Imaging Mass Spectrometry

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Imaging Mass Spectrometry with a Low-Temperature Plasma Probe for the Analysis of Works of Art**

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Advanced analytical techniques are essential for the understanding, restoration, conservation, dating, and authentication of paintings.[1–6] Several X-ray-based spectroscopic techniques, such as X-ray fluorescence (XRF),[7–9] X-ray diffraction (XRD),[10,11] and proton-induced X-ray emission (PIXE)[12,13] are attracting great interest. The limitations of these techniques are that they can provide little structural information and that they are not sufficient to allow precise identification of chemical compounds contained in paintings. Some spectroscopic imaging techniques, such as FTIR[14] and Raman imaging,[15] provide good spatial resolution under ambient conditions and are nondestructive and can be used in situ. However, they give much lower sensitivity for trace components and poorer chemical specificity.[16]

Imaging mass spectrometry (IMS) is currently receiving a significant amount of attention owing to its ability to generate molecular images from a large variety of surfaces and powerful structural identification capacity.[17–20] Currently IMS methods, including matrix-assisted laser desorption/ionization (MALDI),[21,22] secondary-ion mass spectrometry (SIMS),[23–25] and desorption electrospray ionization imaging mass spectrometry (DESI-IMS)[17,26,27] are emerging as powerful tools for investigating the distribution of molecules within biological systems through the direct analysis of thin tissue sections.[20] Although the first two methods can provide spatially specific chemical composition information with respect to the surfaces,[26,28] their drawback is that the samples must be introduced into the vacuum environment, which is not convenient for valuable artwork owing to their large size.[29] DESI-MSI allows direct analysis of the sample with little or no sample pretreatment under ambient conditions. However, for analysis of paintings, the electrosprayed organic solvents may result in contamination and damage to the sample.

Herein, we present a novel IMS method that uses a low-temperature plasma (LTP) probe as an ion source for the analysis of paintings and calligraphy. For the evaluation of artworks, the following requirements should be satisfied. First, to avoid damage and contamination of sample, no solvent or matrix should be introduced into the ion source or samples during the experiment. Second, analysis by the probe should be carried out in a preparation-free approach, and therefore an ambient ionization technique amenable to direct analysis is desirable. Finally, the spatial resolution of the IMS technique should be sufficiently high to allow characterization of the spatial distribution of analytes. The experimental setup and the configuration of LTP probe are shown in Figure 1 A and B, respectively (painting study provided in the Supporting Information, Figure S1). The probe is an improved version based upon the previously reported dielectric barrier discharge ionization (DBDI) source.[30–32] It consists of a fused capillary and two aluminum foil electrodes. A high-voltage alternating current (AC) is applied to both electrodes. Helium gas is introduced through the capillary for microplasma generation. Experimental parameters are optimized for highly sensitive detection (detailed parameters are provided in Supporting Information, Figure S2).

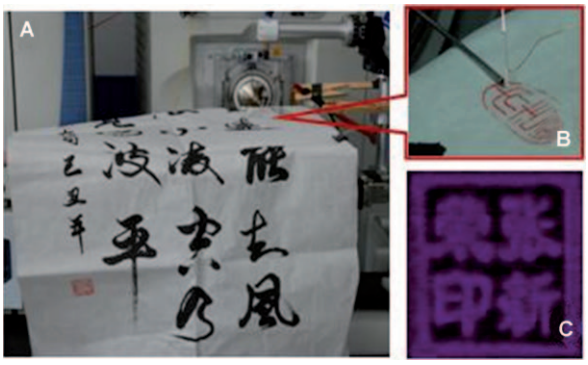

Figure 1. The experimental setup and the configuration of the LTP probe for imaging mass spectrometry. A) Analysis of the calligraphy patterns using LTP-IMS. B) Expanded view of the LTP probe scanning the pattern. C) Imaging of the inkpads of seals on rice paper by using the LTP probe.

An important aspect for the investigation of paintings and calligraphy is to prevent damage to samples during analysis. In our experiment, the temperature of the microplasma was around 30 °C (Supporting Information, Figure S3). Notably, the temperature of plasma could be controlled by adjusting the temperate of discharge gas. The temperature could be

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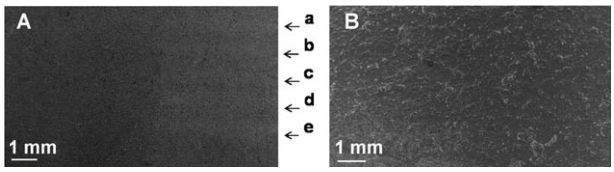

Figure 2. SEM images of rice paper after scanning by the LTP probe. A) A line ink-jet printed onto rice paper and scanned at different speeds: a) 400, b) 300, c) 270, d) 200, and e) 100 $\mu m\,s^{-1}$. B) Inkpad pressed onto rice paper after line-by-line scanning.

further lowered to −30 °C by using liquid nitrogen to cool the helium gas (Supporting Information, Figure S4). Rice paper printed with a black ink jet was chosen as a typical case. Experimental evidence confirmed the feature by comparing the depth of the trace of lines scanned at different speeds (about 100, 200, 270, 300, and 400 $\mu m\,s^{-1}$). Scanning electron microscope (SEM) micrographs (Figure 2 A) show that the trace of lines sputtered at different speeds is hardly observed. In real IMS contexts(Figure 2 B), the inkpad was scanned at a speed of about 270 $\mu m\,s^{-1}$. No damage was observed for the surface induced by the plasma. These results strongly suggest the nondestructiveness of the probe for real-world sample analysis.

The spatial resolution of the probe is inspected in the subsequent phase, which is critical for the imaging quality. The resolution of this technique was related to the capillary size, the flow rate of the discharge gas, and the surface scanning rate. Of these factors, the inside diameter of the capillary is crucial. A typical photograph of microplasma generated by different diameters of capillaries is shown in Figure 3 A. The plasma plume extends from inside to beyond the exit of the capillary. As the inner diameter of the capillaries decreases (530, 320, 150, and 100 μm), the length and diameter of the plasma plume decreases accordingly. The conical plume, which vertically impacts the surface of the artwork (Figure 1 B), forms a circular desorption/ionization region. It should be noted that the actual resolution is influenced not only by the size of LTP probe but also by the ion yield. For this reason, and based on our preliminary experimental results, a capillary with an inner diameter of 150 μm was selected. A black ink containing 3-aminoquinoline printed on a plastic film was chosen as a typical case (Figure 3 B). Lane 1 and lane 2 represent a spacing of 175 μm and 765 μm, respectively. Each lane was scanned with a 3D moving stage at a rate of about 270 $\mu m\,s^{-1}$, and the extracted ion current (*m*/*z* 145) was recorded (Figure 3 C,D). The lateral resolution of the probe is approximately 250 μm in the horizontal direction, which is comparable to that of DESI.[27]

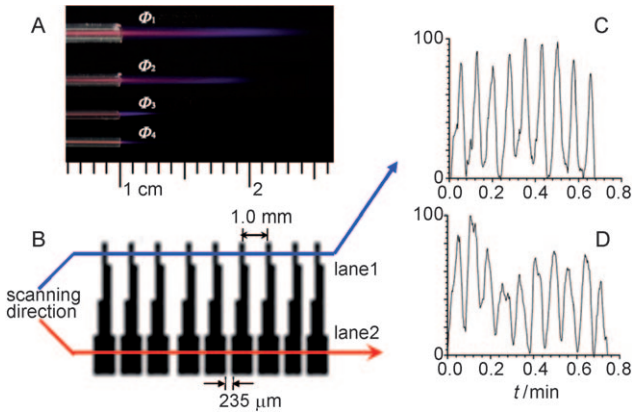

Figure 3. Spatial resolution studies of the LTP probe. A) True-color photographs of LTP probe jets generated using the two electrode configurations for DBD with four different inside diameters Φ of the capillaries. $\Phi_1 = 530$, $\Phi_2 = 320$, $\Phi_3 = 150$, $\Phi_4 = 100$ μm inside diameter. B) Ink-jet pattern generated on glossy photographic paper to test lateral spatial resolution. The blue and red line represents lanes 1 and 2, respectively. C,D) Extracted ion current of the mass spectrum for 3-aminoquinoline (at *m*/*z* 145) from lanes 1 and 2, respectively.

Based on these two advantages, we applied this method to the analysis of a typical case, namely inkpads, which were frequently used in Chinese paintings and calligraphy. All the inkpads are composed of the same basic components: pigments, binders, and addictives. One genuine seal and two counterfeits were subject to analysis with present method. The size of the pixel is 150 μm by 150 μm, and the MS images clearly show the outline of the characters in seals. The total area scanned by the probe was approximately $38 \times 20\ mm^2$; the area was scanned in both the horizontal and vertical directions to generate an array containing 253 × 133 (33 649) pixels. The spatial distributions of compounds from different types of inkpads, together with the specific intensity of each pixel, are shown in Figure 4 A–I (detailed experimental procedures and data processing are provided in the Supporting Information). The three mass spectra in Figure 4 J–K show that the relative intensities of most peaks are similar, and differences exist only among the peaks with relatively low intensities. For example, the peak at *m*/*z* 116 only appears in the mass spectrum in the genuine seal, rather than in the mass spectra of the two counterfeit seals (Figure 4 J–L). By extracting ions of *m*/*z* 116, a relatively good image is obtained for the genuine seal, whereas almost no image can be found in the corresponding extracted images for the counterfeit seals (Figure 4 B, E, and H). This feature is unparalleled in that it means we can tell the genuine sample apart from the other two by simply comparing the extracted images for ions of a

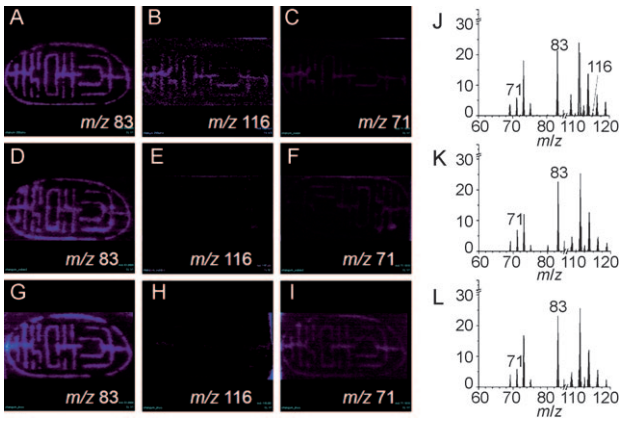

Figure 4. Imaging of inkpads of the seals on calligraphy using the LTP probe. A–C) Genuine calligraphy, D–F) counterfeit 1, G–I) counterfeit 2. J–L) Mass spectra of J) genuine calligraphy, K) counterfeit 1, L) counterfeit 2.

specific m/z value. The three patterns can also be distinguished by comparing the MS images (Figure 4C, F, and I), even though the relative intensities of m/z 71 were similar at some point (Figure 4J–L). Therefore, MS images can provide considerably more information in terms of spatial distribution as one mass spectrum only represents one specific point of the pattern. Furthermore, we carried out an experiment by using Q-TOF mass spectrometer coupled with the LTP probe to interpret the mass spectra (Supporting Information).

The results presented herein are the first application of a LTP probe in the two-dimensional imaging of paintings and calligraphy. The probe offers unique advantages in terms of ease of implementation, direct ambient imaging capability, and absence of sprayed solvent. These virtues are especially useful in the imaging analysis of paintings and calligraphies, in which a nondestructive and in situ technique is highly desirable. The spatial resolution of the probe is approximately 250 μm, and it could be further improved by taking a capillary with a smaller inner diameter. Results clearly show that IMS with a LTP probe can distinguish genuine seals from counterfeit ones by giving out different imaging patterns and mass spectral fingerprints. As some components in paintings are organic compounds, the present imaging technique should be potentially applied to the analysis of western paintings. It is anticipated that this simple-to-fabricate, yet powerful technique can contribute to the identification, conservation, and restoration of precious artworks.

Experimental Section

All the experiments were performed on a commercial Thermo Fisher LTQ (San Jose, CA) linear ion-trap mass spectrometer equipped with a home-built LTP probe ion source. Xcalibur software 1.4 SR1 was used for data acquisition. An extended ion-transfer line made of metal was connected to the orifice of the MS as required. A 3D automate moving stage was fixed to MS. For analysis, the probe was fixed perpendicular to the sample. For the examination of spatial resolution of IMS, the ink-jet cartridge was doped with 3-aminoquinine (molecular ion, m/z 145) and two lines of 175 and 765 μm in width were printed onto plastic film separated in each case by distances of 1.0 mm (center-to-center). Positive-ion detection was used for the all the ink-jet experiments and helium was used as the discharge gas at a pressure of 0.5 MPa. Subsequently, the surface of the plastic film was scanned over one set of lines (one lane) at a time at a rate of about 270 $\mu m\,s^{-1}$ in the direction indicated by the arrows. For the detection of paintings and calligraphy, the whole sample was placed on the 3D automate moving stage under LTP probe. After experiment is completed, software (Aston lab, Purdue University) was used to convert the XCalibur mass spectral files. BioMap was used for visualization and basic enhancement of the images. For nondestructive investigation, the thin layer of black ink was printed on the rice paper and then a set of lines on the surface was scanned at different speeds. A sheet of rice paper was pressed onto the inkpad several times until the inkpad was evenly covered on the rice paper. The rice paper was then scanned line by line using the probe at a speed of 270 $\mu m\,s^{-1}$. Finally, the surface was scanned by the probe using a scanning electron microscope.

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