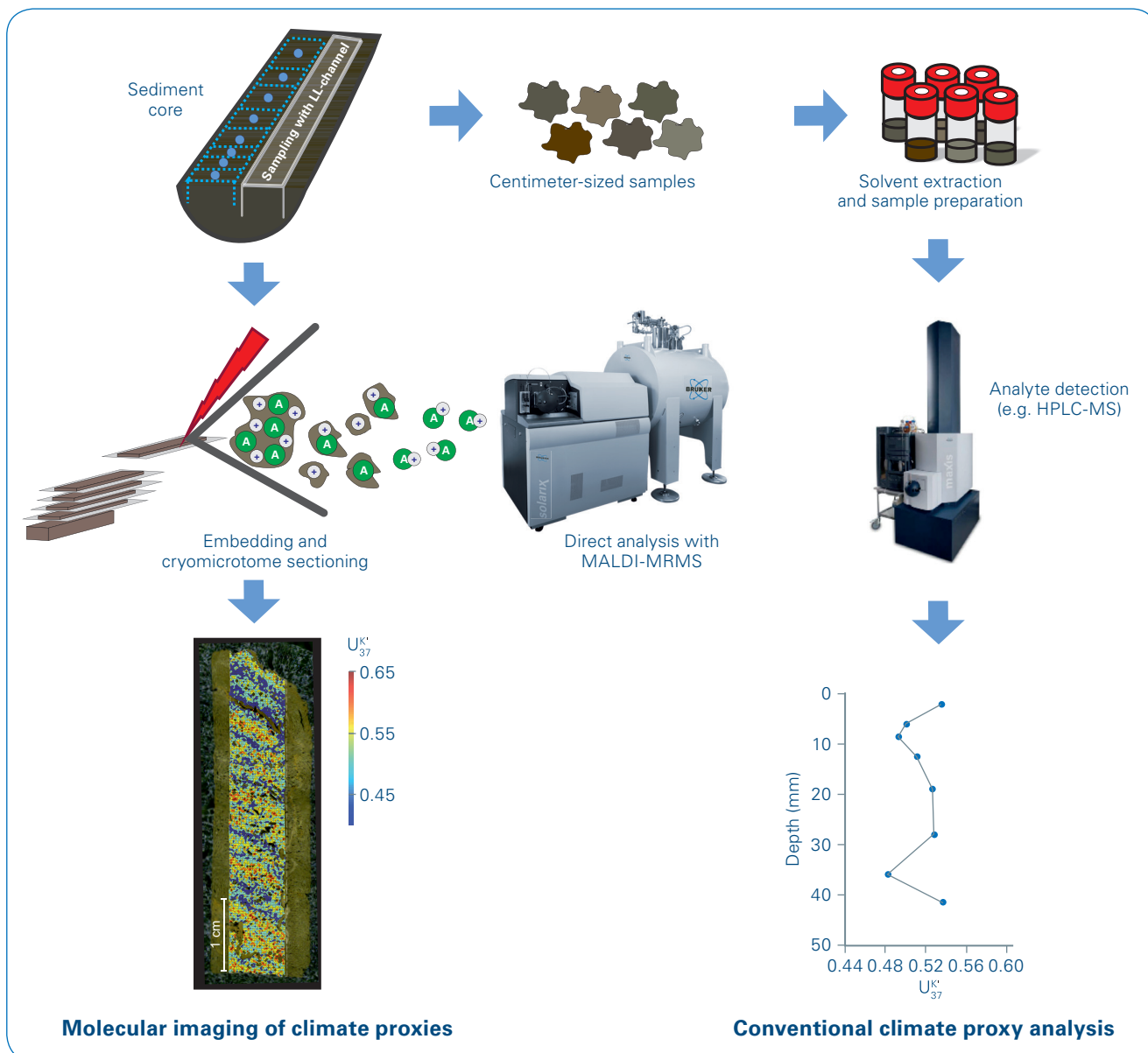


Figure 1: Schematic workflow of molecular imaging of marine sediments with MALDI-MRMS compared to conventional extraction based analysis by HPLC-MS.

Introduction

Gaining insights into past climate variability is essential to classify its current state and future trend. Marine sediments have shown to be valuable climate archives, as they can be accurately dated and record the prevailing environmental conditions under which they were formed. One approach of reconstructing paleoclimate uses molecular tracers originating from the lipid membrane of microorganisms that

once inhabited the water column. These biomarkers provide information on properties such as temperature, nutrient availability or pH in their biological producers' habitat. Conventionally, biomarker analysis uses gram-sized samples from a sediment core to account for the amount of material needed to generate a lipid extract using organic solvent-based extraction methods. The subsequent analysis is usually performed by gas- or liquid chromatographic methods coupled to mass spectrometry (Figure 1).

Besides the time-consuming procedure for the analysis, the extraction-based approach entails a loss of information as several millimeters to centimeters of the sediment are combined into one sample. Depending on the sedimentation rate, this method limits the temporal resolution of paleoclimate studies to decades or even centuries. Consequently, short-term climate oscillation operating on (sub)annual time oscillations remain inaccessible by this approach.

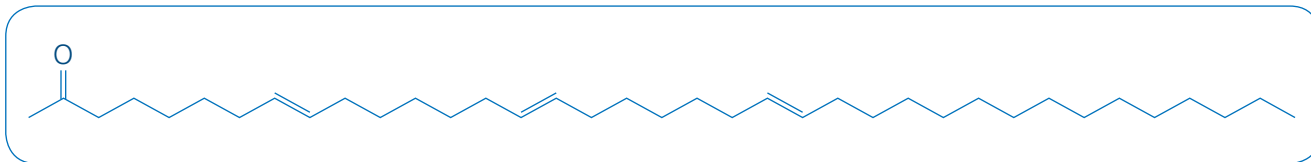


Figure 2: Structure of a $C_{37,3}$ alkenone.

Long-chain alkenones from haptophyte algae are widely applied biomarkers to assess past sea surface temperature (SST) changes. The degree of unsaturation in these compounds is correlated to the temperature in the haptophyte's habitat, i.e. higher (lower) degree of unsaturation with lower (higher) temperatures. This relation is expressed in the SST proxy U_{37}^K as formula (equation 1 and 2) [1,2].

Here we show how molecular imaging by mass spectrometry can be used to reveal the micrometer-scale distribution of this proxy, allowing for high-resolution reconstruction of past SST, and provide an example from varved (i.e. annually laminated) sediments recovered from the Santa Barbara Basin (CA, USA). Molecular imaging is ideally suited to overcome the limitations of the extraction-based method and has the potential to set the stage for paleoclimatology at unattained temporal resolution.



Figure 3: Scanning electron microscope picture (JEOL JSM-6330F) of the long-chain alkenone producing coccolithophore *Gephyrocapsa oceanica*. Colors are artificial. Scale bar = 1.0 μm . (source: <https://en.wikipedia.org/wiki/Coccolithophore>).

Methods

Sample preparation

Sediment cores recovered from areas with a strong preservation of the organic matter due to high-sedimentation rates and oxygen depleted bottom waters are ideally suited for high-resolution molecular imaging. To analyze sediments with MALDI MRMS, spatially intact subsections of the sediment core are sampled with double L-channels and further sectioned into 5 cm pieces. The following sample preparation of the 5 cm section from a sediment box core recovered from the center of the Santa Barbara Basin in 2009 includes freeze-drying of the sediment and a subsequent embedding in a mixture of gelatin (5%) and carboxymethyl cellulose (2%) (Figure 1) [3]. Composition of embedding media might be modified in densely packed material. A 60 μm slice was prepared from the embedded and frozen sediment block using a cryotome and placed onto the conductive indium tin oxide glass slide for imaging mass spectrometry. No MALDI matrix was added to the sample in accordance to Wörmer et al. [4] who proposed that the natural occurring NaCl in marine sediments as well as the organic matter in the sediment may facilitate ionization.

Additionally, 8 sediment samples covering ~ 4.5 cm adjacent to the intact section were extracted using a modified Bligh and Dyer protocol [5].

MS analysis

Molecular imaging of the 60 μm sediment slice was performed using a solarix XR MRMS system equipped with a 7T magnet and a MALDI source. Measurements were performed in the continuous accumulation of selective ions (CASI) mode in order to enhance sensitivity in the m/z range of targeted molecules (Wörmer et al. 2019). For the alkenone analysis, an isolation window for CASI with a mass to charge ratio (m/z) of 550 ± 30 Da was chosen. Mass spectra were acquired in positive ionization mode with a data size of one megaword and a data reduction of profile data of 95%. For the spatially resolved analysis a ~ 8 mm wide area covering the entire length of the sediment section was defined and scanned at a special resolution of 200 μm using a large laser focus. The number of laser shots per spot was set to 700 with a frequency of 1 kHz and a laser power of 35%. External mass calibration was performed in electrospray ionization mode with sodium trifluoroacetate prior to the MALDI Imaging experiment, followed by an internal lock mass calibration of each single spectrum in DataAnalysis (Bruker Daltonik GmbH, Bremen, Germany) using the highly abundant Na^+ -adduct of pyropheophorbide *a* at m/z 557.2523.

$$1. U_{37}^K = \frac{C_{37:2}}{C_{37:2} + C_{37:3}}$$

$$2. U_{37}^K = 0.033T + 0.044$$

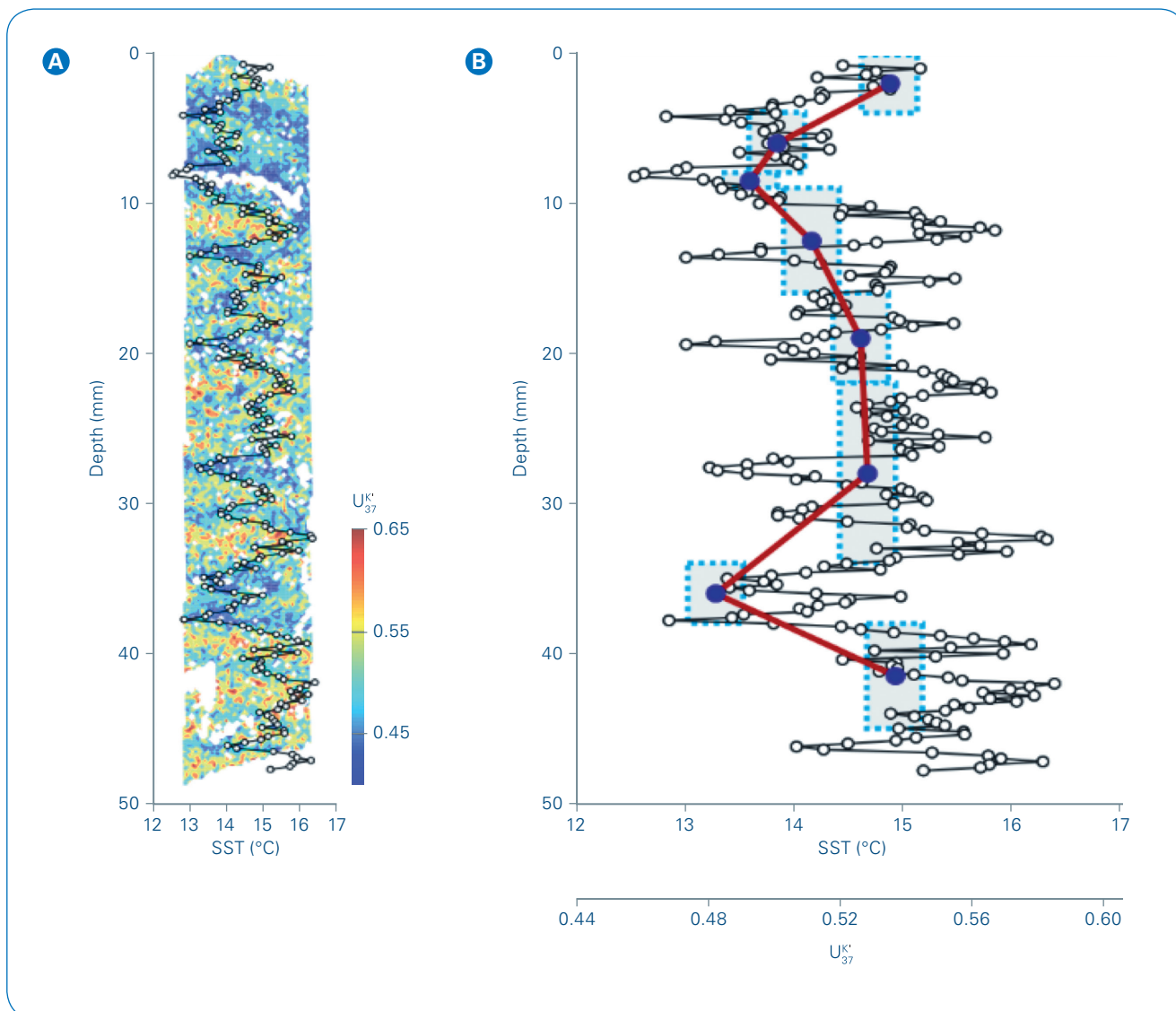


Figure 4: (A) Distribution of U_{37}^K corrected for inclined lamination and overlaid with 200 μm resolution averaged profile. (B) Comparison of conventional, extraction-based U_{37}^K values (blue dots, red line) with MALDI-MRMS-derived high-resolution profile. Dotted rectangles indicate sample amount used for extraction.

Data processing

The alkenone mass range of the calibrated profile spectra for each spot position were exported and further evaluated with Matlab R2016a (Natick, MA: The MathWorks Inc.). Proxy data of each individual spot were calculated using the intensities of the di- and tri-unsaturated C_{37} alkenones as Na^+ -adducts identified by accurate m/z values. To assess information of the proxy-derived reconstructed

temperature over time, the mapped data were averaged along each individual horizontal line resulting in a 200 μm spatial resolution profile. The goal was to pool data from coeval horizons, therefore correction for tilting or disturbance of laminae were required. In the present case, a simple correction for tilting was applied.

The alkenone unsaturation index U_{37}^K was calculated from the absolute intensities of the detected $C_{37:2}$ and

$C_{37:3}$ alkenones (Figure 2). In the SBB, these long-chain alkenones are produced, i.e., by the coccolithophore *Gephyrocapsa oceanica* (Figure 3). U_{37}^K is defined as described by Prahl et al. [2] with the transformation into SST according to Müller et al. [6].

Results

In total an area consisting of 9525 spots was measured with MALDI MRMS. Successful ionization of both alkenones defining the U_{37}^K index was achieved in 8759 spectra, resulting in a success rate of ~92%. Taking into account the small cracks in the sediment slice, it can be assumed that these biomarkers can be detected throughout the sediment. Alkenones predominantly ionized as Na^+ -adducts and successful ionization was achieved without the addition of artificial matrices, such as dihydroxy benzoic acid (DHB).

The visualization of the U_{37}^K reveals the alternating seasonal deposition of the sediment in the Santa Barbara Basin, i.e., colder SST during winter/spring represented by lower U_{37}^K ,

and warm water temperatures in summer/autumn archived by higher U_{37}^K values (Figure 4A). This annual change of SST becomes also visible in the averaged 200 μm resolution downcore profile with reconstructed temperatures ranging from 12.5 to 16.4°C (Figure 4B).

The comparison of the 200 μm resolution MALDI-based analysis to the extraction-based approach shows an overall strong correspondence suggesting that no systematic bias is produced due to the different ionization. Additionally, this overlay emphasizes the strong advantage of the much higher spatial resolution produced by molecular imaging and consequently reveals the loss of information due to extraction-based analysis (Figure 4B).

Conclusion

- Molecular Imaging by MRMS reveals the so far undetectable small-scale distribution of organic geochemical proxies archived in marine sediments.
- Molecular imaging by MRMS paves the way for paleoclimate reconstruction at previous inaccessible resolution.



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