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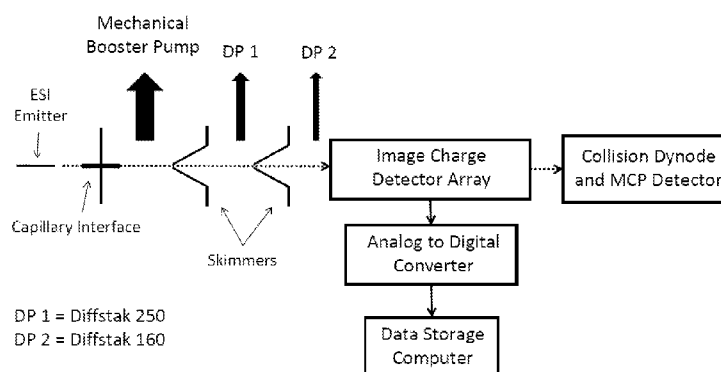
(54) **Title:** CHARGE DETECTION MASS SPECTROMETER WITH MULTIPLE DETECTION STAGES

FIG. 7

(57) **Abstract:** A charge detection mass spectrometer (CDMS) with two groups of image charge detectors that operate at different electrical potentials is described. The device provides an accurate mass measurement for a single ion without prior knowledge of the ion's energy. Further described is a method of analyzing CDMS data using a correlation approach. The method provides a means of reducing the confounding effect of spurious noise signals and enhancing signal to noise. The method further provides more accurate determination of the charge and velocities values than time and frequency domain methods. The method and the device enables advanced analysis of high mass ions from heterogeneous mixtures (e.g., viruses, polymers and nanoparticles).



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CHARGE DETECTION MASS SPECTROMETER WITH MULTIPLE DETECTION STAGES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 USC §119(e) to U.S. Provisional Application Serial No. 61/423,867, filed on December 16, 2010, the entire disclosure of which is incorporated herein by reference.

GOVERNMENT RIGHTS

[0002] This invention was made in whole or in part with support from The National Science Foundation NSF Grant No. 0832651. The United States Government may have certain rights in this invention.

TECHNICAL FIELD

[0003] The present invention relates to devices and methods for analyzing high molecular weight compositions. More particularly, the invention is directed to a charge detection mass spectrometer and methods of using the same to determine the mass and charge of molecular ions.

SUMMARY

[0004] According to the present disclosure, a device and method for analyzing high molecular weight compositions is described. A mass spectrometer and a method of using the same to detect and analyze multiply charged macroions is described which can be used to determine the molecular weight distribution of polymeric distributions, detection and analysis of biological macroions, and the like.

[0005] A mass spectrometer system is described which includes an ion source that generates an ion beam and an image charge detector array. The image charge detector array has n charge detection tubes and m charge sensitive amplifiers. The m charge sensitive amplifiers are electrically connected to one or more of the charge detection tubes. The charge detection tubes include an electrically conducting tube and a grounded shield. The grounded shield comprises an electrical conductor surrounding the tube separated from the electrically conducting tube by an insulator. The image charge detector array has n charge detection tubes, wherein n is an integer greater than or equal to four and m charge sensitive amplifiers, wherein m is an integer greater than

or equal to two. The charge detection tubes are grouped into at least two groups, the groups comprising two or more charge detection tubes. A first group operates at a first voltage and a second group operates at a second voltage. In one embodiment, the first and second voltages are distinct. The charge detection tubes are located in the ion beam path and having a long axis parallel to the flight path of ions in the ion beam. In one embodiment, n is from 4 to about 120. In another embodiment, m amplifiers are JFET transistors operated as high-gain negative feedback amplifiers. In one embodiment, m is from 2 to about 4.

[0006] A method of determining m/z (mass to charge ratio) and z (charge) of a macroion comprises detecting the macroion traveling through a series of charge detection tubes and inferring the m/z from the respective time-of-flight in the subsequent charge detection tubes. The series of charge detection tubes comprises at least a first charge detection tube operating at a first voltage and a second charge detection tube operating at a second voltage. The step of detecting the macroion includes detecting an image charge on the first charge detection tube for establishing a first time-of-flight and detecting an image charge on the second charge detection tube for establishing a second time-of-flight. The step of detecting the macroion also includes detecting an amplitude of the image charge in the series of charge detection tubes. The image amplitude directly provides z and the first time-of-flight and the second time-of-flight provides m/z without knowledge of the initial ion energy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 is a schematic showing an Amptek A250 preamplifier and a low noise JFET for the first amplification stage, the circuit shown uses a $100\text{G}\Omega$ feedback resistor to eliminate thermal resistor noise and to raise the RC time constant that results from the use of very small feedback capacitance;

[0008] FIG. 2 shows the effect on the signal to noise ratio as the number of detection cylinders varies (while keeping all the other parameters fixed), the signal to noise ratio initially increases as the number of detectors increases, and then decreases;

[0009] FIG. 3 shows the dependence of the signal to noise ratio for a 44 detector array on the number of amplifiers, the signal to noise ratio initially increases rapidly as the number of amplifiers increases, but then the rate of improvement slows substantially;

[0010] FIG. 4 shows a plot of the m/z accuracy versus the number of detectors, the peak occurs near 80 detectors, with this number of detectors the mass to charge resolution is approximately 1.3x better than with 44 detectors;

[0011] FIG. 5 shows that for a value of $c = 0.108$ and $r = 0.20$ cm, an increase of L to 67.3 cm, where $b = 0.98$, an increase of m from 4 to 14, and shorten s to 0.075 cm, the optimum performance should be obtained with 106 detection cylinders;

[0012] FIG. 6 shows a schematic diagram of an image charge detector design including a minimized insulator to electrode contact area through the use of raised ridges on the detection cylinder and the use of a groove in which the insulator is located;

[0013] FIG. 7 shows a schematic diagram of an exemplary embodiment of a mass spectrometer;

[0014] FIG. 8 shows a schematic diagram of an exemplary embodiment of a charge detector array;

[0015] FIG. 9 shows a graphical representation of an unprocessed signal for a macroion with a charge of around 2,500 e travelling through the group of detectors shown in FIG. 8;

[0016] FIG. 10 shows a schematic of the response of the correlation analysis routine (with the correct value of w) to a 50% duty cycle square wave that simulates the output from one of the groups of detectors in the charge detector array;

[0017] FIG. 11 shows a graphical representation of the response of the correlation analysis to an ion travelling through one group of detectors at 491 m/s, the plot shows the normalized output amplitude of the correlation analysis against w (plotted in terms of the ion velocity);

[0018] FIG. 12 shows a graphical representation of a plot of typical rms error output from the correlation analysis described in the text, the vertical axis is the rms error in units of elementary charge while the horizontal axis shows the parameter w over the relevant times for ions to travel through the exemplary detector array;

[0019] FIG. 13(A-C) shows a graphical representation of the results for ions with masses less than 1MDa, (A) showing the mass distribution, (B) showing the m/z distribution, and (C) showing the charge distribution;

[0020] FIG. 14(A-B) shows a graphical representation of velocity distributions for ions with masses less than 1MDa, (A) showing the velocity distribution measured

with the first group of detectors and (B) showing the velocity distribution measured with the second group of detectors after the ions have been decelerated; and

[0021] FIG. 15(A-C) shows a graphical representation of the results for ions with velocity shifts less than 1%, (A) showing the mass distribution, (B) showing the m/z distribution, and (C) the charge distribution.

DETAILED DESCRIPTION

[0022] While the invention is susceptible to various modifications and alternative forms, specific embodiments will herein be described in detail. It should be understood, however, that there is no intent to limit the invention to the particular forms described, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

[0023] The present disclosure describes the use of a charge detector array with at least two groups of detectors biased at different voltages to determine the m/z ratio without prior knowledge of the ion energy. Furthermore, the present disclosure describes the use of a correlation method to analyze the output from an image charge detector array.

[0024] One aspect of the present disclosure is that a macroion can be analyzed to determine the mass to charge (m/z) ratio together with the measured charge (z) using the device described herein. From the m/z ratio and the charge, the mass (m) of each ion can be determined. The m/z ratio may be determined from the initial and shifted macroion velocities, along with the offset voltages. Another aspect of the present disclosure is that the device and method allows an accurate determination of the charge for macroions with charges 2.5 times lower than the previously reported for charge detection mass spectrometry. The ability to measure small charges accurately allows the determination of the mass of single ions around an order of magnitude smaller than previously reported using charge detection mass spectrometry. (Fuerstenau et al. "Molecular Weight Determination of Megadalton DNA Electrospray Ions Using Charge Detection Time-of-Flight Mass Spectrometry," *Rapid Commun. Mass Spectrom.* 1995, 9, 1528-1538; Schultz, et al. "Mass Determination of Megadalton-DNA Electrospray Ions Using Charge Detection Mass Spectrometry," *J. Am. Soc. Mass Spectrom.* 1998, 9, 305-313; Fuerstenau, et al. "Mass Spectrometry of an Intact Virus," *Angew. Chem. Int. Ed.* 2001, 40, 541-544; Benner, W. H. "A Gated Electrostatic Ion Trap to Repetitiously Measure the Charge and m/z of Large

Electrospray Ions,” Anal. Chem. 1997, 69, 4162-4168; Gamero-Castaño, M. Induction Charge Detector with Multiple Sensing Stages. Rev. Sci. Instrum. 2007, 78, 043301.)

[0025] In illustrative embodiments, the mass spectrometer includes an image charge detector. The image charge detector electrically responds to the presence of an ion in two different ways. First, the electrical response is of a magnitude induced by and dependent upon the ion charge. Second, the electrical response is for a time related to the time-of-flight of the macroion through the image charge detector. Accordingly, an image charge detector detects the magnitude of the ion charge and the velocity of the ion charge through the detector. In one embodiment, the image charge detector comprises one or more conductive tubes or plates. In another embodiment, the image charge detector comprises one or more materials that are inductively manipulated by the passage of the microion through a nearby location. Illustratively, the image charge detector comprises a conductive tube having two ends and a length sufficient to provide time-of-flight information for an ion passing through the tube. In one embodiment, the tube is cylindrical. In another example, the tube is rectangular. In another example, image charge detector comprises a pair of plates. In one embodiment, the plates are arranged in parallel pairs. The plates may be of any shape, but illustratively, the plates are rectangular.

[0026] In illustrative embodiments, the entrance to the image charge detector may be a narrower tube or orifice that limits the number of entering macroions, such as to one at a time. For example, this narrow tube or orifice may be used to ensure that the macroions entering therein have trajectories that pass through the image charge detector according to a central path so as to not become too close to any or either side of the detector. As a charged macroion enters the image charge detector, it induces a charge on the image charge detector nearly equivalent to its own charge. The charge induced is detected and amplified with an amplifier circuit that is electrically connected to the image charge detector. That amplifier enables the quantification of the induced charge and a determination of the charge on the macroion. The charge of the macroion may be calculated from the induced potential and the capacitance of the tube.

[0027] Another aspect of the present disclosure is that current techniques and instruments used for mass spectrometry are inadequate when used to determine the mass of large objects (i.e., masses in the 1MDa to 1GDa range). Furthermore, current techniques and instruments used for mass spectrometry are inadequate for determining mass distributions for multicomponent mixtures such as polymers and nanoparticles.

In the case of large objects, detector sensitivity and mass heterogeneity are the major stumbling blocks. In the case of mixtures, it is the complex spectrum of overlapping peaks resulting from different masses and charge states that provides the road block. In principal, this problem could be overcome by very high resolution. The present disclosure addresses both of these challenges using charge detection mass spectrometry (CDMS).

[0028] As described herein, image charge detectors permit the simultaneous measurement of the charge and velocity of a macroion. If the energy is known, this can be used with the measured velocity to determine the m/z ratio. The m/z ratio can then be combined with the measured charge to yield a mass for each individual ion. This approach can be contrasted with conventional mass spectrometry where a m/z spectrum is recorded. Then, in order to determine the mass, the charge must be deduced from the m/z spectrum. For small molecules and low charge states, this approach is effective. However, with high charge states, high masses, and heterogeneous molecular distributions, determining the mass by deducing the charge becomes very difficult. For macroions, this deduction can be accomplished by analyzing the series of peaks in the m/z spectrum resulting from different charge states. The separation between the peaks provides the charge. However, heterogeneous samples and multiple charge states cause the peaks to overlap and the approach becomes problematic.

[0029] In illustrative embodiments, an image charge detector comprises a conducting tube connected to a charge sensitive preamplifier. As a charged object enters the cylinder it impresses an image charge onto the cylinder which is detected by the preamplifier. If the cylinder is long enough, the image charge provides a measure of the charge on the object, and the time between when the object enters the cylinder and when it leaves provides a measure of the velocity. One aspect of this approach is that it depends on directly measuring the charge on a single macroion. The charge on a single macroion is small and thus the measurement is susceptible to error. In particular, a device useful for accurately determining the charge on a single microion would be accurate to within better than 0.4 elementary charges (e) to achieve quantitative charge accuracy with a confidence of greater than 99%.

[0030] The present disclosure uses an image charge detector such as those disclosed in art. For example, the first use of an image charge detector to determine mass was in 1960 when this approach was used to determine the masses of microparticles for hypervelocity impact studies. (Shelton, et al, "Electrostatic

Acceleration of Microparticles to Hypervelocities,” J. App. Phys. 1960, 31, 1243-1246.) According to those disclosures, microparticles were charged and accelerated through an image charge detector. The measured velocity and charge along with the known acceleration voltage provided the mass of each microparticle. Hendricks used a similar approach to measure the charges and masses of liquid droplets generated by electrospray in the vacuum. In the mid 1990s, Fuerstenau, Benner, and their collaborators used image charge detection to perform mass measurements on Megadalton molecular ions such as large DNA fragments and electrosprayed viruses. (Fuerstenau, et al. “Molecular Weight Determination of Megadalton DNA Electrospray Ions Using Charge Detection Time-of-Flight Mass Spectrometry,” Rapid Commun. Mass Spectrom. 1995, 9, 1528-1538.) In their implementation, the ions were generated by an electrospray source and accelerated by a voltage gradient before travelling through an image charge detector.

[0031] One aspect of the present disclosure is that electrical noise limits the accuracy of the charge measurements. Illustratively, Fuerstenau and Benner used a Gaussian differentiation peak shaping technique and reported a rms noise of 150 e. Another aspect of the present disclosure is that a more accurate value for the charge can be obtained by averaging over a series of measurements. For example, this approach was implemented by Benner who used a linear ion trap to repetitively measure the charge of a trapped macroion. The uncertainty in the charge measurement is expected to decrease as $n^{-1/2}$ where n is the number of measurements. Benner reported as the rms noise of 50 e which is reduced to 2.3 e for an ion that oscillates 450 times (the maximum number observed). However, for these oscillatory experiments, it was important that the signal be large enough to know when an ion passes through the image charge detector so that it can be trapped. For example, Benner required the macroion possess a charge of at least 250 e. This high detection limit is problematic because it limits the utility of the detector.

[0032] One aspect of the present disclosure is that multiple image charge measurements may be used as a linear array of charge detectors. Previous attempts to utilize this approach were described by Gamero-Castaño. He used a detector consisting of six collinear tubes with tubes 1, 3, and 5 connected to one amplifier (1) and tubes 2, 4, and 6 connected to another (2). He utilized this array by subtracting the output from the amplifier 2 from amplifier 1. Using this approach, Gamero-Castaño realized the detection limit in the time domain that was $2^{1/2}$ times lower than a single

detector and the noise is $n^{1/2}$ lower (where n is the number of detectors). A noise level of around 100 e was reported for analysis in both the time and frequency domains for typical signals.

[0033] Conventional mass spectrometry (where only the m/z ratio is measured) is limited to problems where the charge can be determined from the m/z ratio. This is not an issue for small singly charged ions but for large multiply charged ones it is necessary to resolve the charge states to determine the charge. While it is easy to resolve the charge states for a 10 kDa protein, it becomes increasingly difficult to resolve them as the mass increases. There are only a few cases where charge states have been resolved for ions with masses larger than 1 MDa. When there is heterogeneity in the mass (i.e., polymers and nanoparticles) the problem emerges at much smaller masses. The present disclosure describes a manner in which charge detection mass spectrometry (CDMS) can be used to fill this void. Using this approach, m/z and z are measured for each ion, so that the mass (not just m/z) is determined for each ion. Mass distributions can then be measured for objects from kilodaltons to Gigadaltons, and in cases where complexity renders conventional mass spectrometry ineffective. In illustrative embodiments, the mass spectrometer of the present disclosure can determine the mass m/z and z are measured for ions having a mass from 10 kDa to 100 GDa. In one embodiment, the mass spectrometer of the present disclosure can determine the mass m/z and z are measured for ions having a mass from 100 kDa to 10 GDa. In another embodiment, the mass spectrometer of the present disclosure can determine the mass m/z and z are measured for ions having a mass from 1 MDa to 1 GDa.

[0034] According to another aspect of the present disclosure, analysis of an individual macroion by CDMS utilizes the fact that the macroion traveling through a conducting cylinder impresses an image charge on the cylinder which is directly detected. As described herein, the length of the signal provides the time of flight, which combined with the ion energy yields a value for m/z , and the amplitude of the signal provides z , and so m can be directly determined for each ion. One challenge with this approach is to measure the charge with sufficient accuracy. Because of the small signal to noise ratio, it is useful to perform multiple charge measurements. Illustratively, this can be accomplished using a recirculating trap or a linear array of charge detectors.

[0035] According to one example of the present disclosure, we describe an image charge detector array comprising 22 detectors divided into two groups operated at different voltages. The m/z ratio is determined from the different macroion velocities in the two groups of detectors without knowledge of the ion energy. For example, the initial ion velocity may be set by the aerodynamic acceleration that occurs in a capillary interface. According to this approach, the ions would start with a constant velocity rather than a constant energy. We determined that this was advantageous because the accuracy of the charge measurement is proportional to the time spent in the detectors. Using the capillary interface, we established that the constant velocity could be set at a pre-determined value which was sufficiently low so that the time spent in the detectors is sufficiently long for accurate determination. In illustrative embodiments, the mass spectrometer includes a capillary interface that provides macroions having a constant velocity. In one embodiment, the constant velocity is sufficiently low so that the time spent in the detectors is sufficiently long for accurate determination of the time-of-flight of the ions.

[0036] Another aspect of the present disclosure is the fact that the accuracy of the mass determined by an image charge detector is dependent on the signal to noise ratio of the measurement. The accuracy of the image charge detector directly affects the accuracy of the charge measurement and indirectly affects the accuracy of the velocity measurements. If the signal was too noisy, it was not possible to determine the velocity accurately. For the charge, an uncertainty of less than 0.4 e is required to assign the integral charge with an accuracy of 99%. Prior techniques and apparatus have achieved 2.3 e using a recirculating trap and 10 e using a detector array. One aspect of the present disclosure is that we have discovered that the signal to noise ratio for a detector array can be significantly decreased using the design described herein.

[0037] **The Main Contributors to the Signal to Noise Ratio**

[0038] The signal to noise ratio of a charge detector system depends upon five basic elements: A) the overall noise baseline of the amplification electronics; B) the net gain of the amplification electronics; C) the intrinsic noise of the detectors themselves; D) the quality of the signal produced by the detectors upon passage of an ion; and E) the noise scaling of the data processing algorithm. Putting all of these components together yields an equation of the form:

$$SNR \propto \frac{BDE}{\sqrt{(BC)^2 + A^2}} \quad (1)$$

where the letters refer to the contributions outlined above. While not bound by theory, a discussion of each factor will be included herein so that the present disclosure can be ascertained in its entirety.

[0039] A) Noise baseline of amplification electronics

[0040] The noise of the amplification electronics contribute to the overall SNR of an image charge detector. In illustrative embodiments, a low noise amplifier is used for the first amplification stage. In one embodiment, the amplification electronics includes a low noise junction gate field-effect transistor (JFET). In another embodiment, a low noise preamplifier is used with a low noise JFET for the first amplification stage. In one embodiment, a hybrid charge sensitive preamplifier is used. In another embodiment, the preamplifier and the charge sensitive preamplifier are electrically connected but spatially separate so that the JFET may be cooled separately from the preamplifier to reduce noise. In one embodiment, the noise performance of the preamplifier is such that its contribution to JFET and detector noise is negligible in all charge amplifier applications, i.e., it is essentially an ideal amplifier. In one embodiment, the amplification electronics includes an Amptek A250 preamplifier and a low noise JFET. Referring now to FIG. 1, shown is a schematic of the amplification electronics showing a preamplifier, a JFET, and a 100GΩ feedback resistor to eliminate thermal resistor noise and to raise the RC time constant that results from the use of very small feedback capacitance. In one embodiment, the feedback resistor is at least about 1GΩ. In another embodiment, the feedback resistor is about 10GΩ. In yet another embodiment, the feedback resistor is between about 1GΩ and 10TΩ. In illustrative embodiments, the feedback capacitance is as small as possible to maximize the gain of the first amplification stage. One aspect is that the feedback capacitance is set by the capacitance of the board traces. In one embodiment, the feedback capacitance is less than 100 femtofarads. In another embodiment, the feedback capacitance is between about 0.1 and about 100 femtofarads. In another embodiment, the feedback capacitance is between about 1 and about 20 femtofarads. The noise of the amplification electronics can be estimated by analyzing the noise density without any detectors present.

[0041] B) The net gain of the amplification electronics

[0042] One aspect of the present disclosure is that the, JFET is selected so that the input gate capacitance is matched to the total capacitance of the detector array to ground. As the capacitance to ground is increased the gain decreases. This gain decrease also lowers the gain of the noise from other sources external to the electronic circuit, but does not affect the noise which is intrinsic to the electronic circuit. Measuring the noise contribution which is from the electronic circuit and not the surrounding circuit board and other insulators is challenging and is partially accounted for in the value of C. The gain can be estimated by measuring the relative gain upon addition of a single detector unit. The relative gain, b , can be represented by:

$$b = \frac{\text{Gain with 1 detector present}}{\text{Gain with no detectors present}} \quad (2)$$

For a detector array this becomes

$$B = b^{\frac{n}{m}} \quad (3)$$

where n is the number of detector units and m is the number of amplifiers present.

[0043] C) The intrinsic noise of the image charge detectors

[0044] One aspect of the present disclosure is the discovery of a detector structure having lower intrinsic noise compared to those previously described. In one embodiment, a detector comprises a conductor surrounded by a grounded shield. It was determined that the intrinsic noise of the detector, within the scope of the present disclosure, depends on both the design of the detector and the insulator used to isolate the detector from the shield. The intrinsic noise for one detector (c) is determined by measuring the background noise level of the amplifier without a detector attached and then measuring the noise level of the amplifier with one detector attached. In the alternative for a group of detectors, the noise contribution from a single detector can be deduced by assuming the noise adds as the sum of the squares. The total noise contribution from a group of detectors becomes

$$C = c \sqrt{\frac{n}{m}} \quad (4)$$

where, as before, n is the number of detectors in the array, and m is the number of amplifiers used.

[0045] Another aspect of the present disclosure is the appreciation that the value of c may be minimized to provide SNR benefits. Factors contributing to c

include the surface area of insulator contacting the pick-up electrode, the volume of insulator used to isolate the electrode, and nature and/or identity of the material being used as the insulator.

[0046] Another aspect of the present disclosure is the discovery that the selection of an appropriate insulator minimizes the SNR. Illustratively, the following tests were done to establish an appropriate insulator. Various insulators, configured as two flat pieces of insulator, were clipped, one each side, to a flat electrode connected to an amplifier input. On the other side of each insulator, a second electrode matching the first electrode was placed to sandwich the insulator. The outside electrodes were connected to ground. The nominal thickness of each material was 0.125 cm with a surface area of 4 cm². The measured values were corrected to subtract the noise contributed by the amplifier. Measurements of the spot noise density at 20kHz were made on a Stanford Research Systems SR760 FFT spectrum analyzer. All measurements were taken within 5 minutes of clamping the sample into the holder. The results are shown in Table 1.

Table 1: The relative noise levels measured for a variety of different insulation materials using the approach described in the text.

Material	Relative Noise
TEFLON®	1.00
UHMW	1.18
Polyethylene	
VESPEL®	2.38
MACOR®	2.38
PLEXIGLAS®	2.75
Polycarbonate	2.75
DELRIN®	2.75
Polyether ether ketone	2.90
TECHTRON®	3.90
KEL-F®	4.10
Polyvinyl chloride	6.26
NYLATRON®	11.6
TORLON® 4301	13.0
ULTEM®	>28

[0047] It was determined that a wide range of relative noise values can be attributed to the various insulators. The relative noise values ranged from the lowest polytetrafluoroethylene (PTFE) (*e.g.* TEFLON®) to amorphous thermoplastic polyetherimide (PEI) (*e.g.* ULTEM®), where the noise was too large to measure. ULTEM® performs more than 28 times worse than TEFLON® as an insulator within the scope of the present disclosure. According to Table 1, some general inferences were determined.

[0048] In illustrative embodiments, the image charge detector includes an insulator that is non-polar. In one embodiment, the image charge detector includes an insulator that is PTFE. In another embodiment, the image charge detector includes an insulator that exhibits an amount of noise less than or equal to TEFLON®. Within the present use, polar polymers, amorphous materials, and heterogeneous polymer mixtures exhibit greater relative noise than non-polar, crystalline, and homogeneous polymers. It was surprising that TEFLON® and KEL-F® would exhibit such divergent insulative utility within this application considering they differ only by a chlorine atom on every other carbon. This observation is believed to be based on polarity in part by the similar observation between the ultrahigh molecular weight polyethylene (UHMWPE) and the polyvinyl chloride (PVC) which also differ primarily in their polarity. It was determined that the noise could be decreased by approximately five fold by using an insulator that is non-polar.

[0049] In illustrative embodiments, the image charge detector includes an insulator that is a homopolymer. It was determined that a homogeneous insulator performs better as an insulator within the scope of the present disclosure than a heterogeneous polymer. For example, it was determined that polyimide homopolymers (VESPEL®) and polyamide homopolymers (NYLATRON®) exhibit significantly better performance within the scope of the present disclosure than a polyamide-imide copolymer (TORLON®).

[0050] In illustrative embodiments, the image charge detector includes an insulator that is free of internal stress. In one embodiment, the image charge detector includes an insulator that has been annealed so as to remove internal stresses. It was determined that the internal stress and thermal history of the insulator can affect its performance within the scope of the present disclosure. For example, a piece of TORLON® was test the effect of internal stress on performance. According to this test, TORLON® was machined to a 0.125 cm thick disk with a 2 cm diameter and the

noise was measured as described above. The sample was then placed in an oven and heated to 350°C. This temperature is above the glass transition point for TORLON® so it was held in a mold to maintain its shape. After heating for 3 hours it was quickly cooled to room temperature and the noise was re-measured. The noise had decreased by approximately 18%. After an additional 20 minutes, noise dropped to 76% of the initial noise level.

[0051] In illustrative embodiments, the image charge detector includes an insulator that has external stresses minimized. In one embodiment, the image charge detector includes an insulator that has been incorporated into the image charge detector so as to not place stress on the insulator. One aspect of the present disclosure is that stress placed on the insulator can increase the noise. For example, it was determined that some polymeric materials (*e.g.* ULTEM®, TECHTRON®) are highly sensitive to clamping force and clamping duration.

[0052] In illustrative embodiments, the image charge detector includes a pick-up electrode. In one embodiment, the pick-up electrode is in contact with a second insulator. In another embodiment, the second insulator comprises an insulator that is homogeneous, non-polar, internally stress free, and/or externally stress free. One aspect of the present disclosure is that was determined that the insulator in contact with the pick-up electrode influenced noise within the scope of the present disclosure. In particular, it was investigated whether using bilayers of TORLON® 4203 and TEFLON® influenced the SNR. For these tests the thicknesses of the insulators were nominally 0.25 cm thick. Noise measurements were taken with TORLON® on the inside, contacting the pick-up electrode that is connected to the amplifier. The pieces were then swapped so that the TEFLON® was in contact with the electrode and the TORLON® was next to the grounded plates. With these arrangements the capacitance between the electrodes remains constant, but the material which contacts the pick-up electrode changes. The results, normalized to TEFLON® and corrected for the contribution to the noise from the amplifier, are summarized in Table 2.

[0053] **Table 2:** The relative noise levels for TEFLON®, TORLON® 4203, and TEFLON®/TORLON® and TORLON®/TEFLON® bilayers.

Material	Relative Noise
TEFLON® Alone	1.00
TORLON® 4203 Alone	4.57

TEFLON® Inside, TORLON® 4203 Outside	2.30
TORLON® 4203 Inside, TEFLON® Outside	2.55

[0054] The results show that the material contacting the pickup electrode makes a difference to the noise.

[0055] D) Quality of Signal Output

[0056] Another aspect of the present disclosure is that the quality of the output signal will affect how well the data processing method identifies the signal. In illustrative embodiments, for the processing method employed here (see below), the optimum gain is achieved with a square wave function with a 50% duty cycle. The real signal departs from this ideal by some amount. The relative gain of the signal can be calculated in the following way:

$$D = \frac{\sqrt{\sum f(t) \times f(t + \tau)}}{\sqrt{\sum g(t) \times g(t + \tau)}} \quad (4)$$

Where $f(t)$ is the normalized intensity of the real waveform and $g(t)$ is a normalized intensity of the idealized waveform which the data processing algorithm is optimized for, both with a period equal to τ . The end result if we assume our real signal, $f(t)$, to maintain a shape which is similar to a square wave, but with an altered duty cycle is the following:

$$D = \frac{\text{Duty Cycle of } f(t)}{\text{Duty Cycle of } g(t)} \equiv \frac{L - ns}{L} = 1 - \frac{ns}{L} \quad (5)$$

Where n is the number of detectors in the array, s is the distance between adjacent detection cylinders, and L is the total length of the detection array. This approximation is accurate until the length of the pick-up cylinder is close to the inside diameter of the pick-up cylinder. Including a simple $\sin$ function for the finite rise time of the signal results in the approximate expression:

$$D = 1 - \frac{ns}{L} - \frac{2nr}{5L} \quad (6)$$

where r is the radius of the pickup cylinder.

[0057] E) Data Processing Algorithm

[0058] Another aspect of the present disclosure is that the data processing algorithm used influences SNR. In illustrative embodiments, the following auto-correlation algorithm is used:

$$g(t, w) = c(w) \sum_{k=k_{\min}}^{k_{\max}} b(k) \left| \sum_{n=0}^w (f(t+n) - a(t, w)) * \left(f\left(t+n+\frac{wk}{22}\right) - a(t, w) \right) \right|^{1/2} \quad (7)$$

[0059] It was determined that this approach is advantageous to those alternative approaches (*e.g.* Fourier transform or correlation to a square wave) used for CDMS within the prior art. In particular, it was determined that this method is superior to Fourier transform or correlation to a square wave at rejecting non-gaussian noise contributions. This is significant because, the noise profiles coming from certain materials within the scope of the present application are non-gaussian in nature. Furthermore, the auto-correlation algorithm described herein provides better rejection ratios for shot noise (by around 10^3) and step functions (by around 10^1) than provided by a correlation to a square wave. Since the non-gaussian nature of the noise is the largest impediment to determining when an ion is in the detector, this is an important feature of any data analysis method which uses short time spans to detect a lowly charged ion (less than about 200 electrons).

[0060] Assuming that the quality of the waveform is unchanged, the SNR scales as the square root of measurement time and as the square root of the number of k values utilized. This is because each summation over any particular value of k has a random noise contribution which is then averaged with all other k values and so it scales as the root of the number of averaged values. The number of k values which are available to be averaged is linear with the number of detectors such that if we have n detectors then there are $2n$ values of k that can be used.

[0061] The SNR scales as the root of measurement time since doubling the time provides twice as many data points to average over. Accordingly failure to change the ion source conditions when increasing the total length of the detector array will increase the measurement time in a linear fashion. The net result of this analysis method is captured in the following:

$$E = \sqrt{Ln} \quad (8)$$

where L is the total length of the detector array and n is the number of detection cylinders.

[0062] **Equation for the Overall Signal to Noise Ratio for an Image Charge Detector Array**

[0063] With the contributions outlined above the overall signal to noise ratio is given approximately by the following equation:

$$SNR = \frac{b^{\frac{n}{m}} \times \sqrt{Ln} \times \left(1 - \frac{ns}{L} - \frac{2nr}{5L}\right)}{\sqrt{\left(b^{\frac{n}{m}} \times c \sqrt{\frac{n}{m}}\right)^2 + A^2}} \quad (9)$$

[0064] One aspect of the present disclosure is that the equation shows that if the total length of the detector array is increased by adding more cylinders and amplifiers, as opposed to increasing the length by elongating each cylinder, then the signal to noise ratio becomes linear with L , i.e. if the length is doubled then the signal to noise ratio is also doubled.

[0065] The equation can be used to evaluate the performance of an existing charge detector array, which was described herein as Example 1. For this detector array, $A = 1.0$, $b = 0.98$, $c = 0.2283$, $L = 58.2$ cm, $s = 0.292$ cm, $n = 44$, $m = 4$, and $r = 0.20$ cm. In particular, it has 44 detectors ($n=44$) and 4 amplifiers ($m=4$). FIG. 2 shows the effect on the signal to noise ratio as the number of detection cylinders varies (while keeping all the other parameters fixed). The signal to noise ratio in FIG. 2 initially increases as the number of detectors increases, and then decreases. As can be seen from Equation 9 the dependence of the signal to noise ratio on the number of detectors (n) is complex. However, the factor mainly responsible for the increase in the signal to noise ratio at small n is E (the $\sqrt{Ln}$ term), and the term mainly responsible

for the decrease in the signal to noise ratio for large n is D (the $1 - \frac{ns}{L} - \frac{2nr}{5L}$ term).

The peak in the signal to noise ratio occurs at 32 detectors which is less than the 44 used in the existing detector array. The dependence of the signal to noise ratio for the existing 44 detector array (with the parameters outlined above) on the number of amplifiers is shown in FIG. 3. The signal to noise ratio initially increases rapidly as the number of amplifiers increases, but then the rate of improvement slows substantially. There is about a 33% gain in the signal to noise ratio if the amplifier count is raised from 4 to 20 and about a 22% decrease in signal to noise ratio on going from 4 to 2 amplifiers. This system with 44 detectors described above yields a charge error of 5 electrons rms.

[0066] **Extension to m/z Accuracy**

[0067] The principal figure of merit in charge detection mass spectrometry is the accuracy of the mass measurement. The accuracy of the charge measurement

(which we focus on above) is only one piece of this problem. The other main contributor to the overall mass accuracy is the accuracy of the m/z measurement. The m/z ratio is determined from velocity measurements in the two groups of detectors that are operated at different potentials. Assuming the ion can be detected, the accuracy of the m/z ratio measurement is the product of the SNR and the number of detectors. The reason for this is that the velocity accuracy is linearly related to the number of detectors, while the ability to identify the ion's true velocity is approximately proportional to its signal to noise ratio. Altogether, this yields the following equation for the accuracy of the m/z determination:

$$m/z \text{ accuracy} \propto n \times SNR = n \times \frac{b^{\frac{n}{m}} \times \sqrt{Ln} \times \left(1 - \frac{ns}{L} - \frac{2nr}{5L}\right)}{\sqrt{\left(b^{\frac{n}{m}} \times c \sqrt{\frac{n}{m}}\right)^2 + A^2}} \quad (10)$$

[0068] FIG. 4 shows a plot of the m/z accuracy versus the number of detectors. The peak occurs near 80 detectors. With this number of detectors the mass to charge resolution is approximately 1.3x better than with 44 detectors. The tradeoff in this case is a decreased SNR for the charge measurement. This decrease lowers the SNR by approximately 1.3x, which adversely affects the minimum charge state ion that can be detected.

[0069] **Implications for Detector Design**

[0070] Accordingly, the present disclosure relates to a number of ways in which the SNR of m/z ratio and m determination can be enhanced by the structure of an image charge detector array and the methods of using that array, in particular the applied correlation functions. In illustrative embodiments, the SNR is minimized by minimization of the amount of insulator used in any particular detector design, especially taking into consideration the area nearest the pick-up cylinders. It was determined that this lowers the white noise of the system as well as lowering the non-gaussian contribution to the noise. As discussed herein, the insulator should be selected to have the lowest relative noise contribution and should not be mounted in a stressed configuration. Furthermore, the detector should be cooled. It was determined that cooling lowers the noise contributions from thermal noise both in the electronics and in the detector itself within the scope of the present disclosure.

[0071] In illustrative embodiments, the structure of the image charge detector is shown in FIG. 6. This is one solution to these design criteria described above. Noteworthy aspects of this design include the minimized insulator to electrode contact area through the use of raised ridges on the detection cylinder and the use of a groove in which the insulator is located. In particular, the groove is important in compensating for the different thermal expansion coefficients of the insulator and the conductor. It enables the insulator to remain well located and vibration free even as it shrinks more than the surrounding conductor does upon cooling.

[0072] The image charge detector of FIG. 6 was constructed and achieved a c value of 0.157 with TEFLON® as the insulator and 0.108 with polycarbonate as the insulator versus a relative value of 1.0 for the optimized electrical circuit utilizing a 2SK152 JFET. If we take this value of 0.108 for c and use the same r value of 0.20 cm, increase L to 67.3 cm, use $b = 0.98$, increase m from 4 to 14, and shorten s to 0.075 cm then we find that optimum performance should be obtained with 106 detection cylinders (*see* FIG. 5).

[0073] The value of A can be improved through the use of the IF140 JFET instead of the 2SK152. The improvement in A from a different JFET is roughly 40%, though this results in a slightly lower value of b . Further improvements such as cooling the detector array are expected to increase A by an additional 80% and decrease C by a factor of 2 as well. These improvements, when combined, are expected to yield a detector with approximately 1 electron of error when measuring an ion for approximately 2 ms. In illustrative embodiments, the detector has less than about 10 electron of error when measuring an ion for approximately 2 ms. In one embodiment, the detector has less than about 5 electron of error when measuring an ion for approximately 2 ms. In another embodiment, the detector has less than about 2 electron of error when measuring an ion for approximately 2 ms. In another embodiment, the detector has less than about 1 electron of error when measuring an ion for approximately 2 ms.

[0074] The following examples illustrate specific embodiments in further detail. These examples are provided for illustrative purposes only and should not be construed as limiting the invention or the inventive concept to any particular physical configuration in any way.

[0075] Overview of the Experimental Apparatus:

[0076] The experimental apparatus is shown schematically in FIG 7. Ions were generated with an electrospray source and transferred into the vacuum through a capillary interface. The electrospray needle was pulled from a 1 mm OD 700 μm ID borosilicate glass capillary to an ID of around 100 nm. The end of the tip was broken off when it became clogged or if the spray became unproductive and so the ID used in the experiments could be larger than 100 nm. The electrospray voltage was applied to the solution through a stainless steel wire. A syringe pump provided a nominal flow rate of 20 $\mu\text{L/hr}$. The electrospray solution was 49.75% water, 49.75% methanol and 0.5% acetic acid with poly(ethylene oxide) (PEG) (MW 300,000, Polysciences, Inc) added to a concentration of 1.0 μM . The gas flow into the vacuum chamber is limited by a 15 cm long stainless steel capillary with an internal diameter of 0.75 mm. The copper block holding the capillary was heated to around 110°C by cartridge heaters. Two conical skimmers were optically aligned co-axially with the capillary tube to provide two differentially pumped regions. The first skimmer (1.0 mm diameter opening) was located 2.5 cm behind the exit of the capillary tube. The second skimmer (0.8 mm diameter opening) was located 5 cm behind the first.

[0077] The first differentially pumped region (between the capillary exit and the first skimmer) was pumped by an Edwards EH-500 mechanical booster pump and operated at around 0.4 torr, the second differentially pumped region (between the two skimmers) was pumped by a pair of Edwards Diffstak 250/2000M diffusion pumps and operated at around 1×10^{-5} torr. The final chamber (beyond the second skimmer) where the charge detector array was located operated below 1×10^{-6} torr with pumping provided by an Edwards Diffstak 160/700M diffusion pump.

[0078] The expansion of the gas as it traveled through the capillary caused an aerodynamic acceleration of the ions entrained in the gas flow. The aligned skimmers, when combined with the 0.5 mm diameter aperture at the beginning of the charge detector, only allowed ions to enter the charge detector if they were within an acceptable angle to pass cleanly through the entire array (i.e. without colliding with the detector walls). An ion detector, consisting of an orthogonal collision dynode and a pair of microchannel plates, was located after the charge detector to assist in optimizing the electrospray source. It only detected ions that passed through the detector array and it did not detect objects with m/z greater than around 1 MDa/e (in order to eliminate

signals from solvent droplets). Both ions and droplets were accelerated in the capillary interface, but the desolvated ions (with their smaller masses) have much lower kinetic energies than the droplets and so they were much easier to deflect onto the collision dynode than droplets.

[0079] The Charge Detector Array

[0080] The exemplary charge detector array consisted of 22 image charge detector tubes in series. The detector tubes were separated by grounded tubes. The 22 detector tubes were divided into two groups of 11 as illustrated in FIG 8. Each set was connected in parallel to a single amplifier and the sets were electrically isolated from each other, and from ground. A potential difference was applied between the sets by floating the potential of one set relative to the other. The ions traveled at different velocities in the two sets of detectors when they were set at different potentials and the velocity difference, along with the potential difference between the two sets of detectors allowed the m/z ratio to be determined without knowledge of the ions initial kinetic energy. With charge detection technology an important performance parameter to control is measurement time, which was set by the ion's velocity. In a heterogeneous mixture of ions with different masses and m/z values, different kinetic energies were required to achieve the same velocity. By making the experiment agnostic with regard to the initial kinetic energy, the ion velocities were set by the aerodynamic expansion at the capillary interface. For the results reported here, the second set of detectors was floated while the first is set to ground.

[0081] The individual tubes in the charge detector array (Figure 2) were modeled in COMSOL Multiphysics® (COMSOL, Inc) and SIMION® to optimize their performance. The important parameters in the design of a single unit for use in an array are: capacitance to ground, signal rise time, cross-talk to neighboring cylinders and electrical shielding, quantity of insulator material, and dead space. The final dimensions of the detectors were as follows: 12.7 mm wide grounded shield, 10.16 mm detector image detection tube length with 4.75 mm O.D. and 4.11 mm I.D., 0.13 mm thick end shield plates, and 0.51 mm thick insulation separating neighboring detectors. This choice of parameters yields rise times that are typically around 1 μ s with a capacitance per tube on the order of 1 pF while allowing a reasonable angle for the ions to traverse without colliding with individual detector tubes. The thin insulator that separates the shields at the interface between the two sets of detectors does allow for some noise contamination, but it is compensated by the use of extremely low

impedance ($< 0.01 \text{ Ohm}$) four stage low pass filter that connects the shields of the two detector groups together.

[0082] The amplifiers were designed and constructed using custom printed circuit boards. The charge detection amplifier was located in the vacuum chamber as close to the array as possible. The first circuit board contained a preamplifier based around an Amptek A250 preamplifier and an Interfet IFN152 FET. This board outputs a signal directly to another board that contains a local op-amp based active voltage filter and passive voltage filter circuitry and a differential amplifier based on a pair of Analog Device AD797B op-amps. The output of the differential amplifier is passed out of the vacuum chamber to the differential input of an analog to digital converter which sampled at approximately 2 MHz with 15 bits of resolution. The ADC units output the digital data over fiber optic connections to a computer for data storage and offline processing.

[0083] The power supply for the charge detection amplifiers was provided by rechargeable NiMH battery packs. A voltage regulator, a passive low voltage filter stage, an op-amp based active filter network, and another passive voltage regulation low pass filter were utilized to clean up the battery power before it passed into the vacuum chamber to power the detectors. In addition to the steps required to smooth the supply voltages, the effective ground potential between the detectors was set by the use of a Hewlett-Packard E3612A which was filtered through a four stage low pass passive circuit.

[0084] An example of the raw output signal is shown in FIG. 9. This signal is the unprocessed output of a single charge detection amplifier (one set of detectors) for a highly charged macroion. The macroion has a charge of around 2,500 e and a velocity of around 580 m/s. In this example, it is easy to identify the responses from the 11 detection tubes as the macroion travels through them.

[0085] Data Processing

[0086] A number of approaches were considered to process the data. Previously point averaging (Benner, 1997) and FFT (Fuerstenau, 2001; Benner, 1997) methods have been used to analyze repetitive CDMS signals. The use of time domain signal averaging is only appropriate for signals that significantly extend above the noise floor (because it is necessary to locate the signals precisely before averaging them together) and the FFT method suffers from poor frequency resolution on medium size signals, like that resulting from the 22 detector setup used in this experiment. It was

determined that the approach that yielded the highest charge and frequency accuracy was to selectively auto-correlate the data and then correlate the output of this signal to an expected output pattern. This method affords very accurate velocity and charge values. A Fortran program was written to process the data using this approach.

[0087] The first step in processing the data is to locate a signal. This is accomplished by stepping an auto-correlation function across the signal. We take $f(t)$ to be the raw signal from the digitizer, $a(t, w)$ to be a rolling average from t to $t+w$, and w to be the total length of the signal (the time the ion spends in the detector array). Note that w is not known (it needs to be determined from the data) and so we step through reasonable values of w consistent with the time resolution to get a reconstructed signal given by:

$$g(t, w) = c(w) \sum_{k=k_{\min}}^{k_{\max}} b(k) \left| \sum_{n=0}^w \left(f(t+n) - a(t, w) \right) * \left(f\left(t+n+\frac{wk}{22}\right) - a(t, w) \right) \right|^{1/2} \quad (11)$$

If $\left(n + \frac{wk}{22}\right) > w$, $\left(n + \frac{wk}{22}\right)$ in the second term in the summation is replaced with

$\left(n + \frac{wk}{22} - w\right)$ so that it wraps around to small values. $c(w)$ in Equation 11 are

normalization factors. The value of $b(k)$ is set to +1 for even k and -1 for odd k values.

For the correct value of w , Equation 11 preferentially amplifies repetitive in-phase signals. FIG. 10 shows its response (with the correct value of w) to a 50% duty cycle square wave that simulates the output from the charge detector array. The output is a triangle with an amplitude that is 18 times the amplitude of the original square wave (the factor of 18 comes from the sum over k which runs from 3 to 20 in this implementation). The width of the triangle is $2w$ (twice the total length of the signal). For values of w that are larger or smaller than the correct value, the triangular waveform is truncated to yield a trapezoid.

[0088] One advantage of the function described herein is its response to sharp spikes and steps in the baseline. In other approaches that we tried, we found that false positives from spikes and steps to be a significant problem. With the function used here the spikes and steps are attenuated by around 10^6 and 10^2 , respectively. Information on the sign of the charge on the ions is lost in this analysis, however, this is easily recovered from whether the ions are accelerated or decelerated by the voltage

change between the two sets of detectors. In the studies reported here we used positive mode electrospray and all the detected ions were positively charged.

[0089] After the function $g(t,w)$ is generated, it is passed through a low pass filter to remove unwanted high frequency components. For the correct value of w , $g(t,w)$ is expected to be a triangular waveform, and so the best performance should be obtained from a triangular smoothing function. However, we found that smoothing with a triangular function to be computationally expensive, and so instead we employed a filter consisting of four boxcar filters to approximate the triangular shape:

$$G(t,w) = \sum_{m=1}^4 \sum_{n=-\frac{w+m}{10}}^{\frac{w+m}{10}} g(t,w) \quad (12)$$

[0090] Accordingly, the filter is much faster than a full triangular smoothing function, with a performance that is almost as good.

[0091] $g(t,w)$ and the smoothed $G(t,w)$ both yield triangular waveforms with the correct value of w . Away from the correct value of w , both functions are truncated to a trapezoid. The correct value for w is determined by doing calculations with a range of different w values (consistent with the experimental time resolution) and then selecting the value that has the largest amplitude. The amplitude of the triangular waveform provides the charge and the width is related to the velocity of the ion. FIG. 11 illustrates the response of the correlation analysis to an ion travelling through one group of detectors at 491 m/s. The figure shows a plot of the normalized output amplitude of the correlation analysis against w (plotted in terms of the ion velocity). The peak at 491 m/s indicates the correct velocity. The small features at around 300 m/s are due to overtones.

[0092] A typical noise profile for the output of the $G(t,w)$ function is shown in FIG. 12. This was generated by analyzing a blank data set (i.e., a data set without a signal). The plot shows the rms deviation of the signal as a function of the time w . For pure white noise the rms deviation should decrease as $t^{-1/2}$. The broad peak in the noise at around 1.2 ms results from ~4 kHz interference present in the room (from an unknown source). Beyond around 2 ms, 1/f noise becomes dominant and the noise ramps up to be 20-30 e at ~4 ms. In the 0.6 ms to 1.8 ms region of interest the rms noise is 9-11 e. This limits the accuracy of the charge measurement. The noise can be reduced further by calculating more than just the peak values in the auto correlation as

well as using a full triangular smoothing function. These improvements will be implemented in the future.

[0093] Data Analysis

[0094] The experimental parameters obtained from the data processing are the initial velocity, the shifted velocity, and the charge. In addition, we know the voltage change used to shift the velocity. From conservation of energy:

$$\frac{1}{2}mv_{SHIFTED}^2 = \frac{1}{2}mv_{INITIAL}^2 - qV \quad (13)$$

where m is the mass of the ion, q is the charge, $v_{SHIFTED}$ and $v_{INITIAL}$ are the shifted and initial velocities, and the effect of the voltage change V is to decelerate the ions.

Deceleration provides better mass resolution than acceleration. For the results reported here, the voltage on the second group of detectors was set to +1 V with respect to the first group. This value is optimized for the detection of ions with relatively small m/z values. A larger offset voltage could be employed for ions with larger m/z values.

Equation (13) can be rearranged to yield an expression for the mass:

$$m = \frac{2qV}{v_{INITIAL}^2 - v_{SHIFTED}^2} \quad (14)$$

[0095] Inserting the measured values into this equation yields a mass for each ion which can then be binned into a histogram to yield a m spectrum (in contrast to the m/z spectrum usually obtained from mass spectrometry measurements).

[0096] The results from the electrospray of 300 kDa PEG are divided into two parts. First, we show the results for ions with a mass of less than 1MDa. Then, we show all the data where the velocity is shifted by at least 1%.

[0097] FIG. 13 (A-C) shows mass, m/z ratio, and charge state distributions for ions with a mass of less than 1MDa. The mass distribution (FIG 13A) shows a broad peak centered around 320 kDa with a FWHM of around 240 kDa. The width of the peak in the mass distribution is mainly due to the heterogeneous nature of the sample (see below). FIG. 13(B) corresponds to the measured m/z distribution. The peak in this distribution is centered at around 2,000 Da/e. FIG. 13(C) shows the charge distribution which extends from 100 to 400 e. Here, the charge distribution was truncated and results where the charge is less than around 100 e were not analyzed. This truncation is responsible for the sharp lower bound in the charge distribution in each of FIG. 13(A-C).

[0098] FIG. 14(A-B) shows the velocity distribution measured in the first and second groups of detectors. FIG. 14(A) shows the velocities measured in the first group, and FIG. 14(B) shows the velocities recorded in the second group which is floated to a positive potential to decelerate positive ions. The initial velocities have a peak centered around 425 ms^{-1} , with a high velocity tail extending to almost 600 ms^{-1} . All of the ions' kinetic energy results from the expansion at the end of the capillary interface. After deceleration the peak in the velocities is shifted to around 280 ms^{-1} . The substantial shift in the velocities ensures that the m/z values deduced from the velocity shift are accurate. The uncertainty in the m/z values is around 5%. As we discuss in more detail below, this relatively large uncertainty in the m/z values for ions with masses less than 1 MDa results mainly from their relatively low charge. The low charge leads to a low signal to noise ratio which makes it difficult to determine the velocities accurately.

[0099] The data where the velocity was shifted by at least 1% was also analyzed. FIG. 15(A-C) shows the mass, m/z , and charge distributions respectively. FIG. 15(A) shows the mass distribution which contains at least three components. The peak at lowest mass (close to the origin) consists mainly of the relatively low m/z and low charge ions with masses around 300 kDa (which are discussed above). In addition there are two other peaks at around 100 MDa and 500 MDa. There may be another peak at around 1.25 GDa but it is too poorly defined to be sure. Masses are observed to beyond 2 GDa. FIG. 15(B) shows the m/z distribution for ions with velocity shifts of at least 1%. This distribution shows two components: the lower charge one with a peak near the origin corresponds to the low m/z ions that contribute the mass peak at around 300 kDa. The large peak in the m/z distribution at around 40 kDa/e is due to the higher mass features in the mass distribution (i.e., the peaks at around 100 MDa and 500 MDa. FIG. 15(C) shows the charge distribution for ions with velocity shifts of at least 1%. The charge distribution looks similar to the mass distribution because the charge shows a correlation with the mass. Thus the peak in the charge distribution at around 3,000 e is due to ions with masses around 100 MDa, and the broad peak in the charge distribution at around 12,500 e is due to ions with masses of around 500 MDa.

[00100] The velocity shifts for the 100 MDa and 500 MDa ions are much smaller than for the 300 kDa ions. Thus the m/z values derived from the velocity shifts are less reliable than for the smaller ions. The uncertainties in the m/z ratios for the heavier ions are around 10-20%. More reliable m/z values could be obtained for the

larger ions by using a higher voltage on the second group of detectors (the higher voltage will lead to a larger velocity shift and hence more reliable m/z values). An increase in the voltage for the results reported here, although possible, was not attempted because our main focus was on the lighter ions (around 300 kDa), and significantly raising the voltage on the second detector would discriminate against the lighter ions.

[00101] There were some ions with velocity shifts that are less than 1%. These ions have m/z ratios that are around 100 kDa/e and masses that extend up to 10 GDa. However, because of the small velocity shift, the uncertainty in the m/z ratio can approach 100%.

[00102] The combined uncertainty of the mass measurements

[00103] The uncertainty in the masses obtained from the image charge detector array is a combination of the uncertainties in the m/z ratio and the charge. The relative uncertainty in the m/z values is given by the following equation:

$$\frac{\sigma_{m/z}}{m/z} = \frac{1}{v_{INITIAL}^2 - v_{SHIFTED}^2} \sqrt{(2v_{INITIAL}\sigma_{VINITIAL})^2 + (2v_{SHIFTED}\sigma_{VSHIFTED})^2} \quad (15)$$

where $\sigma_{VINITIAL}$ and $\sigma_{VSHIFTED}$ are the uncertainties in the initial and shifted velocities, respectively. The uncertainty in the m/z values increases when the difference between the initial and shifted velocities decrease, and when the uncertainties in the initial and shifted velocities increase. The uncertainty in the velocities depends on the charge of the ion. When the charge on the ion is small, the unfavorable signal to noise ratio means that it is difficult to determine the velocity accurately. The uncertainty in the velocities from this source can be estimated from Figure 5 which shows the normalized response of the digital filter used to analyze the signals from the image charge detector array plotted against ion velocity. If the signal to noise ratio approaches infinity then the velocity is precisely defined at the maximum in the plot, but if the signal to noise ratio is 2 then the uncertainty in the velocity error can be estimated from half of the FWHM of the peak, which is 4.5%. For the ions examined here the uncertainty in the charge is 10% or less. The corresponding uncertainty in the velocity can be estimated from half the width of the response peak in Figure 5 at a normalized intensity of 0.9 (or more). Thus the uncertainty in the velocity determinations from this source is, at worst, 1.4%.

[00104] Most of the ions with m/z values centered around 2,000 Da/e have charges of 100-200 e and so maximum uncertainty in the charge is approximately 10%. This yields a maximum mass to charge uncertainty of approximately 5% and a maximum combined mass uncertainty of 11%. For the ions with m/z values centered about 40 kDa/e, the charge is around 10,000 e, and so the relative uncertainty in the charge is much smaller (around 0.1%). Thus the uncertainty in the velocities from the noise on the charge is also much smaller than for the ions with m/z around 2,000 Da/e. However, the velocity change for the 40 kDa/e ions is much less than for the 2,000 Da/e ions since the offset voltage on the second set of detectors to shift the velocity was optimized for the lower mass ion measurements. So the uncertainty in the m/z values for these ions is approximately 15% and quickly becomes larger as the velocity shift decreases further. For the highly charged ions the combined uncertainty in the mass is dominated by the small change in velocity and it is therefore 15%. With a velocity shift voltage of 20 V (instead of the 1 V used in these measurements) the combined uncertainty in the mass would drop below 1%.

[00105] The m spectrum measured for 300 kDa PEG

[00106] The m spectrum measured for 300 kDa PEG shows a broad peak centered around 300 kDa and then peaks at around 100 MDa and 500 MDa. All of these peaks are reproducible and were observed in multiple runs. The 300 kDa peak is broad, however, this is not due to uncertainty in the mass measurements because (as outlined above) the maximum uncertainty expected in this mass regime is 11%. Most of the width of the measured distribution is intrinsic to the PEG sample. The distributor (Polysciences) quotes a distribution that extends from 0.5 to 1.5 times the average mass. The quoted average mass is deduced from solution phase viscosity measurements. Our measured distribution starts at around 150 kDa, peaks at around 320 kDa, and tails off at around 600 kDa. The slight excess of high mass ions could: 1) be intrinsic to the sample; 2) result from incomplete desolvation; or 3) result from the presence of a small amount of dimer. The small maximum at ~900 kDa may result from a trimer.

[00107] The origin of the peaks in the m spectrum at 100 MDa and 500 MDa was uncertain. One explanation that comes to mind is residual water droplets, in which case they would have diameters around 70 nm and 120 nm respectively. The problem with this explanation is that droplets of this size should evaporate away very quickly, and it is not clear why these particular sizes should persist. Furthermore, the 100 MDa

and 500 MDa peaks were not observed when we electrosprayed other solutions. For example, with a highly diluted PEG solution (around 1 nM) we hardly observed any ions. We also did not observe the 100 MDa and 500 MDa peaks when we electrosprayed a BSA (bovine serum albumin) solution. In this case the largest ions observed were less than 10 MDa.

[00108] Another plausible explanation for the high mass peaks is that they result from aggregates of PEG (around 330 PEG molecules for the 100 MDa peak and around 1670 PEG molecules for the 500 MDa peak). It is possible that the aggregates are present in solution. Alternatively, if the PEG is completely dispersed in solution, the aggregates could be the residue from large electrospray droplets. Assuming that the ions are generated by the charge residue model, (Yang, et al. "Zeptogram-Scale Nanomechanical Mass Sensing," *Nano Lett.* 2006, 6, 583-586; Shelton, et al, "Electrostatic Acceleration of Microparticles to Hypervelocities," *J. App. Phys.* 1960, 31, 1243-1246) then the electrospray droplets from which the aggregates originate must be at least 1.0 μm diameter for the 100 MDa peak and at least 1.7 μm diameter for the 500 MDa peak (droplets of 1.0 μm and 1.7 μm diameter contain 330 and 1670 PEG molecules at the concentration employed here). These droplets are around 2 times and 3.4 times larger than expected from the scaling laws for the electrospray conditions (flow rate and solution conductivity) employed here. (Keaton, et al. "A Hypervelocity-Microparticle-Impacts Laboratory with 100 km/s Projectiles," *Int. J. Impact Eng.* 1990, 10, 295-308; Stradling, et al. "Ultra-High Velocity Impacts: Cratering Studies of Microscopic Impacts from 3 km/s to 30 km/s," *Int. J. Impact Eng.* 1993, 14, 719-727) So while it seems likely that the 100 MDa and 500 MDa peaks result from PEG aggregates, it is not clear whether the aggregates are the residues of large electrospray droplets or result from incomplete dispersion of the PEG in solution.

[00109] The mass, m/z , and charge distributions recorded for 300 kDa PEG were presented. The mass distribution shows a peak at around 300 kDa with a width close to that expected from the polymer size distribution. In addition, there are broad peaks in the mass distribution at around 100 MDa and 500 MDa. The 300 kDa ions have m/z ratios around 2 kDa/e and the 100 and 500 MDa ions have m/z ratios around 40 kDa/e. We suggest that 100 and 500 MDa ions probably result from PEG aggregates that are either present in solution or the residue of large electrospray droplets.

WHAT IS CLAIMED IS:

1. A mass spectrometer system comprising an ion source that generates an ion beam and an image charge detector array having n charge detection tubes and m charge sensitive amplifiers electrically connected to one or more charge detection tubes, the charge detection tubes including an electrically conducting tube and a grounded shield comprising an electrical conductor surrounding the tube separated from the electrically conducting tube by an insulator; wherein

n is an integer greater than or equal to four,

m is an integer greater than or equal to two,

the charge detection tubes are grouped into at least two groups, the groups comprising two or more charge detection tubes, a first group operating at a first voltage and a second group operating at a second voltage,

the n charge detection tubes are located in the ion beam path and having a long axis parallel to the flight path of ions in the ion beam.

2. The mass spectrometer system of claim 1, wherein n is from 4 to about 180.

3. The mass spectrometer system of claim 1, wherein n is from 20 to about 120.

4. The mass spectrometer system of claim 1, wherein n is from 30 to about 110.

5. The mass spectrometer system of claim 1, wherein m is from 2 to about 30.

6. The mass spectrometer system of claim 1 wherein, the m amplifiers are JFET transistors operated as high-gain negative feedback amplifiers.

7. The mass spectrometer system of claim 1, wherein the image charge detector array comprises a detector that includes an insulator that is non-polar.
8. The mass spectrometer system of claim 7, wherein the insulator is polytetrafluoroethylene.
9. The mass spectrometer system of claim 1, wherein the image charge detector array comprises a detector that includes an insulator that is a homopolymer.
10. The mass spectrometer system of claim 7, wherein the insulator is a polyimide homopolymer or a polyamide homopolymer.
11. The mass spectrometer system of claim 1, wherein the image charge detector array comprises a detector that includes an insulator that is free of internal stress.
12. The mass spectrometer system of claim 11, wherein the insulator has been annealed.
13. The mass spectrometer system of claim 1, wherein the image charge detector array comprises a detector that includes an insulator that has external stresses minimized.
14. The mass spectrometer system of claim 13, wherein the insulator is incorporated into the image charge detector so as not to place stress on the insulator.
15. The mass spectrometer system of claim 1, wherein the image charge detector array comprises a detector that includes a pick-up electrode.
16. The mass spectrometer system of claim 15, wherein the pick-up electrode is in contact with a second insulator.

17. The mass spectrometer system of claim 16, wherein the second insulator comprises an insulator that is homogenous, non-polar, internally stress free, and/or externally stress free.

18. The mass spectrometer system of claim 17, wherein the second insulator comprises an insulator that is non-polar.

19. The mass spectrometer system of claim 17, wherein the second insulator comprises an insulator that is internally stress free.

20. A method of determining m/z and z of a macroion comprising:
detecting the macroion traveling through a series of charge detection tubes to generate data, the series of charge detection tubes comprising at least a first charge detection tube operating at a first voltage and a second charge detection tube operating at a second voltage; wherein

(a) the step of detecting the macroion includes detecting an image charge on the first charge detection tube for establishing a first time-of-flight;

(b) the step of detecting the macroion includes detecting an image charge on the second charge detection tube for establishing a second time-of-flight; and

(c) the step of detecting the macroion includes detecting an amplitude of the image charge in the series of charge detection tubes, the image amplitude providing z ;

analyzing the data with an auto-correlation function; and

inferring the m/z from the first time-of-flight and the second time-of-flight without knowledge of the initial ion energy.

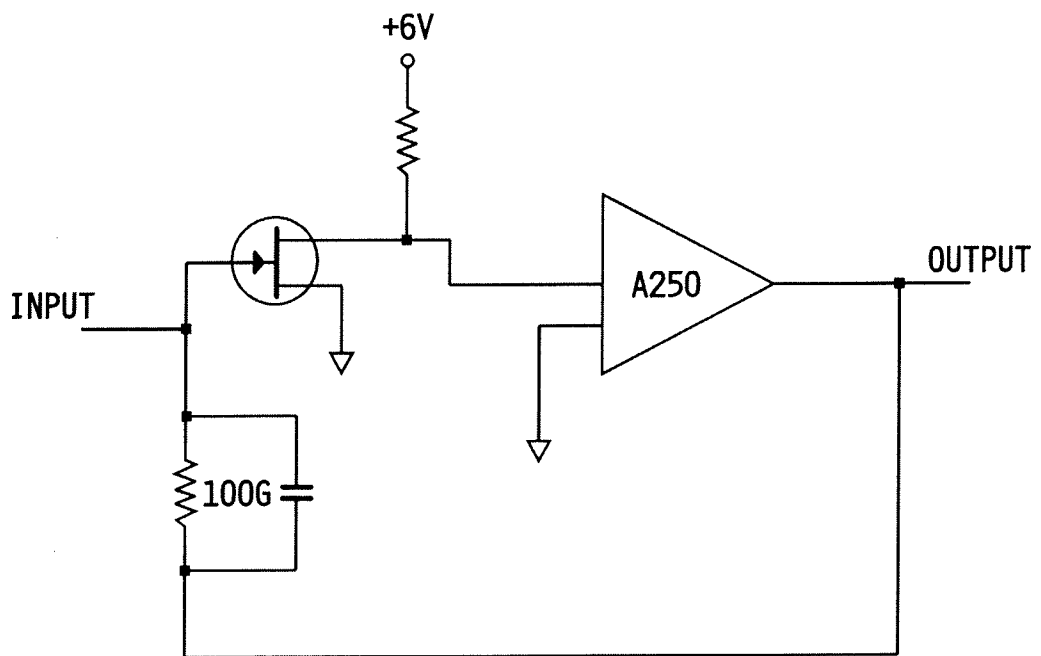


FIG. 1

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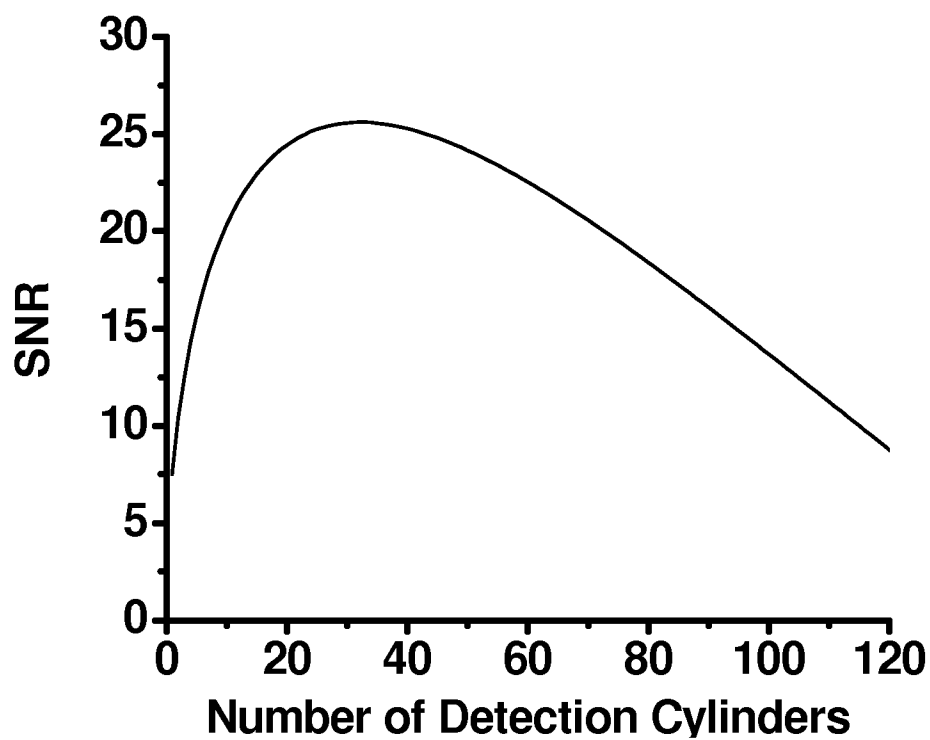


FIG. 2

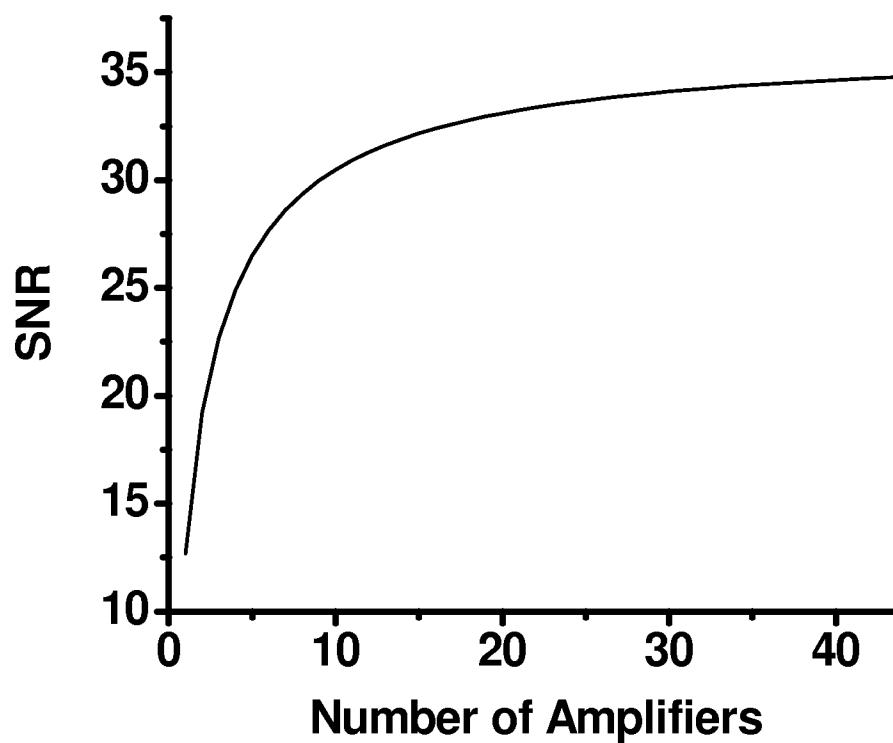


FIG. 3

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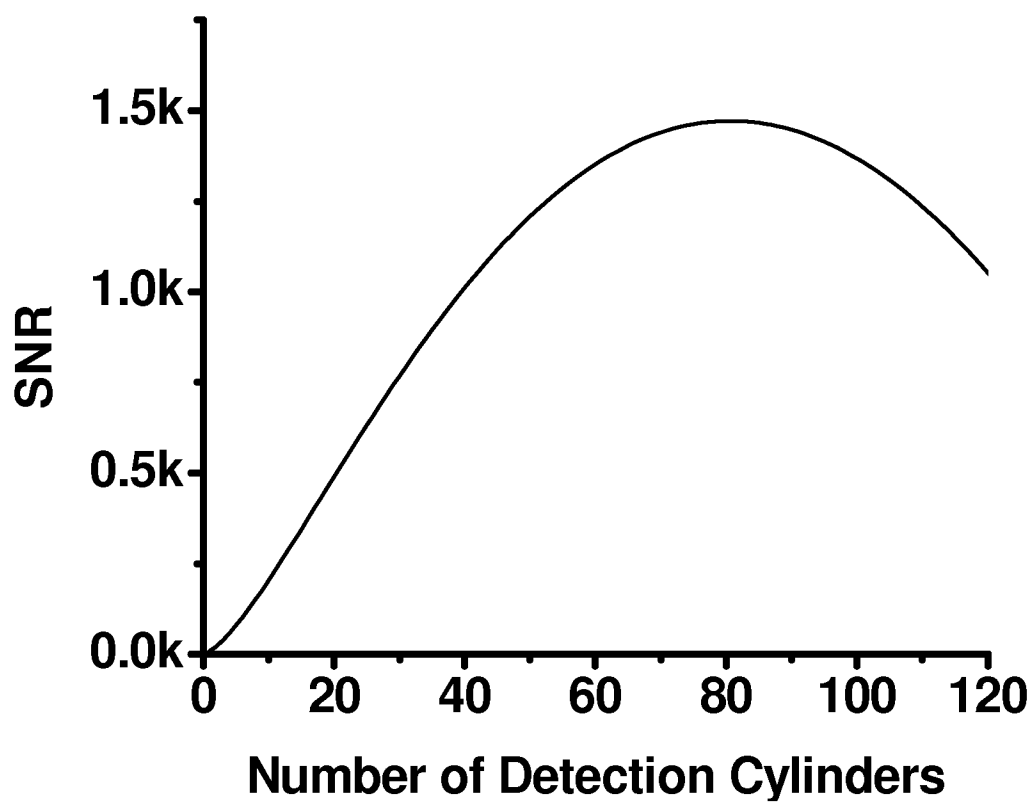


FIG. 4

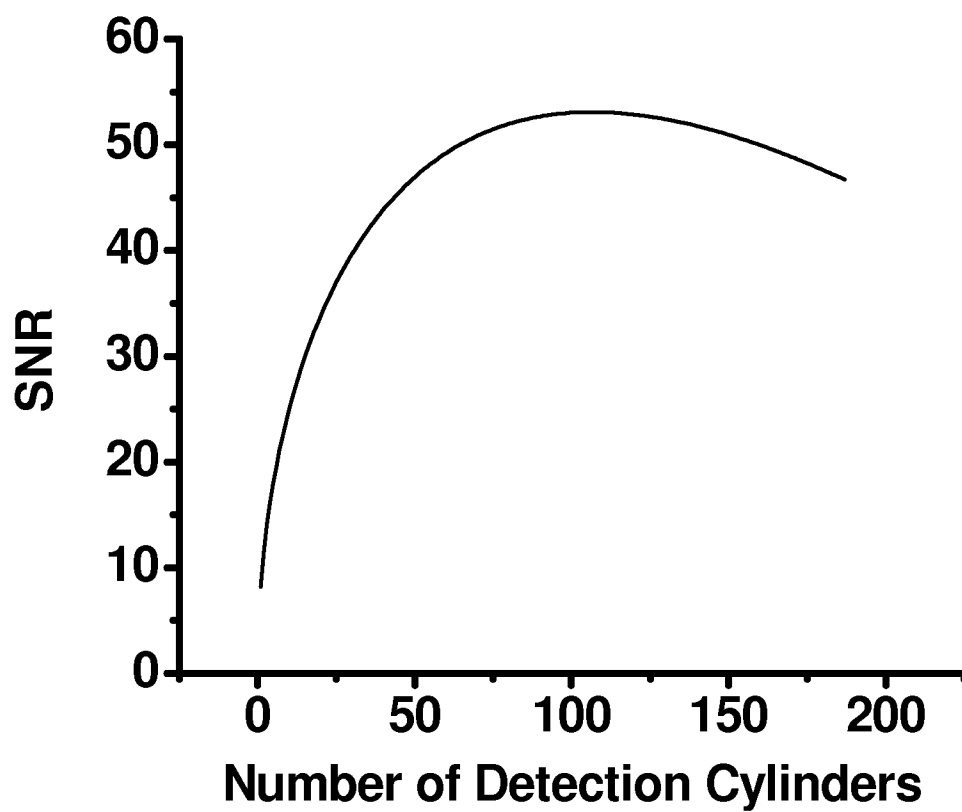


FIG. 5

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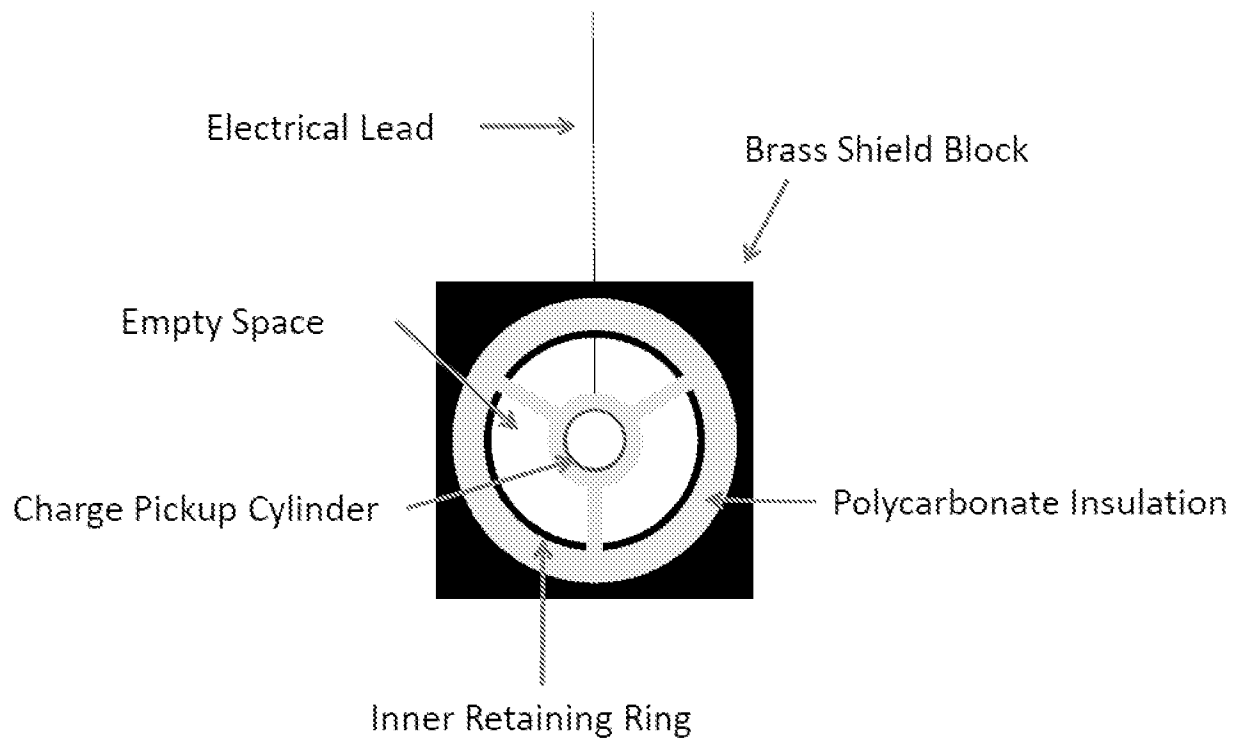


FIG. 6

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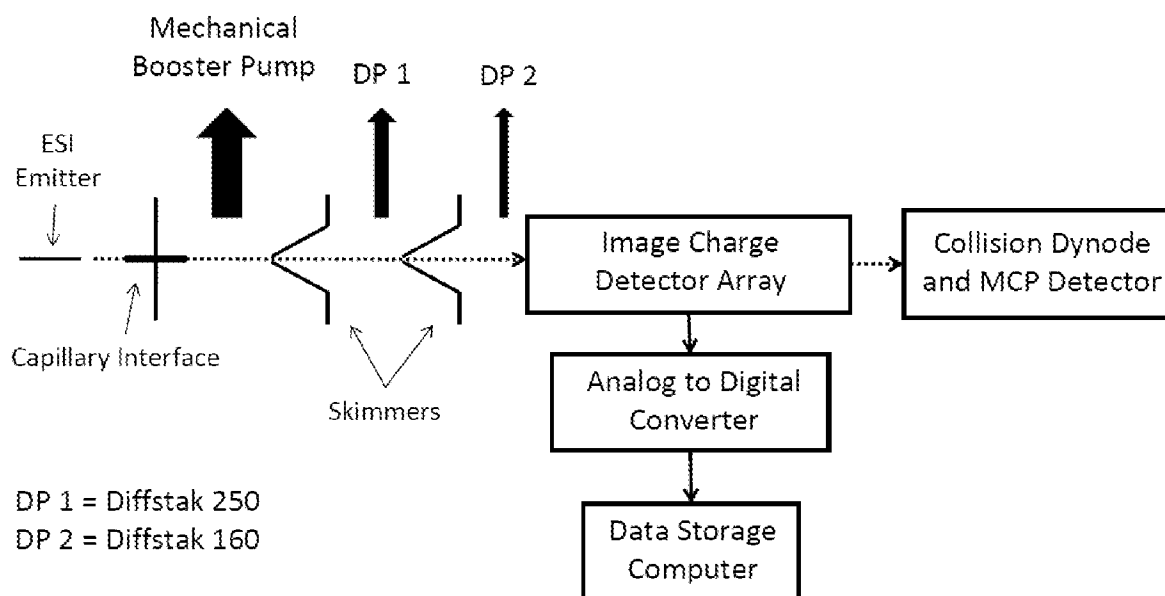


FIG. 7

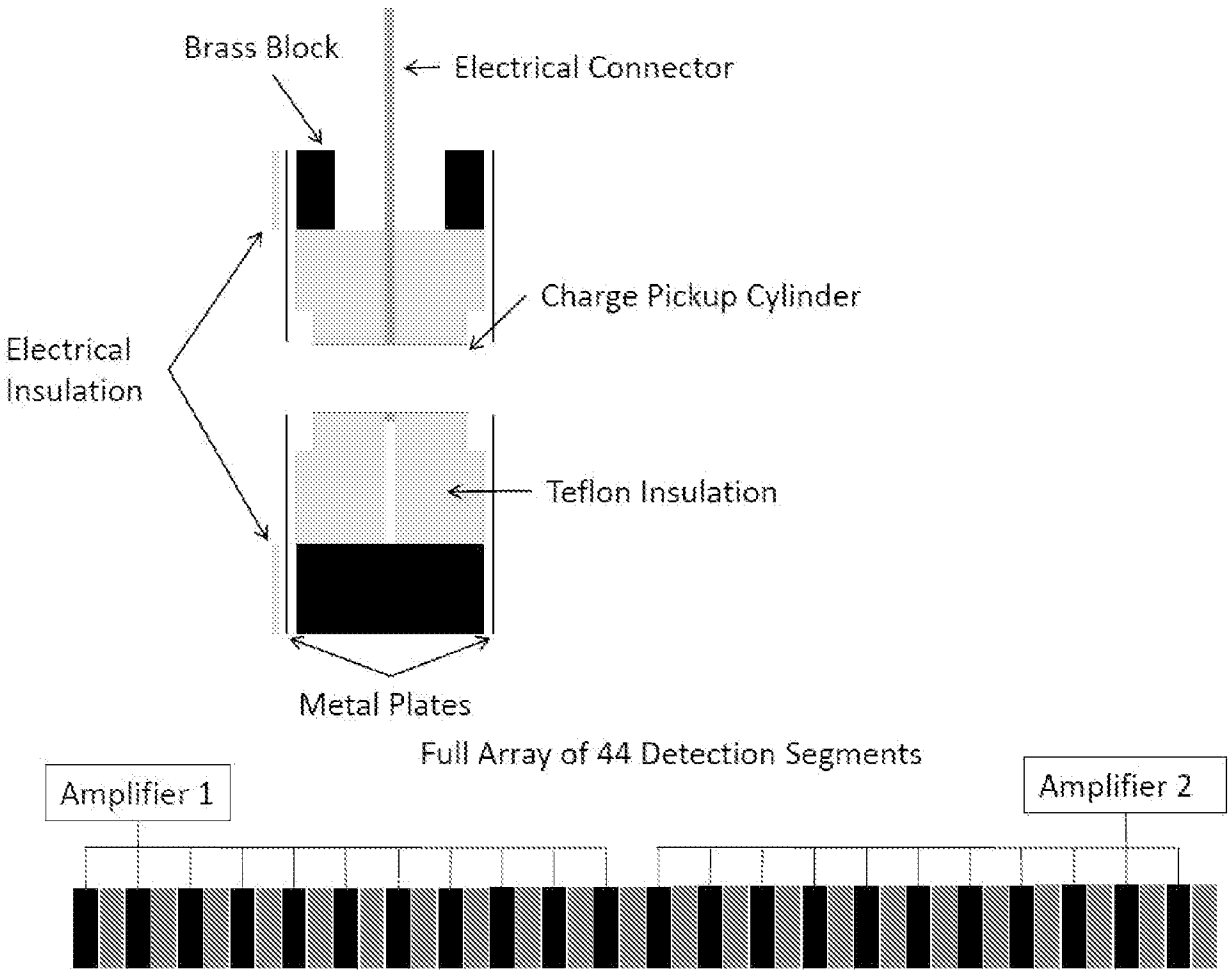


FIG. 8

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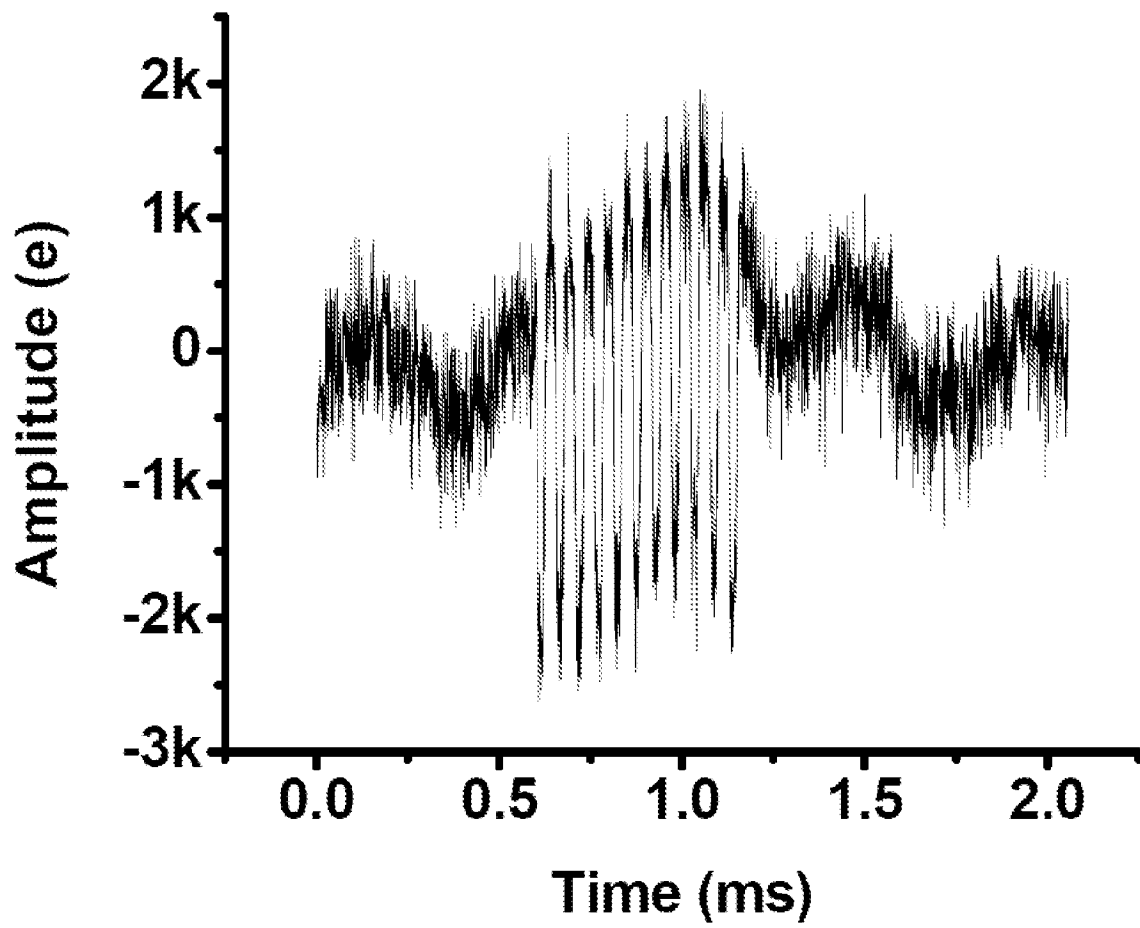


FIG. 9

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INPUT



RESPONSE

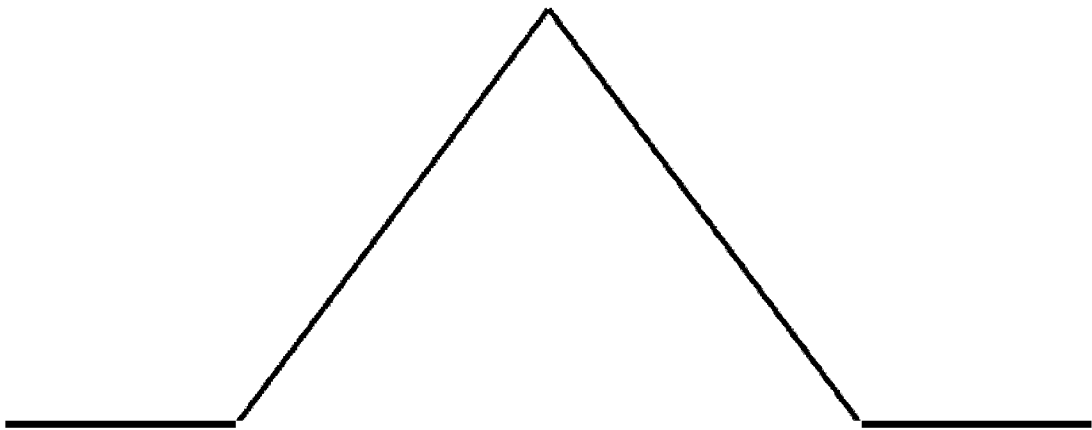


FIG. 10

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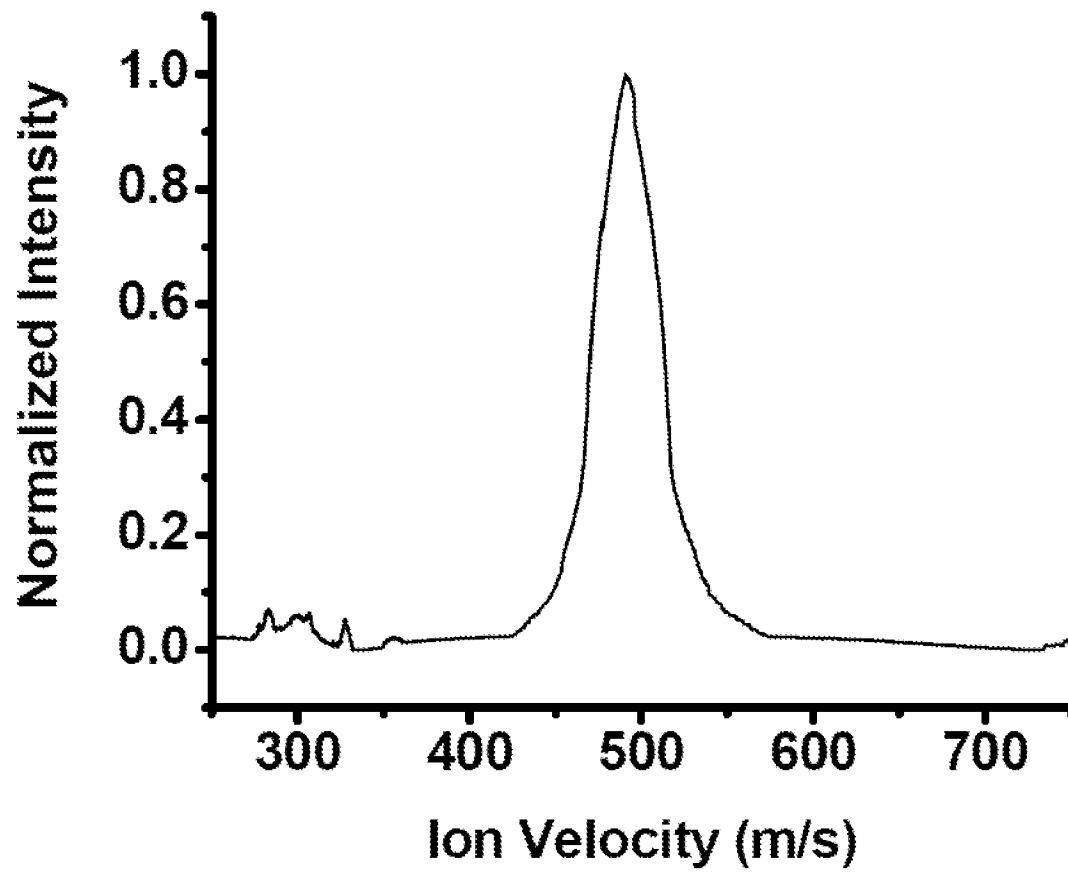


FIG. 11

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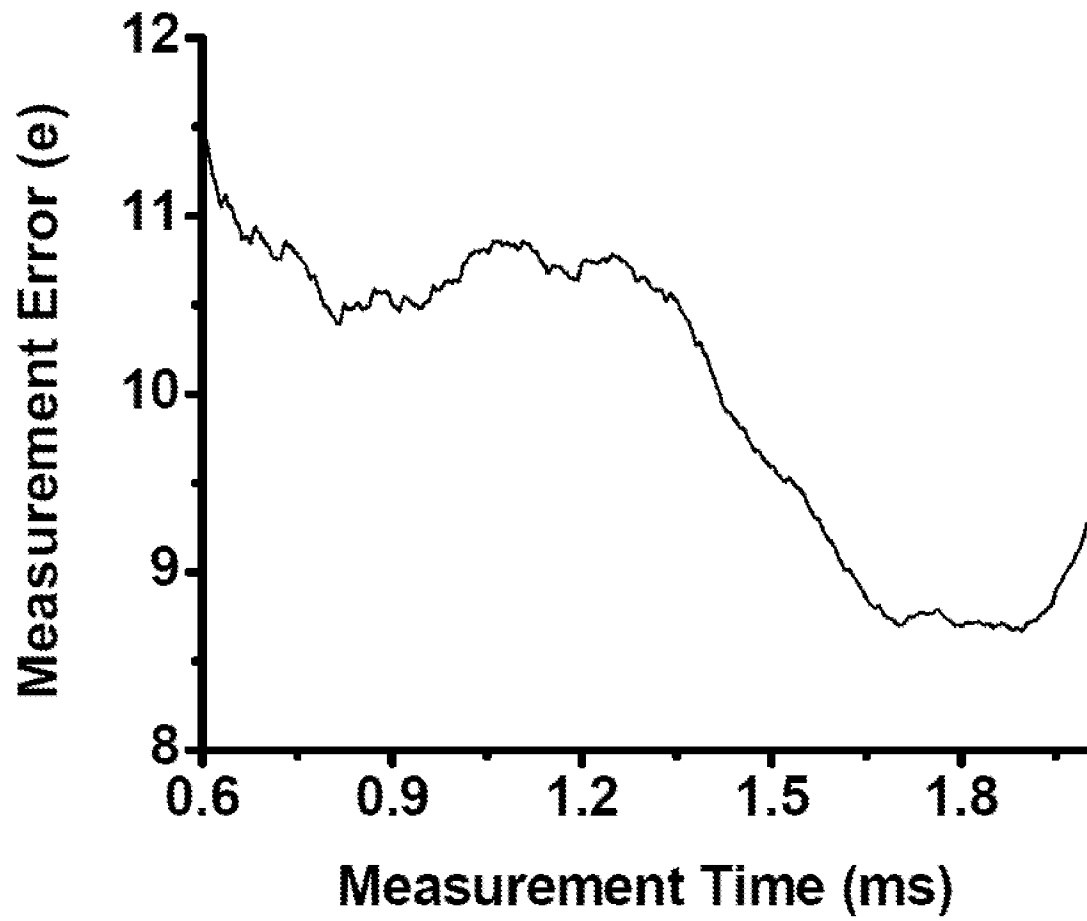
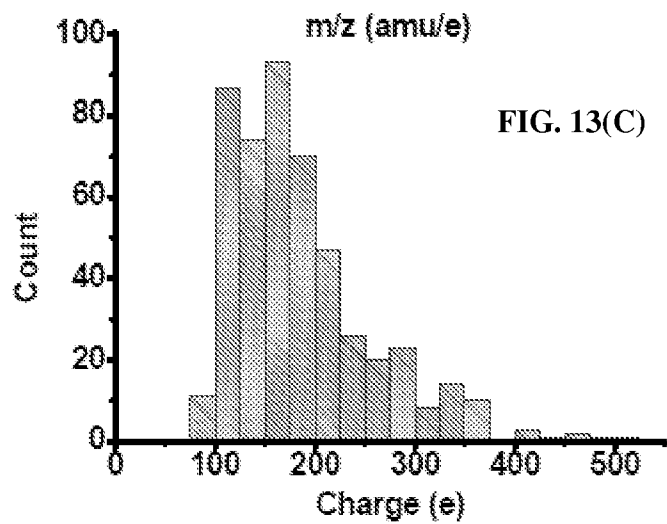
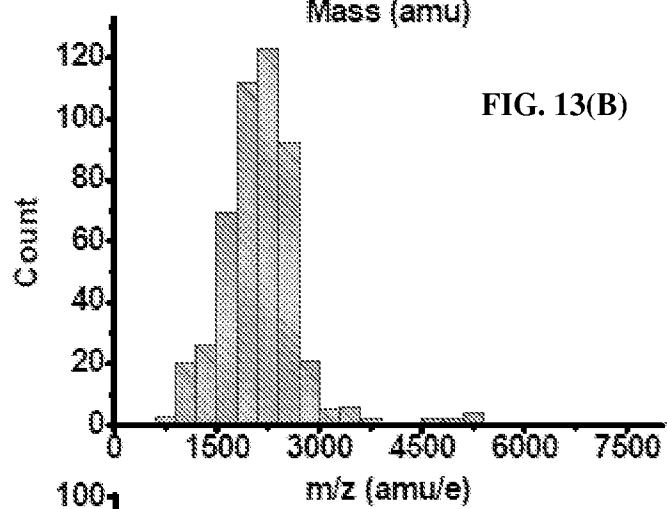
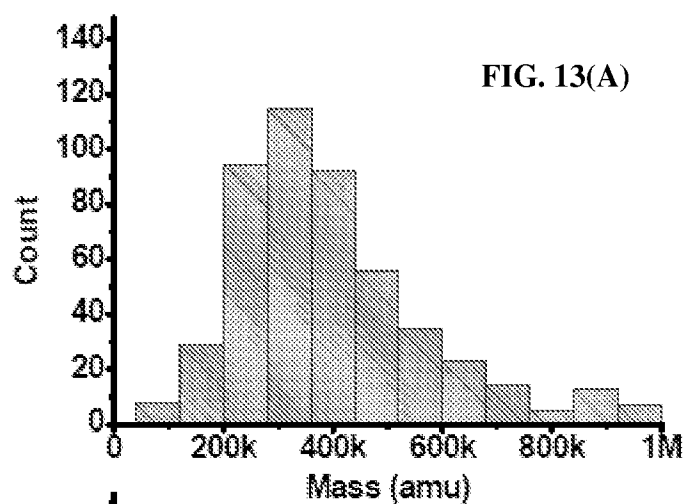
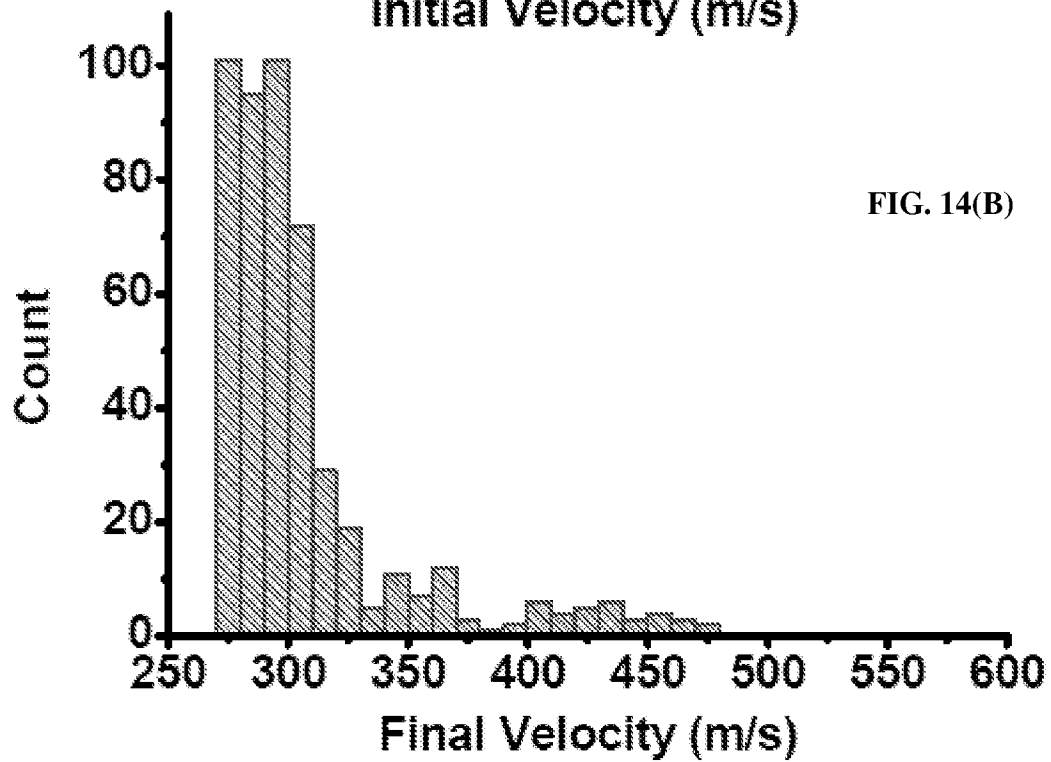
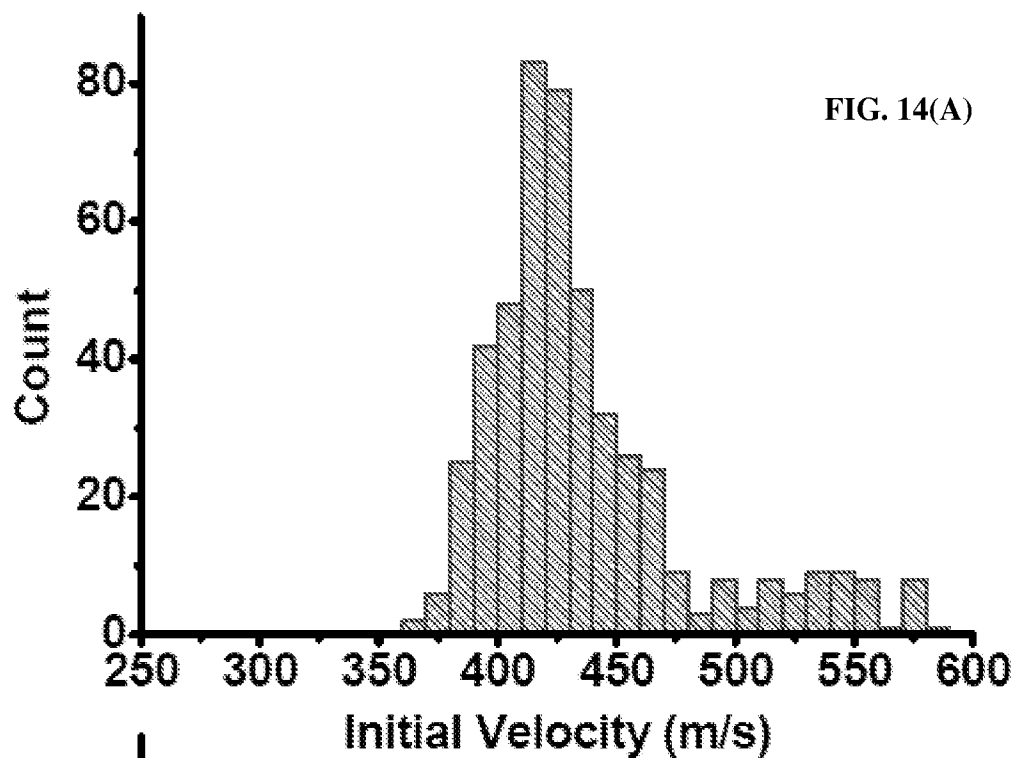


FIG. 12

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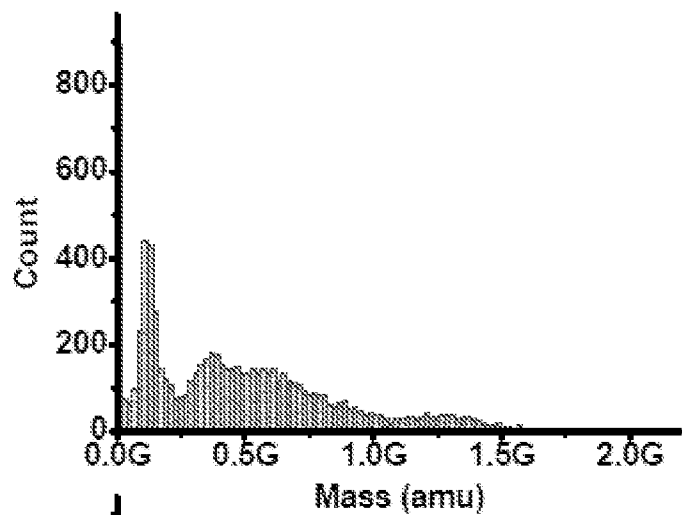


FIG. 15(A)

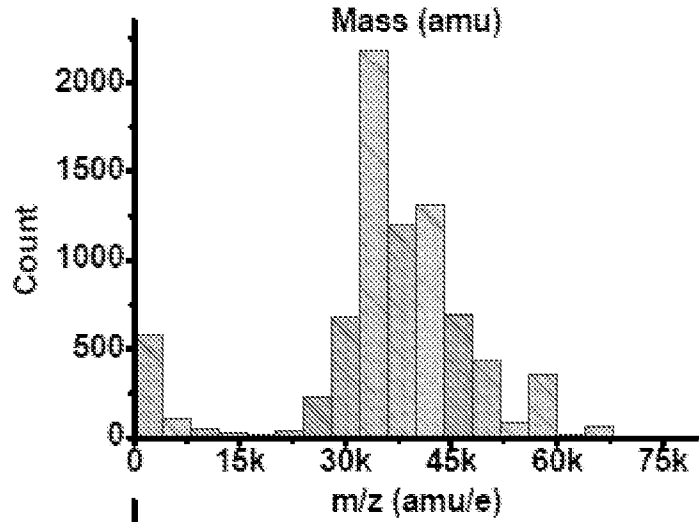


FIG. 15(B)

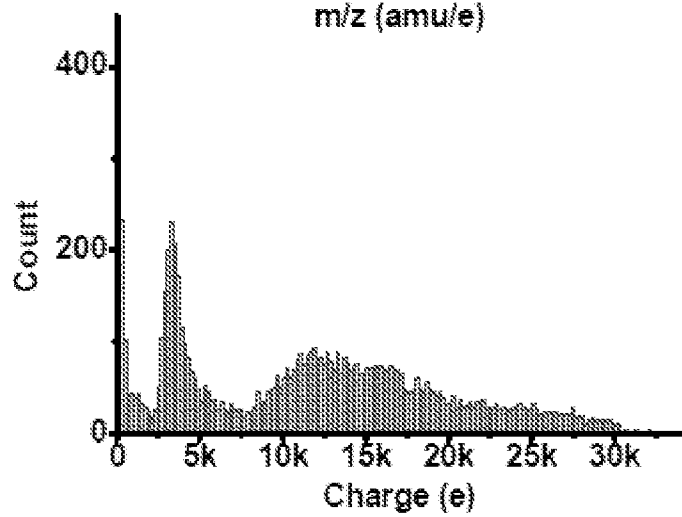


FIG. 15(C)

FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/65185

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01D 59/44, H01J 49/00 (2012.01)

USPC - 250/283

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): B01D 59/44, H01J 49/00 (2012.01)

USPC: 250/283

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8): B01D 59/44, H01J 49/00 (2012.01)

USPC: 250/281-283, 286

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PubWEST; PGPB, USPT, EPAB, JPAB; Google Scholar; Google Patent; Search Terms: JFET amplifier mass spectrometer insulator dielectric Teflon inner outer coaxial concentric hollow preamplifier noise array ion beam tube charge voltage TOFMS time flight m/z electrode conductive brass metal ion mobility macroion linear array

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0256070 A1 (Nagano et al.) 15 October 2009 (15.10.2009) para. [0019] through [0093], Fig. 1-7	1-20
Y	WO 2010/072137 A1 (Ding) 01 July 2010 (01.07.2010) abstract, Fig. 1-10	1-20
Y	US 2009/0078866 A1 (Li et al.) 26 March 2009 (26.03.2009) para. [0024] through [0064], Fig. 1-6	1-19
Y	US 5,770,857 A (Fuestenau et al.) 23 June 1998 (23.06.1998) col. 2 ln. 31 to col. 11 ln. 45, Fig. 1, 2	6-19
Y	US 6,419,985 B1 (Ishizuka) 16 July 2002 (16.07.2002) col. 6 ln. 30-39, Fig. 6	12

☐ Further documents are listed in the continuation of Box C.

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 April 2012 (11.03.2012)

Date of mailing of the international search report

24 APR 2012

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