Advancing analytical frontiers in molecular organic biomarker research through spatial and mass resolution

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ABSTRACT

Analytical developments have been crucial for the advancement of molecular biomarker research in the earth sciences. In this chapter, we focus on two areas where recent technological developments profoundly impact the use and relevance of molecular organic biomarkers: (1) the increase in spatial resolution heralded by imaging techniques, which results in biomarker-based paleoenvironmental reconstruction with unprecedented temporal resolution, and (2) the accurate, simultaneous characterization of a wide range of compounds in complex matrices enabled by ultra-high resolution mass spectrometry.

KEYWORDS: FT-ICR MS, hyperspectral imaging, Fourier transform, mass spectrometry imaging, molecular stratigraphy, sedimentary pigments

INCREASING SPATIAL RESOLUTION IN MOLECULAR BIOMARKER ANALYSIS

Conventional biomarker analysis relies on the extraction of targeted organic compounds from samples that are typically cubic-centimeter-sized. This means that the information stored in this sample volume is averaged, minimizing variability. With bacterial cell volume being generally less than 1 μm^3, a 1 cm^3-sized sample could average the information stored in more than a trillion (10^{12}) cells. The effect of such averaging is also evident when

attempting to reconstruct paleoenvironmental conditions from sedimentary archives, where spatial resolution translates into temporal resolution. Typical sedimentation rates in the marine realm are a few micrometers per year, while rates as high as a millimeter per year are restricted to high productivity areas and lacustrine systems. A sample spanning one centimeter of depth can thus contain the information deposited during decades, centuries, or much more time. Therefore, increasing spatial resolution for physically undisturbed biomarker records is required to assess abrupt or short-term environmental changes, and high frequency climate variability.

Subannual to decadal climate reconstructions are especially relevant, given that the durations of extreme events (e.g., heat waves, cold spells, droughts and floods) range in time scales from days to months; high time resolution is also needed to study seasonal cycles or high frequency climate fluctuations such as El Niño Southern Oscillation (ENSO). All these phenomena take place at human time scales and are relevant from societal and ecosystem perspectives. Fortunately, certain marine and lacustrine sedimentary archives capture this variability: for instance, varved sediments contain at least two distinct micro- to millimeter-thick layers deposited in every single year and thus offer an archive with subannual resolution (Zolitschka et al. 2015, Schimmelmann et al. 2016).

Conventional, wet-chemical approaches to extracting molecular biomarkers from sediments and rocks have sought to maximize spatial, and thereby temporal resolution by increasing the sampling effort (more and smaller sample sizes) but are ultimately limited to the centimeter- or millimeter-scale (e.g. Kennedy and Brassell 1992). However, it has recently become possible to approach these materials using techniques that obtain molecular information directly from intact sediment samples, without previous sub-sampling or extraction. These techniques not only provide unmatched spatial resolution in the micrometer-range, but also the possibility to explore the two-dimensional spatial distributions of characteristic biomarkers in a sample. Here, we focus on two spatially-resolved techniques that are based on spectrophotometric and mass spectrometric biomarker detection.

Hyperspectral imaging to detect and quantify sedimentary organic pigments at micrometer-scale resolution

Hyperspectral imaging (HSI) is a widely used method in remote sensing and other fields that has recently been developed for biogeochemical analyses of sediment cores. The technique relies on interpretation of spectral profiles of light reflected from the target sample in the visible to near-infrared (Vis-NIR) range (400 – 2500 nm). Here, we focus on the use of HSI to measure photosynthetic pigments in sediment cores at extremely high spatial (micrometer-scale) and temporal (subannual) resolution.

The principles and development of HSI core scanning are based on simpler methods of point-based reflectance spectroscopy. Rein and Sirocko (2002) were the first to show that reflectance spectroscopy scanning of fresh sediment cores could be used to generate relatively high-resolution (2 mm) semi-quantitative measurements of pigments with much less time and cost compared to conventional wet-chemical techniques (e.g., high-performance liquid chromatography, HPLC). The measurement principle relies on the fact that groups of pigment compounds absorb light in specific wavelengths, and the amount of that absorption, quantified by a relative absorption band depth (RABD) index, is proportional to pigment concentrations in the sediments. For many years, the hand-held Gretag-Spectrolino or Minolta 2600d spectrophotometers were the state-of-the-art instruments for reflectance spectroscopy core-scanning. Pigment records from these devices were used to reconstruct aquatic productivity in lake and marine sediments, which could be related to oceanographic, climatic and ecological conditions in studies spanning hundreds of years to over 10,000 years. However, the spatial (2-8 mm) and spectral resolutions (10 nm) of these instruments remained limited.

HSI builds upon the principles of reflectance spectroscopy by connecting an imaging lens with a spectrograph. Hyperspectral images of sediment cores are acquired using a "pushbroom" method; this means that as the core passes under the hyperspectral camera and light source, rows of pixels are acquired and stacked to form a complete image of the core surface (**Figure 1**). Each pixel within the hyperspectral image contains an individual spectral profile of light reflected from the sediment surface.

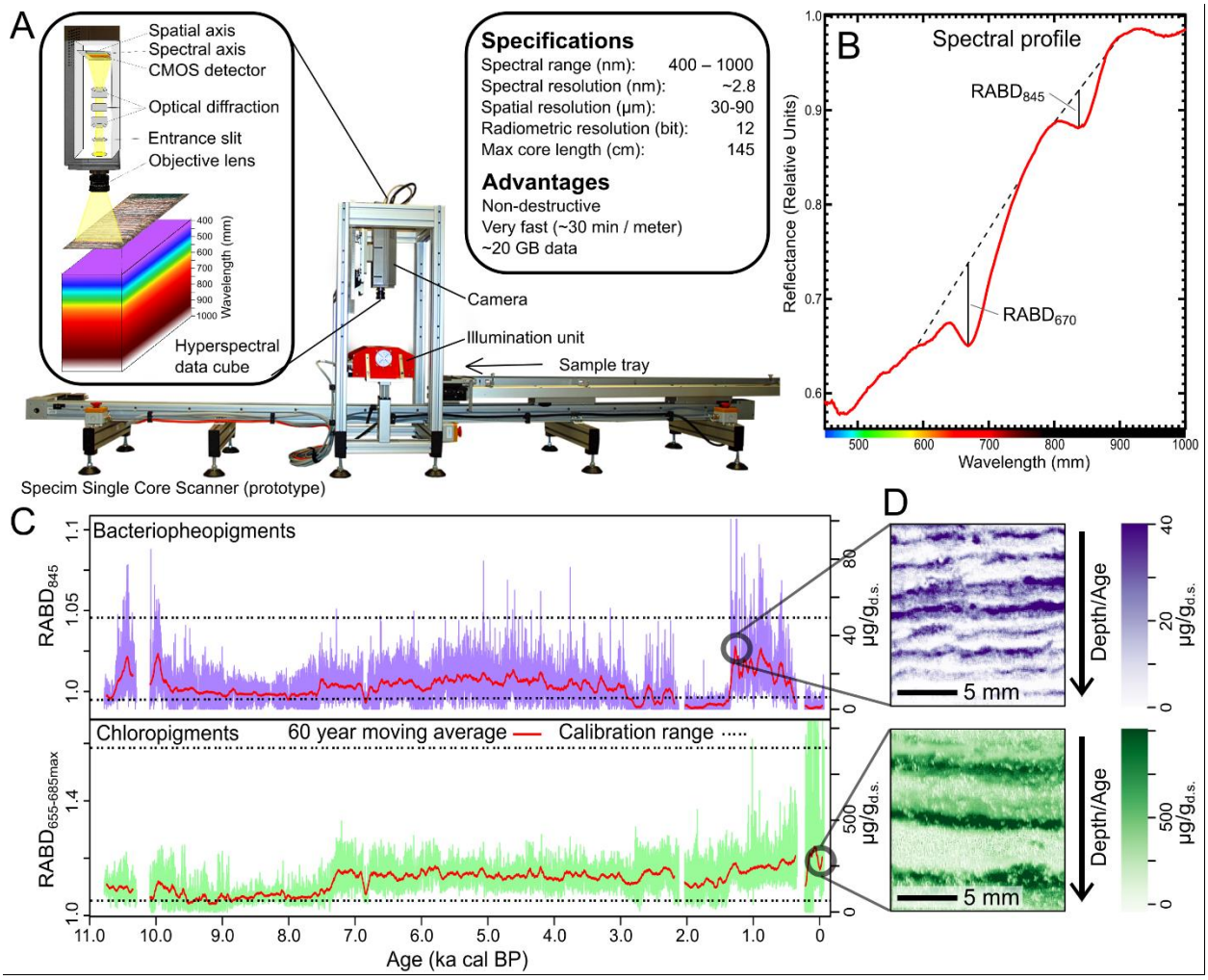

Figure 1. A) Specim Single Core Scanner and technical specifications (image adapted from Butz et al. 2015). B) Example spectral profile showing pigment absorption troughs and relative absorption band depth index measurement principle. C) Example hyperspectral imaging (HSI) pigment data from Lake Żabińskie Poland (Zander et al. 2021a) showing a 10,800 year-long record of bacteriopheopigments and chloropigments used to reconstruct aquatic anoxia and primary productivity. D) Close-up of spatial distribution of pigments showing seasonal pigment variability in annually laminated sediments. Note: $g_{d.s.}$ = grams dry sediment.

HSI was first applied to sediment cores by Butz et al. (2015) who used a prototype of the Specim Single Core Scanner (**Figure 1**). Images are acquired in the Vis-NIR wavelength range (400-1000 nm) with a spectral resolution of 2.8 nm, and a scanning resolution of 30-90 µm (pixel size). Scanning a one-m-long split core takes ~30 minutes. The following workflow is used to quantify pigment concentrations from HSI data: First, raw spectral data are

normalized to standardized units based on dark (camera shutter closed) and white ($BaSO_4$ plate) references. Next, spectral endmembers (the spectral profiles that are most distinct) are identified to understand the variation in the spectral data within a sample, and to identify spectral features suitable for quantification (i.e., pigment absorption troughs). Then, RABD indices are calculated for each individual pixel, creating maps of the spatial distribution of pigments within a core. Typically, down-core profiles of pigment abundance are obtained by taking row averages (perpendicular to depth) of RABD values across a representative 2-mm-wide subset of the image data. RABD indices can be calibrated to pigment concentrations measured using chemical extraction techniques and HPLC or spectrophotometry, with typical errors less than ~15 %. Concentrations of pigments as low as 5 µg/g dry sediment are detectable in fresh sediment cores. This method enables measurements of pigment concentrations in sediment records that are tens of meters long (ca. 10-20,000 years) at 60-90 µm resolution in a few weeks.

To date, HSI has been shown to effectively measure distributions and concentrations of bacteriopheophytin-*a* (a derivative of bacteriochlorophyll-*a*) and total chloropigments (chlorophyll-*a* and degradation products). Bacteriochlorophyll-*a* is produced by anoxygenic phototrophic bacteria, and therefore is a biomarker for anoxia in aquatic systems (Butz et al. 2015). Chlorophyll-*a* is ubiquitous in photosynthetic organisms and is often used to reconstruct total algal production. HSI tracks all degradation products of chlorophyll-*a* that remain colored, which means HSI-based reconstructions of algal production are less affected by pigment degradation than studies based only on chlorophyll-*a*. Detection of other pigment groups in sediments, including carotenoids, phycocyanin, and other bacterial chloropigments, remains possible, but has not been conclusively demonstrated.

The primary advantages of HSI are rapid data acquisition and extremely high spatial resolution that cannot be achieved with conventional methods of biomarker analysis in sediments. In varved sediments, it is possible to resolve the seasonal cycle of pigment production by HSI (**Figure 1**). However, interpretations based on spectral data should be validated with conventional techniques. Spectral signatures of pigments are non-specific, so pigments that absorb light in the same spectral range cannot be differentiated using this

technique (i.e., chlorophyll-*a* and -*b*). In addition to providing calibration and validation of RABD indices, combining HSI with HPLC techniques is advantageous to obtain more detailed information about photosynthetic communities and pigment degradation processes. Other high-resolution core-scanning techniques, such as micro-X-ray fluorescence (μXRF) scanning, provide the opportunity for paleoenvironmental studies utilizing subannual measurements of both inorganic and organic proxies (Zander et al., 2020b).

HSI-inferred pigment records from Poland, Switzerland and Greece have documented how natural and human-caused changes to catchment land-cover have affected lake productivity and redox conditions since the Late Glacial (**Figure 1**). For example, at Lake Moossee, Switzerland, changes in pigment production were linked to climatic changes, and cycles of Neolithic/Bronze Age deforestation and afforestation related to early human activities at the site (Makri et al. 2020).

More advanced numerical and computing methods may improve the quantification of pigments or other substances in sediments based on HSI data. In a recent study, random forest regression (a machine learning technique for regression and classification) was applied to HSI data to infer grain size distributions in six Canadian lakes (Ghanbari et al. 2020). These types of techniques also could be used to improve the analysis of organic sedimentary components using HSI. HSI and other high-resolution spectroscopic techniques are expected to yield new insights in several scientific disciplines, particularly for questions that can only be answered with ultra-high spatial resolution data.

Micrometer-scale resolution images of biomarker distribution and ultra-high-resolution molecular stratigraphy via Mass Spectrometry Imaging

Most organic compounds are not detectable with photometric techniques. In contrast, mass spectrometry can detect and quantify an immense diversity of ionizable molecular species. Spatially resolved mass spectrometric analyses are now feasible with ionization probes that scan across a sample in a predefined pattern, including focused solvent sprays, lasers or ion beams. At each nanometer to micrometer-sized raster spot, these probes generate an ion cloud that is transferred to the mass analyzer. Thus, a spatially referenced collection of thousands of complete mass spectra is obtained for every square centimeter. In each

spectrum, single molecular species can be identified, and their relative abundances quantified. By documenting the distribution of single compounds, compound ratios, or more complex molecular signatures, an image of their spatial distribution is obtained.

This approach, termed mass spectrometry imaging (MSI), is firmly established in biomedical and microbiological research (e.g. Watrous and Dorrestein 2011). In the earth sciences, however, its implementation has been slower, probably because of the complexity encountered in samples that combine organic material and minerals, and because of the low polarity and concentration of targeted compounds. Pioneering work with time-of-flight secondary ion mass spectrometry (ToF-SIMS) detected lipid biomarkers in modern and fossilized marine microbial mats (e.g., Thiel et al. 2007). These developments, as well as the use of nanospray desorption electrospray ionization (Lanekoff et al. 2013), evidenced the possibility that MSI can reveal the spatial distributions of geologically relevant compounds in environmental samples. Compared to these techniques, matrix-assisted laser desorption/ionization (MALDI) offers some crucial advantages. First, it provides the opportunity to analyze relatively large, centimeter-sized sample surfaces, as opposed to the micrometer-sized areas typical for ToF-SIMS. In addition, MALDI is a soft ionization technique, preventing the fragmentation of labile compounds upon ionization and allowing the detection of the intact biomarker molecules. When coupled to ultra-high-resolution mass spectrometry, such as Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS; see the following section, below), excellent mass resolution and sensitivity are attainable. These properties make MALDI-based MSI ideally suited for the exploration of sedimentary archives, as demonstrated in the early publication by Wörmer et al. (2014) (**Figure 2**).

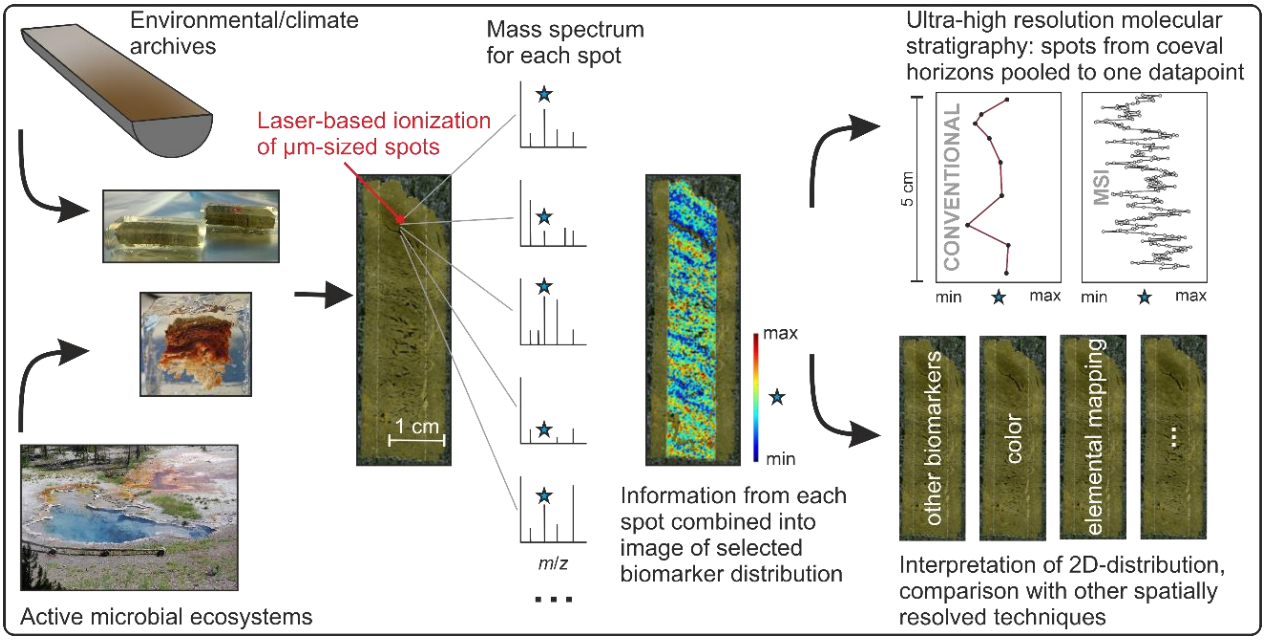

Figure 2. Summary of analytical process and applicability of MALDI-based molecular biomarker mass spectrometry imaging (MSI) in the earth sciences. After proper preparation, spatially resolved biomarker distributions can be obtained from the surface of thin sample slices. In paleoenvironmental archives, the micrometer-scale raster allows for ultra-high- resolution molecular stratigraphy. The interpretation of the 2D biomarker distribution, in combination with other imaging techniques, can inform on characteristic spatial patterns in a diversity of sample types. Picture of the Octopus Spring (Yellowstone National Park) is courtesy of R. Summons (MIT, USA); pictures of embedded sediment, sediment slices, and map of biomarker distribution are courtesy of S. Alfken (Univ. Bremen, Germany).

Following this initial development, a dedicated facility at the University of Bremen has been responsible for major advances in MSI of sedimentary molecular proxies, starting with the development of an optimized workflow for sample preparation, and data processing, analysis, and visualization (Alfken et al. 2019, Wörmer et al. 2019, Alfken et al. 2020). Given the need to maintain the original sediment structure, samples are stabilized by embedding, for example into aqueous solutions of gelatin and carboxymethyl cellulose. After solidification at -20 °C, embedded pieces are sectioned into 60-100 µm thin slices on a cryomicrotome. Each slice has an approximate area of 5 x 1.5 cm and is suitable for MSI and complementary elemental mapping by µXRF. High-quality images of these slices are made to guide MSI and to record sediment color as an additional parameter. Solid rock archives can

be cut into suitable size, with a maximal thickness of a few millimeters, and analyzed, while the use of thin sections remains to be explored.

Measurement of molecular biomarkers in sedimentary archives typically does not require application of an additional artificial matrix, and the slices can be directly analyzed after completely drying in a vacuum chamber. However, signal enhancement due to matrix application is observed in some cases. Besides the use of typical MALDI matrixes (e.g., 2,5-dihydroxybenzoic acid, DHB), unconventional matrixes such as fullerite or silver may be useful in the detection of nonpolar biomarkers (Wörmer et al. 2019). MSI requires the definition of measurement area and spatial resolution and tuning of laser power and ion optics for each sample and analyte to maximize signal intensity. A spatial resolution of 100-200 µm is recommended for sedimentary archives, while active microbial ecosystems can be analyzed with ten-fold higher spatial resolution (10-20 µm). To increase sensitivity for individual compounds, analyses are carried out in restricted mass spectral 'm/z' windows, ideally spanning less than 100 mass units. Given the minute amounts ionized from each micrometer-sized spot, samples with concentrations of the target compounds in the range of µg per g dry weight are ideal. Alkenones, archaeal glycerol ether lipids, sterols, fatty acids and pigments have been detected in sediments by MALDI-MS and enable reconstructions of sea surface temperature (SST), redox conditions or changes in terrestrial vegetation and aquatic primary productivity. However, these compounds represent only a minor fraction of the molecular signatures revealed by MSI of sediments and other environmental samples.

The images of molecular biomarker distribution obtained by MSI can be interrogated by averaging them across horizontal layers and evaluating them against a single dimension (depth, age) or by exploiting their two-dimensional nature. In the first case, biomarker or proxy information from a micrometer-sized coeval horizon is pooled, providing a data point that can be assigned to a certain depth (age). This approach is most consistent with conventional molecular stratigraphy, but temporal resolution is improved by several orders of magnitude. Therefore, we refer to it as ultra-high-resolution molecular stratigraphy. It relies on properly referencing the data obtained by MSI to the original core depth (and thus age), and benefits from image processing: in laminated sediments, non-horizontal deposition

of the sediment can be corrected and data points corresponding to the same horizon aligned (Alfken et al. 2020). MSI records from ~the last century can be compared to monthly or seasonal records of instrumental data describing the water column (for example SST, nutrient concentration, redox conditions) or to historical climate data to understand how these conditions are recorded in the sedimentary proxy signal (Alfken et al. 2020). In older records, and when compared with other high-resolution archives (e.g., ice cores), MSI can inform on changes in high frequency climate oscillations, on short-lived environmental disruptions, or on leads and lags in the expression of abrupt climate change events and the forcing factors behind them (Obreht et al. 2020).

The second approach to MSI measurements explores both spatial dimensions of the dataset. When combined with complementary measurements of the same sample surface, each micrometer-sized spot is described by information such as sediment color, elemental composition and a suite of molecular biomarker or proxy signatures. In sedimentary archives, this provides a means to better understand biomarker transfer to and preservation in the seafloor, but also to deconvolute the proxy signal according to other sedimentary parameters. For example, in varved sediments seasonal changes can be evaluated by binning molecular SST proxies according to sediment color. In active microbial systems, fine spatial structure can be assessed, documenting variations in the abundances of molecular biomarkers to microscale environmental gradients (Wörmer et al. 2020). Moving forward, it might be possible to identify potential new biomarkers by non-targeted approaches. Thereby MSI data are searched for compounds with distinct spatial patterns that correlate to other spatial clues in the sample. Such clues include distinctively shaped or colored regions, or regions with specific elemental compositions, and could indicate a particular depositional environment or specific microbial communities and biogeochemical processes.

MSI can be applied to a variety of environments, such as marine and lacustrine sediments, soils, rocks or microbial mats. It provides an extremely rich data set, with typically more than 10,000 spatially referenced mass spectra for each 5-cm piece of sample, each spectrum containing the signatures of hundreds or thousands of compounds. We are only starting to

understand how challenging and rewarding it is to simultaneously access such high information density at both the spatial and molecular level.

ULTRAHIGH MASS RESOLUTION: FT-ICR MS

The first step in identifying biomarker molecules in environmental samples by mass spectrometry is critical - the mass detector must be capable of separating targeted molecules from the tens of thousands of other compounds present in the sample (e.g., background species, unintended contaminants, or components of complex mixtures that are intentionally analyzed without purification). Conventionally, identification of biomarkers in geochemical samples requires a mass resolution sufficient to separate species that differ in mass by roughly the mass of an electron (0.0005 u); given that many common biomarkers have molecular weights in the range ~200-500 u, this means the mass resolving power required to separate peaks of equal height is on the order of hundreds of thousands to millions (**Figure 3, upper left mass inset;** resolution is often expressed as $m/\Delta m$, where m is ion mass to charge ratio and Δm is the smallest difference in mass to charge ratio that can be discriminated). The mass resolutions achieved by routine detectors in mass spectrometry (e.g., quadrupoles and magnetic sectors) range from hundreds to a few thousand — clearly insufficient for biomarker identification.

FT-ICR MS is the only mass analyzer capable of routinely achieving mass resolutions >1,000,000 across a wide molecular weight range (~150-1500 Da). These devices trap ions in cylindrical detection cells, confining them with intense magnetic fields generated by superconducting magnets that force ions into regular orbits with periods inversely proportional to the mass-to-charge (m/z) ratio. Each ion 'packet' with a given m/z orbits with a single frequency (cyclotron frequency) detected as induced current as the ion packet passes nearby detection electrodes. Although the time-dependent induction current can be complex when multiple ion packets (tens of thousands) of different m/z are present, a Fourier transform analysis of the record of that current (the 'transient') yields the frequency of motion of each detected ion packet. These frequencies are in the radio (RF) range (~10^6Hz) and can be measured with exquisite precision – down to parts per billion. Because frequency is a simple function of m/z, this translates to similarly exquisite mass resolution.

Finally, it should be noted that a newer generation of mass spectrometers, 'Orbitraps', uses similar principles to FT-ICR-MS, but traps ions within small, egg-shaped electrostatic traps, at reduced maximum mass resolution (<200,000). Orbitraps are challenged in resolution for complex mixtures where many species close in mass (several electron mass units) are prevalent. Orbitraps are challenged in resolution for complex mixtures due to peak coalescence behavior and are best suited to lower molecular masses (up to ~300 u) (Birner-Gruenberger et al. 2017).

FT-ICR MS has provided major advances in several fields, including studies of organic molecular composition of crude oil, dissolved organic matter (DOM), soil organic matter (SOM), sediment extracts, and weathered oil from natural and anthropogenic releases. It is particularly useful that FT-ICR MS achieves high mass resolution, high mass accuracy, and high dynamic range (ratio of highest and lowest peak in a spectrum) and can simultaneously detect both abundant and less abundant species across a wide mass range, separating species by mass alone in highly complex sample matrices. Therefore, FT-ICR MS can analyze mixtures without prior chromatographic isolation— a great advantage in studies of exceptionally complex natural materials. One example is shows in **Figure 3** from a Mesoproterozoic shale extract from the Taoudeni Basin and asphalt volcano from Santa Barbara, California (McKenna et al. 2014, Gueneli et al. 2018). First, all the species must be resolved from one another and assigned elemental compositions (Figure 3, A). Next, elemental compositions can be used to calculate the number of rings plus double bonds to carbon (calculated from the elemental composition, DBE = $C + h/2 + n/2 + 1$). Figure 3 (B) shows the DBE values and representative core structures for vanadyl porphyrins. Finally, compositional images of DBE versus carbon number (Figure 3, C) show the three dominant vanadyl porphyrin classes detected, and (D) shows the relative abundance for each porphyrin class (DBE value).

A major complication of any mass spectrometry-based application to biomarker research is that the potential target molecules vary greatly in molecular structure and chemical properties. FT-ICR MS can separate these species in mass space, but they first must be transferred to the gas phase through ionization. One single, soft ionization technique cannot

efficiently ionize all the species present in geochemical samples because of the immense polydispersity and polyfunctionality of these samples. For example, atmospheric pressure chemical ionization (APCI) or atmospheric pressure photoionization (APPI) are applied to oil samples to selectively ionize non-polar and aromatic compounds, whereas electrospray ionization (ESI) targets acids and bases (Qi et al. 2020). Often, multiple ionization techniques are applied to selectively target specific chemical and structural functionalities and capture the widest compositional information, resulting in multiple mass spectral analyses for one single sample. Qi et al. (2020) assessed the selectivity of three ionization techniques (APPI, APCI, and ESI) and vastly increased the number of identified novel biomarkers in lignin through compilation of species identified from three separate mass spectral analyses.

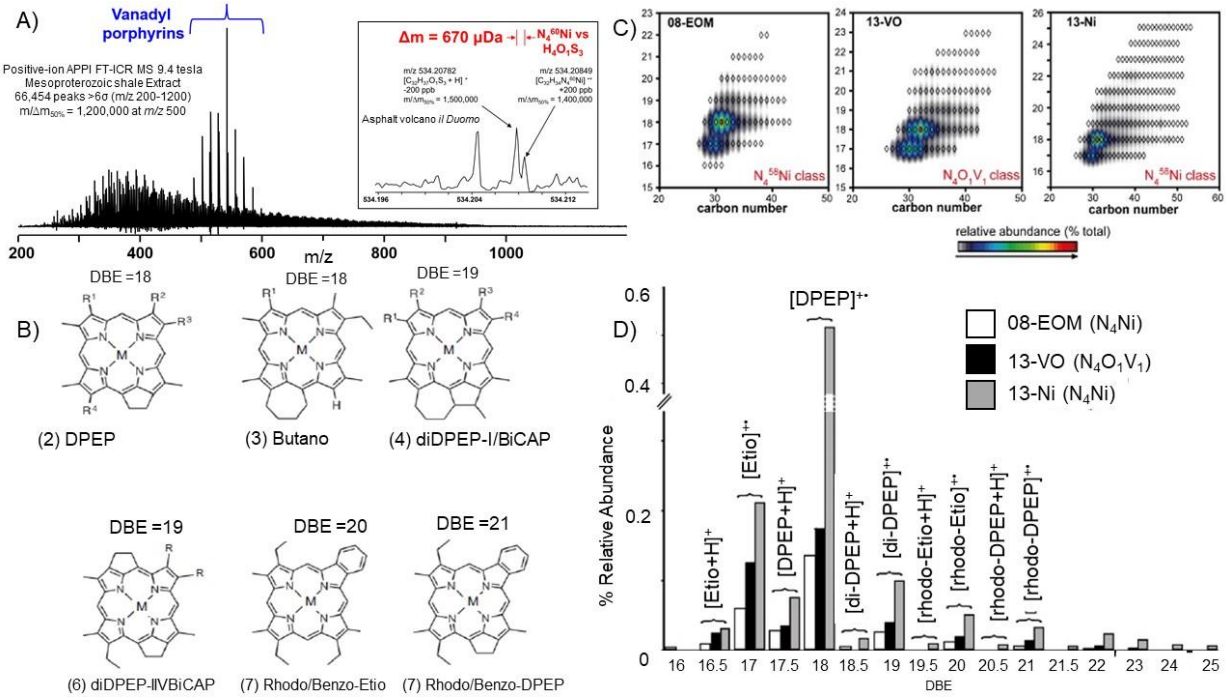

Figure 3. A) Broadband positive-ion Atmospheric Pressure Photoionization (APPI) 9.4 tesla FT-ICR mass spectrum from a Mesoproterozoic shale extract from the Taoudeni Basin that detects more than 66,000 unique elemental compositions across a molecular weight range from *m/z* 200-1200 with achieved mass resolving power ($m/\Delta m_{50\%}$) of 1,200,000 at *m/z* 500 (Gueneli et al. 2018). A mass-scale zoom inset of an asphalt volcano sample illustrates the ultrahigh resolving power of two species that differ in mass by roughly the mass of an electron (McKenna, et al. 2013). B) Some structures of the nickel and vanadyl porphyrins identified by FT-ICR MS that extended the geological record of photosynthesis on Earth by 600 million years. The structures are arranged by the number of double-bond equivalents (DBE), defined as the number of aromatic rings plus

the number of double chemical bonds in each molecule. C) Identification of two classes of Ni- and VO-porphyrins by FT-ICR MS, plotting relative abundance versus DBE and carbon number. D) The structure of C_{30}-C_{32} porphyrins identified supports phototrophs as dominant photosynthesizing organisms on Earth 1.1 billion years ago. Identified porphyrins likely derived from simpler cyanobacteria *versus* phototrophic sulfur bacteria

These advances in mass spectral instrumentation and ionization techniques have been led by petroleum research focused on more comprehensive characterization of global energy reserves. Such advances have expanded the application of FT-ICR-MS to important petrochemical, environmental and geochemical systems and have identified new biomarkers at the level of elemental composition assignment (Kim et al. 2020). In the following paragraphs, we highlight a few selected applications of FT-ICR MS for detection of biomarker species in geochemical systems and recognize many other great research areas (e.g., marine and terrestrial organic matter).

Petroporphyrins.. Applications to target porphyrins for demetallation strategies have largely utilized APPI FT-ICR MS in a host of geochemical systems, including 1.1 Ga old sediments from the Taoudeni Basin (Gueneli et al. 2018), natural petroleum seeps (McKenna et al. 2014), bitumen (McKenna et al. 2009), and tar balls (Lima et al. 2020). A new high-mass-resolution tool for routine analysis of complex metalloporphyrin distributions in geological sample extracts couples HPLC to an Orbitrap mass spectrometer detector, and quantitates Cu, Ni, VO, Zn and Mn porphyrins simultaneously (Woltering et al. 2016).

*Lipids***.** Radović et al. (2016) identify complete series of core glycerol dibiphytanyl glycerol tetraethers (GDGTs with 0 to 8 alicyclic rings), including the complete resolution of GDGT-4 and the unexpected detection of GDGTs with more than 5 rings, in sediments from mesophilic marine environments. These capabilities establish geochemical relationships between archaeal ether lipids, overcomes the limited ability of chromatography to resolve the polydispersity of the sample, and leverages the mass resolving power of FT-ICR MS.

Hydrocarbon biomarkers. In a combined technique approach, FT-ICR MS analysis of an unusually blue crude oil causing deposition issues in an offshore production platform in the Gulf of Mexico identified perylene as the source of the oil's blue color (Juyal et al. 2011). Negative-ion ESI FT-ICR MS identified an enrichment of C_{27}-C_{32} hopanoic acids (DBE of 5-9)

in the field deposit. Collectively, FT-ICR MS, GC-MS and accelerator mass spectrometry (AMS) results suggest that the oil originates from the hydrothermal alteration of algal and bacterial detritus and that the oil is the youngest known hydrothermal petroleum.

Polar biomarkers. New parameters based on oxygen classes ($O_{>2}$) detected by ESI ion source coupled to FT-ICR MS are reported as new paleoenvironmental proxies of terrestrial organic matter input in oxic waters, whereas S_1O_X compounds indicate a sulfidic depositional environment (Orrego-Ruiz et al. 2020). Organic compounds extracted from deep fracture waters in billion-year-old rocks in South Africa characterized by negative-ion ESI FT-ICR MS correspond to microbial metabolites, and indicate geomicrobiological reports of chemolithoautotrophic microbial ecosystems functioning in isolation from photosynthetic primary production (Kieft et al. 2018).

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