

## Mass Spectrometry Imaging of Organic Biomarkers in Geological Samples

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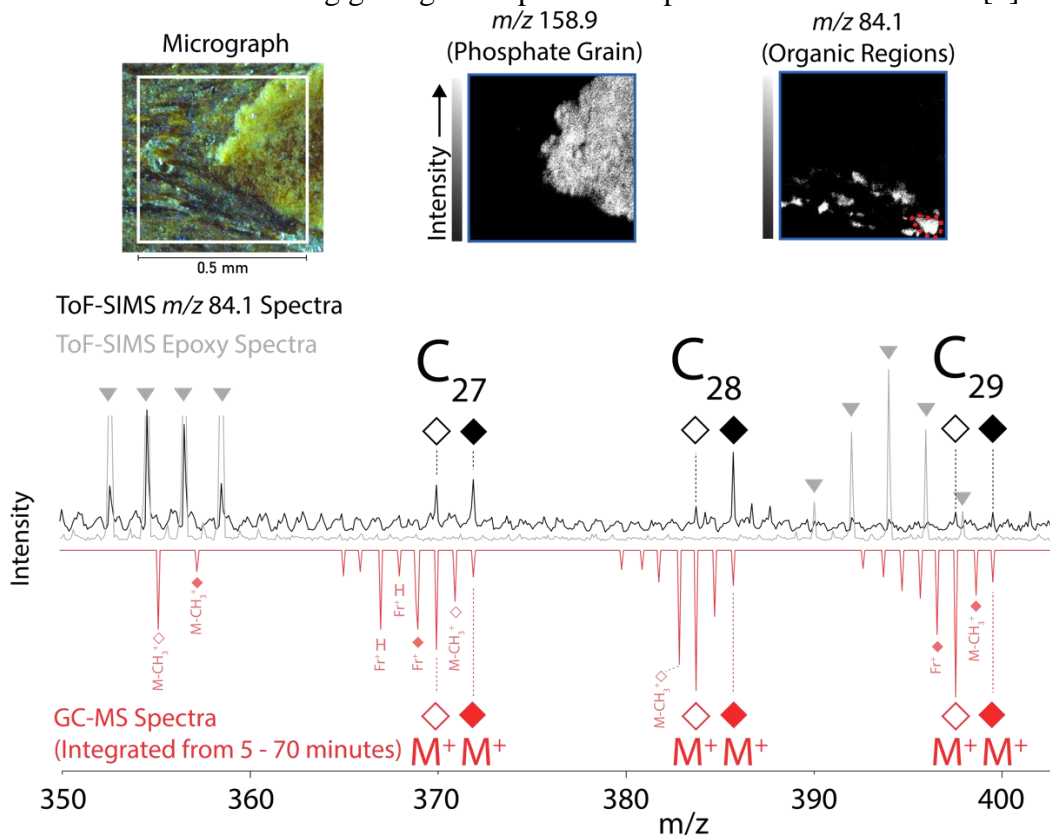
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Organic biomarkers are biologically produced molecules found in the geosphere that retain enough chemical structural information to be attributable to a defined biological source [1]. In the geologic context, biomarker analyses are useful for understanding the chemical and biological evolution of life on Earth. In the context of astrobiology, organic biomarkers (also referred to as organic molecular biosignatures) or a pattern of organic biomarkers observed within extraterrestrial samples could provide evidence for the existence of extraterrestrial life [2].

Gas chromatography mass spectrometry (MS) can detect organic biomarkers within bulk extracts of geological samples, but it is difficult to identify and preclude organic contamination [3]. MS imaging can overcome these shortcomings by identifying organic biomarkers within intact geological samples and correlating such MS data with petrographic and fluorescence images. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging is currently the premier method for identifying organic biomarkers within geological samples [4], so ToF-SIMS is compared here with a novel strategy for MS imaging: femtosecond laser desorption postionization mass spectrometry (fs-LDPI-MS) [6]. An entirely custom-built fs-LDPI-MS instrument utilizes 800 nm, ~75 fs pulses for ultrafast laser ablation of neutral organic molecules from a thin prepared slice of a geological sample, followed by postionization using 157 nm (7.9 eV) laser pulses and subsequent detection of ions by a time-of-flight mass analyzer [5,6]. A TOF.SIMS 5 NCS instrument (IONTOF GmbH, Münster, Germany) was used for the ToF-SIMS data collection: Samples were sputter-cleaned using a 20 keV Ar<sub>2200</sub> gas cluster ion beam to expose areas of 800 μm × 800 μm then areas of 500 μm × 500 μm were analyzed with 25 keV Bi<sub>3</sub><sup>+</sup> primary ions [6]. An ultraclean sample preparation method was developed to avoid organic contamination of geological samples [6]. The entire procedure was demonstrated on a thin slice of the kerogen-rich Oxford Clay formation (UK) [6].

Optical and fluorescence microscopies were used to identify organics including aromatic-rich regions consistent with kerogen [6]. Figure 1 shows an optical micrograph of the sample with correlated ToF-SIMS images of inorganic ( $m/z$  158.9) and organic-rich ( $m/z$  84.1) regions. ToF-SIMS data of the red-dotted region of the organic regions included higher mass ions interpreted as C<sub>27</sub>, C<sub>28</sub> and C<sub>29</sub> steranes (black spectra in Figure 1) that were also observed by gas chromatography MS (red spectra). ToF-SIMS and fs-LDPI-MS showed multiple common peaks in the organic region, with the latter also displaying ions from pyrite [6]. However, the photoionization strategy used in fs-LDPI-MS cannot detect steranes as it is sensitive only to species with lower ionization energies. This custom-build fs-LDPI-MS instrument also suffers from relatively low mass resolution and accuracy compared to the commercial ToF-SIMS employed here, but the former's performance metrics can be improved further.

One major advantage of fs-LDPI-MS is that the laser ablation step can rapidly remove micrometers of material to expose buried material within a geological sample [6]. By contrast, the common strategy in ToF-SIMS of sputtering with a gaseous cluster beam is several orders of magnitude slower. ToF-SIMS and fs-LDPI-MS combined with direct laser desorption ionization MS could be a path forward for the analysis of natural material including geologic samples or samples returned from Mars [7].



**Figure 1.** ToF-SIMS spectra of steranes in kerogen-rich regions of the Oxford Clay formation (UK). Top, from left to right: optical micrograph and ToF-SIMS images of the spatial distributions of  $m/z$  158.9 (phosphate grain) and 84.1 (organic region). Expanded mass spectra from the red-dotted region of the  $m/z$  84.1 image indicates the presence of steranes: middle shows ToF-SIMS spectra (black) overlaid on ToF-SIMS spectra from silver epoxy used for sample mounting (triangles indicate  $\text{Ag}_n^+$  ions) while bottom shows gas chromatography-mass spectra (red). Sterane molecular ions are indicated with diamonds (open diamonds correspond to unsaturated steranes, filled diamonds to saturated).

#### References:

- [1] K Peters, C Walters and J Moldovan, "The Biomarker Guide", 2<sup>nd</sup> ed., (Cambridge University Press, Cambridge, 2004). Doi:10.1017/CBO9780511524868
- [2] M Neveu, LE Hays, MA Voytek, MH New, and MD Schulte, *Astrobiology* **18** (2018), p. 1375. doi:10.1089/ast.2017.1773
- [3] GT Ventura, et al. *Proceedings of the National Academy of Sciences U.S.A.* **104** (2007) p. 14260. doi:10.1073/pnas.0610903104
- [4] V Thiel and P Sjövall *Annual Review of Earth & Planetary Science* **39** (2011) p. 125. doi:10.1146/annurev-earth-040610-133525

[5] L Hanley, RC Wickramasinghe, and YP Yung, *Annual Review of Analytical Chemistry* **12** (2019) p. 225. doi: 10.1146/annurev-anchem-061318-115447

[6] R.C. Wickramasinghe, M.J. Pasterski, A.V. Ievlev, M. Lorenz, J.M. Gross, F. Kenig and L. Hanley *Analytical Chemistry* **93** (2021) p. 15949. doi:10.1021/acs.analchem.1c03275

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