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Gas cluster ion beam for the characterization of organic materials in submarine basalts as Mars analogs

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The solar system contains large quantities of organic compounds that can form complex molecular structures. The processing of organic compounds by biological systems leads to molecules with distinctive structural characteristics; thus, the detection and characterization of organic materials could lead to a high degree of confidence in the existence of extra-terrestrial life. Given the nature of the surface of most planetary bodies in the solar system, evidence of life is more likely to be found in the subsurface where conditions are more hospitable. Basalt is a common rock throughout the solar system and the primary rock type on Mars and Earth. Basalt is therefore a rock type that subsurface life might exploit and as such a suitable material for the study of methods required to detect and analyze organic material in rock. Telluric basalts from Earth represent an analog for extra-terrestrial rocks where the indigenous organic matter could be analyzed for molecular signatures. This study focuses on organic matter in the basalt with the use of surface analysis techniques utilizing Ar gas cluster ion beams (GCIB); time of flight secondary ion mass spectrometry (ToF-SIMS), and x-ray photoelectron spectroscopy (XPS), to characterize organic molecules. Tetramethylammonium hydroxide (TMAH) thermochemolysis was also used to support the data obtained using the surface analysis techniques. The authors demonstrate that organic molecules were found to be heterogeneously distributed within rock textures. A positive correlation was observed to exist between the presence of microtubule textures in the basalt and the organic compounds detected. From the results herein, the authors propose that ToF-SIMS with an Ar GCIB is effective at detecting organic materials in such geological samples, and ToF-SIMS combined with XPS and TMAH thermochemolysis may be a useful approach in the study of extra-terrestrial organic material and life. © 2016 American Vacuum Society. [http://dx.doi.org/10.1116/1.4954940]

I. INTRODUCTION

One of the most fundamental questions in science currently is whether we are alone in the universe. All the terrestrial planets, such as Mercury, Venus, Earth, and Mars, show evidence of volcanic activities in their past.1,2 Mars is the only location where budgetary and technological constraints permit access and is also considered as having had the most likely planet for sustaining life.1,3–5 The surface conditions of Mars presently appear hostile to life,5 and thus we rely on evidence that the planet has been more hospitable to life in the past,7 such as the recent discovery of liquid water by NASA’s Mars Reconnaissance Orbiter.8

The change to colder and drier conditions may have forced any ancient life to retreat into the subsurface.9,10 Alternatively, while Mars experienced significant volcanic activity, biological remnants may have been absorbed into ancient rocks, and some organic molecules as an indicator of life on Mars may still remain. Thus, the organic matter within the rock potentially contains information that can be used to determine the past or extant presence of life. On this assumption, if we could show that the production of new living organisms or organelles has occurred independently on Mars, it would be possible to infer that life has independently arisen twice. In this case, since life has arisen twice in
the same solar system, it may be common throughout the universe.

In the case of Mars, a practical solution would be to analyze complex organic materials to search for those indicative of biological processing. The search for evidence of life on Mars is however not an easy task because of the scarcity of pristine Martian meteorite samples. The most convincing approach would be to correlate the presence of molecules indicative of biological processes with structures deep within such Martian samples, perhaps in locations or distributions that make it extremely unlikely that they arise from terrestrial contamination. For this, spatially resolved analysis methods are needed (i.e., “chemical imaging”) in favor of homogenized analysis, however sensitive the analysis method.

Basalt represents the dominant form of geological material in the solar system, and large igneous provinces are formed by basalt in all the terrestrial planets. The composition of basalt on the terrestrial planets is sufficiently similar to permit the use of analogs on Earth as a reasonable proxy for the terrestrial rocks. Recently, basaltic glass has been detected on Mars; therefore, a logical approach is to investigate a terrestrial basalt analog on Earth upon which at least the possibility of life occurs. This allows us to evaluate methods that will be required to detect past or present life on Mars. In this study, we use analogs on Earth and develop a test procedure with the aim of arriving at a method suitable for analyzing Martian crust samples.

Earth basalts often contain broken glass fragments which have crystallized structures called clasts. Many basaltic clasts contain features that have been interpreted as tunneling by microbial activity. These are localized features that originate around the outer edge of the clast and develop inward to the center of the clast. The microtubule textures are associated with enrichments of carbon and other biologically important elements. An extensive and easily identifiable example of these is the microtubule textures within basaltic clasts embedded in the volcaniclastic matrix of the Ontong Java plateau (OJP). The OJP formed from volcaniclastic submarine flood basalt, which is estimated to be approximately 120 × 10^6 years old, is the largest igneous province in the world. Staudigel et al. and references therein showed microtubule textures in 3.5 × 10^9 years of basaltic clasts using optical microscopy, and the size of microtubule textures was around 20–200 μm. Organic elements such as carbon and nitrogen associated with microtubule features were also observed in the OJP with energy dispersive spectroscopy in a scanning electron microscope and carbonates by the synchrotron-based technique scanning transmission x-ray microscopy. Preston et al. investigated a rock from the OJP using Fourier transform infrared spectroscopy microprobe. This study suggested that the sample showed a correlation of the tubular bioalteration textures observed by optical microscopy with the locations of organic materials, consisting of aliphatic hydrocarbons, amides, esters, and carboxylic groups.

Nevertheless the nature of the organic compounds associated with microtubule textures in the OJP has not yet been fully characterized in the existing literature. Additionally, biological activity or molecular biosignatures from past activity has yet to be directly associated with these features; thus, the geological biomarkers of these features remain a subject of debate. It therefore seems logical to search for any molecular biomarkers in the OJP samples where at least the possibility of geological biomarkers exists. It is anticipated that any molecular biomarkers may provide further evidence for the origin of these structures.

Surface analysis techniques such as x-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS) are promising techniques that provide potential biomarker information with high spatial resolution and sensitivity. In recent years, Ar gas cluster ion beam (GCIB) sources have become popular in XPS and ToF-SIMS systems due to the minimal chemical damage that they typically introduce, especially in the analysis of polymer and organic materials. We have also previously considered the total sputter yield of GCIB depth-profiling of organic/inorganic interfaces and organic materials. With these studies, GCIB is now available to “clean” a sample surface with less damage of potential organic information. In addition, as a primary ion beam in SIMS, Ar GCIB gives more mass information with lower damage than the other ion sources in common use. In this work, we used XPS and ToF-SIMS to further elucidate the nature and distribution of the organic compounds associated with the microtubule textures in the OJP. Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH), otherwise known as TMAH thermochemicalysis was also used to support identification of the fragments from ToF-SIMS analysis.

II. EXPERIMENT
A. Ontong Java plateau 13 sample

The basalt in this study was retrieved from Leg 192 Site 1184A Core 13R (5°0.6653’S 164°13.9771’E) of the Ocean Drilling Programme. This basalt was obtained from 224.6 m floor overlaying the basement crust. This is equivalent to 23.5 m beneath the top of the basement crust described hereafter as Ontong Java plateau 13 sample (OJP13). To remove environmental contamination, specimens were removed from the internal volume of the parent rock samples and trimmed to ~20 × 30 × 0.5 mm using an IsoMet 1000 precision sectioning saw (Buehler Illinois, USA), and then mounted on a glass slide with an epoxy resin prior to surface analysis by ToF-SIMS and XPS. A separate sample was prepared for TMAH thermochemicalysis analysis which was not embedded in epoxy resin. Several precautions were taken to avoid unnecessary organic contamination of the specimen. Steel apparatus used in this study was heat-treated to 800 °C for 8 h. All of the specimens were handled with flame-forged forceps and stored in a shelf wrapped in clean aluminum foil after 30 min ultraviolet ozone cleaning of the specimen using a UVO-Cleaner Model 144AX (Jelight

Company, Inc., Irvine, USA). The efficiency of this cleaning procedure has been reported previously.28

The photomicrograph images of OJP13 thin sections were produced using a Leica DM2700P Upright Polarization Microscope with a light-emitting diode illumination (Leica Microsystems, Ltd., Milton Keynes, UK) coupled with an inline Axioimager camera using transmitted light. The images as shown in Fig. 1 confirmed the presence of microtubules18 in OJP13 specimens obtained for this study.

B. Instrumental

1. XPS analysis

The XPS analyses were performed using a K-Alpha XPS instrument (Thermo Scientific, East Grinstead, UK) equipped with a monochromatic Al Kα x-ray source (1486.6 eV). Pass energy was set at 40 eV for high resolution spectra with 0.1 eV step size. A 30 μm x-ray spot size was used for obtaining chemical distribution images. A 4 keV Ar1000 GCIB source was rastered over analysis areas for 90 s to remove adventitious carbon and any organic contamination by the preparation process. This decontamination approach was previously validated by XPS analysis of deliberately contaminated surfaces exposed to different GCIB raster times.29 XPS spectra were processed using THERMO AVANTAGE DATA SYSTEM software v 5.944 (Thermo Scientific, East Grinstead, UK). Charge neutralization using a dual ion (electron and positive Ar ion) was utilized throughout the analysis.

2. ToF-SIMS analysis

The microtubules section in OJP13 was analyzed using a PHI TRIFT V nanoTOF instrument (Physical Electronics, Inc., MN, USA) and a J105 SIMS instrument (Ionoptika, Ltd., Southampton, UK). The PHI TRIFT V nanoTOF instrument was equipped with a bismuth liquid metal ion gun for analysis and a low energy electron flood gun for charge compensation. The bismuth primary ion gun was operated at 60 keV using the Bi3+ beam delivering a 1.0 nA ion current in the HR2 mode with a spatial resolution of around 500 nm.30 Charge compensation was also achieved, and data-processing was carried out using WINDAENCE ver.4.3 (Physical Electronics, Inc., MN, USA). The J105 SIMS instrument was equipped with an Ar gas cluster ion beam source for analysis, and the analysis was performed with a 40 keV Ar4000 cluster primary ion source operating in direct current mode with a spatial resolution of around 3 μm. Mass spectra and a large area image were acquired in a positive mode with a charge neutralizer. The tremendous volume of data obtained from this instrument necessitated the use of multivariate analysis for data processing.31 As a background, a complete and detailed mass spectrum containing detected ion intensities over a half million mass channels can be obtained in ToF-SIMS. The

![Fig. 1. (Color online) Photomicrograph images of OJP13 sample. A section through OJP13 sample showing (A) sectioned OJP13, (B) glass clast 20×, the orange area, size: approximately 200 × 200 μm, alteration textures (gray area in the glass clast with the arrow in (B)), noncrystallized area (the perimeter of the glass clast), (C) microtubules 64× (e.g., a microtubule with the arrow in (C)).](image)
volume of ToF-SIMS spectra in a single data-set, therefore, easily increases to more than 1 GB in file size, and much greater in the case of imaging. In this study, 35 GB was acquired as the SIMS imaging data for a mosaic image; four tiled images with 512 × 512 pixels in total area 2000 × 2000 µm as a field of view. When one has any target mass fragments for a material of interest, it is not too prohibitive to process the data using univariate analysis and built in software with the instrument. Whereas when one is uncertain of mass fragments in the material, multivariate analysis becomes a far more powerful processing technique from the perspective of analysts to identify the most meaningful and important fragments within a reasonable duration. To assist the process of ToF-SIMS imaging data quickly and easily, one of the most powerful multivariate analysis techniques, principal component analysis (PCA), was carried out to provide better contrast of characteristic peaks in spectra. The data matrix was exported into MATLAB R2012a version 7.14 (The Mathworks, Inc., MA, USA), and in-house scripts were used to perform image PCA. There are a number of methods for scaling the data to compensate for spectral characteristics prior to PCA such as mean-centring and autoscaling. However in this study, no preprocessing has been applied to the dataset. No binning was performed with this data to avoid losing any information from the huge amount of imaging and mass spectra data.

3. TMAH thermochemolysis analysis

The TMAH thermochemolysis analysis of the OJP13 specimens was adapted from Abbott et al. The sample for this analysis was not the same piece used for the XPS and ToF-SIMS analyses; however, the sample came from the OJP13 rock and contained the same textural features. On-line THM in the presence of TMAH was performed using a CDS Pyroprobe 1000 pyroprobe unit (CDS Analytical, Inc., USA) fitted with a platinum coil and a CDS 1500 valved interface. TMAH of 2 µl was added immediately prior to pyrolysis. The pyroprobe interface was maintained at 320°C with the pyrolysis products passing into a Hewlett-Packard 6890GC split injector (320°C) linked to a Hewlett-Packard 5973MSD (electron voltage 70 eV, filament current 220 µA, source temperature 230°C, quadrupole temperature 150°C, multiplier voltage 2200 eV, and interface temperature 320°C). The acquisition was controlled by a HP kayak xa Chemstation computer, in full scan mode (50–650 amu). Separation was performed on a fused silica capillary column (30 m × 0.25 mm i.d) coated with 0.25 µm 5% phenyl methyl silicone (HP-5). Initially, the GC was held at 50°C for 1 min and then the temperature was ramped from 50 to 310°C at 5°C/min and held at the final temperature for 15 min, for a total time of 65 min, with helium as the carrier gas (constant flow 1 ml/min, initial pressure of 50 kPa, split at 30 ml/min). Peaks from the TMAH thermochemolysis analysis were identified and labeled after comparison of their mass spectra with those of the NIST/EPA/NIH Mass Spectral Library (NIST 05) (National Institute of Standards and Technology, Gaithersburg, USA).

III. RESULTS AND DISCUSSION

A. XPS

To quantify the chemical states of OJP13, XPS analysis was conducted upon 13 different locations on the sample. All areas for the analysis were observed and defined by the optical microscope at first. These areas were classified into three groups, namely, inside (glass clast), the unaltered texture of the areas in the center of a clast; tubule rich (noncrystallized area), the altered texture around the perimeter of the clasts area; and matrix, the surrounding lapilli nonclast matrix.

Table I shows the average surface concentrations from the 13 different areas of the OJP13 sample. C, N, O, Si, Al, Fe, Na, K, Mg, and Ca were observed on each area of OJP13, and the elements (Si, Al, Fe, Na, K, Mg, and Ca) obtained by XPS analysis are typical elements for basalts.

Elemental mapping analysis was also conducted using XPS on the area of the OJP sample containing the 13 different analyzed locations. The target area of the clast for this analysis is shown as the green square in Fig. 2, and the area was 1.4 × 1.4 mm bounding the clast object, labeled as area 1. Figure 3 shows atomic concentration maps as elemental maps of area 1, highlighting the contrast between the inorganic element (Si) and organic elements (C and N). Si distribution was observed in the matrix area and the inside of the clast object (glass clast hereafter); meanwhile, higher intensities of C and N were observed than that of Si on the tubule rich area in area 1.

B. ToF-SIMS

1. Bi3+ primary ion source

To investigate molecular distributions of OJP13, ToF-SIMS analysis was conducted using the PHI TRIFT V nanoTOF instrument. The positive ion images as shown in Fig. 4 were obtained by the mosaic mapping mode over a 1.6 × 1.6 mm analysis area covering the same clast as the XPS analysis. The distribution of inorganic fragments such as Si7+, Al7+, and K7+ was observed in the matrix area, as well as organic fragments on cracklike textures (fracture after here) and noncrystallized areas. The distribution of the inorganic

<table>
<thead>
<tr>
<th>Area</th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>Si</th>
<th>Al</th>
<th>Fe</th>
<th>Na</th>
<th>Mg</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside</td>
<td>9.2</td>
<td>0.3</td>
<td>53.6</td>
<td>19.2</td>
<td>6.5</td>
<td>1.7</td>
<td>1.9</td>
<td>3.6</td>
<td>0.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Tubule rich</td>
<td>10.5</td>
<td>0.3</td>
<td>53.5</td>
<td>18.7</td>
<td>6.2</td>
<td>1.7</td>
<td>1.9</td>
<td>4.2</td>
<td>0.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Matrix</td>
<td>11.2</td>
<td>0.6</td>
<td>52.9</td>
<td>17.2</td>
<td>5.3</td>
<td>2.0</td>
<td>1.8</td>
<td>6.1</td>
<td>0.2</td>
<td>3.1</td>
</tr>
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</table>

<p>| | | | | | | | | | | |</p>
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</table>

Table I. Surface concentrations (at. %) of the texture of the clast and the matrix areas in OJP13.
fragments suggests that they were localized in the matrix, and the glass clast area possesses other components more than the inorganic fragments. The distribution of the 30 m/z fragment assigned to CH$_4$N$^+$ shows an inverse correlation to that of the inorganic fragments with the exception of the glass clast. This correlation is in good agreement with the XPS mapping images of Si 2p, N 1s, and C 1s, as shown in Fig. 3. The data indicate that carbon–nitrogen compounds were localized around the noncrystallized area and within fractures in the matrix, whereas the presence of CH$_4$N$^+$ on the fractures was not clearly observed in the N 1s and C 1s elemental mappings by XPS due to the lower spatial resolution.

2. Ar$_{4000}$ cluster primary ion source

To obtain further organic molecular information from the sample, an additional analysis was conducted on a second area utilizing an Ar$_{4000}$ GCIB as the primary ion beam on the Ionoptika J105 instrument. The Ar$_{4000}$ cluster primary ion source in the J105 was used for the analysis because the GCIB is able to provide information on higher mass fragments than Bi$^{3+}$ ion source. The purpose of the selection of the fresh second area was to detect less damaged organic molecules than any of damaged organic molecules by the x-ray and/or the Bi ion beam from the previous XPS and ToF-SIMS analyses. The second area where microtubule textures were identified by the optical microscope was selected for the J105 analysis and named as area 2 as shown in the red square in Fig. 5. Unlike the positive ion images taken using the Bi$^{3+}$ ion source as shown in Fig. 4, the positive ion images using the Ar$_{4000}$ GCIB source do not show a good contrast between inorganic fragments and organic fragments for inside (glass clast), tubule rich (noncrystallized area), and matrix. However, higher molecular fragment peaks between 40 and 400 u were observed to be using the Ar$_{4000}$ GCIB source than for the Bi$^{3+}$ ion source.

To characterize molecules of clasts including the fractures and the noncrystallized areas in area 2, PCA was carried out using the Ar$_{4000}$ GCIB data; PCA was carried out using the Ar$_{4000}$ data; PCA was expected to explain organic chemical differences clearly between the target area (the fractures and the noncrystallized areas) and the other areas (the matrix and glass clast areas). The first principal component (PC1: 24.9%) as shown in Fig. 6 was obtained from the
analysis, and the PC1 represents the total ion image of the analysis area with the result of nonmeancentring. This PC1 is useful to determine fragmental components on the entire analysis area. Therefore, in this study, the PC1 data were used to confirm the assignment of the fragments. No fragments which strongly corresponded with the epoxy medium were observed on the analysis area. This absence confirms that the sample surface targeted for analyses was decontaminated.

In Fig. 7, the second principal component (PC2: 8.8%) describes a good contrast between the areas. Hence, it is regarded that PC2 is able to separate key chemical environment within the surface. The positive values in the score image of the PC2 correspond to the matrix and glass clast areas, and both organic and inorganic fragments were observed on the positive loadings. The main organic fragments were assigned to aromatic hydrocarbons such as C_6H_5^+, C_7H_7^+, C_9H_7O^+, and C_{10}H_{13}O^+. In addition, some inorganic fragments which were assigned as K^+, Ca^+, Fe^+, Si^+, and Al^+ are also loaded on the loadings plot, although the loading weights are not strong enough to see clearly in the plot shown. These elements on the positive loadings are also in a good agreement with the typical basalt mineral components\textsuperscript{34} and the XPS results as discussed above as well. In contrast, the negative score area corresponds to the noncrystallized and the fracture areas in the object. The negative loadings show more organic fragments containing nitrogen atoms characteristically than the positive loadings, and they were identified as alkaloids; for instance, they were as assigned as CH_3N^+ (m/z 30), C_5H_8N^+ (m/z 82), and C_8H_{16}N^+ (m/z 126). Furthermore, more alicyclic hydrocarbons, alkene, or alkane were also assigned as characteristic fragments on the negative loadings, such as C_4H_7^+ (m/z 55), C_5H_9^+ (m/z 69), C_7H_{13}^+ (m/z 97), C_8H_{13}^+ (m/z 109), C_{11}H_{23}^+ (m/z 155), C_{12}H_{25}^+ (m/z 169), and C_{14}H_{19}^+ (m/z 187).

TMAH thermochemolysis is a bulk analytical technique; however, this technique can identify organic compounds within the sample. The molecular information from TMAH is complementary to ToF-SIMS analysis, and enables correlation between ToF-SIMS fragments and chemical structures.
identified from TMAH results. Therefore, TMAH analysis was also conducted on another piece of the OJP13 sample used in this study. The mass fragments detected by TMAH thermochemolysis were assigned to compounds shown in Table II. The assignment was then used as supporting data for the identification of the fragments by Ar4000 GCIB obtained from the PCA loadings (Fig. 7). The suggested chemical structures of the SIMS fragments are illustrated in Table III. For example, the fragments from the PCA loadings of the noncrystallized and the fractures areas are supposed to form similar chemical structure of the following chemicals: cyclooctene (m/z 109, C₈H₁₃⁺), indolizidine (m/z 126, C₈H₁₆N⁺), and undecane (m/z 155, C₁₁H₂₃⁺) using the TMAH thermochemolysis results. Although organic fragments were observed across all of the areas, the suggested fragments on the matrix and glass clast areas were different from those on the noncrystallized and the fracture areas. While alicyclic hydrocarbons, alkanes, and alkaloids were observed as dominant components on the noncrystallized and the fractures areas, aromatic compounds and ethers were more on the matrix and the glass areas.

### Table II. Suggested molecules and chemical formulae identified by TMAH thermochemolysis.

<table>
<thead>
<tr>
<th>Molecular weight (g mol⁻¹)</th>
<th>Name</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.11</td>
<td>Phenol</td>
<td>C₆H₆O</td>
</tr>
<tr>
<td>108.14</td>
<td>Cresol</td>
<td>C₇H₈O</td>
</tr>
<tr>
<td>118.14</td>
<td>Benzofuran</td>
<td>C₈H₈O</td>
</tr>
<tr>
<td>120.20</td>
<td>Trimethylbenzene</td>
<td>C₉H₁₂</td>
</tr>
<tr>
<td>122.17</td>
<td>Xylenol</td>
<td>C₁₀H₁₀O</td>
</tr>
<tr>
<td>134.21</td>
<td>Durene</td>
<td>C₁₀H₁₄</td>
</tr>
<tr>
<td>137.22</td>
<td>Cyclopentenyl pyrrolidine</td>
<td>C₉H₁₅N</td>
</tr>
<tr>
<td>148.20</td>
<td>Dimethylphenyl ethanone</td>
<td>C₁₀H₁₆O</td>
</tr>
<tr>
<td>148.25</td>
<td>Pentamethyl benzene</td>
<td>C₁₁H₁₆</td>
</tr>
<tr>
<td>162.22</td>
<td>Methylphenyl propanal</td>
<td>C₁₁H₁₄O</td>
</tr>
<tr>
<td>256.33</td>
<td>Bisphenol C</td>
<td>C₁₇H₂₀O₂</td>
</tr>
</tbody>
</table>

### C. Optical microscopy

To examine the correlation between the molecular information obtained by ToF-SIMS and physical information obtained by the optical microscope, the ion images of area 1 and area 2 were compared to optical microscope images of the same areas (Fig. 8).

In Fig. 8, the orange rectangle was imaged using the optical microscope at 40× magnification for area 2. The observation area in the orange rectangle was the edge of the specimen, and this edge area, we suppose, is the result of the removal of material during the sample sectioning. The edge area appears cloudy in color; therefore, it is likely to be non-crystallized and the cloudiness is likely to be a result of a light scattering effect due to the amorphous nature of the noncrystallized glass. Coincidentally, the Ar4000 PCA results (Fig. 7) indicate that the cloudy edge area shows strong correlations with alkaloids, alkanes, and alicyclic hydrocarbons. These alicyclic hydrocarbons, alkanes, and alkaloids are typical components in micro-organisms such as bacteria and fungi. Alkaloids such as indolizidine, which was assigned from the Ar4000 PCA data in Sec. III B 2, occur in terrestrial and marine organisms. Thus, any noncrystallized area in the sample is supposed to contain more alkaloids, alkanes, and alicyclic hydrocarbons, and the cloudiness is considered a result of losing its crystallinity with the organic molecules which are potentially products of bioactivity.

The image of area 1 in the black rectangles in Fig. 8 also shows a similar cloudiness to the edge area in area 2, i.e., the nitrogen rich area identified by XPS and ToF-SIMS. This was identified as the noncrystallized area that included the microtubule textures shown in Figs. 3 and 4. It appears colocated with the cloudy area optically, with the cloudiness seeming to occur by light scattering. The nitrogen rich area is therefore assumed to be compositionally similar to the cloudy edge area in area 2 (the orange rectangle). This cloudiness at the noncrystallized area including the microtubule texture as shown in Fig. 1(b) is considered the result by microbial...
Whereas the other area (the inside and outside of the cloudy area) shows clear, glasslike appearance, it seems to be well-crystallized. In this area, more inorganic glass clast components were observed than the noncrystallized area using ToF-SIMS as described in Subsection III B 1. The well-crystallized area, where is the unweathered basaltic glass area, is therefore confirmed as crystalline, containing inorganic elements such as Si, Al, and Fe. However, while some organic molecules were also observed in the region using the J105 SIMS, these organic molecules probably did not influence the crystallization. Following this, the well-crystallized area likely correlates with aromatic hydrocarbons appearing in with the J105 SIMS PCA data results (Fig. 7). Aromatic hydrocarbons have been discovered in many species of algae and also as compounds within plants, such as antifungal compounds or accelerators of growth, but not the main components of “live” organisms. Therefore, the fragments on the matrix and glass clast areas are interpreted as taphonomic or by-products of organisms such as algae, plants, and planktons, and some fragments may originate from accelerators of growth.

Trends in both chemistry and physical structures are the key to understanding the potential remnants of life in ancient rocks, and hence possibly the mechanism of the origin of life. Here, we propose a hypothesis that some biologically productive activities occurred involving alkanes, alicyclic hydrocarbons, and alkaloids in the noncrystallized areas, including microtubule areas and the fractures areas. Micro-organisms such as bacteria or fungi in these regions have been metabolized and are responsible for the formation of the microtubule textures within the glass clasts. This is supported on the proposals by Saccone et al. and Smits that the tubular textures in basaltic glass clasts are the result of excavation by fungi. Meanwhile, the well-crystallized areas, where the matrix and the glass clast areas form with more inorganic and aromatic hydrocarbons, does not host biological activity but may contain resources that micro-organisms require.

### IV. CONCLUSIONS

Using XPS, ToF-SIMS, and TMAH thermochemolysis within a multiple analytical approach, we have demonstrated the presence of bioactivity in the basalt core sample. In this study, Ar GCIB was used to remove adventitious surface contamination in XPS and also as the analytical beam in ToF-SIMS. The data obtained in this work successfully differentiated between carbonaceous species in the well-crystallized and noncrystallized areas in the OJP13 sample. In the amorphous regions, biologically relevant chemical signatures appeared, such as alicyclic hydrocarbons, alkanes, and alkaloids. On the other hand, aromatic hydrocarbons and inorganic elements were observed more in the matrix and the glass clasts than the amorphous regions. The distribution of the different types of organic molecules indicates that a positive correlation exists between the presence of microtubule textures and the higher concentration of specific classes of organic molecules, implying the presence of bioactivity in the basalt rock.

<table>
<thead>
<tr>
<th>Mass (u)</th>
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<th>Chemical structure</th>
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<td>K⁺</td>
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<tr>
<td>51</td>
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<tr>
<td>57</td>
<td>KOH₂⁺</td>
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<tr>
<td>155</td>
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We propose that ToF-SIMS using GCIB is effective at detecting organic molecules in geological samples, and also the combination with XPS, ToF-SIMS, and GC-MS may prove crucial in the study of extra-terrestrial organic materials and life. In addition, the application of PCA for processing ToF-SIMS data is strongly recommended, especially for large imaging datasets like we have demonstrated here, to reduce data-processing time and to help to suggest meaningful fragments for further analysis.

The question as to the origin of the organic molecules is still open, but this study represents a step forward in our understanding of what organic molecules were preserved in the tubule alteration textures which were assumed to be resulting from micro-organisms in the OJP13 sample. Thus, continued application of these multiple techniques will help elucidate the bioalteration of the basalt. In future, these techniques with GCIB cleaning and analysis as a primary ion beam could also be applied to samples returned from Mars and other extraterrestrial bodies in the search for life.

ACKNOWLEDGMENTS

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FIG. 8. (Color online) Optical microscope images of OJP13: The black rectangle areas in area 1 are nitrogen rich area observed by XPS (Fig. 3). The orange rectangle area in area 2 is the edge of the rock matrix. The blue and red squares are the areas analyzed using the ToF-SIMS instruments. Note: The area below the edge is a void, and it is considered the result of the removal of material during the sample sectioning.