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Determination of absolute ion yields from a MALDI source through calibration of an image-charge detector

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Abstract

MALDI (matrix-assisted laser desorption/ionization) is one of the most important techniques used to produce large biomolecular ions in the gas phase. Surprisingly, the exact ionization mechanism is still not well understood and absolute values for the ion yields are scarce. This is in part due to the unknown efficiencies of typical detectors, especially for heavy biomolecular ions. As an alternative, charged particles can be non-destructively detected using an image-charge detector where the output voltage signal is proportional to the total charge within the device. In this paper, we report an absolute calibration which provides the voltage output per detected electronic charge in our experimental arrangement. A minimum of 3×10^3 ions were required to distinguish the signal above background noise in a single pass through the device, which could be further reduced using filtering techniques. The calibration results have been applied to raw MALDI spectra to measure absolute ion yields of both matrix and analyte ions.

Keywords: MALDI, image-charge detector, absolute ion yield, pickup ring, bovine insulin, F20TPP, particle detection

(Some figures in this article are in colour only in the electronic version)

1. Introduction

In any scientific or commercial instrument which involves the measurement of small numbers of particles, the detection efficiency is naturally very important. For instance in a mass spectrometer, this will in part determine the sensitivity of the instrument. Also, to make an absolute determination of interaction cross sections in an atomic or nuclear physics experiment, the absolute detection efficiency must be carefully measured. For these experiments, various detectors exist to fulfil this role, such as scintillators [1], calorimeters [2], cryogenic detectors [3], multi-channel plates (MCP) and single channel electron multipliers (CEM) [4]. However, if the device is small, e.g. table-top size, particle energies are restricted to a few tens of keV and the use of bulky and expensive cryogenics is limited. This rules out the use of high particle-energy and cold solid-state detectors respectively and means that electron emission detectors such as MCPs and CEMs are the workhorses for such devices. One major problem with

these detectors is that for particles with a given kinetic energy, the detection efficiency drops dramatically as the particle mass increases ($\sim m^{-\frac{7}{4}}$ [5]). Whilst in many cases this variation can be determined by calibrating the detector using a Faraday cup to determine the total charge of the ions, this adds to the complexity of experiments.

This problem is witnessed in matrix-assisted laser desorption and ionization—time of flight (MALDI-ToF) mass spectrometry. The conventional approach in MALDI is for a light ‘matrix’ to be mixed with a heavy ‘analyte’ and irradiated with a nanosecond laser to generate ions for ToF analysis (a full description of this technique can be found in [6–8]). As the detection efficiency reduces with increasing mass, small yields of heavy analyte ions might not be detected. Additionally, as the light matrix ions reach the detector earlier, detection of the analyte ions may be further suppressed due to saturation effects. To reduce or avoid these saturation effects modification of the experimental setup may be necessary, resulting in increased experimental complexity.

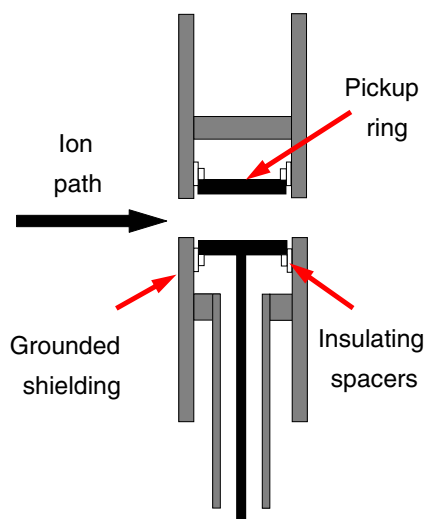


Figure 1. Cross-sectional schematic of the pickup ring.

These factors have no doubt contributed to the fact that there are to our knowledge no absolute ion yields from MALDI in the literature.

As an alternative to CEM and MCP detectors, image-charge detectors are increasingly being used in mass spectrometers [9]. This technique has the advantage of being mass independent, non-destructive, simple and inexpensive. While the sensitivity of this detection technique for low masses is low compared to using an MCP, it is superior for heavy masses ($>10^4$ amu) [10]. These benefits have been demonstrated in work ranging from Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/FTMS) [11] (where mass resolutions of up to 10^7 can be achieved) and ion trapping in devices such as linear electrostatic traps [13] and the Orbitrap [14]. In these devices the non-destructive signal is crucial in attaining very high mass resolution since this scales linearly with the observation time.

In this paper we demonstrate the experimental implementation of an image-charge pickup-ring, on which an absolute calibration was performed using a well-characterized ion beam. This enabled the determination of the absolute number of ions produced from a MALDI sample, and the minimum achievable charge sensitivity of the device. This provides a simple template for replacing electron multipliers in mass spectrometers, e.g. MALDI-ToF [15], and determining absolute ion yields from pulsed ion sources.

2. Image-charge detector

The image-charge detector (or ‘pickup ring’) used in the experiments consisted of a hollow stainless steel ring (internal diameter 10 mm, length 16 mm) encased inside a grounded conducting shield to reduce electronic noise. 8 mm entrance and exit apertures ensure that the image charge is low when the ions are outside the assembly. To prevent contact between the ring and shielding, insulating spacers are used to keep a separation of 1 mm between the grounded aperture and the ring. A schematic of the pickup can be seen in figure 1.

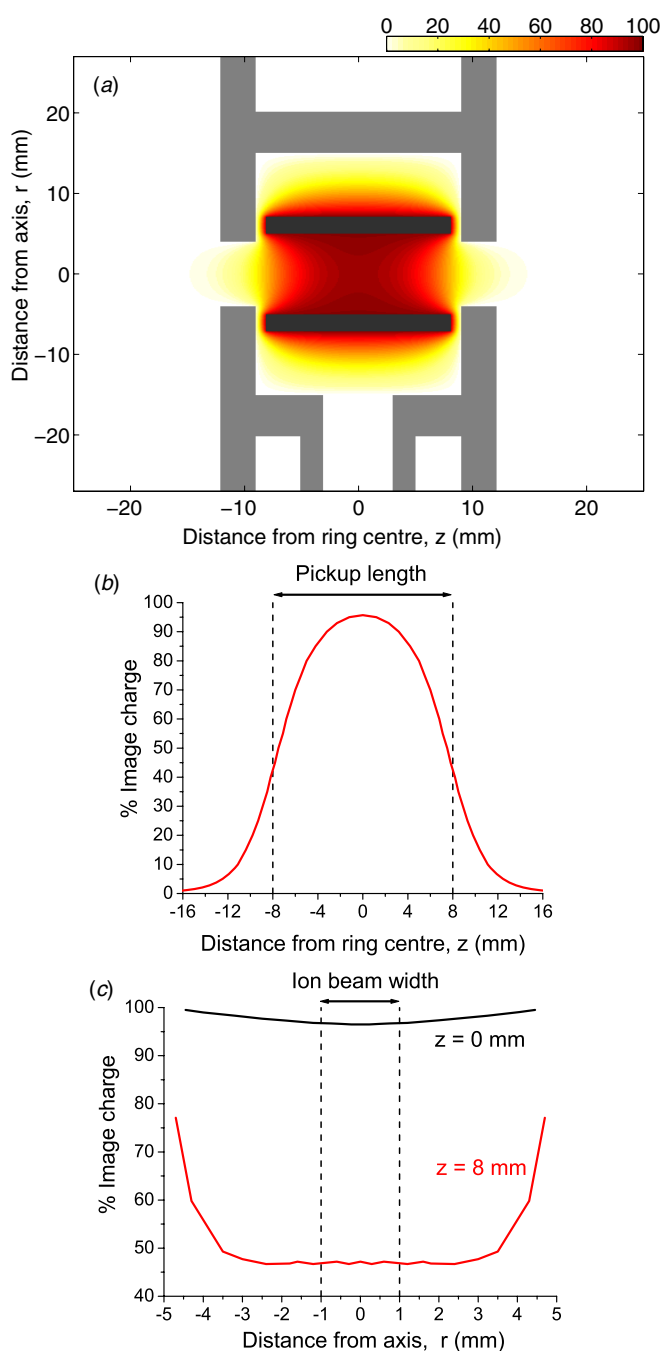


Figure 2. (a) Spatial variation in the percentage image-charge response as a function of r and z . (b) Fraction of ions imaged at $r = 0$ mm (pickup axis). (c) Fraction of ions imaged at $z = 0$ mm (pickup centre) and $z = 8$ mm (pickup edge).

The potential surface generated by applying a potential to the ring is shown in figure 2(a) (as modelled using SIMIONTM 7 [16]). Green’s reciprocity Theorem [17] states that the ratio of the potential at a point P in space, relative to the ring potential, is equal to the negative of the ratio of charge imaged on the ring by a charge present at P. In essence, this means that the image-charge detector has a spatially dependent response function. Figure 2(a) shows that the image-charge is substantial when the charged particle is inside the ring, reaching a peak of ~ 0.96 at the ring centre (figure 2(b)). The dependence on

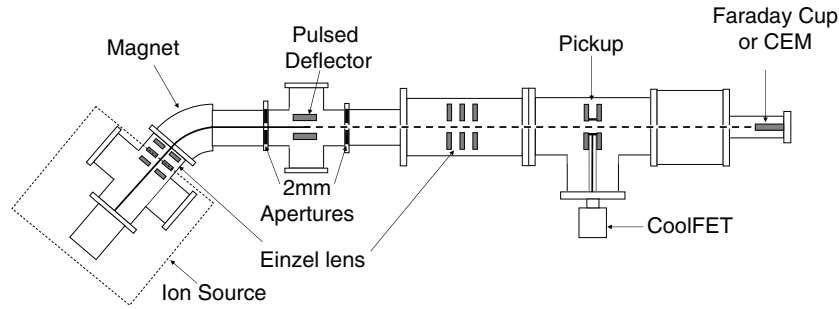


Figure 3. Schematic of the experimental apparatus.

the ion's radial position was found to be relatively small (figure 2(c)).

Assuming that the radial dependence can be neglected, the detected image-charge (Q) is given by

$$Q = -q \int_{-\infty}^{\infty} n(z) \times f(z) dz, \quad (1)$$

where $n(z)$ is the number of ions (each of charge q) per unit length in the bunch and $f(z)$ is the axial response of the pickup (figure 2(b)). If the bunch length is much larger than the ring length ($5 \mu\text{s}$ corresponds to a bunch length of 0.5 m for 2 keV Ar^+), and if the bunch is homogeneous in z , this can be simplified to

$$Q = -nq \int_{-\infty}^{\infty} f(z) dz. \quad (2)$$

For a beam of ions of mass m , kinetic energy E , and beam current I , the image-charge is given by

$$Q = -I \sqrt{\frac{m}{2E}} \int_{-\infty}^{\infty} f(z) dz, \quad (3)$$

where the integral of $f(z)$ is 1.5×10^{-2} m in the present arrangement.

3. Experimental calibration

A schematic of the pickup ring calibration apparatus is shown in figure 3. A plasma discharge ion source was used to produce a beam of argon ions. The beam was passed through a bending magnet to select Ar^+ ions with a beam energy of 2 keV. Before reaching the ring, the ion beam was collimated to a beam diameter of 2 mm and passed through a set of deflection plates. By pulsing these plates, a $5 \mu\text{s}$ bunch of ions was injected through the pickup ring. A Faraday cup placed after the pickup detector enabled the absolute ion current of a continuous beam to be measured using an electrometer. The pickup ring was connected to an Amptek CoolFET [18] via a conducting rod and BNC feedthrough. This arrangement was measured to have an 8 ± 2 pF capacitance to earth.

The Amptek CoolFET consists of a field effect transistor circuit connected to a charge sensitive preamplifier (CSP) (figure 4). This arrangement gives a voltage output proportional to the image charge (Q) on the pickup ring. Using a feedback capacitor (C_F), the system is able to remove the charge present at the gate of the FET, mitigating any

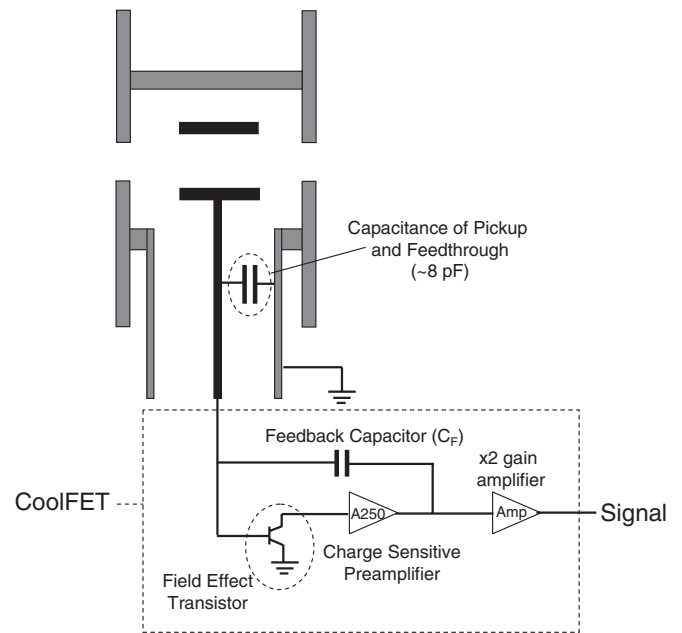


Figure 4. Electrical schematic of the detector and the CoolFET electronics.

influence the detector's capacitance has on the output voltage and resulting in an output voltage V of

$$V = \frac{2Q}{C_F}, \quad (4)$$

where a factor of 2 accounts for an additional amplification stage after the CSP. This capacitance cancelling design has been verified in recent work by Benner *et al* [19].

To calibrate the device a continuous ion beam was initially passed through the device and the ion current measured. The beam was then pulsed and the resulting signal from the pickup was recorded against this ion current. This process was then repeated for different ion beam currents.

4. Calibration results

The pulse heights obtained for various currents are shown in figure 5, from which the gradient yielded the voltage output per nA of beam current. From equation (3) it was calculated that, at any given time, approximately 950 electronic charges are effectively measured for each nA of continuous current. Using this value, each singly charged ion that is imaged contributes

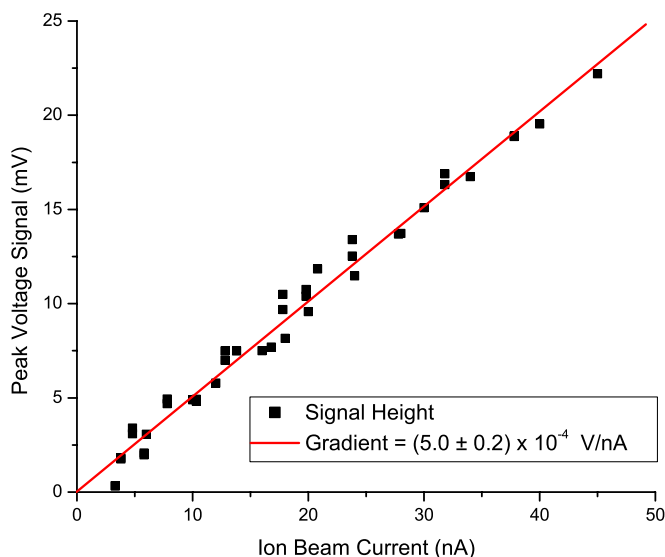


Figure 5. Variation of the signal height as a function of the ion beam current.

$(5.3 \pm 0.2) \times 10^{-7}$ V to the CoolFET output (for $z = 0$), in the present setup. From equation (4), this gives a value of 0.6 ± 0.02 pF for the capacitance of the feedback capacitor, which is within the tolerances expected for this component (nominally 0.5 pF).

Observation of the output signal when no ions are passing through the ring allows the electronic noise in the system to be assessed. This acts to limit the minimum number of ions detectable by the pickup ring. In the current setup this voltage noise per experimental cycle followed an approximately normal distribution, with a standard deviation of 0.84 mV. An ion bunch signal of twice this value was determined as the minimum required for an ion bunch to be detectable. This corresponds to 3100 singly charged ions being present in the centre of the ring. To determine if the noise was correlated with the experiment or arose from random background interference, a series of 10 000 noise spectra were summed and the resulting noise level was found to increase by a factor of ~ 300 . For N iterations, the uncorrelated noise should increase by a factor $N^{\frac{1}{2}}$, and by N for correlated noise. From the results it was determined that $\sim 98\%$ of the noise was uncorrelated with $\sim 2\%$ correlated (most likely due to the ion beam pulsing). The relative noise reduction from the averaging consequently increased the sensitivity of the device reducing the minimum detectable bunch charge to < 100 electronic charges per observation (when 10 000 observations were combined). It should be noted that this method of noise reduction can be limited in certain applications, as the number of observations may be restricted (e.g. in MALDI, each shot will deplete the sample).

In the current setup, amplifier noise at a fundamental frequency of 1.5 MHz, plus harmonics were observed to make a significant contribution. This could be reduced by using a low pass (< 1.5 MHz) filter, adjacent averaging, or convolution functions to the point where approximately 1000 ions could be detected per pass. The applicability of these filtering techniques is dependent on the temporal properties of

the induced pulse (and hence the velocity of the ions). For example, the signal from shorter bunches is more strongly influenced by the filtering techniques due to their increased bandwidth.

An alternative means of enhancing sensitivity was demonstrated by Fuerstenau *et al* [20], where current was measured instead of charge. A ‘pulse peaking-time’ filter was applied to the detected current, resulting in a noise level equivalent to 150 electrons. This is obviously desirable for pure detection purposes but is unsuitable for absolute ion number calculations and for the detection of large masses, as current flow decreases with ion velocity.

5. MALDI-ToF spectra

To study MALDI produced ions, a sample plate at a potential of +2.7 kV, followed by a series of aperture plates with gradually reducing voltages, was installed just before the einzel lens nearest the pickup. This was initially tested using a matrix-only sample of meso-Tetrakis-(pentafluorophenyl) porphyrin (F20TPP). To form this sample, a solution of 1 mg F20TPP (mass 974 amu) [21] was dissolved in 0.1 mL chloroform and deposited on the sample plate. The chloroform was then allowed to evaporate forming F20TPP crystals. The resulting crystals were exposed to a UV laser pulse (337 nm, 150 μ J, 4 ns, focused to a fluence of 8 mJ cm $^{-2}$), to form a plume of neutral and ionic species.

The positive ions were accelerated by the electric field and passed through an einzel lens to reduce the angular divergence of the ion bunch. The ions then passed through a field-free region, containing the pickup ring, before being detected by a CEM (which replaced the Faraday cup used in the previous experiment), thus enabling time-of-flight analysis to be carried out using both detectors.

The signals obtained from a single laser shot are shown in figure 6. While the absolute ToF values in each spectrum are different due to the different detector locations, five dominant peaks can be observed in both. It can be seen that the relative peak heights are significantly different when the spectra are compared. In the pickup ring spectrum (figure 6(a)) it can be observed that the ratio of the peaks corresponding to singly and doubly charged F20TPP ions is approximately 8 (as the pickup ring responds linearly to the charge, this means that there are approximately 16 times more singly charged ions than doubly charged). For the CEM spectrum of the same ions (figure 6(b)) this ratio has reduced to approximately 0.5. The failure of the CEM to detect the ratio correctly is due to the higher kinetic energy of the doubly charged ions leading to better detection.

The calibration results obtained in section 4 can be used to make an estimate of the number of ions produced from MALDI. If the ion bunch is much shorter than the pickup length, at the pickup centre ($z = 0$) the peak signal is $0.53 Nq \mu$ V, where N is the number of ions in the bunch and q is the charge state. For a bunch with a finite length, the relationship is more complicated depending on the pickup response $f(z)$ and the bunch density $n(z)$, resulting in a lower peak signal than that obtained for the zero length case. In

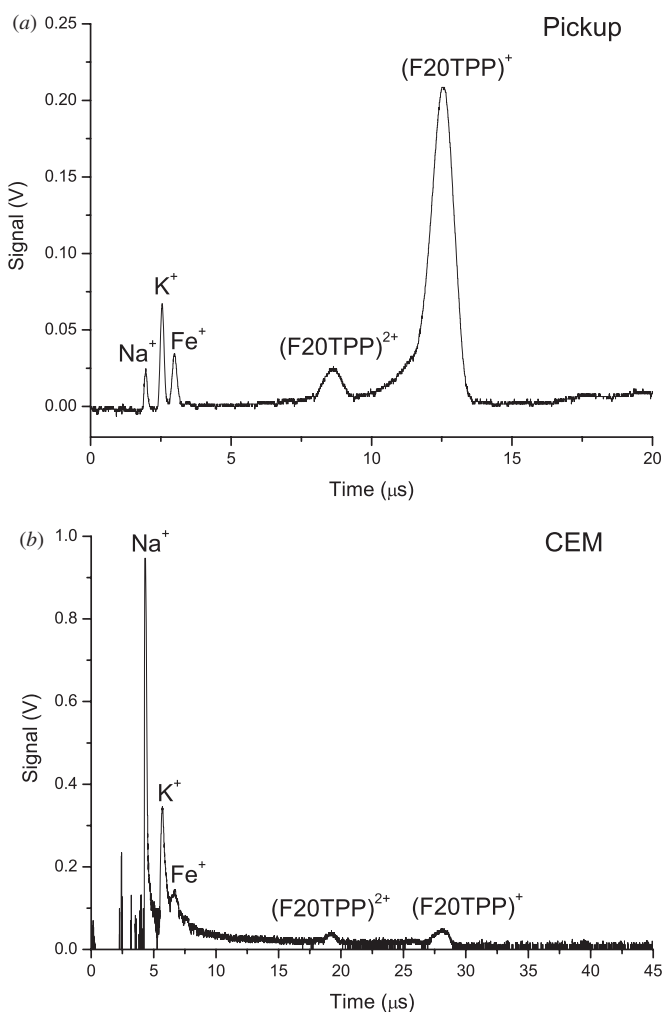


Figure 6. Single-shot MALDI-ToF spectra of F20TPP obtained using (a) a pickup ring and (b) a CEM detector.

Table 1. Lower limit in the number of ions produced by UV-MALDI on a sample of F20TPP.

Species	Mass/charge (amu/e)	No of ions ($\times 10^3$)
Na ⁺	23	51
K ⁺	19	120
Fe ⁺	56	62
(F20TPP) ²⁺	487	21
(F20TPP) ⁺	974	380

table 1 a lower limit on the number of MALDI produced ions has been calculated by assuming a zero length bunch. The width of the peaks in figure 6(a) indicate that the bunch lengths are comparable to the pickup length, which would, at most, increase the table 1 values by 50%. It should also be noted that these results are highly dependent on the exact MALDI parameters used (e.g. laser fluence [22]), and that by adjusting these settings, the absolute and relative yields of the peaks could be changed.

Also exhibited in the spectrum are some saturation effects caused by light ions (contamination of K⁺ and Na⁺ from salts and Fe⁺ from the sample plate) hitting the detector. This is

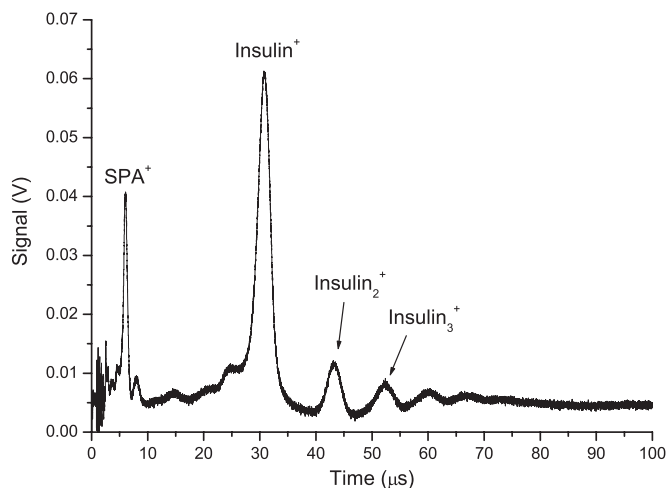


Figure 7. MALDI-ToF spectrum of bovine insulin in a sinapinic acid (SPA) matrix using a pickup detector.

observed as a tail (the decay function between 7 and 15 μs) in the peaks due to recharging of the CEM's resistive coating. Whilst the amplitude of this tail has dropped by the time the later ions arrive at the detector, it is still non-zero indicating that the detector is still not fully recharged and hence not at its maximum detection efficiency. This would potentially prove a much larger problem if the yield of these low mass ions was much higher and highlights some of the difficulties encountered in MALDI-ToF when a heavy analyte is mixed with a light matrix. While this saturation effect can be reduced by removing low mass matrix ions from the spectrum (by, for example, using pulsed deflection techniques), this can also remove low mass ions of interest and still does not alleviate the reduced detection efficiency for high masses.

Further to this work on a matrix-only sample, a specific study of a typical MALDI-ToF sample (including analyte) was carried out using an analyte of bovine insulin embedded within a matrix of sinapinic acid. To form this sample, 10 mg of sinapinic acid and 0.2 mg of bovine insulin (mass 5731 amu) were dissolved in a solution of 0.6 ml acetonitrile and 0.4 ml 0.1% v/v trifluoroacetic acid and allowed to evaporate. This was irradiated by a UV laser pulse, with the same parameters as were previously employed.

The ToF spectrum of the resulting ions produced is shown in figure 7. In this spectrum, alongside the expected sinapinic acid (SPA) and insulin peaks, small clusters of insulin molecules are observed. Detection of $>20\,000$ amu is easily achievable, even at relatively low ion kinetic energies (2.7 keV). Table 2 shows that in excess of 10^5 bovine insulin ions are produced per laser shot, with similar numbers of matrix ions.

It should be noted that not all the MALDI ions produced have necessarily passed through the pickup. To determine the extent of the loss, SIMIONTM 7 was used to model the ion trajectories. The initial conditions were determined from the review article by Zenobi *et al* [23] which demonstrated that the vast majority of the neutrals and ions produced in UV MALDI are emitted with initial energies of a few eV. In the simulations, the ions were therefore given an initial velocity

Table 2. Lower limit in the number of ions produced by UV-MALDI on a sample of bovine insulin and sinapinic acid.

Species	Mass/charge (amu/e)	No of ions ($\times 10^3$)
SPA ⁺	224	68
Insulin ⁺	~5700	105
Insulin ₂ ⁺	~11 400	16
Insulin ₃ ⁺	~17 100	9

equivalent to this kinetic energy, with a random initial direction of motion. The results of these simulations showed that all the ions produced should successfully pass through the pickup, giving us confidence that the majority of the ions generated lay within the angular acceptance of the detector.

6. Conclusion

An image-charge detector, coupled to a field effect transistor and charge sensitive preamplifier, has been assembled and calibrated using a well-characterized ion beam. This mass-independent charge detector was found to be capable of detecting ion bunches with a total of more than 3000 electronic charges, from a single pass. The sensitivity could be further enhanced by a factor of 3 using filtering techniques to reduce high frequency electronic noise. As most of the limiting noise was uncorrelated with the signal, the detection limit also scaled inversely with the square root of the number of passes. The instrument was also used to quantify the saturation effects and reduction in detection efficiency with increasing mass of a CEM used in a MALDI-ToF setup. The absolute calibration of the pickup detector showed that the number of MALDI ions which could be injected into a ToF analyser or storage device is of the order of 10^5 ions per laser shot for laser fluences of 8 mJ cm^{-2} . This information will be valuable in the understanding and applications of the MALDI technique and for instruments where very heavy ions (e.g. DNA, proteins) are analysed.

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