

AC2: Response to reviewers' comments

Colors:

Reviewer comments

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Response to Reviewer #2: RC2

(Comment on egusphere-2025-5089) by an Anonymous Referee

Reviewer #2

Review of Viola et al. 2025

This study offers a valuable sensitivity test for a promising new tool in alkenone paleothermometry, mass spectrometry imaging (MSI), which currently offers the highest possible resolution for Uk'37 measurements in sediment. Here, MSI is applied to a well-characterized, finely-laminated sediment core from the Santa Barbara Basin (SBB). The authors describe heterogeneities in Uk'37 measurements across horizontal laminae and vertical depth, using estimates of SNR to infer the upper resolution limit at which a climate signal is preserved. The use of SBB sediments offers an opportunity for excellent age constraints and the description of signal preservation in temporal terms. This approach has a number of valuable applications in alkenone paleothermometry and is helpful for those looking to apply MSI in a statistically rigorous way. My background is in organic geochemistry and my comments focus mainly on the use of the alkenone Uk37 proxy in sediment and the overall structure and clarity of the paper. I cannot provide in-depth commentary on the quality/validity of the scanning methods used or the applied statistical analysis.

We thank the reviewer for their careful and constructive evaluation of our work. The comments and numerous corrections were very helpful in improving the quality and overall clarity of the manuscript, and precisely pointed out inconsistencies or confusing communication. They also highlighted sections where explanations relied on implicit assumptions and were therefore potentially unclear to a broader readership (outside of MSI working groups), so we could improve the text and schematic figure.

The reviewer also commented on the brevity of the section on age control of the sediment section. We initially kept this section short because the study does not aim to link the sediment section to a specific time interval or other cores, and comparisons are made within the same sediment core and thus our conclusions are not sensitive on the age-model. The reference to specific flood layers provides general orientation and supports the validity of the first-order depth-to-time conversion based on average sedimentation rates. We improved the clarity of this section in the main text body and added a supplemental figure showing stratigraphic alignment.

Lastly, the reviewer commented on our discussion of the causes of the spatial heterogeneity of the MSI UK'37 values and pointed out that *if* these variations could be shown to be a robust, environmental signal, they could have the potential to provide valuable information. We fully agree that these variations may contain valuable environmental information. However, we decided to decouple this aspect from the present study in order to keep the manuscript focused and cohesive.

Reviewer #2

1. The clarity of the manuscript could be improved in a few ways. First, I would recommend that the authors be much more specific about directionality when describing depth intervals, particularly given that this study explores both horizontal and vertical variability in UK'37. For example, I find the methods section beginning at line 69 quite confusing. A “30-cm long section” of the core is used, as well as three “5-cm replicated MSI measurements” of boxcore SPR0901-05BC. Later, “5-cm subsections” are “cut into 100-um slices” (line 78). I have a hard time visualizing this workflow, and I think that the schematic in Figure 1 could be introduced sooner, referenced more, and also made clearer. I don't quite understand what each step represents, particularly with respect to the direction of each core section and the direction of scanning. Arrows and labels would help.

We agree that further clarification of the workflow, particularly regarding the directions, would be helpful for readers. We therefore updated the schematic figure 1 and its caption, **extended the description in the method section**, as well as worked through the manuscript to improve the descriptions.

Updated figure 1 and caption:

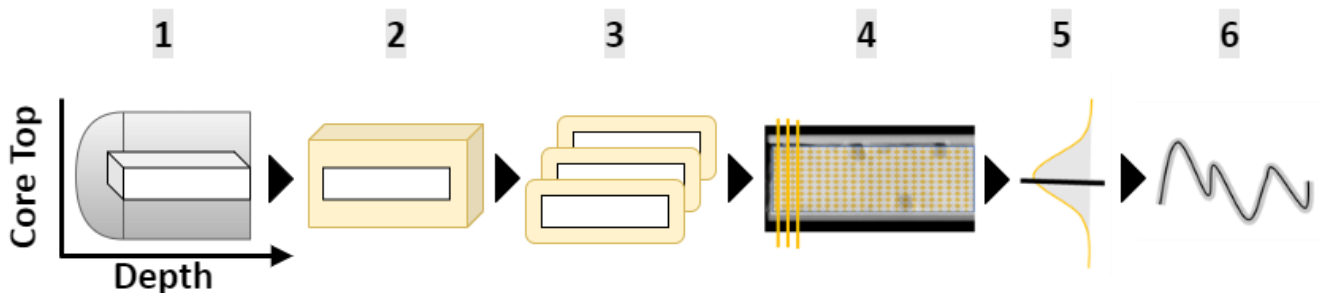


Figure 1: Schematic representation of the triplicate workflow. 1) Subsampling from sediment cores using 30cm LL-channels (Suigetsu 2006 Project Members and Nakagawa, 2014), used for X-ray photography; 2) freeze-drying and embedding of 5-cm subsections (“pieces”), 3) cryomicrotome cutting onto ITO slides of 3 subsequent 100-μm “slices” per piece; 4) MSI scanning; 5) data processing, horizon-wise aggregation & conversion to time series; 6) time series analyses. Orientation of sediment samples and time series is left to right: core top (“recent”) to greater depth (“past”).

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2. The discussion of methods for spot-measuring alkenone compounds should be described more fully. The equation for UK'37 should be given somewhere, and I'm a bit confused about how swUk is calculated for the shot measurements. The authors say in Line 166 that "On average, at 62% of the shots at least one of the three compounds was detected...while at least one alkenone was detected in 46% and both in 34%." Were swUk calculated for shots with one alkenone compound present? If so, would these values not just go to 0 or 1? Is it that the concentrations are near the detection limits of the instrument, or is it actually possible to have a relatively high concentration of C37:2, for example, and no C37:3? If the former is true, is there any size effect bias on swUk?

We thank the reviewer for this remark and agree that these aspects were not sufficiently clear in the initial submission. We have revised the relevant sections accordingly. Different approaches of handling individual ("incomplete") detections, as well as to aggregate shot-wise spectra to horizon data have been tested. However, under the sediment and temperature conditions of the Santa Barbara Basin, these methodological choices have only a minor influence and do not affect the key results of this manuscript.

While it is possible to calculate swUk for every shot by filling in "0" into the equation for the peak that is missing - which indeed would lead to swUk values of 0 and 1 across the map - we decided to calculate swUk values only calculated were both alkenones were reliably detected within the same shot/spectrum for several reasons. Using only spectra where both compounds have been detected simultaneously with sufficient peak quality (FT_{SNR}) is a constraint also employed by earlier studies (Alfken et al., 2020; Napier et al., 2022; Obreht et al., 2022; Wörmer et al., 2014, 2022) to avoid spurious detections or misattributions. While we are not aware of any compounds appearing in the close m/z range of C37:2 and C37:3 sodium adducts, and the FT-ICR-MS based UK'37 results have been validated with GC-FID measurements and SST data, we use this as an additional safety precaution due to the inability of our current setup to distinguish isomers and the lack of MS2 spectra.

Second, and related to the reviewer's question on detection limits (and partly to comment #4), this approach minimizes potential bias. For example, if the less abundant compound falls below the detection limit but the shot were still treated as a valid full detection, horizon-wise aggregated UK'37 values could be artificially skewed. The effects of such processing choices are being explored in ongoing work. For this study, we followed the approach of Alfken et al. (2020), which is appropriate given the <1 km distance between the sediment cores analyzed here (SPR0901-05BC and MV1012-001KC).

We rarely detect unaccompanied high-intensity alkenones. To show this in more detail, figure R1 compares peak intensities for both alkenones as density contours when they were detected simultaneously within the same shot (spectrum), while unaccompanied detections of either C37:2 or C37:3 alkenones are shown as "rug stripes" on the respective side of the plot. Most of the detections where only one alkenone is present fall in the lower range of observed intensity values. The lower end of the intensity range is truncated due to our cutoff using the FT_{SNR} threshold. We observe strong correlation between peak intensities of simultaneously detected alkenones (see contours in fig. R1 below). This and that the correlation increases with peak intensity (less scatter), while the offset from the dotted 1:1 line (= UK'37 of 0.5) remains stable throughout the range of intensities indicates, to us, that swUk values are not biased by absolute peak intensities.

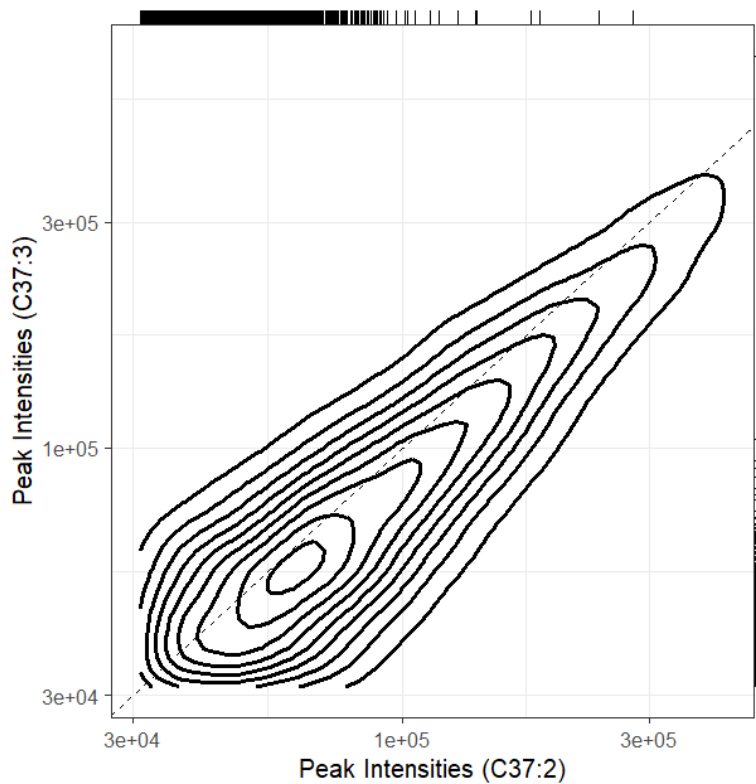


Figure R1: Density plot of peak intensities of both alkenones for an exemplary slice of the KC1 sediment section (rel. depth 5-10, replicate 1). Only showing peaks that crossed our FT_{SNR} quality threshold. Contour lines bin counts of datapoints into equal sized groups. Black lines outside of the plotting area (“rug”) show peak intensities of single alkenone detections, individually occurring C37:2 peaks on the top, C37:3 to the right. The black dotted 1:1 line indicates where both intensity values amount to swUk values of 0.5, the offset of point cloud matches the measured average Uk’37 of ~ 0.53 for this depth interval.

We extended the method section for spot-measuring alkenone compounds and subsequent formation of horizon-wise data, as well as added the equation for Uk’37.

Reviewer #2

3. The paper needs the age controls on the sediment core to be discussed more thoroughly. The authors, for example, identify their sediments as containing the 1761 and 1532 CE gray flood layers (Line 115), but do not provide further information on how these specific floods were identified. A supplemental figure showing stratigraphic alignment of core KC1 with previously characterized SBB sediments, or a more thorough description of the method the authors used, would be helpful for demonstrating the validity of these flood ages to the reader. Further, the authors describe that SBB sediments are generally “varved, laminated, or bioturbated” (Line 112), but do not thoroughly describe the banding characteristics of the core section KC1. This creates confusion when the authors translate from depth to temporal scale.

We thank the reviewer for highlighting the need for clearer description of the age control and sediment characteristics of core KC1. As noted above, all time-series analyses in this study were performed on the depth scale and were only translated to an approximate age scale to aid interpretation. We clarified this now in the revised text.

The mentioned flood layers served as orientation and to establish that the used sediment samples lie in the SBB sediment sequence, for which relatively stable, linear sedimentation rates of ~1.4 mm/yr have been reported (Du et al., 2018; Hendy et al., 2013, 2015; O’Mara et al., 2019; Thunell et al., 1995; Zhao et al., 2000). The flood layers were identified by visually comparing the line scan images and X-ray density plots of core MV1012-001KC with those of neighboring cores and published event stratigraphies, as in Du et al. (Du et al., 2018; Hendy et al., 2013, 2015; O’Mara et al., 2019; Thunell et al., 1995; Zhao et al., 2000). Flood layers are also clearly visible in the MSI data because of their drastically different (lower) number of successful detections of any compound class, for example, in Fig. 3 A-C, where the middle section of each of the pieces is basically void of valid detections (shots with valid detections of any of the three used compounds are shown as dark grey).

We agree that a proper age model, for example, using the flood layers as tie points to published chronologies, would be required for studies aiming to integrate multiple cores or reconstruct SST time series for specific calendar intervals. In this manuscript, we clarified the age control paragraph in the main text body and added a supplementary figure showing the alignment of our KC1 section to linescan images of neighboring core MV1012-014TC with published age control.

Reviewer #2

4. How do Uk'37 values derived from MSI scanning methods compare with measurements from traditional laboratory extractions and GC-MSD/GC-FID/HPLC measurements? I think that this technique is new enough that it warrants more thorough discussion/citation of previous validation studies.

We agree that this aspect deserved more detailed discussion. As also noted in the general comment by Reviewer #1, we have now expanded this section accordingly.

MSI U_{37}^K results have been extensively verified using extraction-based GC-FID data. However, differences between the GC-FID and other mass spectrometry techniques have been reported (Liao et al., 2023; Rama-Corredor et al., 2018), which each seem to suffer from individual biases.

Given the similarity of this specific request from both reviewer we decided to answer both comments jointly:

Issues regarding the ionization efficiencies of C37 alkenones are reported for several mass spectrometry approaches (Chaler et al., 2000, 2003; Liao et al., 2023). Unfortunately, to our knowledge, systematic studies addressing this for (MA)LDI FT-ICR MS workflows are currently lacking. Studies in warm SST areas (Napier et al., 2022; Obreht et al., 2022; Wörmer et al., 2022) reported systematic offsets between GC-FID and MSI results and introduced site specific correction factors. Most likely these effects are due to a bias towards individual measurement spots (“shots”) representing relatively colder temperatures. Shots with very high temperatures would lead to C37:3 more likely falling below the detection limit, and hence being excluded. In general, the potential influence of data processing choices is part of an ongoing project by the author. An alternative explanation could be related to lower alkenone concentrations at these areas in an effect similar to the “injection amount-effect” described in Liao et al. 2023a, or a non-linear behavior at the end of the UK37 temperature range (Liao et al. 2023b), especially given the still open questions around ion formation and matrix effects in (MA)LDI (Fuchs et al., 2010; Knochenmuss, 2006).

In contrast, these potential biases are expected to be much less important in the temperate Santa Barbara Basin (SBB), where both alkenone species occur in similar abundances and overall concentrations are high. In fact, the comparisons to GC-FID data in sediments from the SBB showed high agreement between MSI and GC-FID based Uk'37 values (Alfken et al., 2020) (see Fig. R2 below). The MSI results for SBB sediments were additionally verified with instrumental SST data. The temporal overlap of boxcore SPR0901-05BC utilized by Alfken et al. 2020 and CalCOFI buoy station data (California State Department of Fish and Game; NOAA Fisheries; Scripps Institution of Oceanography, 2001) allowed to estimate the correlation between sediment MSI uk'37 based temperature reconstructions and buoy SST data of the modern era. On interannual timescale estimated from 1984 to 2009 the MSI based temperatures resulted in spearman's rank correlations of up to ~0.6 with upper 0-30m water column data at the closest CalCOFI station 81.8 46.9. Similar average temperatures for this period (MSI ~0.3C warmer) lead us to believe that differences in ionization efficiencies are not influential or relevant compared to the overall uncertainties and that MSI based uk37 in the middle of the Uk'37 temperature range and especially in SBB to be robust recorders of SST variability.

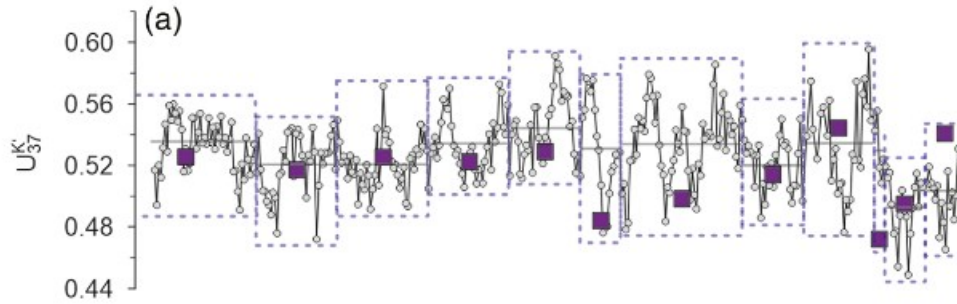


Fig. R2: Comparison of MSI-based and conventional extraction-based data of UK'37. Grey points and line are MSI derived UK'37 estimates, purple squares are GC-FID based estimates. Dotted rectangles indicate the corresponding depth range averaged for one sample used for extraction and horizontal lines display the corresponding mean value of the MSI data. Based on fig. 2 of Alfken et al. (2020).

In the revised manuscript we added the following part:

During the development of the MSI workflow by Wörmer et al. (Wörmer et al., 2014) and in subsequent studies, the resulting U'_{37} values were verified using GC-FID measurements (Alfken et al., 2020; Napier et al., 2022; Obrecht et al., 2022; Wörmer et al., 2022). Differences in U'_{37} values obtained by different mass spectrometry techniques have been reported and attributed to varying ionization efficiencies of alkenones, the co-elution of other compounds, and additional factors (Chaler et al., 2000, 2003; Liao et al., 2023; Rama-Corredor et al., 2018). Notably, data derived from MSI in warm SST regions have shown a cold bias compared to GC-FID data, leading to the introduction of site-specific correction factors. However, in the temperate SST regime of SBB, MSI and GC-FID data did not exhibit significant differences and correlated well with CalCOFI buoy SST data at the site (Alfken et al., 2020; California State Department of Fish and Game; NOAA Fisheries; Scripps Institution of Oceanography, 2001).

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5. In line 301, the authors speculate as to the causes of spatial heterogeneity in alkenone swUk and posit that this signal may be reflecting real variability (as opposed to measurement error), given that “water column and sediment trap data display a broader range of UK'37 values than core tops, and lab cultures have been shown to exhibit even greater variability...” I would appreciate a quantitative comparison between the variability captured by the MSI scanning techniques and those in the aforementioned settings. Is the degree of heterogeneity captured by MSI physically reasonable? I would particularly appreciate a direct comparison to sediment trap data, given that the authors are investigating individual laminae which would accrete on somewhat comparable timescales. Based on previous studies, what is the maximum amount of noise that might reasonably be explained by variability in alkenone production about the same SST? What might be the approximate range of SST values that might be reasonably integrated over a single season? How do you expect water column and sedimentation processes to smooth (or not smooth) variability of alkenone production in the photic zone? It seems that if the observed spatial heterogeneity is not a product of measurement error, signal noise may contain valuable

information that would be of interest to paleoclimatologists looking to apply MSI. I think that the paper could be improved if these possibilities were explored more thoroughly. I would recommend that Supplementary Figure 5 be brought into the main body of the paper and discussed.

We thank the reviewer for this thoughtful comment. We agree that if the within-horizon heterogeneity of MSI-derived UK₃₇ (or swUk) can be shown to be robust, it could contain valuable environmental information rather than being purely measurement noise. In principle, this type of “distributional” proxy information could reflect variability in alkenone production and export under near-constant mean SST, seasonal sampling biases, or episodic events (e.g., heat-waves), and it is therefore an exciting avenue for future MSI applications

In the revised version, we better discuss the observed variability in relation to instrumental and regional SST data.

However, a detailed quantitative analysis would go beyond the scope of the present manuscript, which is focused on replicability, workflow validation, and horizon-scale time-series behavior.

Reviewer #2

Corrections

The manuscript contains a number of grammatical and typing errors and should be proofread, paying particular attention to comma and hyphen usage, spelling, and the use of “that” versus “which.” Examples are presented for the first 100 lines and the Figure 2 caption, along with one citation recommendation.

L30 - I suggest the authors use “longer” timescales rather than “slower”

L31 - There is an extra space between in “dynamics of”

L31 - Grammatical error in “Similarly, understanding of the long-term dynamics of phenomena like monsoon or El Nino-Southern Oscillation (ENSO), results...”

L38 - Hyphen needed for “long-enough”

L40 - Needs an additional comma. “This might, however, not be the case...”

L42 - Extra space between carriers and the following period

L43-44 - Needs additional comma. “Such heterogeneity may arise during signal production, for example, due to...”

L46 - I would drop the apostrophes/quotations for ‘stratigraphic noise’

L46 - Correct to “archives”

L52 - Should cite Brassell et al., 1986

L56 - Remove comma in “annual varve couplets, and”

We thank the reviewer for their detailed corrections and thoroughly checked for similar errors (typo, grammar, punctuation, etc.) in the rest of the manuscript.

The corresponding lines have been adjusted accordingly.

L57-59 - “for their preservation under low-oxygen conditions that reduce bioturbation” should be amended to “for their preservation under low-oxygen conditions, which reduce bioturbation” unless the authors mean to say that some low-oxygen conditions do not reduce bioturbation. Similarly, “from minimal disturbances that preserved varves...” should be clarified.

We thank the reviewer for pointing out these inaccuracies. We rephrased to better convey that the basin’s bathymetry and other factors lead to low oxygen conditions and that this setting is the main cause for the good sediment preservation due to reduced large scale (> 1mm) bioturbation. However, we also want to convey that the notion of “no bioturbation at all under low oxygen conditions” seems to not hold true at SBB, because submillimeter bioturbation has been reported even under lowest oxygen conditions (Bernhard et al., 2003). Although reworking at these scales and intensities does not appear strong enough to destroy lamination, as evidenced by the continued formation of laminae and varves in the upper SBB

sediment section, we wanted to emphasize this point. It seems irrelevant for many typical proxy workflows and resolutions, especially extraction-based U^k37 reconstructions, but it might be a significant factor at the high 100µm scanning resolution of MSI data (or e.g. µXRF scans). The original authors even directly state: “Thus, physicochemical conditions along any given lamina should not be expected to be consistent and, given the likely activity of the SBB inhabitants, these laminated sediments should not be considered postdepositionally pristine.”

The section now reads as:

Seasonal runoff, high primary productivity, and the basin's bathymetry allow for the formation of laminae or annual varve couplets and for their preservation under low-oxygen conditions due to drastically reduced bioturbation (Schimmelmann and Lange, 1996; Thunell et al., 1995). Past variations in bottom-water oxygen levels resulted in varying bioturbation intensities, ranging from merely submillimeter disturbance, which allows for the preservation of varves (Bernhard et al., 2003), to complete mixing following colonization by larger fauna (Anderson et al., 1989).

Reviewer #2

L64 - Need a comma before “which”... “individual MSI-based reconstructions, which indicate...”

L69-70 - Opening line of the paragraph is not a full sentence and needs to be clarified: “Sediment core MV1012-001KC was retrieved by research vessel Melville during cruise MV1012 in 2012, stored and accessed at Scripps Institution of Oceanography’s cored sediment and microfossil collection.”

L70-71 - Needs an additional hyphen for “30-cm-long”. Comma usage with “named “KC1”” is incorrect. Need to change wording for the second clause of the sentence ending in “...in the following.” For example: “...referred to as KC1 for the remainder of this manuscript.”

L72 - Correct to “three 5-cm-replicated measurements” for clarity

L72 - Need commas for “of boxcore SPR0901-05BC, originally published by Alfken et al. (2020), as an example...”

L76 - Correct to “freeze-dried”

L76 - Correct spelling of “embedded”

L79 - Need a comma for “(Medite Cryostat M630), and”

L81 - “Finally, the slices were measured”... what was measured exactly?

L81 - Need a comma for “FT-ICR-MS, coupled to”

L84 - Need to adjust “in the supplements section S1”... e.g. “in section S1 of the supplemental materials.”

L84 - “For each 5 cm depth”... In some places you use a dash with 5-cm, and other times you do not. Stay consistent with one or the other; both are correct.

L88 - “adducts of the di- and triunsaturated alkenones” should be adjusted to “adducts of the diand triunsaturated alkenones.”

L93 - Need a hyphen for “first-order estimation”

L184 - Need to describe the Xs in panel A in the figure caption

L184-185 - This part of the figure caption is not grammatically clear: “Maps are shown as measured, on the MSI coordinate grid, before affine transformation onto Xray coordinates, values below 1% and above 99% quantiles were removed for optimal color scaling during plotting.

We thank the reviewer for their detailed corrections and helpful suggestions.

The changes have been applied accordingly.

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