High-Resolution Ion Microscope Imaging over Wide Mass Ranges Using Electrodynamic Postextraction Differential Acceleration

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ABSTRACT: A time-dependent postextraction differential acceleration (PEDA) potential was used to temporally focus increasingly heavy ions in a stigmatic imaging mass spectrometer, allowing them to be imaged with high mass and spatial resolutions over a broad mass-to-charge (m/z) range. By applying a linearly rising potential to the ion extraction electrode, sequential m/z ratios were subjected to a changing electric field, allowing their foci to coincide at the detector. Using this approach, at least 75% of the maximum mass resolution was obtained over a 300–600 Da range when the ion microscope was focused around 450 Da, representing more than a 10-fold increase over the conventional single-field PEDA method.

INTRODUCTION

High-throughput imaging of spatially localized chemical distributions plays a central role in many disciplines. Pathology and drug discovery particularly benefit from the rapid identification of biomarkers or proteins in assays and tissues. These and similar experiments often employ mass spectrometry imaging (MSI) to record and assign mass spectra to defined spatial coordinates on a sample.1–4 MSI provides a wealth of information: results can be integrated over mass to extract images of distinct compounds or over position to compare mass spectra from different regions of a sample.

MSI is conventionally performed by raster scanning a focused ion or laser beam across a surface to acquire mass spectra at different coordinates. Ion images are constructed pixel by pixel, with spatial resolutions defined by the spot size of the microprobe, which can be as narrow as 50 nm or 1 μm when using secondary ion mass spectrometry (SIMS) or matrix-assisted laser desorption ionization (MALDI), respectively.5,6 The acquisition time of an ion image is therefore inversely proportional to the square of the desired spatial resolution, meaning that the latter comes at the expense of throughput.

Stigmatic ion microscope imaging is an alternative MSI technique that decouples acquisition time from spatial resolution by using a defocused ionization source to simultaneously ionize relatively larger sample areas, typically 0.05 mm² to 1 cm². An image of the sample plane is then electrostatically projected onto a position-sensitive detector at the end of a time-of-flight mass analyzer.7–11 This allows ion images to be recorded sequentially for any mass-resolved compounds in the sample using multimass imaging sensors.12–22 In this approach, the spatial resolution depends on the distribution of initial velocities of the ions emitted from the surface as well as the strength of the extraction field. In typical instruments, the achievable spatial resolution is 1–10 μm.

Ion optical focusing with fixed voltages optimizes the mass and spatial resolutions for a specific mass-to-charge ratio m/z, with the consequence that both, particularly the former, decrease at higher or lower m/z. As in other time-of-flight measurements, the mass resolution also depends on the distribution of initial ion velocities along the time-of-flight axis. Introducing a time lag between ion generation and the onset of the extraction field corrects for this by allowing the ions to move to new initial positions before acceleration, which focuses their spread of arrival times at the detector.23 Delayed extraction techniques of this kind are used for energy focusing both parallel and orthogonal to ion beams but necessarily sacrifice the initial spatial information of the ions, which limits their value for microscope MSI.24 In other words, it is crucial to detect where an ion hits in addition to when. To resolve this, a postextraction differential acceleration (PEDA) technique has been developed to retain high mass resolution through energy focusing, without compromising the achievable spatial resolution.25

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The PEDA technique immediately extracts surface ions following ionization to retain their spatial information and corrects for their initial velocity dispersion by raising the potential of the extraction electrode after the ions have passed through it. In this way, ions with slower initial velocities gain more kinetic energy from the voltage pulse than faster ions, creating a temporal focus point that can be superimposed on the detector to improve the mass resolution. Acceleration fields of this type are frequently used in mass spectrometry for energy focusing, for example in “LIFT” cells. In the original PEDA configuration, the effective mass range is centered around a single m/z focused by the pulsed acceleration field. Using this method, we showed that the mass range over which the mass resolution of our ion microscope reached at least half that of the focused m/z (410 Da) was just ~8% of the 300 Da range that could simultaneously be spatially resolved.

In order to apply the benefits of PEDA over wider mass ranges, multiple m/z must be focused during an experiment. For example, the mass-resolved range of the above experiment can be extended to cover ~20% of the spatially resolved window using the double-field PEDA method, where the acceleration region is divided in two by an electrode that is raised to a higher potential at the same time as the extraction electrode. The potentials of the two electrodes were set to create a cusp in the potential energy surface between the two acceleration regions, such that heavier ions in the “early” region achieved temporal overlap at the same drift distance as lighter ions in the “late” region. This approach could in principle be extended to a many-field method using additional electrodes to focus broader mass ranges.

This paper instead presents a more robust PEDA technique that electrodynamically adjusts the m/z focused during an experiment by applying a time-dependent voltage pulse to the extraction electrode after a set delay. The concept parallels the use of dynamic acceleration fields for m/z-correlated energy focusing following delayed extraction but retains the stigmatic imaging ability of the mass spectrometer. Electrodynamic PEDA results in increasingly heavy ions being sequentially focused as they pass through the ion microscope, broadening the mass range that can be mass-resolved by an order of magnitude (expanding it to 100% of the spatially resolved 300 Da mass range described above). Every imageable compound can therefore be acquired with high mass resolution, facilitating multimass and high-resolution stigmatic particle imaging.

**THEORY**

PEDA narrows the time-of-flight spread observed when isobaric ions are emitted from a surface with a distribution of initial velocities. The technique is illustrated in Figure 1 and is applied by raising the potential of an extraction electrode, placed parallel to the surface, after the ions have passed through it. In this way, the spatial information of the ion packet is retained due to the electric field in the extraction region, while the mass resolution is increased by the differential acceleration and energy focusing imparted by the voltage pulse. Ions with slower initial velocities will be closer to the extraction electrode when the pulse is applied and accelerate to overlap with faster ions, creating a temporal focus point.

When the initial velocities of the generated ions are independent of mass, which is often the case in MALDI imaging experiments where they tend toward the average velocity of the ejected matrix ions, then heavier m/z will exhibit wider time-of-flight distributions than lighter ions. This will in practice remain true even when the initial velocities are mass-dependent, as their relative contributions to the final velocities will still be much larger for heavier m/z. The PEDA pulse voltage must therefore be chosen to focus a particular m/z, with heavier ions requiring more acceleration to reach a temporal focus.

Electrodynamic PEDA applies the benefits of single-field PEDA over a wider mass range by continuously raising the potential of the extraction electrode, such that different m/z are sequentially focused as they pass through the acceleration region. For a time-of-flight imaging mass spectrometer operating in a Wiley–McLaren configuration, the ideal pulse shape should be a quadratic rise, as the time a particular ion spends in the extraction and acceleration regions is proportional to \( \sqrt{m/z} \). In the experiments reported here, a linear pulse is used instead. Taylor’s theorem demonstrates this will be approximately valid over 300–600 Da when the mass spectrometer is optimized to record ion images around 450 Da. This approach also circumvents the technical challenge of developing a fast-rising quadratic pulse.

**SIMULATIONS**

The ion optical system described in Figure 1 creates separate ion extraction and differential acceleration regions. In the former, an ion generated from the sample plate at \( x = 0 \) with mass \( m \), charge \( q \), and velocity \( v_0 \) is accelerated through a

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**Figure 1.** (a) Electrode potential energy surface (solid black line) of an ion microscope along its time-of-flight axis. In single-field PEDA (dashed black line), the extractor potential is raised by \( V_{0x} \) at time \( \tau \) so that ions (white circles) of a particular m/z are focused when they arrive at the detector plane at time \( \tau \). Electrodynamic PEDA sequentially focuses additional m/z by continuing to raise the extractor potential as a function of time to a maximum \( V_0 \) (blue dashed line). These ions are swept through different electric field strengths, as represented by the blue arrow. Additional details are provided in the *Simulations* section. (b) A generalized schematic of the corresponding ion optics, demonstrating stigmatic imaging (dotted violet lines) of a sample embedded in the repeller electrode. The image plane magnification depends on the distance between the ion crossover point and the detector.
potential difference $V_{\text{ex}}$ toward the extraction electrode at $x = L_i$. At this point, its velocity $v_i$ and flight time $t_i$ are

$$v_i = \sqrt{\frac{2}{m} \left( \frac{m v_0^2}{2} + qV_{\text{ex}} \right)}$$  \hspace{1cm} (1)

$$t_i = \frac{mv_{0i}^2}{qV_{\text{ex}}} \left( 1 + \sqrt{1 + \frac{2qV_{\text{ex}}}{mv_0^2}} \right)$$  \hspace{1cm} (2)

The ion then enters the acceleration region, where it passes through a potential difference $V_A$ as it travels toward the grounded entrance of an einzel lens at $L_i + L_s$. After a set time $\tau$, the ion is located within this domain at $L_i + \Delta x$ and has a velocity $v_p$

$$\Delta x = v_i (\tau - t_i) + \frac{qV_A}{2mL_s} (\tau - t_i)^2$$  \hspace{1cm} (3)

$$v_p = \sqrt{\frac{2}{m} \left( \frac{1}{2}mv_0^2 + q \left( V_{\text{ex}} + \frac{V_A}{L_s} \right) \right)}$$  \hspace{1cm} (4)

At $\tau$, electrodynamic PEDA can be initiated by applying a time-dependent potential $V_D(t, k)$ to the extractor. In the following experiments, the electrode is raised by a PEDA step potential $V_D$, and continuously increased until it reaches a maximum $V_{D0}$.

The rate constant $k$ associated with this change is defined as the inverse time constant of the resistor–capacitor circuit that creates the pulse, such that

$$V_D(t, k) = V_{D0} + V_D (1 - e^{-k(\tau - t_i)})$$  \hspace{1cm} (5)

Ions in the acceleration region are therefore subjected to shifting PEDA conditions, which initially focus a particular $m/z$ at $V_D$, and gradually focus larger $m/z$ as the electric field strengthens. $V_D(t, k)$ will effectively be linear at times close to $\tau$ for small $k$, as required. Governed by this environment, a given
ion will leave the acceleration region with a velocity $v_2$ after a flight time $t_2$

$$v_2 = v_0 + \frac{q}{mL_2} \int_{t}^{t_2} V_A + V_D(t, k) \, dt$$

$$= v_0 + \frac{q}{mL_2} \left[ V_\Delta t - \frac{V_D}{k} (1 - e^{-k\Delta t}) \right]$$

(6)

In the above equation, the total acceleration potential $V = V_A + V_D$ and PEDA flight time $\Delta t = t_2 - t$ were defined for convenience. Integrating $v_2$ with respect to time demonstrates that $t_2$ satisfies the following equation, which can be solved numerically or through a Taylor expansion to determine $\Delta t$ and hence $v_2$

$$L_1 - \Delta x = \left( v_0 - \frac{q}{mL_2} \right) \Delta t + \frac{q}{mL_2} \left[ \frac{V(\Delta t)^2}{2} + \frac{V_D^2}{k^2} (1 - e^{-k\Delta t}) \right]$$

(7)

After leaving the acceleration region, the ions travel through a conventional three-electrode einzel lens and field-free drift distance $L_D$ before impacting the detector. The central potential of the lens is $V_{\text{Ein}}$ and each electrode is separated by a length $L_2$. The ions exit the einzel lens after a flight time $t_3$ with the same velocity $v_2$ that they entered with. As a consequence, the total time-of-flight $t_f$ for a particular ion is

$$t_3 = t_2 + \frac{2mv_2L_3}{qV_{\text{Ein}}} \left( 1 + \frac{1 - 2qV_{\text{Ein}}}{mv_2^2} \right)$$

(8)

$$t_f = t_3 + \frac{L_D}{v_2}$$

(9)

The PEDA pulse ensures that, for a given $m/z$, ions with slower initial velocities leave the einzel lens with a larger $v_2$ than ions with faster initial velocities. An $m/z$ packet will therefore reach a temporal focus within the field-free region, and this position can be tuned to correspond with a position-sensitive detector. Electrodynamic PEDA should additionally allow larger $m/z$ to be consecutively focused to the same point. As the instrument parameters and potentials are fixed for a given experiment, the performance of electrodynamic PEDA can be assessed by calculating the expected times-of-flight for different $m/z$ across a range of initial velocities and comparing them to flight times determined using an average initial velocity $v_0$. The resulting time-of-flight difference $t_d$ provides a measure of the expected energy focusing and time-of-flight resolution at the detector. A smaller $t_d$ corresponds to higher mass resolution and vice versa.

$$t_d = t_f - \frac{m}{z} v_0 - t_f \left( v_0 - \frac{m}{z} \right)$$

(10)

Figure 2 compares the calculated time-of-flight differences of four PEDA variants: single-field, double-field, linear electrodynamic, and quadratic electrodynamic (Figure 2a–d, respectively). Each was determined relative to an average initial ion velocity of 500 m s$^{-1}$ over $v_0 = 0$–1000 m s$^{-1}$ and $m/z = 300$–600 Da. The chosen instrument parameters were $L_1 = 1.45$ cm, $L_2 = 3.85$ cm, $L_3 = 0.79$ cm, $L_D = 91.02$ cm, $V_{\text{Ein}} = 400$ V, $V_A = 4550$ V, $V_{\text{Ein}} = 1728$ V, and $\tau = 2.59 \mu s$. Figure 2a,b were modeled using the calculations developed by Aoki and Guo.25,28 The extractor step potential was 930 V for single-field PEDA and 920 V for the double-field case. The second PEDA electrode of the latter was situated at the midpoint of the acceleration region and raised by 410 V. For Figure 2c, $\tau$ was changed to 2.67 $\mu s$ to optimize the approximately linear electrodynamic PEDA pulse $V_D(t, k)$ over the 300–600 Da window. This was modeled using $V_D = 544$ V, $V_{\text{Ein}} = 2590$ V, and $k = 2.3 \times 10^5$ s$^{-1}$. The quadratic electrodynamic PEDA pulse used for Figure 2d was shaped using

$$V_D(t) = V_{\text{Ein}} + a(\Delta t)^2 + b\Delta t$$

(11)

The constants $a$, $b$, and $V_{\text{Ein}}$ were set to 1.814 $\times 10^{13}$ V s$^{-2}$, 4.990 $\times 10^8$ V s$^{-1}$, and 580 V following their optimization by a genetic algorithm that minimized $t_d$ over the range of $v_0$ and $m/z$ considered. For consistency with Figure 2a–c, only $V_D$, $\tau$, $a$, and $b$ were allowed to vary. Substituting $V_D(t)$ for $V_D(t, k)$ in eq 6 allows $\Delta t$ to be determined following integration in a similar manner to the linear electrodynamic case.

The simulations in Figure 2 are scaled to $\pm 1$ ns. This corresponds to a resolution of 7500 for an ion flight time of 30 $\mu s$, which is typical for the parameters used. Figure 2a,b demonstrates that single- and double-field PEDA meet or exceed this resolution over relatively small ranges, just 15 and 22% of the 300 Da window shown. The linear electrodynamic pulse in Figure 2c, which can be thought of as a many-field adaptation of the double-field method, improves this result to 96%, indicating that it will significantly broaden the overall mass range that can be experimentally imaged with high mass resolution. As expected, the quadratic pulse modeled in Figure 2d provides even better performance, focusing the entire window to at least $\pm 1$ ns. This behavior holds even when the window is extended to 900 Da, whereas the focusing ability of the linear pulse decreases sharply after 600 Da.

The linear electrodynamic pulse can only match quadratic behavior at two points. It should therefore create two temporally focused maxima with reduced performance in between. This effect can be seen in Figure 2c, where the two focused regions around 345 and 540 Da are connected by a curve of focused ions with faster than average initial velocities. This curve occurs because, between these two focal points, the linearly rising pulse overcompensates for the kinetic energy needed to create a temporal focus at the detector. Relatively fast ions in this range will spend less time in the PEDA differential acceleration region and counteract this effect.

Figure 3 depicts the time-dependent PEDA potentials $V_D(t, k)$ (blue dotted line) and $V_D(t)$ (black solid line) used for Figure 2c,d. A linear fit of the latter (gray dashed line) demonstrates that each pulse is essentially linear between its onset and the time at which ions heavier than 600 Da exit the acceleration region (blue shaded area). The applied pulse $V_D(t, k)$ is therefore expected to exhibit similar performance to the quadratic pulse $V_D(t)$ over a 300 Da range, matching the window over which ion images can be recorded with high spatial resolutions experimentally. It is worth noting that due to the 0.08 $\mu s$ difference in $\tau$, $V_D(t, k)$ does not cross $V_D(t)$ as described above. This is a consequence of positioning the focal points of the linear pulse near the center of the simulated mass range in Figure 2c, whereas the optimized quadratic pulse begins to focus ions at lower $m/z$.

**METHODS**

Electrodynamic PEDA was tested using a time-of-flight microscope imaging mass spectrometer. The instrument has

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an adaptable chamber that can be configured as an approximately linear ion microscope or as a two-stage system where the temporal PEDA focus is used as a pseudosource for a reflectron.59 The linear configuration was used here to better compare the data with previous experiments, which shared the same ion optical system but a shorter drift distance.28 The ion optical dimensions are therefore the same as those used for Figures 1b and 2 but with $L_{b} \approx 240$ cm. The instrument is also not perfectly linear; the ions were deflected by 3° toward the detector using two parallel plate electrodes held at ±157.2 V and placed 72.1 cm from the exit of the einzel lens. Despite these differences, the optimal electrode potentials and pulse timing were similar to those used in the simulations, with $V_{Es} = 439$ V, $V_{A} = 4561$ V, $V_{Ei} = 1950$ V, $V_{B1} = 262$ V, $V_{D} = 2877$ V, and $\tau = 2.60 \mu$s. The most noticeable discrepancy is the drop in the PEDA step voltage, which is simply a consequence of the longer flight distance. The shape and magnitude of the PEDA pulse $V_{D}(t, k)$ were experimentally verified, as described elsewhere.54 Using the above settings, SIMION 8.1 predicts the mass and spatial resolutions of the instrument to be about 3800 $m/\Delta m$ (under single-field PEDA conditions) and 20 $\mu$m, respectively.

The mass and spatial resolutions established by electrodynamic PEDA were determined as functions of $m/z$ by recording mass spectra and ion images for five dyes (Auramine O, Exalite 404, Kitsun Red, Rhodamine B, and Rhodamine 6G chloride). Each sample was electrosprayed through a nickel mesh onto conductive 25 × 25 mm indium tin oxide plates to produce grids with 38.1 $\mu$m gaps and 102 $\mu$m pitches. These were individually placed into a recess in the repeller electrode and desorbed by 9 mJ pulses delivered at 10 Hz by a 355 nm Nd:YAG laser (Continuum Powerlight 8010) focused to cover 10 × 10 mm of the sample. The resulting ion images were then electrostatically transferred through the optics described in Figure 1 and projected onto a position-sensitive detector comprising dual-stack microchannel plates and an Exalite 404 screen. Mass spectra and ion images were recorded from the ensuing flashes of light by a photomultiplier tube and by an intensified 768 × 568 pixel CCD camera (Photonic Science) gated on the $m/z$ of interest.

**RESULTS**

**Mass Resolution.** Figure 4 illustrates the mass resolution dependence of the electrodynamic PEDA method on $m/z$,
over each resolved grid yields an average spatial resolution of 22 μm. The spatial resolution was determined by gating the CCD camera over each resolved grid pattern to determine the Gaussian standard deviation σ. The ion image of the 371 Da Rhodamine B fragment shown as an example. Fitting the right-hand peak (purple line) returns a spatial resolution of 15 μm. Repeating this calculation over each resolved grid yields an average spatial resolution of 22 ± 8 μm at this m/z. 

with the simulation (red trace) and with the expected resolution of the ion microscope with and without a PEDA pulse applied.21,28,33

The spatial resolution was determined independently for each recorded ion by gating the CCD camera over the m/z range of interest to produce grid-patterned ion images. The 20–80% intensity rises obtained from these images were then used to determine the Gaussian standard deviation σ and hence the spatial resolution. The ion image of the 371 Da Rhodamine B fragment is shown as an example. Fitting the right-hand peak (purple line) returns a spatial resolution of 15 μm. Repeating this calculation over each resolved grid yields an average spatial resolution of 22 ± 8 μm at this m/z. 

\[
R_x = \frac{1.68\sigma}{M}
\]

The image magnification was determined to be about 21.6 using the 140.1 μm pitch of the nickel mesh, which corresponds to an image diameter of ∼1.16 mm.

**CONCLUSION**

Electrodynamic PEDA method preserves the stigmatic imaging condition. (b) For each m/z, the spatial resolution was determined by gating the CCD camera over the arrival times of interest to produce grid-patterned ion images. (c) The 20–80% intensity rises obtained from these images were then used to determine the Gaussian standard deviation σ and hence the spatial resolution. The ion image of the 371 Da Rhodamine B fragment is shown as an example. Fitting the right-hand peak (purple line) returns a spatial resolution of 15 μm. Repeating this calculation over each resolved grid yields an average spatial resolution of 22 ± 8 μm at this m/z. 

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**Notes**

The authors declare no competing financial interest.

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