Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization in Transmission Geometry

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In both atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI) and vacuum MALDI, the laser typically illuminates the analyte on the front side of an opaque surface (reflection geometry). Another configuration consisting of laser illumination through the sample backside (transmission geometry) has been used in conventional MALDI; however, its use and the number of reports in the literature are limited. The viability of transmission geometry with AP MALDI is demonstrated here. Such a geometry is simple to implement, eliminates the restriction for a metallic sample holder, and allows for the potential analysis of samples on their native transparent surfaces, e.g., cells or tissue sections on slides.

With the widespread application of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry for high-volume biomedical analysis, there is strong motivation to increase the throughput of the method. While large-capacity sample holders go a long way to speed up the analysis, the need to evacuate the sample presents a significant impediment. Atmospheric pressure (AP) MALDI promises to eliminate the need for this step.[1,2] Various implementations of AP MALDI have proved the feasibility of this approach in combination with both time-of-flight (TOF)[1,2] and ion trap (IT)[3,4] mass analyzers.

The predominant design in both vacuum and AP MALDI is reflection geometry; i.e., the laser irradiation and the ion extraction take place on the same side of the sample. The alternative configuration, transmission geometry, applies laser illumination of the sample from the back through a transparent sample support. Successful proof-of-principle experiments with transmission geometry vacuum MALDI were reported in earlier studies.[5−8]

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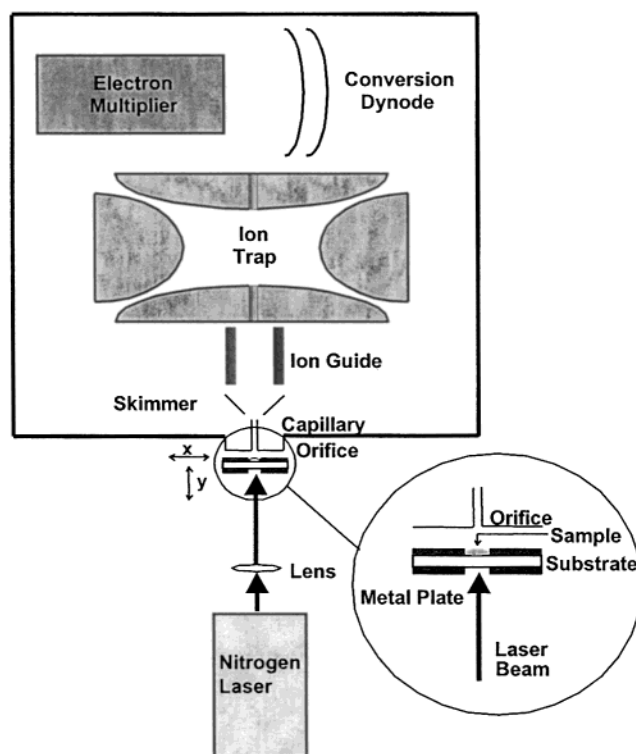

Figure 1. Schematics of transmission geometry AP MALDI IT mass spectrometer showing laser illumination on back of the sample (inset).

Transmission geometry in combination with AP MALDI has not been reported to date. This configuration would expand the capabilities of the technique to samples deposited on transparent substrates, e.g., tissue sections on microscope slides. Due to the coincidence of the sampling orifice axis with the direction of the plume expansion in transmission geometry, there is a potential benefit in terms of improved ion collection efficiency, i.e., sensitivity, in this arrangement.

Differences in sample substrate can affect the MALDI process in various ways. Metallic supports are typically used for reflection geometry, while transparent nonmetallic substrates are employed in transmission geometry. At threshold irradiances, substrate does

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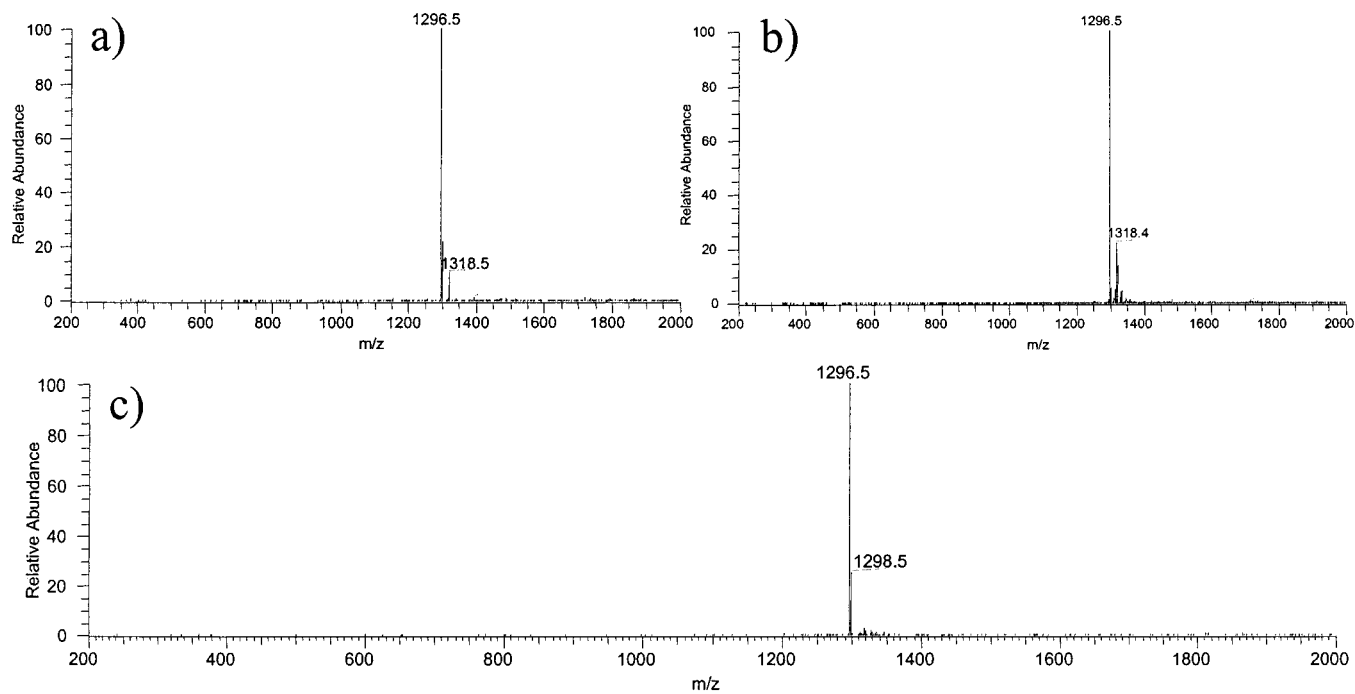
Figure 2. Mass spectra of angiotensin I (AT-I) taken with CHCA matrix using different substrates showing similar results: (a) conductive quartz slide, (b) glass microscope slide, and (c) silane-coated glass slide.

not participate in the MALDI ionization process;[5] therefore, its properties should not affect the ion yield. However, the nature of the substrate may influence other factors, e.g., sample preparation or ion transport. Depositing a sample with the dried droplet method may lead to a different quality sample spot depending on the surface type, e.g., hydrophobic or hydrophilic. In addition, a conductive surface assists in guiding the ions toward the mass analyzer.

Direct comparisons of samples analyzed through both reflection and transmission geometry were used to determine the photochemical and photothermal effects in the MALDI process.[6] In the transmission mode experiments (by use of a quartz sample substrate with a thin gold top layer to eliminate photochemical effects), only $[M + Na]^+$ and $[M + K]^+$ were detected. The lack of protonated analyte species in these spectra suggests that $[M + H]^+$ formation occurs through laser-induced photochemical reactions in the solid or gaseous states. This can be achieved in transmission mode with transparent sample supports.[5,6] In fact, vacuum MALDI spectra obtained with reflection and transmission geometry were found to be virtually identical.[7]

The first transmission geometry AP MALDI experiments are reported in this contribution. Danell and Glish[4b] reported AP MALDI in a configuration in which samples were deposited inside a glass capillary and irradiated by a laser coupled to the capillary with fiber optics. In those experiments, ions were probably desorbed over a wide angular distribution and then directed to the target by an assisting gas flow. In the work described here, we employ a transparent target and desorb the ions directly in line with the sampling orifice of the mass spectrometer. Such a geometry is simple to implement, eliminates the restriction for a metallic sample holder, and allows for the potential analysis of samples on their native transparent surfaces, e.g., cells or biological tissue sections on microscope slides or proteins adhered to surfaces. In particular, with the recent advances in direct write

(DW) technology,[9,10] matrix-assisted pulsed laser evaporation (MAPLE),[11,12] and pulsed laser deposition (PLD)[12–14] of biological[12] and nonbiological materials,[13–14] there is a need for reliable thin-film characterization tools. The purpose of this research is to demonstrate the feasibility of transmission geometry AP MALDI.

EXPERIMENTAL SECTION

A home-built transmission geometry AP MALDI source[15] was interfaced to a Finnigan LCQ IT mass spectrometer as seen in Figure 1. The general operating conditions were set to an applied sample voltage of 2.00 kV, a capillary temperature of 190 °C, and an injection time of 400 ms. The injection time was successfully selected for reflection geometry AP MALDI; therefore, it was also used with the transmission geometry. Different types of transparent slides served as sample substrates, i.e., a quartz slide with an indium−tin oxide (ITO)-coated conductive side and a nonconductive side (Delta Technologies, Ltd., Stillwater, MN), a regular glass microscope slide, and a silanized glass microscope slide.

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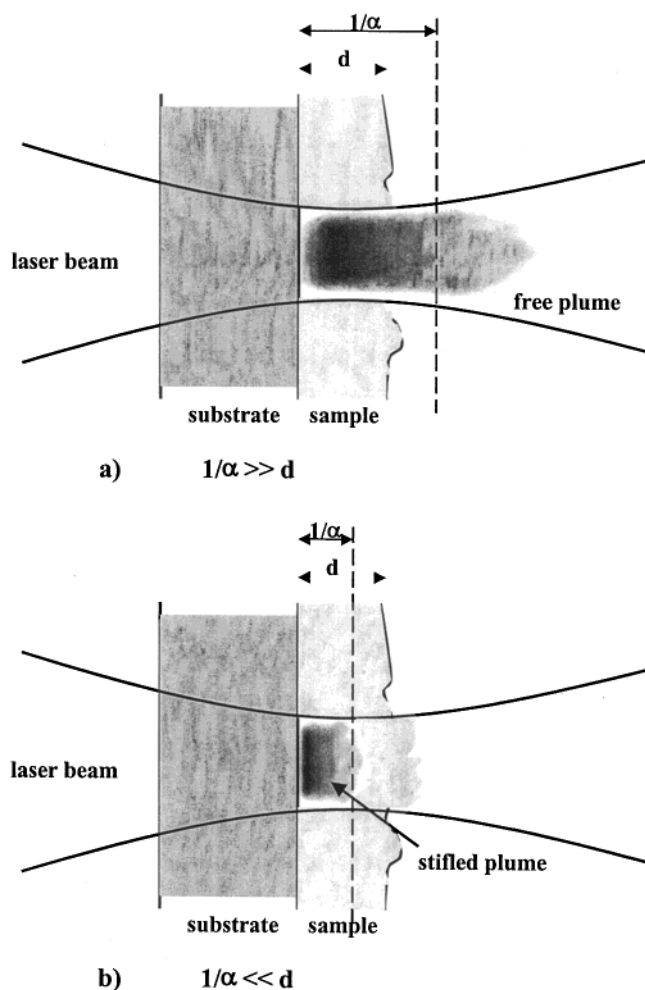
Figure 3. Two dramatically different dynamics of plume expansion depend on the relation between sample thickness, *d*, and absorption length, $1/\alpha$. (a) $1/\alpha \gg d$ leads to free expansion, whereas (b) $1/\alpha \ll d$ results in stifled expansion.

The silanized glass microscope slide was prepared using a method described in the literature.[16] The slide was sandwiched between two metal plates in order to apply the high voltage. Center holes in the metal plates allowed the laser to illuminate the sample. The sample was positioned directly ($\sim$3 mm) in front of the IT orifice. A nitrogen laser beam (VSL-337ND, Laser Science, Inc., Franklin, MA) was focused through a lens (20-cm focal length) to illuminate the sample from behind, causing sample desorption and ionization. Experiments were performed with the laser set to fire at a rate between 5 and 10 Hz.

Instrumental and sample preparation parameters (e.g., applied voltage, matrix type, and deposition method) were varied throughout the experiment. The dried droplet method and the electrospray deposition method were used as sample preparation techniques. The dried droplet method involved either depositing a 1- or 2-μL aliquot of a premixed sample solution (analyte/matrix, 1:5, v/v) onto the substrate or separately depositing 0.5 μL of analyte solution and 1 μL of matrix solution on top (and vice versa) and allowing it to air-dry. For the electrospray deposition, the applied

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voltage was held between 3.5 and 5.0 kV for the 0.5−1.5-min deposition times. The premixed matrix−analyte solution was delivered at a 2.5 μL/min flow rate with a capillary-to-substrate distance of 3.0 cm. The average sample thickness was estimated to be between 1 and 3 μm.

Angiotensin I (AT-I), ASHLGLAR, CDPGYIGSR, and poly-(ethylene glycol) 600 (PEG 600) analytes were obtained from Sigma Chemical Co. (St. Louis, MO). All except for PEG 600 were stored in the freezer. The peptide solutions were prepared in deionized water (18.2 MΩ/cm), whereas the polymer was dissolved in anhydrous methanol (Mallinckrodt Baker, Inc., Paris, KY). The matrix compounds used were α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), sinapinic acid (SA), and 2-(4-hydroxyphenylazo)benzoic acid (HABA). The CHCA required freezer storage, while the SA, DHB, and HABA were kept at room temperature. All matrixes (except HABA) were prepared as saturated solutions in 70% HPLC grade acetonitrile (Fisher Scientific Co., Fair Lawn, NJ) in deionized water. The HABA solution was prepared in anhydrous methanol (Mallinckrodt Baker, Inc.).

RESULTS AND DISCUSSION

Using AT-I as the test compound, the effects of several parameters, e.g., laser power, substrate surface/material, applied voltage, and sample preparation, on the analyte ion yield were investigated to ascertain optimal conditions. To detect analyte in the transmission mode, a relatively high laser power was needed (150−190 μJ/pulse compared to 25 μJ/pulse in reflection geometry). Our experience with reflection geometry AP MALDI showed that laser synchronization to the IT was more advantageous than random laser firing. However, no significant improvements in spectra were observed with the transmission design; therefore, the laser synchronization was omitted for the majority of the experiments. Three types of substrate surfaces/materials, i.e., ITO-coated conductive quartz, nonconductive quartz and glass, and silanized glass, were examined. The laser power transmitted through the quartz and glass (regular and silanized) slides was about 150 and 190 μJ/pulse, respectively. Although glass absorbs more UV radiation than quartz, the conductive coating on the quartz slide decreased the UV transmission efficiency. Figure 2 shows the mass spectra obtained using the (a) conductive quartz substrate, (b) glass microscope slide, and (c) silanized glass target. No significant difference was seen in the mass spectra from the various surfaces and substrate materials. The substrate conductivity, surface, and composition did not affect the ion yield generated by transmission geometry AP MALDI.

Different MALDI matrixes were also tested. The MALDI matrixes CHCA, DHB, and SA were premixed with the AT-I analyte. The CHCA produced better and more reproducible AT-I spectra than the other matrixes. To assess the effect of crystallite size and layer thickness on ion production, dried droplet and electrospray sample deposition methods were utilized. While the dried droplet method typically produces larger crystallites in nonuniform distribution, electrosprayed samples are composed of homogeneously distributed smaller/thinner crystallites. The dried droplet method produced sample spots with the expected characteristics. On the other hand, on insulating substrates, the electrospray preparation did not seem to generate thin layers normally seen on metallic substrates. Instead, the sample seemed
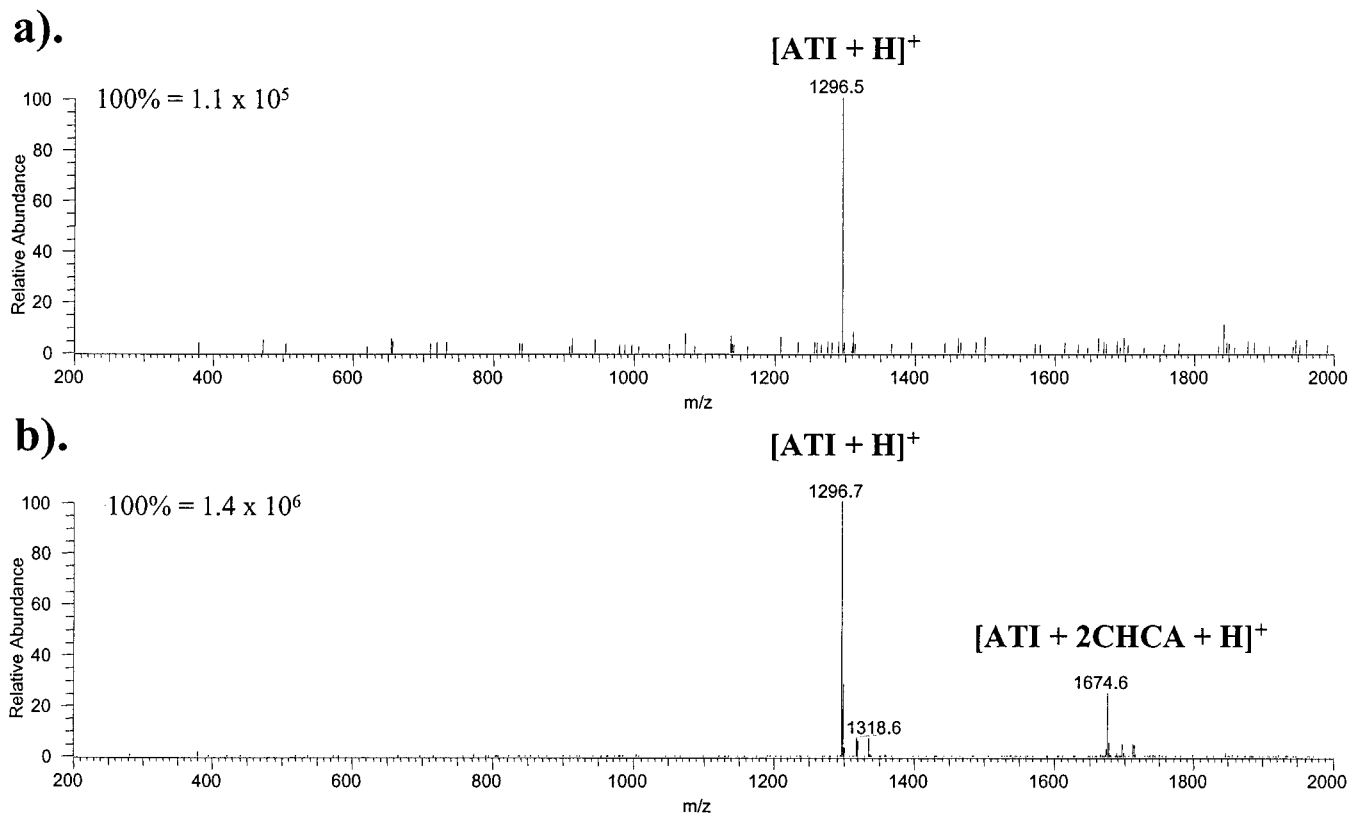
Figure 4. Mass spectra of AT-I comparing (a) transmission and (b) reflection geometry AP MALDI mass spectra from uncoated glass and stainless steel substrate, respectively. The reflection geometry spectrum exhibits more cluster formation.

to deposit as several tiny droplets focused in one region of the slide. Both deposition methods produced similar ion yields and similar quality spectra.

Since ion emission in the transmission configuration occurs from the bottom of the sample,[7] thin sample layers are preferred for efficient ion detection. As seen in Figure 3 the sample thickness, d, along with the absorption length, $1/\alpha$ (where α is the absorption coefficient of the matrix), greatly affects the plume distribution in transmission geometry. For example, if $1/\alpha \gg d$, the laser is able to penetrate through the entire sample layer, allowing the plume to effectively move toward the orifice for detection (Figure 3a). On the other hand, if $1/\alpha \ll d$, the plume is formed but its expansion is stifled by a barrier of solid sample decreasing the ion transport efficiency. Therefore, sample preparation methods that create thin films, e.g., electrospray deposition, may lead to better ion emission from the sample.

In the case of the MALDI matrixes CHCA and DHB, the absorption coefficient of solid samples at the nitrogen laser wavelength of 337 nm are $\sim 2.2 \times 10^5$ and $\sim 7.95 \times 10^4$ cm^{-1}, respectively.[17,18] Thus, the corresponding sample layers must be less than ~ 45 nm for CHCA and ~ 126 nm for DHB for unhindered ion emission to occur. Given the estimated average thickness of the electrospray deposited layers ($1-3$ μm) and the absorption length for CHCA (~ 0.05 μm), it is clear that on the average the $1/\alpha \gg d$ condition was not satisfied. Thus, the plume expansion for the electrosprayed CHCA samples was hindered by a solid

layer (see Figure 3b). Reliable preparation of sufficiently thin sample layers would be needed to see the effects of unobstructed plume expansion. Additional studies with detailed information on sample thickness and sample visualization are necessary.

Other peptides detectable by reflection geometry AP MALDI, i.e., ASHLGLAR, and CDPGYIGSR, were tested by the transmission geometry setup. The CHCA matrix was used for these compounds. Although analyte peaks could be detected in some individual scans, reproducible spectra of stable analyte signals could not be collected. In addition, a synthetic polymer, PEG 600, was tested with the matrix HABA and some PEG oligomer peaks were detected (PEG K$^+$ at m/z 717, 761, 805, 850) with the transmission configuration (spectrum not shown).

As seen in previous studies, AP MALDI IT mass spectrometry is successful in reflection geometry and can be used for the analysis of a variety of compounds and applications.[3,4] Our results show that transmission geometry AP MALDI also generates ions. AT-I was chosen as the test analyte to enable direct comparison with our reflection geometry AP MALDI interface. Even with less than optimal operating conditions, AT-I could often be detected, making it a good candidate for instrument assessment. It is important to note the experimental and spectral differences between the transmission and reflection configurations. Figure 4 shows a spectral comparison between the two geometries. Various differences are observed in the spectra. First, the transmission configuration yields a lower intensity signal (~ 1 order of magnitude lower). Specifically, in Figure 4, the maximum abundance value for transmission mode is 1.1×10^5, whereas it is 1.4×10^6 with reflection geometry. This difference in maximum abundance

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values is typical. The highest maximum abundance values achieved are on the order of 10^7 for the reflection geometry and 10^6 for the transmission design. In reflection mode, analyte−matrix clusters are present; however, these peaks are undetectable when transmission geometry is used. It is important to take into consideration the signal intensities. Analyte−matrix clusters may also be formed in transmission geometry, but since the signal-to-noise ratio is less than that of reflection geometry spectra, the signal may be buried in the noise and, thus, undetected. However, if analyte−matrix clusters are generated with the transmission design, it is clear that the ratio of analyte to analyte−matrix cluster signal is much higher than in the reflection configuration. If the ratios were similar, a low-intensity analyte−matrix cluster signal would have been detected.

Different substrate surfaces and materials resulted in similar mass spectra. We thought it was possible to improve ion transport efficiency to the sampling orifice of the IT by increasing surface conductivity. However, the conductive substrate did not improve the ion signal detection compared to the nonconductive slide. The composition of the slides did not affect the performance as long as they were transparent at the laser wavelength. All the slides allowed for comparable laser illumination of the sample. The silanized surface was prepared to promote beading of the sample solution and concentrate the analyte into a tighter spot. When a sample aliquot was deposited onto the hydrophobic silanized slide, a defined spot was formed. Deposition onto the other surfaces led to larger, less well-defined sample spots. However, no improvement in ion detection was found for the silanized substrate surface compared to the others.

Although substrate conductivity did not play a significant role in improving the analyte signal, the sample voltage was vital to detection. The effect of sample voltage was determined by applying voltages between 0 and 2.5 kV. With voltages of 1.5−2.5 kV, high ion signals (maximum abundance values of 1×10^6 on an arbitrary scale) could be achieved. An applied voltage to the sample was necessary to detect any ions. With as little as 0.1 kV, random ions could be detected; however, with 0 kV applied, no ions were observed. This serves as an indication of voltage-assisted ion transport toward the orifice of the mass spectrometer. Similar to reflection geometry AP MALDI, the optimal sample voltage was found to be ~2.0 kV.

For the direct analysis of samples on their native surfaces, the MALDI matrix must be placed on top of the sample layer. Analyte and matrix solutions were deposited and air-dried separately onto the substrate to simulate the analysis of predeposited analytes.

Ion generation and detection was possible, indicating the prospects of the transmission configuration for a variety of applications. Attainment of ion signal by depositing CHCA on top of a dried peptide layer (AT-I) is the first step in establishing transmission geometry AP MALDI as a potential characterization tool for pedeposited samples.

Other peptides successfully detected by reflection geometry AP MALDI were tested with the transmission configuration to determine the applicability to various compounds. Although spectra could not be collected for these biological compounds, evidence in individual scans shows that ion generation of the peptides does occur. The synthetic polymer−matrix combination of PEG 600 and HABA was also tested. Mass spectra were collected; however, the signal-to-noise ratio was low (maximum abundance values on the order of 10^3). The presence of PEG K^+ oligomer peaks in the spectra indicates the potential for transmission geometry AP MALDI analysis of synthetic polymers. Although these results are preliminary, they suggest that characterization of MAPLD- and PLD-deposited polymer samples may be possible.

We have demonstrated that AP MALDI generates a detectable ion signal in transmission geometry. Ion production was evident through the detection of AT-I by AP MALDI on an IT mass spectrometer. Further optimization is necessary to improve the signal-to-noise ratio. Transmission geometry AP MALDI has a promising potential in the direct analysis of samples predeposited on transparent surfaces, e.g., MAPLE-deposited samples and biological materials on microscope slides. Considering the efficiency of reflection geometry AP MALDI, once ion emission and transport efficiency are improved in the transmission configuration, MS^n analysis can be evaluated, extending the range of possible applications.