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A Gated Electrostatic Ion Trap Provides a Way to Repetitiously Measure the Charge and m/z of Large Electrospray Ions

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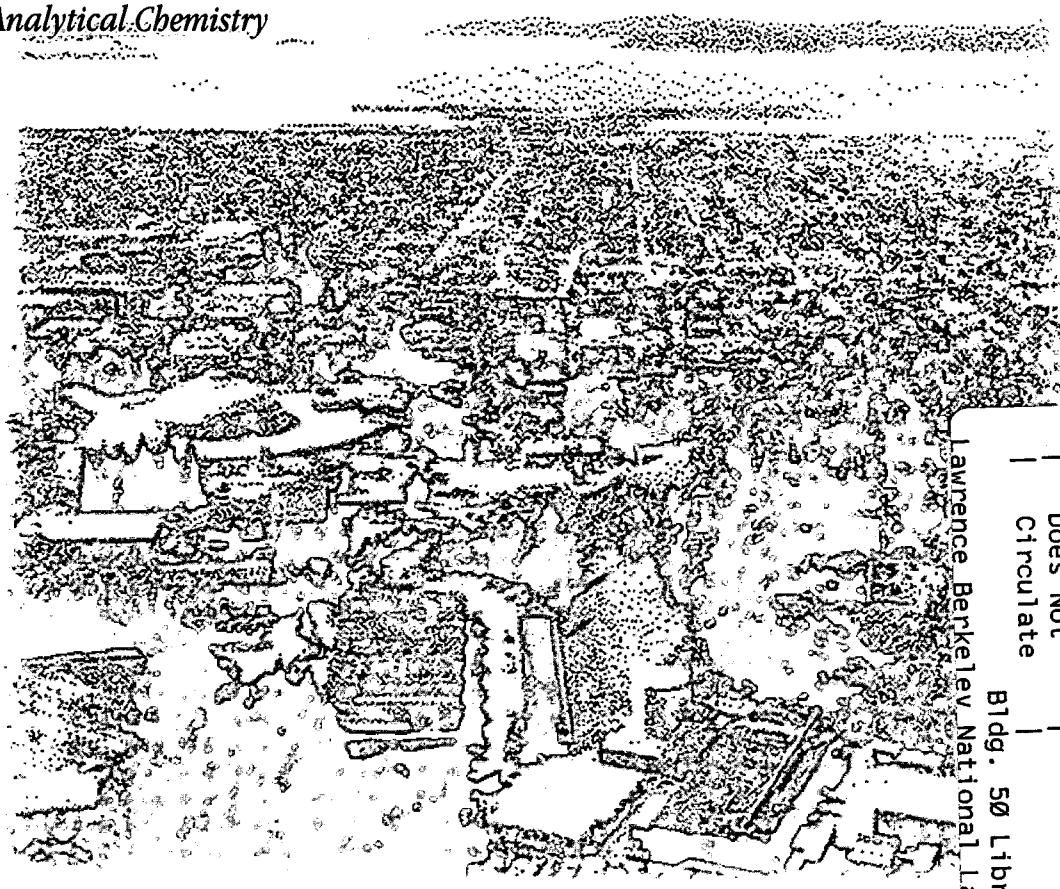

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Abstract

The design and operation of a new type of electrostatic ion trap provides simultaneous measurements of mass, charge, and velocity of large electrospray ions. The trap consists of a detector tube mounted between two sets of center-bored trapping plates. Voltages applied to the trapping plates define symmetrically-opposing potential valleys which guide axially-injected ions to cycle back and forth through the charge-detection tube. A low noise charge-sensitive amplifier, connected to the tube, reproduces the image charge of individual ions as they pass through the detector tube. Ion mass is calculated from measurement of ion charge and velocity following each passage through the detector.

The device does not use magnetic or radio frequency fields but relies on gating the entrance set of electrodes. Voltages on the entrance plates are initially at ground while voltages on the exit plates maintain a potential gradient appropriate for reflecting the ions. When a highly charged electrospray ion enters the detector tube, its image charge triggers a circuit which enables the entrance plates, thus closing the electrostatic gate to the trap. Individual ions carrying more than 250 charges at an energy of 200 eV/charge have been trapped for 10 ms corresponding to 500 cycles through the detector tube. At this level of trapping time, a theoretical precision for charge measurement as small as 2 electrons RMS can be achieved. The operation of the system is demonstrated by trapping 2.88 megadalton ions of DNA.

Introduction

At present, the determination of the mass of electrospray ions larger than about 1,000,000 Da is possible using one of two mass spectrometry techniques. The first relies on Fourier transform ion cyclotron resonance (FTICR) and the second utilizes the simultaneous measurement of charge and time-of-flight.

In the FTICR method, ions are injected into a trapping cell where the resonance condition defined by the magnetic and radio frequency fields definitively resolve the charge/mass ratio of the trapped ions. FTICR has been available for many years and operates at very high m/z resolution. Bruce, et al. commented,[1] that the high resolution achieved with FTICR suggests that the numerous m/z states for electrospray ions exceeding 1 megadalton (MDa) should be resolved. In practice, this goal is confounded by heterogeneity of the population of trapped ions. A number of additional factors addressed in a recent review article by Holliman[2] also degrade resolution in FTICR analyses. The mass analysis of large electrospray ions is nevertheless possible with FTICR. In a series of papers reported by the Smith group[3,4,5,6] a technique was developed for analyzing individual electrospray ions, thus avoiding heterogeneity with respect to the population of trapped ions. With this individual ion analysis technique, they studied the dimer of bovine albumin (133 kDa), poly (ethylene glycol) (5 MDa) and Coliphage T4 DNA (110 MDa). The serial trapping of individual plasmid DNA ions more recently has been used to acquire statistical information about the uniformity of ions from a sample,[7] but this approach is evidently difficult as judged by the observation that only 62 ions were analyzed in order to acquire limited statistical information about the mass of the ions comprising the sample.

Currently, the FTICR technique is not well suited for rapidly analyzing a large number of individual ions sequentially, as is required for determining the average mass of a population of megadalton ions in a sample.

FTICR can also be adapted to the study of the charge state distribution of large electrospray ions. It can also be applied to study the charge states that result when ions are electrosprayed. The determination of charge on individual electrospray ions is an important measurement that has helped to elucidate some aspects electrospray ions formation,[8] including the regime of large ions. It has also been used to examine the influence that solution parameters, most notably surface tension, dielectric constant and conductivity, play in the electrospray charging process.[9] In a study conducted by Chen, et.al.,[3] FTICR was used to measure the charge on large ions by means of an experimentally determined ion ejection curve. The error of the charge measurement was approximately 10 percent.

With much more simple instrumentation, we have determined the mass and charge of individual megadalton electrospray ions.[10] This recent development uses a sensitive low-noise charge sensitive amplifier to capture the image charge as ions pass through a metal detector tube. The transient image-charge signal consists of a pulse with an approximate square-wave shape whose rise and fall corresponds to ion entry and exit times in the tube. By timing the flight of ions with known energy, ion m/z is determined. The amplitude of the resulting differentiated charge pulse is proportional to ion charge and ion mass is calculated simply by multiplying m/z and z.

Currently, we have a detector system that has, at best, a root mean square (RMS) noise of 50 electrons. This is equivalent to a peak-to-peak noise signal of +/- 130 electrons. An amplifier operating at this noise level can readily distinguish ions carrying at least 250 charges from baseline noise. Signals from ions with less charge can also be detected but transients in the background signal interfere with timing and charge measurements. With this detector, we routinely detect and mass analyze DNA ions between 1.5 and 8 MDa, corresponding to an ion charge of 600 and 3200, respectively.

A primary advantage of our approach is the rate at which highly charged individual ions can be analyzed. In our previously reported instrumentation format,[10] ions make a single pass through the tube detector. In this format, several thousand ions can be analyzed in a few minutes, thus supplying enough data for calculating statistically significant measurements of the mass of molecules in a sample population. The cost advantage of this technology, when compared to ICR, is also obvious because large magnets and ultra-high vacuum are not needed. These two advantages are balanced, however, by the low precision of the single-pass charge detection approach. Depending on amplifier noise and the magnitude of the image charge, error in both the amplitude and timing measurements lead to fairly accurate but imprecise mass values.

In the one-pass format, the dominant cause of low mass resolution observed for MDa DNA is due to imprecision of the charge measurement. An estimate of the relative errors associated with charge and velocity measurements can be determined using an electronic pulser to generate charge signals that simulate DNA ions flying through the detector tube. The use of a pulser eliminates measurement variations caused by fluctuations of ion charge and velocity. By introducing 10 μs wide 0.5 mV pulses into the charge-sensitive preamplifier, as typically produced by transiting 3 MDa ions formed by positive mode electrospray, the relative standard deviation (n=100) of the charge measurement is 0.054 compared to a relative standard deviation of 0.013 for the velocity measurement. These values illustrate the relative importance of the charge determination in limiting the precision of the overall measurement.

There are a limited number of options for improving charge measurement precision for the purpose of obtaining better mass measurements. The reduction of noise in the charge measurement circuit will not be very easy. With the current detector operating with a noise level

of 50 electrons RMS, further reduction in the noise level is constrained by fundamental limitations in the charge sensitive circuitry. An approach which bypasses this limitation and which provides a more substantial improvement in the precision and accuracy of the charge measurement is to remeasure the charge on individual ions. Assuming that the source of the electronic noise is uncorrelated with the signal, each additional remeasurement of ion charge reduces the noise associated with the measurement by a multiplication factor of 1/sqrt(n), where n is the number of measurements that are averaged.

The efficacy of signal averaging is shown in Fig. 1 in which the upper trace is a wavelet simulating an ion pulse, generated by introducing test pulses onto a test capacitor connected to the input of the charge sensitive amplifier. The lower trace in Fig. 1 results after 100 of these wavelets are averaged. The noise associated with the upper trace is 50 electrons RMS but after 100 of these pulses are averaged the noise fluctuations associated with the base line is reduced to 5 electrons RMS.

Several approaches might be used to remeasure the charge on an ion repetitively to benefit from signal averaging. A linear series of detectors would accomplish this goal, but for this approach each detector requires its own amplifier and a series of 100 detector tubes is impractical if a ten-fold reduction in noise is targeted. This paper describes a purely electrostatic gated ion trap that recirculates ions through the detector tube so that charge and ion velocity can be measured repetitiously, providing the opportunity for signal averaging.

Experimental

The ion trap, shown in Fig. 2, contains a charge-sensing tube detector that is positioned between two stacks of electrostatic lenses. The lenses create a potential field in which ion velocity is reversed and the ions are guided to pass through the tube detector many times. The stretched ellipse, drawn inside the trap, roughly represents the path of an oscillating ion. This trap design is related to a reflectron or an ion mirror in the sense that ions are reflected out of a potential well. However, this design provides a symmetric restoring force which focuses ions into the detector tube causing them to pass repetitiously through the detector tube. The trap design differs from conventional designs through the introduction of an electrostatic gate on the entrance lens. Operation of this gated trap proceeds as follows: 1) Initially all potentials applied to the lens stack on the entrance side of the detector tube are maintained at ground while the potentials on the exiting lens stack are set to predetermined values designed to reflect and focus ions of a selected energy towards the detector tube. 2) The electrospray interface directs ions through the entrance lens stack into the detector tube. A detectable charge pulse from a single ion triggers a circuit which enables the potentials on the entrance lens stack. These potentials are established in a time interval less than is required for the ion to return through the detector tube. 3) The potentials on each lens stack are held constant over the lifetime of the trapped ion. After a trapped ion has been lost, most likely through collision with the wall of the tube, the entrance plates are returned to 0 volts. The trap is then ready to receive another ion. 4) As ions pass back and forth through the detector tube, the amplified and differentiated image charge pulses are recorded for the duration of the trapping time. The resulting waveform consists of wavelets corresponding to single passes of an ion through the detector tube. A statistically better charge measurement is achieved when the wavelets are parsed and averaged than is obtained from a single-pass measurement obtained in the previously described one-pass format. Fourier transformation of the waveform could also be used to extract amplitude and frequency information from the waveform.

The detector tube (37.5 mm x 6.5 mm id.), shown in Fig 2, is held axially in the bore of a metal block (3 cm diameter, 5 cm long) by two polyethylene disks shown more clearly in the enlarged insert. The metal block provides electrical shielding. The polyethylene disks contain pump-through ports that allow the entire assembly to be evacuated efficiently. End caps on the block,

designed with internal tubes which line up and face each end of the detector tube, provide additional shielding at the ends of the detector tube. Two identical lens stacks are mounted on each end cap. Five square (5 cm x 5 cm, 0.05 cm thick) stainless steel plates separated with insulating spacers (0.2 cm long) comprise the lens stack on each end cap. Centering holes (0.5 cm diam.) were drilled in all of the lens plates and small tabs on the edge of each plate provide locations for attaching power supply wires. A larger tube (4 cm diam, 15 cm long) was attached perpendicularly to one of the longer sides of the metal block and serves as a pedestal for attaching the detector assembly (detector tube, trapping electrodes and the shielding block) to a 6" diameter vacuum flange. Wires leading from electrical feed-throughs in the vacuum flange to the lens stack wrap around the outside of this support tube. A field-effect transistor (FET), along with its feedback resistor and capacitor, is located inside this supporting tube near the metal block. Wires leading to the FET were stretched inside the support tube. The mounting structure design was optimized both to minimize stray capacitance associated with the detector tube and the wire connecting the detector tube to the FET. The mounting structure was optimized to minimize microphonic contributions to the background signal. A picture of the entire assembly is shown in Fig. 3.

The ion optics simulation program, Simion 6.0,[11] was used to study 3D potential gradients that produce ion trapping potential fields. Many different lens geometries were examined as possible trapping fields and those that performed best looked like a valley with a rising valley floor. Figure 4 shows a typical 3D potential gradient that efficiently traps ions. The potential gradient in Fig. 4 was produced with two sets of five electrodes plus an end cap. Each electrode in this model contains a centering hole through which ions travel. The distance an ion travels in the trapping field, in other words, the number of plates through which it penetrates before it turns around, is determined by the relative height of the potential valley with respect to the energy/charge of the ion. For the potential valley in Fig. 4, only ions in a defined range of energy are trapped. Higher energy ions or charged residue particles fly out of the valley and are not captured. Less energetic ions are not trapped because they roll off the potential saddle between lens L1 and lens L2. The lens numbering system progresses from 1 - the end cap, to 6 - the plate farthest from the end cap. The potential valley depicted in Fig. 4 results when the following voltages are applied to the plates in a lens stack: L1 = 0, L2 = -100, L3 = 100, L4 = 200, L5 = 300, L6 = 300. L3 to L5 define a nearly linear gradient. Setting L6 = L5 and applying a negative potential on L2 creates the rising potential valley and the negative potential on L2 additionally prevents the potential gradient from extending into the detector tube.

The slope of the potential valley can be better comprehended by examining a plot of the potential along the centerline of the bore of the trapping plates as presented in Fig. 5. The centerline potential controls ion velocity. As an ion exits the detector tube, it accelerates slightly until it passes through L2 and then rapidly decelerates as it climbs in the potential valley between L2 and L5. The height it attains in the potential valley depends on ion energy/charge. When the magnitude of ion energy/charge equals the magnitude of the local potential (compare for example, 100 eV/charge with 100 V), the ion stops, turns around, and accelerates back down the potential valley. An identical potential valley awaits its arrival in the mirroring lens stack at the opposite end of the detector tube where the ion is forced to turn around again.

Figure 6 shows the waveform created by a single highly-charged electrospray ion of DNA, as it recirculated through the trap. The ion is a 4.3 kilobase long circular DNA molecule of a bacterial plasmid described as pBR322. The entire waveform composes wavelets corresponding to single passes of an ion through the detector tube. The time between a positive and the ensuing negative pulse represents the time the ion spent in the detector tube and the time between a negative pulse and the next positive pulse corresponds to the time it takes an ion to turn around in the trapping field. The shape of each wavelet is roughly the same because its shape does not depend on the direction an ion travels. The amplitude of these wavelets (Fig. 6) provides a measure of ion charge. This particular ion carried an average of 1040 charges and the 1 ms record shows that the

ion recycled more than 51 times through the trap. The actual trapping time was longer than 1 ms but only 1 ms of data is presented. Amplitude and timing data for each cycle through the detector tube was used to calculate ion mass. The open circles above the waveform indicate the mass values calculated from each cycle and these values fall between 2.59 and 3.02 MDa. When these 51 mass values are averaged, the mean +/- sd is 2.79 +/- 0.09 MDa and the 95% confidence interval of the measurement is 0.01 MDa. This value compares favorably to the expected mass of 2.88 MDa for pBR322 DNA in the sodium form. The difference between 2.79 and the expected value of 2.88 MDa appears to be due to cleanup procedures which removed some of the sodium ions from the sample and exchanged them with H+. When ions from this same sample were analyzed with the one-pass method, an average value of 2.9 MDa was obtained when several thousand ions were analyzed.

The length of time an ion can be confined in this gated electrostatic trap determines the precision with which ion mass can be calculated. The time an ion is trapped depends on factors related to the trajectory the ion follows in the trap and detector tube. The most stable trajectory results when an ion follows a radially-centered path through the tube and turns around in the external trapping field without deviating from its centerline position. An ion following a centerline trajectory will remain confined in the trap until it is slowed by gas collisions or spontaneously fragments. An aperture located between the electrospray source and the entrance plates confines ions to +/- 1 mm of the axis of the detector tube. A large fraction of the ions entering the detector tube are trapped. Ions that are more than 1 mm off the centerline or are not traveling parallel to the axis acquire a slightly different trajectory each time they turn around in the trapping field and eventually strike the electrodes or the tube. Gas collisions reduce the energy of the ions and contribute to unstable ion trajectories. The presence of a gas jet flowing through the trap, created by the electrospray source, might be significant. The background gas pressure surrounding the trap was in the 10^{-8} torr range for this experiment.

The longest time an ion has been trapped so far is about 10 ms during which time an ion oscillated nearly 500 times through the detector tube. Trapping times as long as this suggest that charge measurements obtained from this repetitious measurement technique could be as precise as the RMS noise level of the detector (50 electrons RMS) divided by the sqrt 500 or +/- 2.2 electrons RMS. These results demonstrate a mass measurement precision surpassing gel-based analyses for large DNA ions.

It should be noted that the mass measurement technique described here is amenable to direct calibration since it depends only upon the detector tube length, pulse height of the image signal, and ion transit time. The relationship between signal amplitude and induced charge is determined by depositing a known voltage on a 0.215 pF test capacitor. Measurement of tube length is accurate to better than 1 part in 500, although we have preliminary data that indicates that the effective electric tube length is nearly 2 percent longer than the physical tube length. The electric tube length is the length value that is used to calculate ion velocity and it is different from the physical length because of the way the image charge is captured by the detector tube. Pulse amplitude and ion transit time measurements are determined with a self-calibrating digitizing oscilloscope and is accurate to within a fraction of a percent.. As noted earlier, the accuracy of the mass measurement is dominated by the charge-measurement accuracy. Now that ion charge can be measured with improved accuracy with the trapping technique, the relative inaccuracy of velocity and energy measurements will need to be reevaluated.

In this preliminary report describing the development of a gated electrostatic trap, a waveform of a single trapped ion of 2.88 MDa DNA ion is used to calculate the mass of this particular ion. However, the mass analysis of one ion is inadequate to predict the average mass of the population of molecules that exists in a sample. To make such a prediction, numerous molecules need to be analyzed so that statistical parameters can be calculated. Future efforts will be

directed towards developing a data system so that the waveforms produced by sequentially trapped ion can be processed rapidly.

Conclusions

A gated electrostatic ion trap is described which provides repetitious charge and flight-time measurements of single electrospray ions. Charge is determined with a detector tube connected to the input of a sensitive low-noise charge-sensitive amplifier system. The magnitude of the image charge signal is proportional to ion charge. The rise and fall of the image signal provide a method for measuring ion velocity from which m/z is obtained. Ion mass is calculated for each ion simply by multiplying these two values. The operation of the trap has been demonstrated by trapping megadalton ions of DNA. The advantages of the electrostatic trap are: 1) the charge and m/z of individual ions can be measured repeatedly, thus improving the accuracy of the mass calculation over that obtained with a previously described one-pass measurement technique. 2) It operates in a mass and charge regime otherwise accessible only by FTICR. The electrostatic trapping approach, when combined with image charge detection, provides a way to determine the mass of megadalton ions, such as DNA, with much less expensive instrumentation than needed for FTICR. The technique provides a faster and accurate way to size large DNA molecules than is possible with gel electrophoresis.

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Figure Captions

Figure 1 - A waveform generated with a pulser which simulates an ion passing through the detector tube. The vertical scale is 0.5 V/div and the horizontal time scale is 5 μs/div. The upper trace corresponds to a single ion passing once through the detector tube and displays amplifier noise of 50 electrons RMS. The lower trace results when 100 of these waveforms are summed and averaged. Averaging decreases noise to 5 electrons RMS thus improving signal-to-noise by 10-fold and demonstrates the improvement that is gained when the charge on an ion is measured repeatedly.

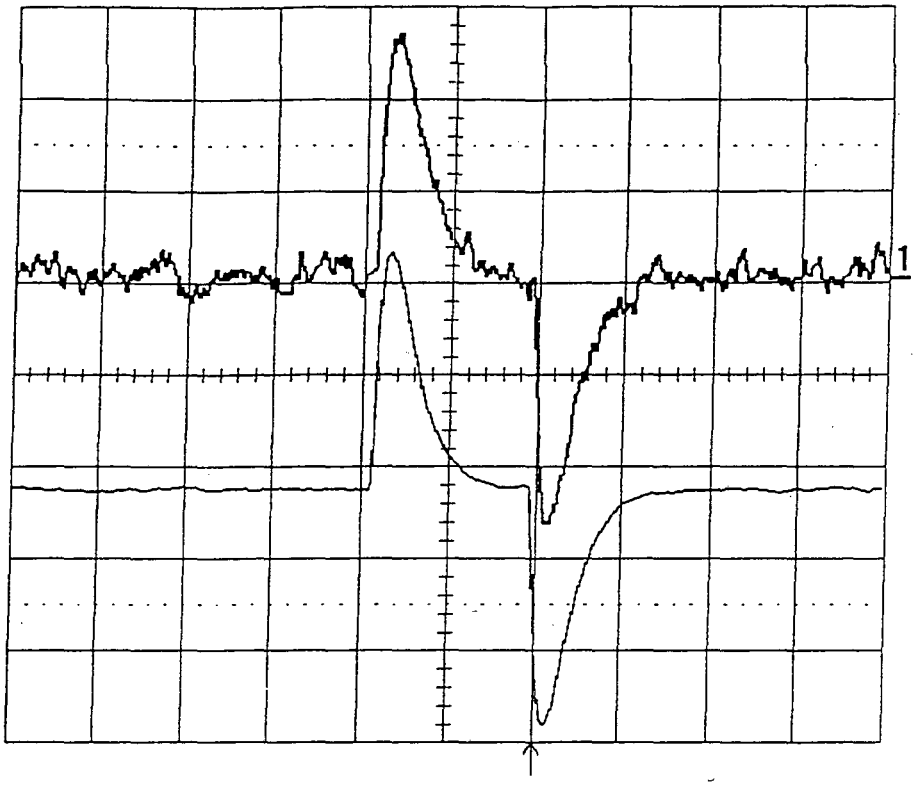
Figure 2 - A diagram of the ion trap. Trapping plates on the left and right sides of the detector module define the potential field which causes ions to cycle back and forth through the detector tube. A support arm, attached to the bottom of the detector block, holds the detector assembly rigidly to minimize vibrations and shields an internal FET from rf noise.

Figure 3 - A photograph of the ion trap attached to a vacuum flange.

Figure 4 - A three dimensional view, produced with Simion 6.0, of the potential valley created to trap positively charged ions. The thick black lines show the location of the electrodes and detector tube. The vertical axis of this plot is voltage. The detector tube and L1 (Lens 1 in the trapping plates) are at ground potential, L2 = -100 V, L3 = 100 V, L4 = 200 V, L5 = L6 = 300 V. The line drawn through the center of the detector tube and extends part of the way up the potential valley shows the path followed by trapped ions.

Figure 5 - The grids represent the electrodes in the trap as drawn by Simion software. Juxtaposed is a plot of the potential along the center of the bore of the trap. As a positive ion travels from right to left, it travels at ground potential in the detector tube and accelerates until it passes L2, then decelerates in the rising positive field. These conditions trap ions possessing about 200 eV/charge.

Figure 6 - The lower oscillatory waveform describes the cycling of a 2.88 MDa DNA ion in the trap. For this waveform, the vertical scale is volts and the displayed trapping time is 1 ms. Pulse height provides a measure of ion charge and the time between a positive peak and the ensuing negative peak is the time the ion is in the detector tube. Ion mass was calculated each time the ion traveled through detector tube and is plotted with open circles. The vertical scale for the mass data is MDa.



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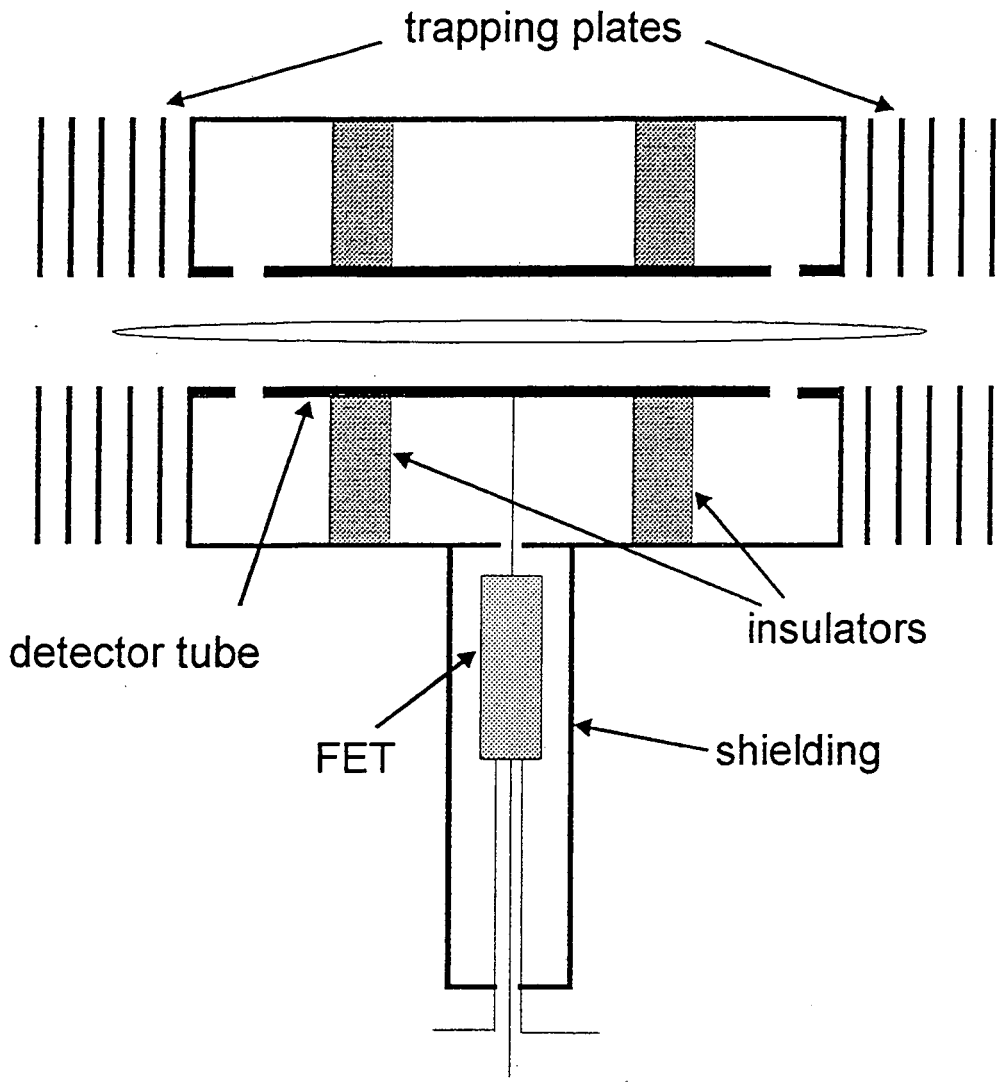

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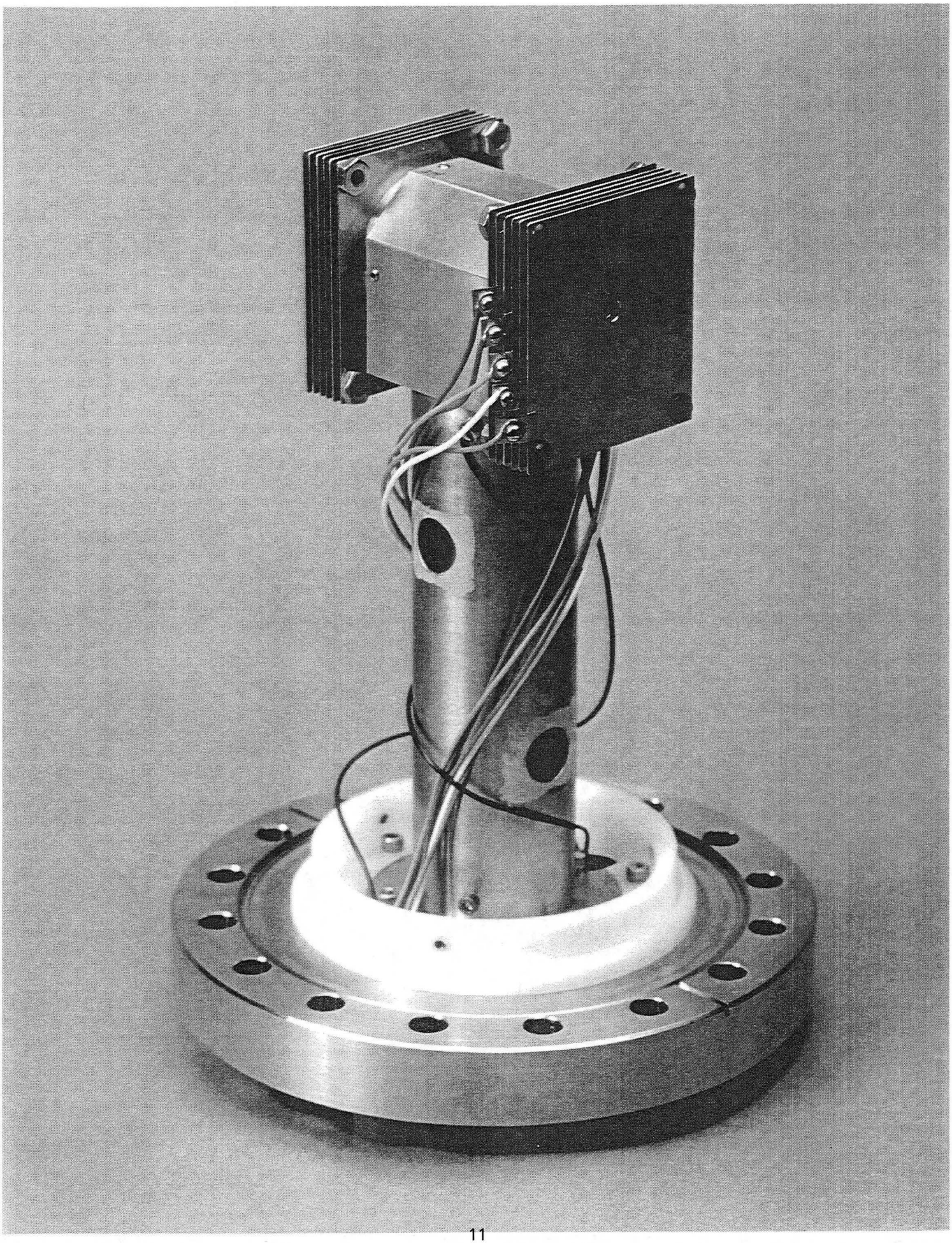

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Figure 3

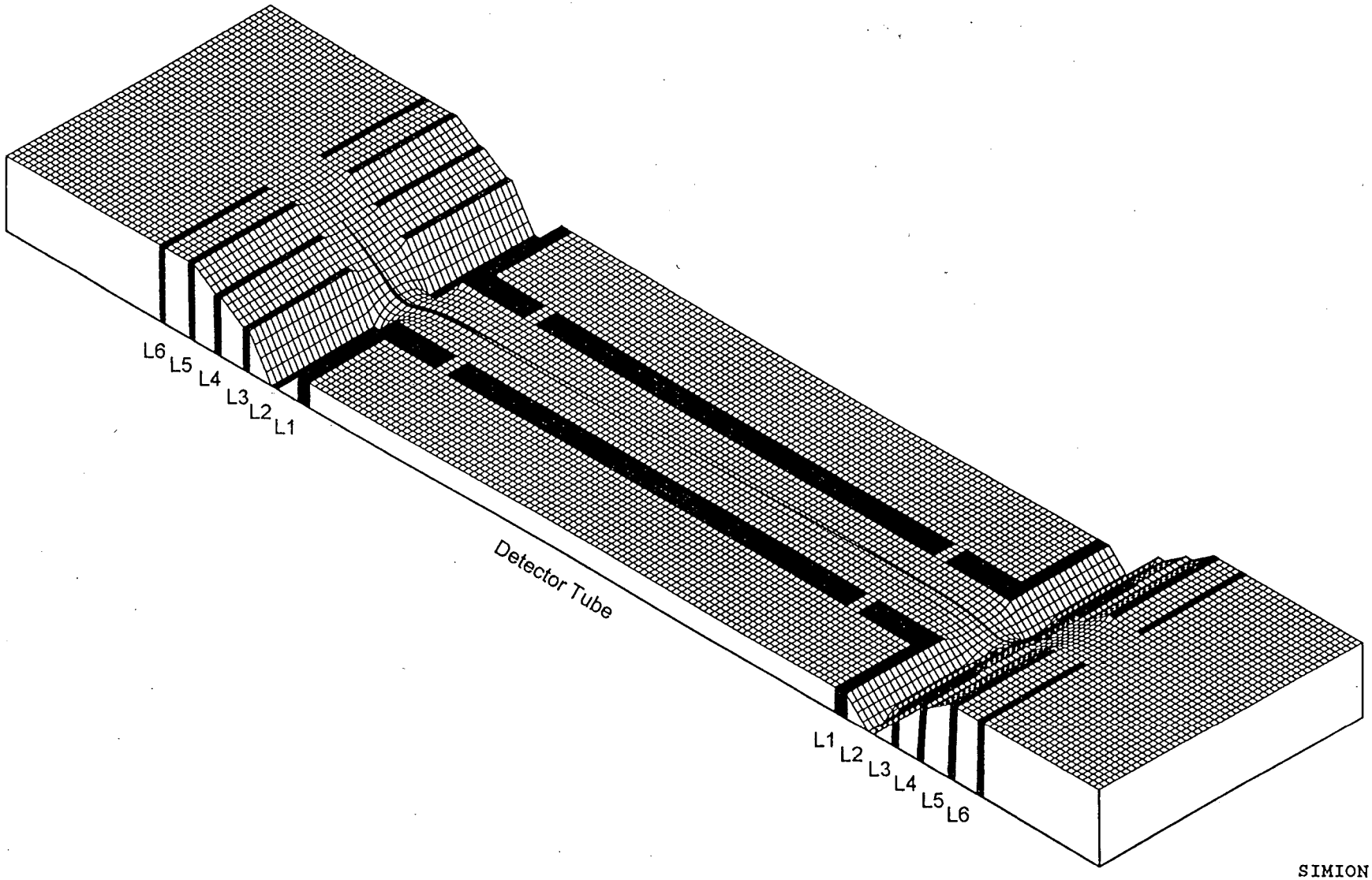

Figure 4.

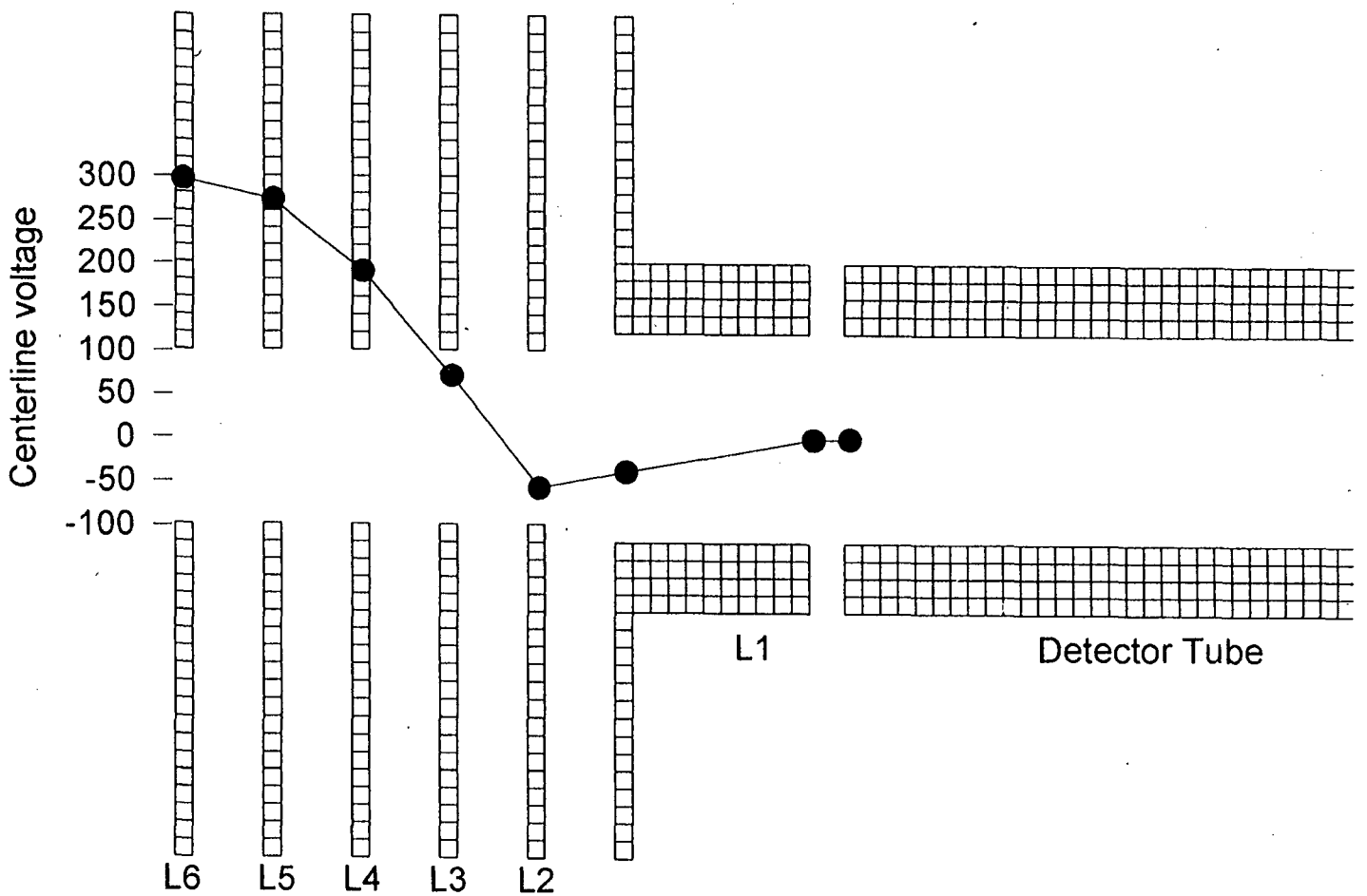
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Figure 5.

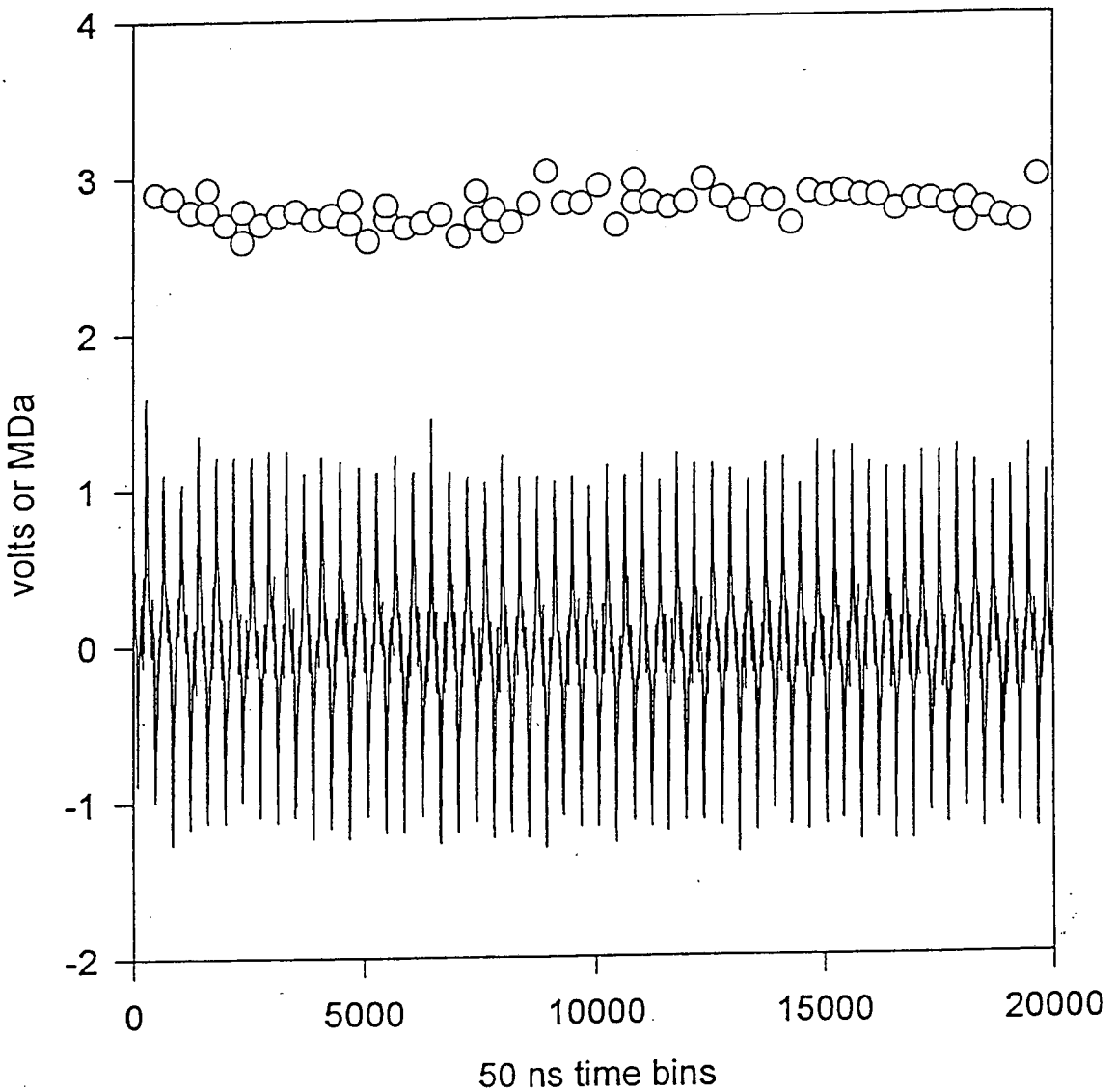

Figure 6.

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