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Atmospheric Pressure MALDI with Pulsed Dynamic Focusing for High-Efficiency Transmission of Ions into a Mass Spectrometer

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The atmospheric pressure (AP) matrix-assisted laser desorption/ionization (MALDI) technique described to date has proven to be a convenient and rapid method for soft ionization of biomolecules. However, this technique, like other AP ionization methods, has so far suffered from a low efficiency in transmitting ions from atmospheric pressure into the vacuum of the mass spectrometer (MS). In this work, a novel technique we termed pulsed dynamic focusing, or PDF, which improves the ion transmission efficiency and sensitivity of AP-MALDI by over an order of magnitude, is described. Pulsed dynamic focusing operates on the basis of pulsing a high-voltage extraction field to zero, when ions are just outside of the MS entrance, to allow the intake gas flow of the MS to effectively entrain the ions into the MS. Results from application of the PDF technique to an AP-MALDI ion trap MS demonstrated that in comparison to static AP-MALDI operation (1) up to 2.1 times more ions from a given laser shot could be transferred into the MS, (2) applying higher voltages in combination with the switching scheme yielded up to 1.6-times-higher ion intensities, and (3) a 3-times-larger laser spot area could be utilized. The combination of these factors produced an enhancement in throughput and sensitivity, as measured by the ions detected per unit time, of over 12 times for a digest sample of bovine serum albumin. In addition, the PDF technique proved to make AP-MALDI less sensitive to laser positioning, creating a more robust ion source in comparison to static AP-MALDI.

Atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) has become a useful technique in a number of important applications, including the identification of proteins,^{1,2} the structural analysis of oligosaccharides,³ and the study of phosphopeptides.^{4,5} With MS/MS capabilities, AP-MALDI is a

powerful tool for confirmation of mass spectral identities. Samples are prepared in a manner similar to that for vacuum MALDI, without the complexity of loading the sample into a vacuum. The operation of MALDI at atmospheric pressure also expands the range of candidate chemical matrixes that may be applied. Thus, AP-MALDI offers a rapid and versatile screening tool with MALDI specificity. However, a limitation in previous AP-MALDI experiments to date has been poor transmission efficiency of ions into the vacuum MS. This restricts analytical throughput and sensitivity performance, which are determined by the number of ions detected per unit time.⁶

The difficulty in transmitting ions into the vacuum of the MS occurs because ions are dispersed once created at atmospheric pressure, and they must enter the MS through an aperture or capillary of limited cross section. The dispersed ions are usually subjected to a constant electric field to aid in ion transport, but a significant portion of these ions are still unable to pass through the aperture and are lost. Efficient transport of ions through a small aperture is even more challenging when the ions are farther removed from the region directly adjacent to the aperture. Minimization of ion losses between the ion source and atmospheric pressure interface is thus an important location where significant improvements to sensitivity can be made.

A simple approach to sample ions from an atmospheric pressure source has been to create the ions on-axis with the mass spectrometer's sampling aperture/tube; however, this requires precise aperture alignment and source positioning, and still the sampling efficiency is generally <1 ion in 10⁴.⁷ In conventional AP-MALDI, described by Laiko et al.,^{8,9} a laser irradiation pulse is used to create ions. Ions created with AP-MALDI are extracted into an atmospheric pressure inlet of a mass spectrometer with the aid of a static electric field and the mass spectrometer's intake airflow. In this AP-MALDI configuration, positioning of the laser beam directly on-axis with the aperture provides the best sensitivity. However, during this on-axis, continuous extraction, many of the ions impact the walls and tip of the mass spectrometer inlet, where they are neutralized and lost.

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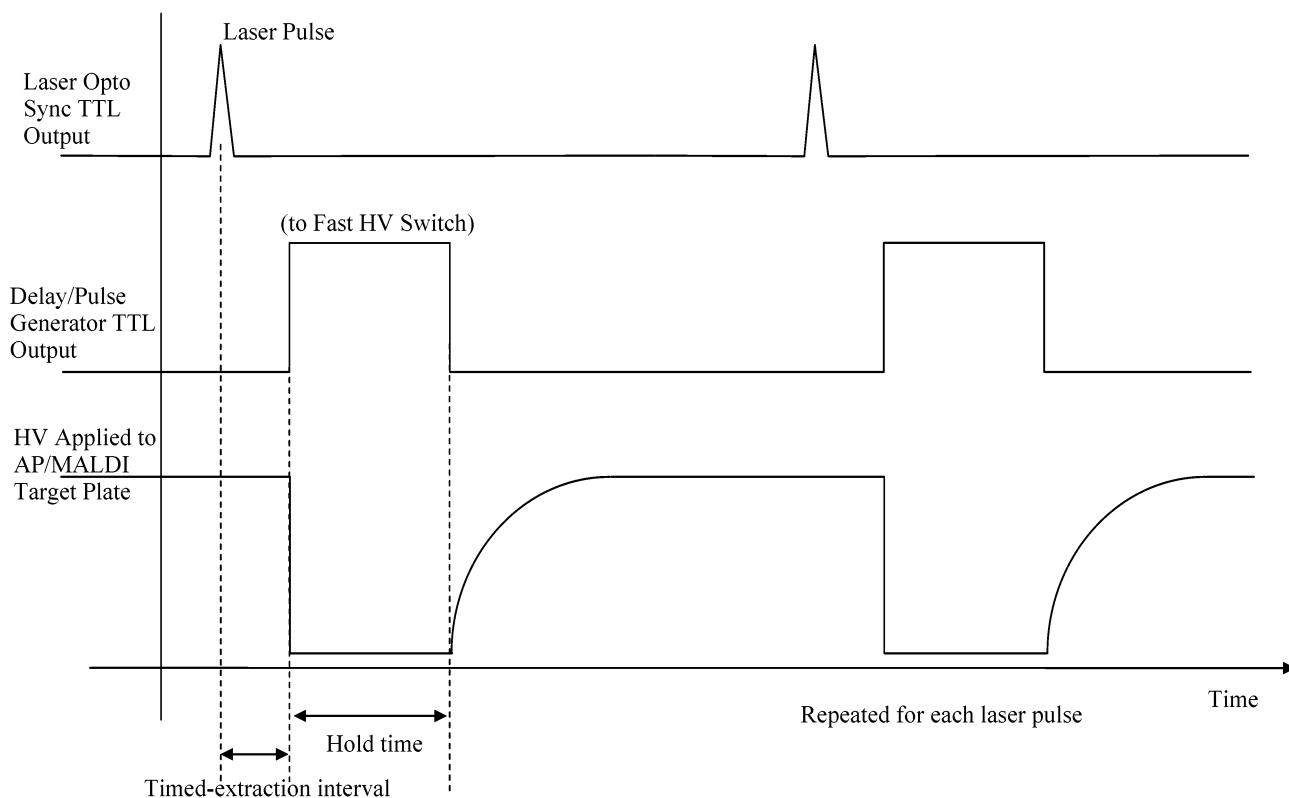


Figure 1. Timing diagram for pulsed dynamic focusing (PDF).

Another approach would be to electrically focus the ions into the sampling aperture. Smith and co-workers developed an ion funnel that consists of a series of elements of decreasing size, where radio frequency (RF) voltages are applied to alternating elements to direct ions at intermediate pressures.¹⁰ Focusing has also been suggested with a plate lens arranged in front of an aperture plate such that electric field force lines point into the MS inlet aperture;¹¹ however, these focusing devices do not eliminate ion losses completely, neither have they been implemented at atmospheric pressure. Consequently, there still exists a need for a simple and robust device to increase the ion sampling efficiency of atmospheric pressure ion sources.

In this work, a new dynamic focusing technique we call pulsed dynamic focusing or PDF, was developed and applied to an AP-MALDI ion source to improve ion transmission into a mass spectrometer. The PDF device, which consists of switching circuitry and a timing apparatus to create a transient high-voltage (HV) extraction field in the AP-MALDI source, is described in detail in this paper. AP-MALDI with the PDF technique is compared to AP-MALDI where a static electric field is applied. The effects of laser position and laser spot size on static AP-MALDI versus AP-MALDI PDF are also investigated. Finally, initial applications of AP-MALDI PDF with a protein digest are presented to demonstrate the enhancement gained from PDF.

EXPERIMENTAL METHODS

Instrumentation. Mass spectrometry measurements were made on either a Thermo Finnigan (San Jose, CA) LCQ Deca XP

ion trap mass spectrometer (ITMS) or a Thermo Finnigan LCQ Classic ITMS. A MassTech Inc. (Columbia, MD) AP/MALDI ion source was utilized on both mass spectrometers and is described thoroughly in the literature.^{12,13} The PDF technique was adapted to both of these systems.

Pulsed dynamic focusing operates on the basis of applying a transient high voltage extraction field to atmospheric pressure ions. The principle of operation for AP-MALDI PDF is illustrated in Figure 1. In this configuration, each laser pulse from the AP-MALDI ion source is used to trigger a delay/pulse generator that appropriately times for the removal of the HV extraction field. Thus, in contrast to continuous extraction, PDF applies a “timed-extraction” for pulses of ions, provided the switch to ground potential is before ions enter the MS. The time duration between the laser pulse and the instant when the HV extraction field is rapidly lowered to ground potential is accordingly termed the “timed-extraction interval”. The time duration that the HV extraction field is removed is termed the “hold time”. The hold time was set to be less than the time between laser pulses, so that the circuitry could return the HV back to the target plate and be ready for the next laser pulse. In these experiments, the hold time was set to 1 ms.

A schematic of the PDF technique interfaced with an AP/MALDI ion source is shown in Figure 2. The HV extraction potential on the sample target plate was provided by the mass spectrometer. The laser output was used to trigger a delay/pulse generator (model DG535, Stanford Research Systems, Inc.) that

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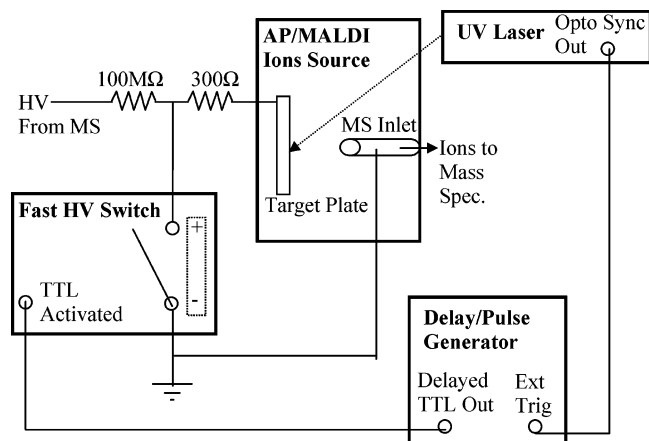


Figure 2. Schematic diagram of applying PDF to an AP/MALDI ion source.

activated a fast HV transistor switch (HTS121, Behlke Electronic GmbH) at a set timed-extraction interval after the laser pulse. According to the circuitry, once the switch was activated, the HV was immediately short-circuited for a given hold time. After the hold time, the circuit reinitialized the target plate to its original high voltage level, with the rise time of this voltage dictated by the 100-M Ω resistor and the load capacitance. The 100-M Ω resistor also served to protect the mass spectrometer in the event of arcing by limiting the discharge current between the target plate and capillary. This allowed voltages above that used in conventional AP-MALDI to be safely experimented. In this paper, the term "conventional AP-MALDI" is used in the specific case when static AP-MALDI was set up in the traditional manner with the target plate voltage at 2.0 kV, the distance between the target plate and capillary of 2 mm, the laser spot size of approximately 0.25 mm², and the laser repetition rate of 10 Hz.

Materials. A standard peptide mixture was obtained from Sigma (MS-CAL2 ProteoMass Peptide MALDI-MS Calibration Kit; St. Louis, MO) and included bradykinin fragment 1–7 (757.4 Da), angiotensin II (1046.5 Da), P₁₄R (1533.9 Da), ACTH fragment 18–39 (2465.2 Da), and insulin oxidized B chain (3494.7 Da). Bovine serum albumin (BSA) digest was obtained from Michrom BioResources (Auburn, CA) and was reconstituted in molecular biology grade water (Cambrex Bioscience, Rockland, ME) with 2.0% acetonitrile (Sigma-Aldrich, St. Louis, MO) and 0.1% trifluoroacetic acid (Applied Biosystems, Foster City, CA). The matrix solution used was α -cyano-4-hydroxycinnamic acid (α -CHCA) obtained from MassTech (Columbia, MD).

AP-MALDI Spot Preparation and Settings. Peptide samples were at a concentration of 100 fmol/ μ L each and diluted in α -CHCA matrix. Peptides were spotted with 2 μ L of peptide-matrix solution and allowed to dry at room temperature. BSA was at a concentration of 10 fmol/ μ L. BSA samples of 1 μ L were mixed on the target plate with 1 μ L of α -CHCA matrix and allowed to dry at room temperature. Sample spot sizes of 2 μ L were analyzed with AP/MALDI Target software set to a spiral speed of 5 mm/min and 0.1-mm spacing between spirals. These settings were important in maintaining a stable ion signal for >6 min.

RESULTS AND DISCUSSION

Both AP-MALDI with PDF and static AP-MALDI (without PDF) analyses were conducted on the same sample spots to directly compare AP-MALDI sensitivity with and without PDF. Static AP-MALDI conditions were created by using a long timed-extraction interval of >1 ms after the laser pulse. Approximating static AP-MALDI with a long timed-extraction interval was justified by experiments (not shown) that demonstrated no significant differences between using a timed-extraction interval of >1 ms and using a setup applying an actual continuous extraction field.

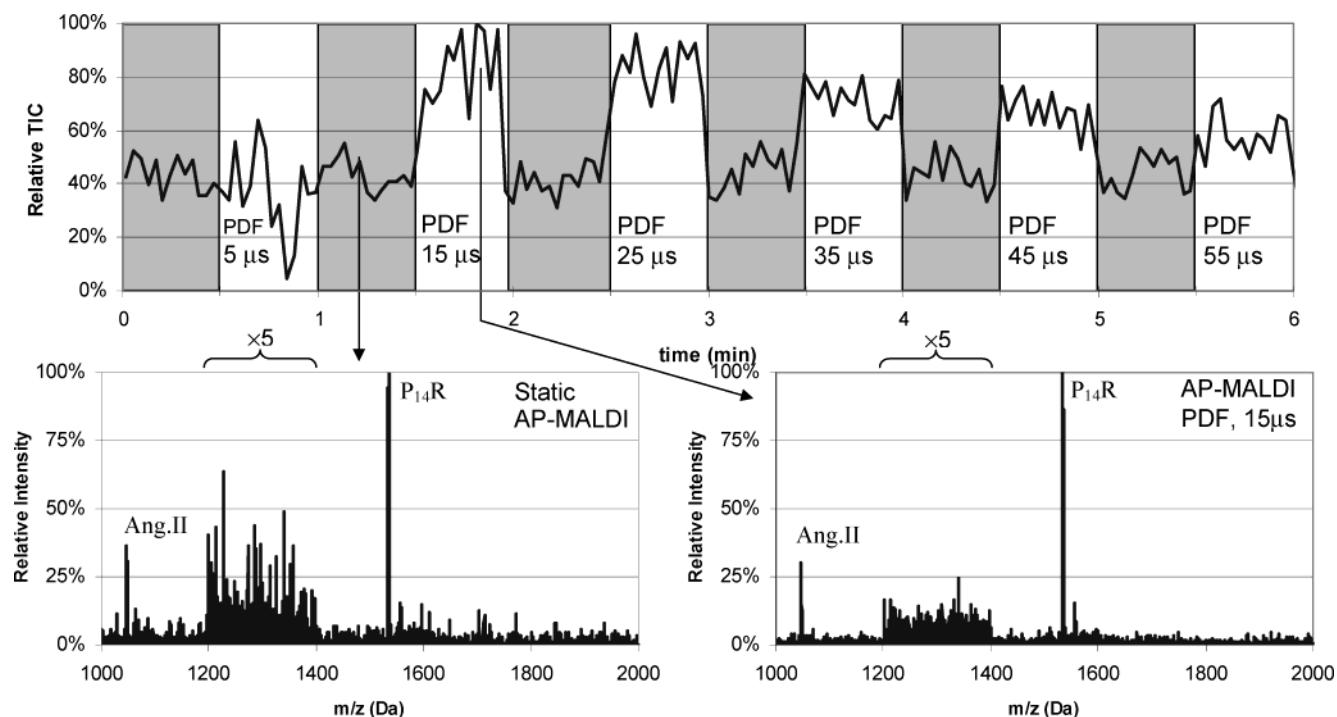


Figure 3. Typical experimental run comparing total ion current (TIC) in static AP-MALDI (shaded regions) with various timed-extraction intervals (unshaded regions) applied to AP-MALDI with PDF. Results were acquired at 4.8 kV applied to the target plate, with 200 fmol of peptide standards spotted.

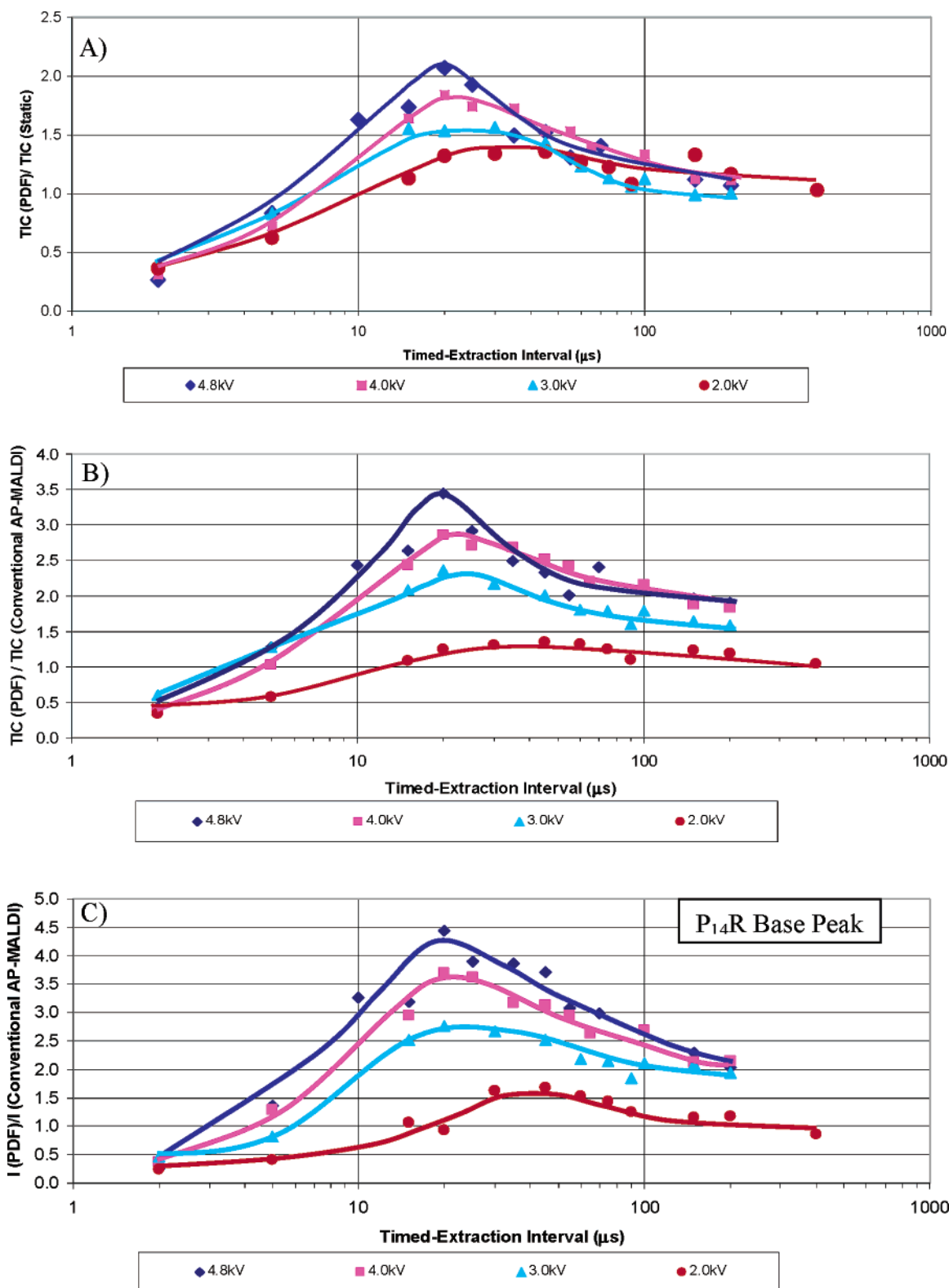


Figure 4. Performance of PDF compared to (A) static AP-MALDI and (B) and (C) conventional AP-MALDI (at 2 kV).

This was logical, because ion mobility calculations show that in the conventional AP-MALDI source, ions spend $\sim 50 \mu\text{s}$ in the atmospheric pressure region prior to entering the MS inlet (based on $t = d/v_{\text{drift}}$, where t = time spent by ions at AP (s); d = target plate to MS entrance distance = 0.2 cm; v_{drift} = drift velocity = $K_0 E$, where K_0 = reduced ion mobility constant = $0.4 \text{ cm}^2/\text{Vs}$ (for $\sim 1000\text{-MW}$ molecule);¹⁴ and E = electric field = $2000 \text{ V}/0.2 \text{ cm}$).

Hence, $>1 \text{ ms}$ after the laser pulse, ions are no longer present in the source to be affected by electric field changes. Thus, experiments to compare AP-MALDI PDF to static AP-MALDI were conducted by simply changing from a short timed-extraction interval ($<400 \mu\text{s}$) to a long time interval ($>1 \text{ ms}$), respectively. A standard peptide mixture was used in all experiments unless otherwise specified.

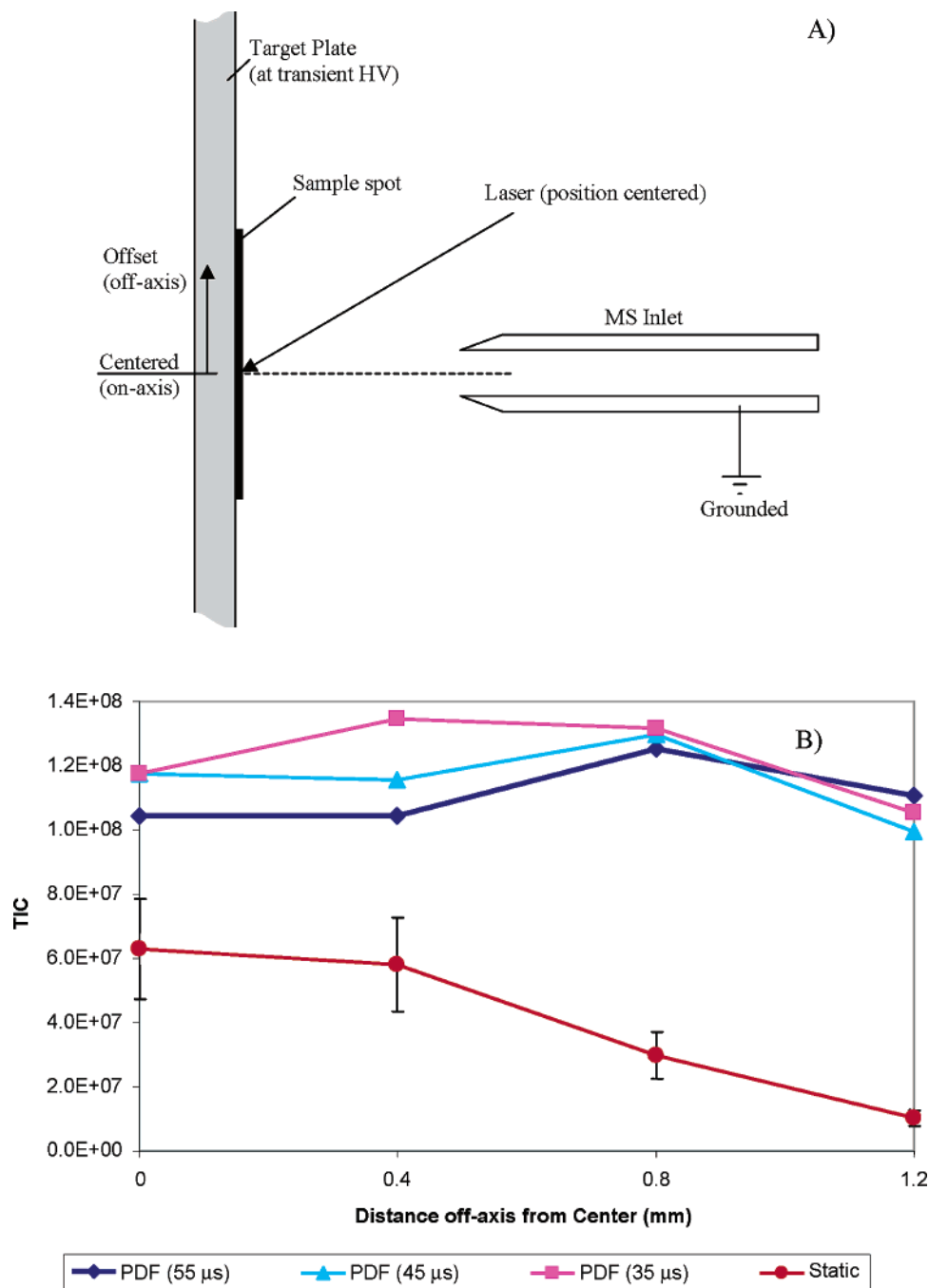


Figure 5. (A) Detailed illustration of sample and laser position arrangement. (B) Comparison of PDF vs static AP-MALDI signal intensities as a function of laser position (all analyzed at 4.0 kV; TIC, 30-s average).

A typical experiment is shown in Figure 3, where static AP-MALDI is run for 30 s, and then PDF at a fixed timed-extraction interval is applied for 30 s. This cycle of static AP-MALDI then PDF at a different timed-extraction interval, was repeated for a total of 6 min. Shaded regions where static AP-MALDI was implemented showed that a stable ion signal within $\pm 25\%$ was achieved for the duration of a 6-min experiment. An example of the individual spectral scans in the Figure 3 total ion current (TIC) chromatogram shows static AP-MALDI with a roughly 2-fold greater noise than AP-MALDI PDF at a 15- μ s timed-extraction interval and 4.8 kV extraction voltage.

AP-MALDI PDF at High Voltages. A compilation of data from experimental runs at various HV fields and various timed-

extraction intervals is shown in Figure 4. The data in Figure 4 were obtained by dividing the average 30-s signal at a PDF setting with the prior 30-s signal at static conditions. In Figure 4a, PDF is compared with static AP-MALDI at the same voltage as applied in the PDF trial. Ion intensity was found to be augmented by a factor of more than 2 over static AP-MALDI when a 4.8-kV target plate voltage and 15–25- μ s timed-extraction interval was used. The highest improvement in Figure 4a was at a level of 2.1 times. Figure 4a results also revealed that the improvement in TIC intensity was best achieved with the combination of higher applied voltages and shorter timed-extraction interval.

The shift in the optimal timed-extraction interval with the applied voltage can be explained by the mobility of ions where

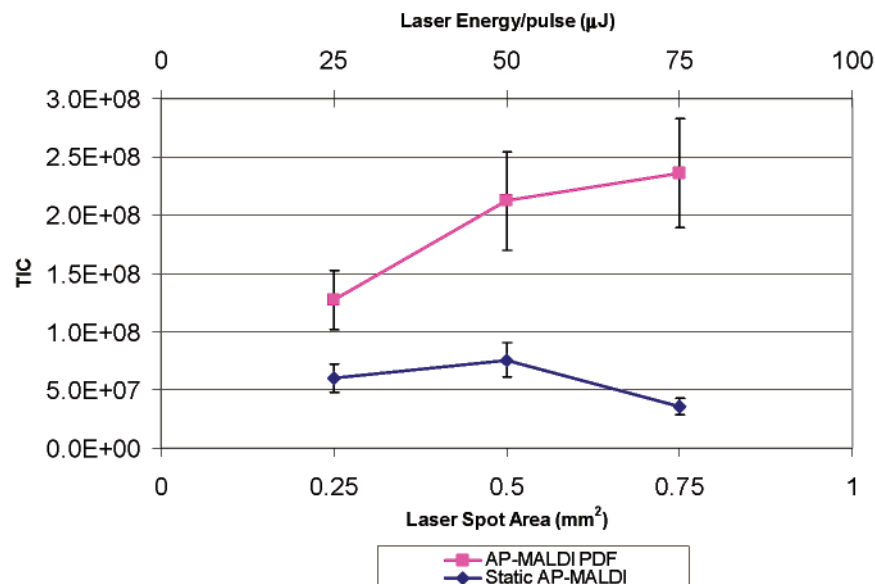


Figure 6. Signal intensities at constant laser fluence for different laser spot sizes. A comparison of AP-MALDI with PDF (20- μ s timed-extraction interval) versus static AP-MALDI (all analyzed at 4.0 kV).

the average drift velocity of ions is proportional to the electric field strength. Thus, based on ion mobility theory, higher extraction voltages require a shorter application time for the ions to reach the same position they would if extracted by lower voltages with longer timed-extraction intervals. At electric potentials >4.8 kV, effects such as corona discharge and arcing became apparent. It is not believed that discharge from operation at higher voltages played any role in increased analyte ion production, as previous work found that corona discharge with AP UV-MALDI only enhanced the matrix signal.¹⁵ The data in Figure 4 were acquired on the LCQ Classic instrument with replicate results showing standard deviations of $<25\%$. The same experiments were also conducted on the LCQ Deca XP with comparable enhancements (not shown).

The best results in Figure 4a were found at a time when the electric field was removed just before the ions reach the capillary entrance (i.e. <50 μ s) and presumably when the ions were close enough to the entrance that airflow can transport the ions the remainder of the distance into the capillary. If the timed-extraction interval was too short, <5 μ s, the electric force required to transfer the ions to the airflow region was not adequate, and fewer ions were detected. When the timed-extraction interval was longer, >200 μ s, the results approached the static, continuous extraction performance.

In computer simulation studies, to be reported in detail in a separate paper, it was evident that the dominant transport force for ions in a static AP-MALDI ion source is governed primarily by the electric field and influenced little by the gas dynamic forces generated by the MS inlet's intake flow field.¹⁶ In static AP-MALDI, the intake gas flow lines funnel inward into the inlet but cannot be taken advantage of because ions follow the stronger electric

force lines that are directed toward the tip and inner walls of the inlet. However, in AP-MALDI PDF, ions could be made to follow the focused gas streamlines by timely exclusion of the strong electric field, allowing ions to follow the gas flow path. This dynamic focusing, collimating ions into the MS inlet, is believed to be the major reason sensitivity is enhanced.

In past applications of AP-MALDI, the optimal high voltage conventionally applied for continuous extraction was ~ 2 kV with a 2-mm coaxial distance between the MS entrance and target plate.¹² A comparison of PDF with this conventional AP-MALDI high voltage of 2 kV is shown in Figure 4b. Sensitivity for the TIC was increased as high as a factor of ~ 3.4 with PDF relative to conventional AP-MALDI settings, and for specific peptide peaks such as the base peak in the spectrum (which was that of P₁₄R, $m/z=1534$), this increase was more than a factor of 4 (Figure 4c). The favorable increase in analyte ion signal in comparison to the TIC suggests that PDF may be used to preferentially transmit ions of similar mobility.

Figure 4b and c indicates that PDF with voltages >2 kV yielded further enhancements in sensitivity, from 2.1 to 3.4 times (i.e., an additional factor of 1.6 times), in contrast to static AP-MALDI, which achieved its best results at 2 kV.¹² It is believed that these differences are due to higher ionization efficiency at increased electric fields, where better charge separation may reduce ion neutralization reactions in the MALDI plume. Such a phenomenon could conceivably be exploited by the PDF technique, but would be likely lost to the MS entrance with large electric forces associated with the continuous extraction case.

PDF Effect on Larger Laser Spot Areas. Experiments were also conducted on the effect of laser positioning on PDF performance. Traditionally, the laser is centrally positioned on-axis with the MS inlet entrance (Figure 5a). Results for TIC presented in Figure 5b show the effect of displacing the laser from a centered position to off-axis positions. When the laser position was offset up to 1.2 mm from a centered position, it was observed

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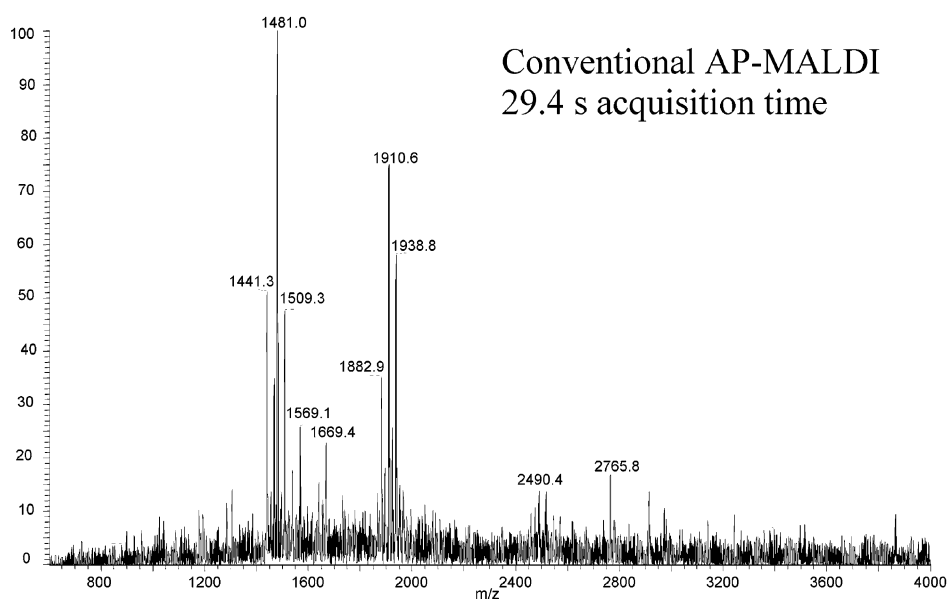
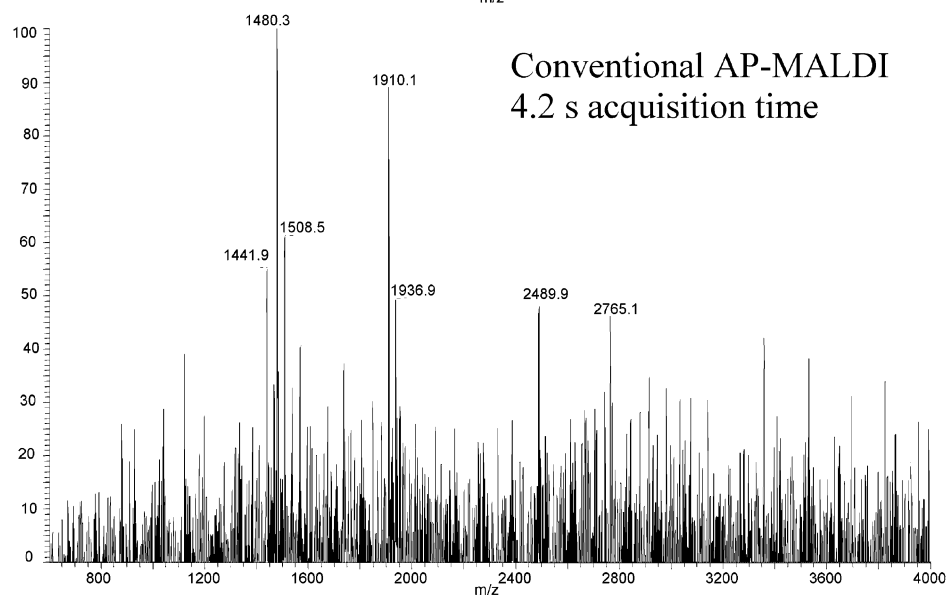
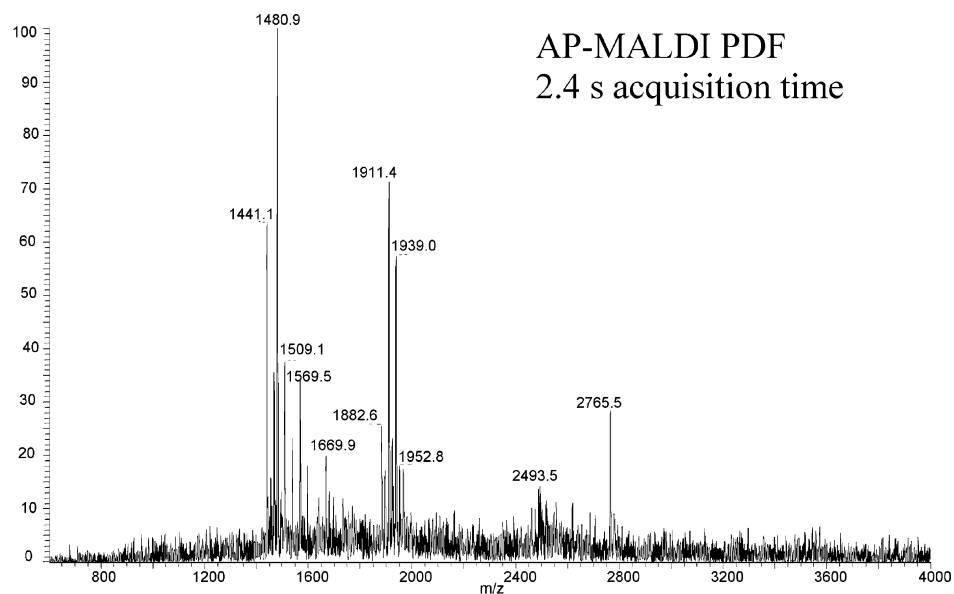


Figure 7. AP-MALDI PDF set to a timed-extraction interval of 20 μ s, with both 0.75 mm² laser spot area and 4.8 kV compared with conventional AP-MALDI settings (0.25 mm² laser spot area and 2.0 kV). Application to BSA digest sample at 10 fmol.

that the signal intensity using the PDF approach did not change significantly (Figure 5b) over a timed-extraction interval between 35 and 55 μ s (4.0 kV). In contrast, for static AP-MALDI, the signal intensity did not change within a 0.4-mm offset from the on-axis position, but dropped significantly when moved 0.8–1.2 mm off-axis. The results indicate the robustness of PDF to fluctuations in laser position, whereas for continuous extraction, a more precise alignment is required for best performance. Furthermore, Figure 5b results suggest that larger laser spot sizes can be effectively used in the PDF approach to allow greater ion current per laser pulse.

A test of the effect of laser spot size for PDF versus static AP-MALDI is shown in Figure 6. The laser fluence was kept constant at 100 μ J/mm² for the three different laser spot sizes tested. It was found that at larger laser spot sizes, PDF continued to show increased ion signal. In contrast, static AP-MALDI did not show any significant signal gains with increased laser spot size. The TIC intensity of PDF over the static AP-MALDI case is shown to be ~ 2.1 times with the use of a conventional 0.25-mm² laser spot area. This factor corresponds reasonably well with results in Figures 4a and 5b. Gains were found to have an almost linear dependence on laser spot area; a ~ 3.1 -times gain was found from a laser spot with 3-times-larger area ($3.1 \times = 6.6 \times$ (for 0.75-mm² spot area in Figure 6, graph)/2.1 \times). The increased sample throughput for larger laser spot sizes makes it conceivable that spot areas >0.75 mm² could be utilized; however, sufficient laser fluence would have to be provided for ionization. On the other hand, it was observed that there can be significant space charge effects when ion currents are too high. Thus, optimizing ion transmission for larger laser spots requires careful setting of the ion trap injection time (in this case, 150 ms) and laser fluence. The ability to increase throughput with PDF while maintaining the use of a simple 10-Hz N₂ laser is a cost-effective alternative to applying an expensive high-repetition-rate laser.¹⁷

AP-MALDI PDF Application. An application of PDF at its best settings of a high electric field and larger laser spot size was

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applied to a BSA digest sample to demonstrate the overall benefit of the PDF technique over conventional AP-MALDI. Figure 7 shows the results of AP-MALDI PDF on a 10-fmol sample of BSA digest for which acquisition time required to achieve a spectrum with reasonable signal-to-noise (S/N) was only 2.4 s. Without PDF, conventional AP-MALDI required at least a 4.2-s acquisition time to achieve some peaks with detectable S/N. But to get the same S/N as PDF, conventional AP-MALDI required >12 times (29.4 vs 2.4 s) the acquisition time. Evidently, AP-MALDI PDF significantly improved the speed at which AP-MALDI can yield a given spectral response by over an order of magnitude. Moreover, when using AP-MALDI PDF, the S/N over similar acquisition times is clearly better than when using conventional AP-MALDI.

Originally, Laiko et al.⁸ noted that conventional AP-MALDI resulted from “consumption of the entire analyte during the recording of a spectrum”, as this was “necessary to compensate for the low efficiency of transfer of the ions from atmospheric pressure into the vacuum system”. Now, with the higher ion sampling efficiency afforded by AP-MALDI with PDF, more sample can be conserved in an analysis. Future developments may include the application of PDF to other AP-MALDI MS systems.

CONCLUSIONS

A pulsed dynamic focusing (PDF) technique was demonstrated to significantly improve the performance of AP-MALDI on an ITMS. With the PDF operated at a high electric field strength and optimal timed-extraction interval, a gain in sensitivity by a factor of 3.4 was observable. An additional benefit of the AP-MALDI PDF setup was that larger laser spot sizes could be robustly utilized. A 3-times-larger spot size was successfully applied to linearly improve sample throughput. The combined throughput and sensitivity improvements of PDF with AP-MALDI were demonstrated to be over an order of magnitude.

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