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(54) Title: IMPROVEMENTS RELATING TO MASS SPECTROMETRY

(57) Abstract: This invention relates to a mass spectrometer including a reaction cell and to a method of using such a mass spectrom-
eter. In particular, although not exclusively, this invention relates to a tandem mass spectrometer and to tandem mass spectrometry.
The invention provides a method of mass spectrometry using a mass spectrometer having a longitudinal axis, comprising guiding
ions to travel along the longitudinal axis of the mass spectrometer in a forwards direction to pass through an intermediate ion store
and then to enter a reaction cell, to process the ions within the reaction cell, to eject the processed ions to travel back along the
longitudinal axis to enter the intermediate ion store once more, and to eject one or more pulses of the processed ions in an off-axis
direction to a mass analyser.



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IMPROVEMENTS RELATING TO MASS SPECTROMETRY

This invention relates to a mass spectrometer including
5 a reaction cell and to a method of using such a mass
spectrometer. In particular, although not exclusively, this
invention relates to a tandem mass spectrometer and to
tandem mass spectrometry.

In general, a mass spectrometer comprises an ion source
10 for generating ions from molecules to be analysed, and ion
optics for guiding the ions to a mass analyser. A tandem
mass spectrometer further comprises a second mass analyser.
In tandem mass spectrometry, structural elucidation of
ionised molecules is performed by using the first mass
15 analyser to collect a mass spectrum, then using the first
mass analyser to select a desired precursor ion or ions from
the mass spectrum, ejecting the chosen precursor ion(s) to a
reaction cell where they are fragmented, and transporting
the ions, including the fragmented ions, to the second mass
20 analyser for collection of a mass spectrum of the fragment
ions. The method can be extended to provide one or more
further stages of fragmentation (i.e. fragmentation of
fragment ions and so on). This is typically referred to as
 MS^n , with n denoting the number of generations of ions.
25 Thus MS^2 corresponds to tandem mass spectrometry.

Tandem mass spectrometers can be classified into three
types:

(1) sequential in space, corresponding to combinations
of transmitting mass analysers (e.g. magnetic sectors,
30 quadrupole, time-of-flight (TOF), usually with a collision
cell in-between);

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(2) sequential in time, corresponding to stand-alone trapping mass analysers (e.g. quadrupole, linear, Fourier transform ion cyclotron resonance (FT-ICR), electrostatic traps); and

5 (3) sequential in time and space, corresponding to hybrids of traps and transmitting mass analysers.

Most tandem mass spectrometers have different stages of mass analysis following each other along a common axis. Such "consecutive" geometry allows installation of an RF collision cell or an additional trapping stage, but
10 precludes other apparatus such as:

- an additional ion source (e.g. for introducing calibrant ions or ions of an opposite polarity);
- a window for introducing laser radiation;
- 15 • a surface for soft landing of ions (as described in WO03/105183);
- a surface for surface-induced dissociation (SID);
or
- an electron source (e.g. for introducing electrons
20 to effect electron capture dissociation (ECD), see WO02/078048 and WO03/102545).

An example of a tandem mass spectrometer having consecutive geometry is provided by WO02/48699. This spectrometer comprises an ion source, a series of ion traps,
25 one of which is operated as a collision cell, followed by a TOF analyser. Tandem mass analysis and MS^n is performed by using multiple reversal of ion movement. This is effected by trapping ions, releasing them in the reverse direction, fragmenting, trapping of fragments, and repeating the cycle
30 to generate the required number of ion generations. However, finally the fragments must pass back through all

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the ion traps to make their way to the consecutively-arranged TOF analyser.

A tandem mass spectrometer with a consecutive, although unusual, geometry is described in WO97/48120. An ion source
5 generates ions that are accelerated orthogonally into a TOF analyser. The ions are reflected by an ion mirror: some of the ions are collected by the TOF detector, whereas some continue to a reaction cell placed after the TOF analyser where they may be fragmented. The fragmented ions are
10 reflected to return along the reverse path into the TOF analyser to be collected by the TOF detector. The reaction cell may fragment the ions in one of three ways: collision induced dissociation (CID), SID or photon induced dissociation (PID). Although this geometry offers greater
15 flexibility in the design and operation of the reaction cell, its utility is limited because of high ion losses caused by the low duty cycle of orthogonal pulsing.

Against this background, and from a first aspect, the present invention resides in a method of mass spectrometry
20 using a mass spectrometer having a longitudinal axis, comprising the sequential steps of: (a) generating ions in an ion source; (b) extracting ions such that they travel along the longitudinal axis of the mass spectrometer in a forwards direction relative to the ion source; (c) causing
25 the ions to enter and then to exit an intermediate ion store as the ions travel along the longitudinal axis in the forwards direction; (d) causing the ions to enter a reaction cell as they travel along the longitudinal axis in the forwards direction; (e) processing the ions within the
30 reaction cell; (f) causing the processed ions to exit the reaction cell to travel back along the longitudinal axis in a backwards direction relative to the ion source;

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(g) causing the processed ions to enter the intermediate ion store once more as they travel along the longitudinal axis in the backwards direction and, optionally, trapping them therein; (h) causing one or more pulses of the processed ions to exit the intermediate ion store in an off-axis direction; (i) causing one or more pulses of the processed ions to enter a mass analyser; and (j) obtaining a mass spectrum of the one or more pulses of processed ions using the mass analyser.

10 It has been realised that efficient mass analysis of processed ions such as fragments requires matching of their emission to the operation of a mass analyser. As most high-performance mass analysers (e.g. FT ICR, TOF, orbitrap, etc.) are of an inherently pulsed nature, it is necessary to store ions for some time prior to injection into the mass analyser as pulses of ions. This could be done either using an additional ion store or incorporating such ion storage into the reaction cell. It has also been realised that the same ion storage could be re-configured not only to inject ions into the mass analyser, but also to do this in a direction that is substantially different from the direction of the ions' entrance (described in more detail below). In addition to simplicity of instrument layout, this arrangement advantageously avoids directing ions into the reaction cell when it is undesirable.

25 Generally, the duration of a pulse for ions of the same m/z should be well below 1 ms, and preferably below 10 microseconds. A most preferred regime corresponds to ion pulses shorter than 0.5 microsecond (this may be used for m/z roughly between 400 and 2000). Alternatively, and particularly for pulses of ions with a spread of m/z, spatial length of the emitted pulse should be less than 1 m,

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and preferably below 50 mm. A most preferred regime corresponds to ion pulses around 5-10 mm or even shorter. The most preferable regime is especially beneficial for electrostatic type mass analysers like the Orbitrap analyser and multi-reflection TOF analysers.

Of course, "ions" should not be construed as all ions within the mass spectrometer. This is consistent with the fact that some ions will inevitably be lost during transport, others will not be processed (i.e. they will remain unprocessed within the reaction cell) and others may not be detected by the mass analyser. However, there will be a group of ions that will undergo all of the above steps and so fall within the terms used.

The longitudinal axis of the mass spectrometer need not be linear, but should extend generally through the mass spectrometer. For example, the longitudinal axis may be curved in one or more parts, e.g. in a serpentine shape to produce a compact spectrometer.

Using such a geometry provides a flexible solution to the problems and the disadvantages of the prior art systems described above. Advantageously, ions are effectively reflected in the reaction cell such that they enter and exit from the same side. However, the ions are reflected back into an intermediate ion store from where they are ejected off axis to a mass analyser. As ions are transported into and out of the intermediate ion store along both axial directions, and are also ejected off-axis, then the intermediate ion store provides a junction in the ion paths through the mass spectrometer. The ions may be trapped in the intermediate ion store prior to ejection. This arrangement allows an effectively free choice of mass analyser, thereby overcoming the narrow applicability of

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WO97/48120 to orthogonal acceleration TOF analysers. Moreover, the benefits of the present invention are not restricted to tandem or MS^n spectrometry, but enjoy wider application.

5 When the present method is used for tandem mass spectrometry, the ions are transported to the reaction cell where they are fragmented according to any known scheme, such as CID, ECD, SID and PID. This may include hard fragmentation, e.g. to fragment ions down to simple elements
10 and their oxides, hydrides, etc. Moreover, the reflection geometry of the reaction cell makes a natural provision of an electron source for ECD straightforward (e.g. it may merely point straight back down the longitudinal axis) and likewise for a laser for PID or a surface for SID.

15 Moreover, the method may optionally further comprise, between steps (g) and (h), the steps of: allowing the ions to exit the intermediate ion store along the longitudinal axis in the backwards direction; optionally, providing additional ion selection; reflecting the ions such that they
20 travel back along the longitudinal axis in the forwards direction such that the ions pass through the intermediate ion store once more and then enter the reaction cell; further processing the ions within the reaction cell; causing the processed ions to exit the reaction cell to
25 travel back along the longitudinal axis in a backwards direction and to enter the intermediate ion store once more.

 The processing and further processing steps may comprise fragmentation, thereby allowing MS^3 spectrometry. Causing further multiple reflections may allow the ions to
30 be returned to the reaction cell for even more stages of fragmentation, such that the method may provide MS^n spectrometry.

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Preferably, step (c) further comprises trapping ions in the intermediate ion store after the ions have entered and then allowing the ions to exit. Trapping ions in the intermediate ion store may afford two benefits. First, ions
5 may be accumulated over a period of time. Second, ions trapped in the intermediate ion store may be squeezed to form a compact bunch. This latter benefit is particularly advantageous where the ions are next ejected to the mass analyser, especially where focussing of the ion bunch is
10 preferred, e.g. ejection from a curved linear quadrupole to an electrostatic analyser such as an Orbitrap analyser.

The intermediate ion store may be operated in a transmit mode such that ions travelling along the longitudinal axis in the forwards direction pass straight
15 through the intermediate ion store.

Although fragmentation has already been described as an example of processing of ions in step (e), the present invention also extends to other forms of processing. Processing ions, in some embodiments of the invention, may
20 be regarded as reacting the ions, i.e. causing an interaction involving ions to cause a change in those ions. In addition to causing a break-up of the ions through fragmentation, other changes to the ions may include their charge state changing. In a broader sense, processing ions
25 may be regarded as changing the ion population in the reaction cell. This may be effected in many different ways. For example a fraction of the ion population may be removed, such as by mass analysis so that only ions within a desired mass range return to the intermediate ion store or by
30 selection based upon ion mobility. Processing may comprise introducing further ions to the ion population. This may be done to introduce calibrant ions or to introduce ions of the

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opposite polarity. Also, processing may comprise altering the energy spread of the ion population. These processing steps may be performed on their own or in any combination.

Optionally, trapping the processed ions in step (h) 5 comprises "cooling" the ions such that they lose energy. There are two preferred ways of achieving this. The first is by collisional cooling where a gas is introduced into the intermediate ion store such that the ions lose energy in low energy collisions (low enough to avoid further fragmenting 10 the ions). The second is by the other well-known technique of adiabatic cooling.

Preferably, the method further comprises, between steps (b) and (c), the step of causing the ions to enter and then to exit an ion trap as they travel along the longitudinal 15 axis in a forwards direction. Such an arrangement is particularly advantageous where the method further comprises trapping ions in the ion trap prior to allowing the ions to exit the ion trap along the longitudinal axis in the forwards direction. In this way, ions may be accumulated in 20 the ion trap to a desired number prior to their release for processing in the reaction cell. The number of ions accumulated may be controlled, for example, using automatic gain control to ensure an optimum number of ions are obtained (there is a trade-off between the desire for as 25 many ions as possible to ensure good statistics in the mass spectra and the deleterious effects of space charge if the concentration of ions is too high). The ion trap may also be used to collect a mass spectrum or spectra, and optionally to perform mass selection of ions passing through 30 the ion trap, either in combination or in the alternative. Of course, the ion trap may be used in any of these ways during any of whatever number of passes the ions make

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through the ion trap. Where multiple passes of the ions along the mass spectrometer are used, the ion trap may be used to reflect the ions at the opposite end to the reflection performed by the reaction cell.

5 From a second aspect, the present invention resides in a mass spectrometer having a longitudinal axis, comprising: an ion source; ion optics operable to guide ions produced by the ion source along the longitudinal axis; an intermediate ion store located downstream of the ion source and having
10 first and second apertures located on the longitudinal axis, such that the first aperture faces the ion source, and a third aperture located off axis; a reaction cell located downstream of the intermediate ion store and having an aperture that faces the second aperture of the intermediate
15 ion store, wherein the reaction cell is operable to process ions; and a mass analyser located adjacent the intermediate ion store having an entrance aperture that faces the third aperture of the intermediate ion store, and wherein the intermediate ion store is operable to eject one or more
20 pulses of ions out of the third aperture to the mass analyser.

The reaction cell may be operable to process ions, as described above. For example, the reaction cell may cause a change in the population of ions, may react the ions so as
25 to cause a change in the ions, or may cause fragmentation of the ions.

With this arrangement, ions may be generated in the ion source, transported along the longitudinal axis in a forwards direction to pass through the first aperture of the
30 intermediate ion store and then the second aperture of the intermediate ion store, and through the aperture of the reaction cell. The ions may then be reflected such that

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they re-emerge through the aperture in the reaction cell in the backwards direction, then to re-enter the intermediate ion store through the second aperture. The ions may then be ejected off axis through the third aperture.

5 The apertures may be provided by any suitable means. For example, the apertures may correspond merely to missing end faces of their associated part. Alternatively, they may correspond to holes provided in an electrode or the like, or by gaps left between electrodes or the like.

10 The ion source may be freely chosen from any commonly available types, such as an electrospray source, an electron impact source, a chemical ionisation source, atmospheric pressure photoionisation, MALDI (at atmospheric pressure, reduced pressure or in vacuum), a secondary ion source or
15 any preceding stage of mass analysis or separation (e.g. DC or field-asymmetric ion mobility spectrometer, travelling wave spectrometer, etc.).

 The intermediate ion store may be implemented in any number of ways. Examples include a 3D quadrupole ion trap
20 or a storage multipole. Where a storage multipole is used, storage may be effected using RF potentials and, preferably, with RF switching as described in GB0413852.5. Whatever is chosen, it must be capable of both axial and off-axis
ejection. Preferably, the off axis ejection is performed
25 orthogonally, such that the third aperture of the intermediate ion store is located to allow such orthogonal ejection.

 Optionally, the intermediate ion store has an associated gas supply for introducing gas into the
30 intermediate ion store. The gas may be used in gas-assisted trapping of the ions.

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In a currently preferred embodiment, the intermediate ion store is a curved linear ion store. Curved linear traps are advantageous in that they allow ejection of pulses of ions (i.e. fast ejection) without requiring further shaping.

5 The curvature of the intermediate ion store may be used to focus ions ejected orthogonally from the ion store through the third aperture. Namely, the ions may be ejected normal to the ion path such that they travel toward the centre of curvature or are radially convergent. The ions may be

10 ejected into an electrostatic mass analyser such as an Orbitrap analyser, optionally through a set of ion optics. The curvature of the intermediate ion store and the positioning of the Orbitrap analyser may be such that the ions are focussed at an entrance aperture of the Orbitrap

15 analyser.

The reaction cell may take many different forms. The reaction cell may correspond to a gas-filled ion-molecule reactor or the reaction cell may have an ion source operable to introduce ions into the reactor cell. Molecules could be

20 delivered in a gaseous state or as a beam of an excited species (molecules or atoms) from an external source (e.g. metastable atom source, or discharge, or spray with charged species eliminated by electric fields). Such a reaction cell could be used for modification or purification or

25 fragmentation of the incoming ions. For fragmentation purposes, the reaction cell may further comprise an electron source for ECD, a surface for SID, ion source for ion-ion reactions (to facilitate, for example, proton transfer or electron transfer dissociation, ETD), a specific collision

30 gas for CID or a beam of photons of any spectral range. Of course, a hybrid cell may be used having any combination of the features above.

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Although electrostatic mass analysers, such as an Orbitrap analyser, have been described above, other types of mass analysers may be used. For example, the mass analyser may correspond to a FT-ICR cell or a TOF analyser.

5 Optionally, the mass spectrometer may further comprise an ion trap located between the ion source and the intermediate ion store and having apertures located on the longitudinal axis. The apertures are positioned to allow the passage of ions along the longitudinal axis. This ion
10 trap may include a further mass analyser. This allows mass spectra of the precursor ions to be collected. The ion trap may be, for example, transporting elongated electrodes, magnetic sector or Wien filter, quadrupole mass filter, storage RF multipole resonant or mass-selective ion
15 selection, 3D quadrupole ion trap or a linear ion trap.

Preferably, the mass spectrometer further comprises a controller operable to perform any of the methods described above. The present invention also extends to a computer program comprising computer program instructions that, when
20 executed by the controller, cause the controller to perform any of the methods described above. The present invention extends still further to a computer storage medium having stored thereon such a computer program.

According to a still further aspect, the present
25 invention resides in a mass spectrometer having a longitudinal axis, comprising: an ion source to direct ions along said axis; a reaction cell having an entrance aperture located on said axis; a mass analyser; and ion optics switchable between a first mode in which ions from the ion
30 source are guided along said axis to said reaction cell and product ions produced in the reaction cell are guided to the mass analyser for analysis, and a second mode in which ions

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from the ion source are deflected from said axis and guided to the mass analyser for analysis without entering the reaction cell.

According to a yet still further aspect, the present invention resides in a mass spectrometer having a longitudinal axis, comprising: an ion source to direct ions along said axis; a reaction cell; a mass analyser having an entrance aperture located on said axis; and ion optics switchable between a first mode in which ions from the ion source are deflected from said axis and guided to the reaction cell and product ions produced in the reaction cell are guided back to said axis and to said entrance aperture of the mass analyser, and a second mode in which ions from the ion source are guided along said axis to the mass analyser for analysis without entering the reaction cell.

In order that the invention may be more readily understood, reference will now be made, by way of example only, to the following drawings, in which:

Figure 1 is a schematic representation showing a generalised mass spectrometer in accordance with an embodiment of the present invention;

Figure 2 is a more detailed representation of a mass spectrometer in accordance with an embodiment of the present invention;

Figure 3 is a representation of a reaction cell for use in the mass spectrometer of Figures 1 and 2, in the form of a gas-filled cell;

Figure 4 is a representation of a reaction cell for use in the mass spectrometer of Figures 1 and 2, having an auxiliary source of ions, or neutral atomic or molecular beams, or beams of photons; and

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Figure 5 is a representation of a reaction cell for use in the mass spectrometer of Figures 1 and 2 for ECD.

A mass spectrometer 100 having a longitudinal axis 110 is shown in Figure 1. Most parts of the mass spectrometer 100 are positioned on the longitudinal axis 110. Starting with an ion source 140 and working downstream, the mass spectrometer 100 comprises the ion source 140, a first mass analyser 180 (an ion trap in this embodiment), an intermediate ion store 220 and a reaction cell 260. A second, pulsed mass analyser 340 is located off axis, adjacent to the intermediate ion store 220. The generalised representation of Figure 1 does not show ion optics that may be used to guide ions between the various parts of the mass spectrometer 100. Moreover, Figure 1 does not show the electrodes of the various parts that are used to guide and/or trap ions within those parts. A controller 360 is used to set potentials on the electrodes of the various parts and perform other control functions that allow the mass spectrometer 100 to function as commanded. The controller 360 communicates with the parts via connections 375.

The passage of ions through the mass spectrometer 100 is also shown in Figure 1. An analyte 120 is introduced into the ion source 140 where it is ionised to form analyte ions 160 that exit the ion source 140. The ions 160 then enter the ion trap 180. This embodiment is described in the context of tandem mass spectrometry, although it is to be understood that the present invention enjoys wider application. Thus, the ion trap 180 provides a mass spectrometer capability to obtain a mass spectrum from the ions 160 under the direction of the controller 360. The

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ions 160 are then mass selected such that only ions 200a,b within a certain mass range exit the ion trap 180.

Advantageously, the mass spectrometer 180 may be used to implement automatic gain control, i.e. to ensure an optimum number of ions are accumulated. This optimum is a compromise between a desire for as many ions as possible to ensure good experimental statistics and the need to limit ion concentrations to avoid space charge effects. Automatic gain control may be used to control the ion abundance in the intermediate ion store 240, the reaction cell 260 or the mass analyser 340. Automatic gain control is described in US 5,107,109 and US 6,987,261.

The next step sees the mass spectrometer 100 used in two different ways. In a first mode, ions 200a are transported into the intermediate ion store 220 where they are trapped. Once a suitable time delay has passed, the controller 360 transports the ions 240 to the reaction cell 260. In a second mode, the intermediate ion store 220 is used merely as an ion guide ("transmission mode"). The intermediate ion store 220 may be filled with gas, thereby reducing the energy of the ions 200b through collisional cooling as they pass through the intermediate ion store 220 and enter the reaction cell 260.

The controller processes the ions 240 once in the reaction cell 260. The processing may take any number of forms, as will be described below. The processed ions 300 are returned to the intermediate ion store 220 by the controller 360. In this embodiment, the intermediate ion store 240 traps the processed ions 300 prior to ejecting the ions 320 off axis such that they are radially convergent. Thus the ions are focussed as they pass from the intermediate ion store 220 to the mass analyser 340.

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Alternatively, the processed ions 300 are merely deflected from the axis 110 to follow path 320 to the mass analyser 340. The controller 360 then uses the mass analyser 340 to collect one or more mass spectra from the processed ions.

5 Figure 2 shows in more detail an embodiment of the present invention, again in the context of a tandem mass spectrometer comprising a first mass analyser provided by an ion trap 180 and a second mass analyser provided by an Orbitrap analyser 340 (an electrostatic analyser). Figure 2
10 is not to scale.

The mass spectrometer 100 is generally linear in arrangement, with ions passing along the longitudinal axis 110. The front end of the spectrometer 100 comprises a conventional ion source 140, supplied with analyte ions 120.
15 Ion optics 150 are located adjacent the ion source 140, and are followed by a linear ion trap 180. Further ion optics 190 are located beyond the ion trap 180, followed by a curved quadrupolar linear ion trap 220 bounded by gates 222 and 224 at respective ends. This ion trap 220 provides the
20 intermediate ion store 220. Ion optics 226 are provided adjacent the downstream gate 224 to guide ions to and from the reaction cell 260.

The curvature of the intermediate ion store 220 is used such that when the ions are ejected off axis, the ions are
25 radially convergent. The ions are ejected off-axis in the direction of the entrance 342 to an Orbitrap mass analyser 340. The ions are ejected through an aperture 228 provided in an electrode 230 of the intermediate ion store 220 and through further ion optics 330 that assist in focussing the
30 emergent ion beam. It will be noted that the curved configuration of the intermediate ion store 220 also assists in focussing the ions. Furthermore, once ions are trapped

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in the intermediate ion store 220, potentials may be placed on the gates 222 and 224 to cause the ions to bunch in the centre of the intermediate ion store 220. This also assists focussing. The curved linear ion trap 220 is inherently
5 useful as it allows rapid ejection of pulses of ions to the mass analyser 340 with little, if any, further shaping required.

In operation, ions 160 are generated in the ion source 140 and transported through ion optics 150 to be accumulated
10 temporarily in the ion trap 180 according to e.g. US20030183759 or US 6,177,668. Ion trap 180 contains 1 mTorr of helium such that the ions 160 lose some of their kinetic energy in collisions with the gas molecules.

Either after a fixed time delay (chosen to allow
15 sufficient ions 160 to accumulate in the ion trap 180) or after sufficient ions 160 have been detected in the ion trap 180, ions 200a are ejected from the ion trap 180 to travel through ion optics 190 and into the intermediate ion store 220. Ions 200b will pass through the intermediate ion store
20 220 into the reaction cell 260 where they are processed before being returned back to the intermediate ion store 220.

Cooling gas is introduced into the intermediate ion store 220. Nitrogen, argon, helium or any other suitable
25 gaseous substance could be used as a cooling gas, although helium is preferred for the ion trap 180 and nitrogen for the intermediate ion store 220 of this embodiment. Typically, 1 mTorr of nitrogen is used in the intermediate ion store 220. The pumping arrangement used, indicated by
30 the pumping ports and arrows 380, ensures that other components are substantially free of gas and kept at the required high vacuum. Transfer of ions into intermediate

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ion store 220 could be realised as described in co-pending patent application GB0506287.2.

Various parts of the mass spectrometer 100 will now be described in more detail.

5 The ion source 140 may be any one of the commonly available types. For example, electrospray, atmospheric pressure photoionisation or chemical ionisation, atmospheric pressure/reduced pressure/vacuum MALDI, electron impact (EI), chemical ionisation (CI), secondary ion, or any
10 preceding stage of mass analysis or ion selection (e.g. DC or field-asymmetric ion mobility spectrometer, travelling wave spectrometer, etc.) would all be suitable choices.

The ion trap 180 may also be chosen from a number of options. The skilled person will appreciate that the choice
15 may be made in accordance with the experiments to be performed. Options include transporting elongated electrodes, magnetic sector or Wien filter, quadrupole mass filter, storage RF multipole with resonant or mass-selective ion selection, 3D quadrupole ion trap, or linear trap with
20 radial or axial ejection.

Suitable types of ion traps/ion stores to use in the intermediate ion store 220 include 3D quadrupole ion traps, storage RF multipoles without RF switching, storage multipoles according to US 5,763,878 or US20020092980A1,
25 storage RF quadrupole with RF switching according to GB0413852.5, ring traps, stack traps or static traps.

The intermediate ion store 220 may be operated in a number of ways. For example, intermediate ion store 220 could operate in ion capture mode (the traditional way of
30 operating ion traps). Alternatively, the intermediate ion store 220 could operate in ion transmission mode to allow ions to reach the reaction cell 260 and in capture mode on

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their way back. A further alternative is for the intermediate ion store 220 to operate in transmission mode for multiple ion bounces between the ion trap 180 and the reaction cell 260, and then could be switched into the capture mode after a pre-determined number of bounces. Each bounce could involve a different type of processing in ion trap 180 or reaction cell 260.

Where ion trap 180 is used to trap ions, multiple ion ejections from the ion trap 180 into the intermediate ion store 220 per each cycle of mass spectrometry in the mass analyser 340 are possible to accumulate a larger ion population.

In the embodiment of Figure 2, a curved or straight gas-filled quadrupole 220 has switchable RF potentials applied to its electrodes and time-dependent voltages on gates 222 and 224. These potentials and RF offsets are changed to switch from one regime of operation to another: sufficiently high potentials reflect the ion beam thus blocking its further propagation. Also, these potentials could be ramped up prior to ion ejection in order to squeeze the ion bunch. Ions are ejected into the mass analyser 340 through the aperture 228 in electrode 230 by switching the RF off and applying a DC gradient between the electrodes.

The mass analyser 340 may be a FT-ICR cell, a TOFMS of any type or an electrostatic trap like an Orbitrap analyser.

The reaction cell 260 will now be described in more detail and with reference to Figures 3, 4 and 5 that show exemplary embodiments. The reaction cell 260 may take one of many forms that effectively operate on the population of ions within the reaction cell 260 to change that population in some way. The ions themselves may change (e.g. by fragmentation or reaction), ions may be added (e.g.

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calibrants), ions may be removed (e.g. by mass selection), or properties of the ions may change (e.g. their kinetic or internal energy, etc.). Thus, the reaction cell 260 may be any one of a number of possibilities to meet these functions.

Figure 3 shows a reaction cell 260 in the form of a gas-filled collision cell for high-energy CID, along with ion optics 226 operable to guide ions into and out from the reaction cell 260. Admission and retention of ions within the reaction cell 260 is controlled by gate electrode 262. Trapping of ions within the reaction cell 260 is assisted by a trapping RF quadrupole 264. A trap electrode 266 located at the opposite end to the gate electrode 262 completes the trapping arrangement. A gas supply 268 is used to introduce a gas into the reaction cell 260.

In operation, ions are mass selected in the ion trap 180, transported through the intermediate ion store 220 at low energies, are then accelerated by the ion optics 226 up to energies of about 30-50 eV/kDa. The ions then enter the reaction cell 260 where they collide with gas molecules and fragment. The fragmented ions are trapped in the reaction cell 260. Preferably, the reaction cell 260 is operated such that the product of gas pressure P (mbar) and ion depth of penetration L (mm) exceeds 0.1 mbar·mm, most preferably 1 mbar·mm. The fragmented ions, and any precursor ions, are ejected from the reaction cell 260 by suitable manipulation of DC voltages (on the gate electrode 262 and trap electrode 266). These ions are then trapped in the intermediate ion store 220, before being transported to the mass analyser.

Operation in this reflection mode allows the following two applications. First, high-energy fragmentation of

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precursor ions, including parallel fragmentation of all ions. Prior to fragmentation, some of the background peaks or wide ranges of the mass spectrum may be excluded using mass selection in the ion trap 180. Second, analysis of
5 low-mass immonium ions and precursor ion scans using the ion trap 180 and mass analyser 340.

The reaction cell 260 of Figure 3 may also be operated as a gas-filled ion-molecule reactor. In this method, ions are introduced into the reaction cell 260 at lower energies.
10 The trapped ions enter into reactions (e.g. charge exchange) with an active reactant introduced with the collision gas. Examples of reactant gas include methane, water vapour (inc. deuteriated), alkyl bromides, alcohols, ethers, ketones, amines (e.g. tri-ethylamine), etc. Another application is
15 specifically reacting an isotopically labelled gas with a specific ion(s) functional group (e.g. a phosphate) to label ions having this group. Labelled ions could be ear-marked for subsequent fragmentation, either at once for analysis in the mass analyser 340 or sequentially in the ion trap 180.
20 In both applications, the extent of the reaction may be regulated by the duration of trapping within the reaction cell 260 and the pressure of reactant. Low-pressure electrical discharge could be also employed to provide activation and fragmentation.

Figure 4 shows a reaction cell 260 fed by ion optics
25 226 (an RF octopole). The reaction cell 260 has a trapping RF quadrupole 264 bounded by a gate electrode 262 at one end and a trap electrode 266 at the other end. A gas supply 268 is also provided, along with an ion source 270. This ion
30 source 270 may be used to introduce further ions of the same polarity to those already trapped in the reaction cell 260. Alternatively, the ion source 270 may be used to introduce

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ions of an opposite polarity ("reactant ions"). Preferably, ions of each polarity are introduced sequentially: e.g. positive ions first, then negative ions. Ions of both polarities are trapped within the reaction cell 260 by
5 applying suitable RF potentials to the gate electrode 262 and the trap electrode 266. Ions of both polarities could be transported to react in the ion trap 180 or in the intermediate ion store 220 (or they could react in the reaction cell 260). An example of such reaction is
10 electron-transfer dissociation, ETD (J.E.P. Syka, J.J. Coon, M.J. Schroeder, J. Shabanowitz, D.F. Hunt, Proc. Nat. Acad. Sci., 101 (2004) 9528-9533).

Reaction might involve more than one stage. For example, positive precursor ions could produce negative
15 product ions in reaction with negative reactant ions. These negative product ions, in their turn, could be transformed into positive further products by reacting with positive reactant ions delivered by an appropriately switched ion source 270. This way, multi-stage reactions are made
20 possible. Among other advantages, this allows an increase in the charge state of resulting ions, thus allowing ECD or ETD on normally singly-charged ions produced by MALDI.

Reactant ions could be delivered also from the original ion source of the mass spectrometer e.g. by switching
25 polarity of the entire ion source and ion path. This allows to mass-selection of desired reactant ions. During polarity switching, precursor ions remain stored in the reaction cell 260 and therefore are not affected. To accelerate polarity switching, it is preferable to have both polarities of ions
30 continuously generated within the ion source (e.g. by having two sprayers at opposite polarities), with only one polarity transmitted at any given time.

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Instead of ion source 270, one could use an emitter producing any other type of beams: excited (e.g. metastable) or cooled molecules or atoms or clusters, etc., photons of any spectral range. In this case, they could be directed not only along the axis, but also at an angle to it. These beams could be pulsed or continuous. Among examples of photon beams, femtosecond UV- or visible-light or IR pulse trains, vacuum UV or nanosecond UV pulses are the most preferable.

Figure 5 shows a reaction cell 260 operated as a collision cell for hard fragmentation to provide elemental analysis of biomolecular ions. The reaction cell 260 is supplied with ions via ion optics 226 (a RF octapole). The reaction cell 260 traps ions using a trapping RF quadrupole 264 bounded by a gate electrode 262 and a trap electrode 268. Gas may be provided through gas supply 268. The reaction cell 260 is also provided with a laser source 272.

Hard fragmentation, preferably down to simple elements or their oxides, hydrides, etc., is achieved by subjecting ions to high-intensity pulses of laser light provided by laser source 272. Alternatively, a glow discharge may be used to cause the hard fragmentation. While irradiated by photons, ions could be stored in the RF quadrupole 264. The mass range of stored ions may be improved in favour of low-mass ions by an additional axial magnetic field that may be provided by a permanent magnet 274.

The reaction cell 260 of Figure 5 may be adapted for use in ECD by substituting an electron source for the laser source 272. The electron source 272 can be used to introduce low-energy electrons into the reaction cell 260 to cause ECD. However, the presence of an RF trapping field is undesirable as it excites the electrons to high energies and

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this materially alters the way in which the ions fragment. To overcome this problem, the ions are trapped in the reaction cell 260 using a magnetic field provided by the permanent magnet 274. Once ECD is complete, electric fields
5 may be used to assist trapping and/or effect ejection of the ions from the reaction cell 260.

In another embodiment, the reaction cell 260 comprises a DC or field-asymmetric ion mobility spectrometer. Preferably, such reaction cell includes RF-only linear ion
10 traps on both sides of the separation tube. In either case, the spectrometer is operated in two passes: first, from the entrance trap towards the back trap, and then in reverse. On one or both passes, only ions with a specific mobility or charge state are allowed to pass (i.e. the spectrometer acts
15 as a filter). The second pass could be also used for fragmenting ions selected on the first pass that is achieved by significantly increasing DC offset of the back trap relatively to the entrance trap (e.g. above 30-50 V per kDa of selected m/z).

20 The reaction cell 260 may also be used for SID. For example, the reaction cell 260 of Figure 3 may be operated such that the trap electrode 266 provides a collision surface to effect fragmentation through SID. For example, self-assembled monolayers of various organic molecules are
25 known to provide good efficiency of fragmentation. The combination of this SID with trapping of ions using the trapping RF quadrupole 264 and collisional cooling (via gas supply 268) ensures better transmission of ions back into the intermediate ion store 220 and better flexibility in the
30 choice of collision energy. Alternatively, the trap electrode 266 may be used as a surface for ion soft-landing and preparatory mass spectrometry, as described in

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WO03/105183. In this case, ions could be deposited on the trap electrode 266 while quality control is carried out by concurrent analysis in the mass analyser 340 or by analysing ions desorbed from the surface (e.g. by a laser).

5 Also, the reaction cell 260 may comprise a further mass analyser. Of course, various combinations of the features contributing to the reaction cells 260 described above may be employed.

As will be appreciated by those skilled in the art,
10 various modifications may be made to the embodiments described above without departing from the scope of the present invention that is defined by the appended claims.

For example, inclusion of the first mass analyser 180 is optional. This part may merely be an ion trap with no
15 mass analysis function, or this part may be omitted entirely.

CLAIMS

1. A method of mass spectrometry using a mass spectrometer having a longitudinal axis, comprising the sequential steps
5 of:
- (a) generating ions in an ion source;
 - (b) extracting ions such that they travel along the longitudinal axis of the mass spectrometer in a forwards direction relative to the ion source;
 - 10 (c) causing the ions to enter and then to exit an intermediate ion store as the ions travel along the longitudinal axis in the forwards direction;
 - (d) causing the ions to enter a reaction cell as they travel along the longitudinal axis in the forwards
15 direction;
 - (e) processing the ions within the reaction cell;
 - (f) causing the processed ions to exit the reaction cell to travel back along the longitudinal axis in a backwards direction relative to the ion source;
 - 20 (g) causing the processed ions to enter the intermediate ion store once more as they travel along the longitudinal axis in the backwards direction
 - (h) causing one or more pulses of the processed ions to exit the intermediate ion store in an off-axis direction;
 - 25 (i) causing the one or more pulses of processed ions to enter a mass analyser; and
 - (j) obtaining a mass spectrum of the one or more pulses of processed ions using the mass analyser.
- 30 2. The method of claim 1, wherein step (c) further comprises trapping ions in the intermediate ion store after the ions have entered and then allowing the ions to exit.

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3. The method of claim 1 or claim 2, wherein processing the ions in step (e) comprises changing the ion population in the reaction cell.

5 4. The methods of claim 3, wherein processing in step (e) comprises removing a fraction of the ion population.

5. The method of claim 4, wherein processing in step (e) comprises removing a fraction of the ion population using
10 mass selection.

6. The method of any of claims 3 to 5, wherein processing in step (e) comprises introducing further ions to the ion population.

15

7. The method of any of claims 3 to 6, wherein processing in step (e) comprises altering the charge of at least some of the ion population.

20 8. The method of any of claims 3 to 7, wherein processing in step (e) comprises altering the energy spread of the ion population.

9. The method of any of claims 3 to 8, wherein processing
25 in step (e) comprises fragmenting at least some of the ion population.

10. The method of claim 9, wherein fragmenting in step (e) comprises: collisions with molecules of gas; collisions with
30 a surface; collisions with reactive stable molecules; collisions with metastable molecules or atoms; collisions with ions of the opposite charge; or irradiation by photon

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beams from the spectral range from vacuum UV to IR and from attosecond to continuous.

11. The method of claim 9, wherein fragmenting in step (e)
5 does not comprise electron capture dissociation.

12. The method of claim 9, wherein fragmenting in step (e) comprises electron capture dissociation.

10 13. The method of claim 12, further comprising trapping the ions in the reaction cell without the use of a magnetic field while fragmenting through electron capture dissociation.

15 14. The method of any preceding claim, wherein step (e) comprises trapping the ions in the reaction cell.

15. The method of claim 14, comprising trapping the ions in two or more trapping regions in the reaction cell.

20

16. The method of claim 14 or claim 15, comprising separating ions while being transferred between the two or more trapping regions according to their mobility, m/z or differential ion mobility.

25

17. wherein step (f) comprises ejecting the processed ions by manipulating DC voltages placed on the reaction cell.

18. The method of any preceding claim, comprising trapping
30 the processed ions in the intermediate ion store in step (h).

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19. The method of claim 18, wherein the processed ions are trapped by cooling the ions such that they lose energy.

20. The method of claim 19, comprising cooling the ions by
5 collisional cooling or adiabatic cooling.

21. The method of any of claims 18 to 20, comprising trapping the ions in a curved linear trap corresponding to the intermediate ion store.

10

22. The method of claim 21, comprising in step (h) causing one or more pulses of the processed ions to exit the intermediate ion store in an off-axis direction such that the one or more pulses travel normally to the longitudinal
15 axis to be radially convergent.

23. The method of any of claims 17 to 22, comprising ejecting the ions as pulses with a duration of less than one of: 1 ms, 10 microseconds or 0.5 microseconds.

20

24. The method of any of claims 17 to 23, comprising ejecting the ions as pulses with a spatial length less than one of: 1 m, 50 mm, 10 mm or 5 mm.

25. The method of any preceding claim, comprising introducing gas into the reaction cell to a pressure such that the product of gas pressure and the length of the reaction cell does not exceed 1 mbar·mm.

26. The method of claim 25, wherein the product does not
30 exceed 0.2 mbar·mm.

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- 27 The method of claim 26, wherein the product does not exceed 0.1 mbar·mm.
28. The method of any preceding claim, further comprising a
5 step of mass selection.
29. The method of any preceding claim, further comprising, between steps (g) and (h), the steps of:
- 10 allowing the ions to exit the intermediate ion store along the longitudinal axis in the backwards direction;
- reflecting the ions such that they travel back along the longitudinal axis in the forwards direction such that the ions pass through the intermediate ion store once more and then enter the reaction cell;
- 15 further processing the ions within the reaction cell;
- causing the processed ions to exit the reaction cell to travel back along the longitudinal axis in a backwards direction and to enter the intermediate ion store once more.
- 20 30. The method of any preceding claim, further comprising, between steps (b) and (c), the step of causing the ions to enter and then to exit an ion trap as they travel along the longitudinal axis in a forwards direction.
- 25 31. The method of claim 30, further comprising trapping the ions in the ion trap prior to allowing the ions to exit the ion trap along the longitudinal axis in the forwards direction.
- 30 32. The method of claim 31, further comprising obtaining a mass spectrum of ions trapped in the ion trap.

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33. The method of any of claims 30 to 32, further comprising using the ion trap as a mass filter such that only ions within a desired mass range are allowed to exit the ion trap along the longitudinal axis in the forwards
5 direction.

34. The method of claim 33, comprising using the ion trap to implement automatic gain control.

10 35. The method of claim 33 or claim 34, comprising using the ion trap as a mass filter on more than one pass of the ions through the ion trap.

36. A mass spectrometer having a longitudinal axis,
15 comprising:

an ion source;

ion optics operable to guide ions produced by the ion source along the longitudinal axis;

an intermediate ion store located downstream of the ion
20 source and having first and second apertures located on the longitudinal axis, such that the first aperture faces the ion source, and a third aperture located off axis;

a reaction cell located downstream of the intermediate ion store and having an aperture that faces the second
25 aperture of the intermediate ion store, wherein the reaction cell is operable to process ions; and

a mass analyser located adjacent the intermediate ion store having an entrance aperture that faces the third aperture of the intermediate ion store,
30 and wherein the intermediate ion store is operable to eject one or more pulses of ions out of the third aperture to the mass analyser.

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37. The mass spectrometer apparatus of claim 36, wherein the intermediate ion store has an associated gas supply for introducing gas into the intermediate ion store.

5

38. The mass spectrometer apparatus of claim 36 or claim 37, wherein the intermediate ion store is a curved linear ion store, the curvature being such as to focus ions ejected radially convergent from the ion store through the
10 third aperture.

39. The mass spectrometer apparatus of any of claims 37 to 38, wherein the reaction cell is a gas-filled ion-molecule reactor.

15

40. The mass spectrometer apparatus of any of claims 36 to 39, wherein the reaction cell has an ion source operable to introduce ions into the ion cell.

20 41. The mass spectrometer apparatus of any of claims 36 to 40, wherein the reaction cell is a fragmentation cell.

42. The mass spectrometer apparatus of claim 41, wherein the fragmentation cell is a gas-filled collision cell for
25 collision induced dissociation.

43. The mass spectrometer apparatus of claim 41 or claim 42, wherein the fragmentation cell further comprises a surface for surface induced dissociation.

30

44. The mass spectrometer of any of claims 41 to 43, wherein the fragmentation cell is arranged for fragmenting

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ions by collisions with reactive stable molecules;
collisions with metastable molecules or atoms; collisions
with ions of the opposite charge; or irradiation by photon
beams from the spectral range from vacuum UV to IR and from
5 attosecond to continuous.

45. The mass spectrometer apparatus of any of claims 41 to
claim 44, wherein the fragmentation cell does not comprise
an electron source for electron capture dissociation.

10

46. The mass spectrometer apparatus of any of claims 41 to
claim 44, wherein the fragmentation cell further comprises
an electron source for electron capture dissociation.

15 47. The mass spectrometer of claim 46, not comprising a
magnet operable to trap ions within the reaction cell.

48. The mass spectrometer apparatus of any of claims 36
to 47 further comprising an ion trap located between the ion
20 source and the intermediate ion store and having apertures
located on the longitudinal axis.

49. The mass spectrometer apparatus of claim 48, wherein
the ion trap includes a mass analyser.

25

50. The mass spectrometer apparatus of any of claims 36
to 49, further comprising a controller operable to perform
the method of any of claims 1 to 35.

30 51. A computer program comprising computer program
instructions that, when executed by the controller of the

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apparatus of claim 50, cause the controller to perform the method of any of claims 1 to 35.

52. A computer storage medium having stored thereon the
5 computer program of claim 51.

53. A mass spectrometer having a longitudinal axis,
comprising:

an ion source to direct ions along said axis;
10 a reaction cell having an entrance aperture located on
said axis;
a mass analyser; and
ion optics switchable between a first mode in which
ions from the ion source are guided along said axis to said
15 reaction cell and product ions produced in the reaction cell
are guided to the mass analyser for analysis, and a second
mode in which ions from the ion source are deflected from
said axis and guided to the mass analyser for analysis
without entering the reaction cell.

20

54. A mass spectrometer having a longitudinal axis,
comprising:

an ion source to direct ions along said axis;
a reaction cell;
25 a mass analyser having an entrance aperture located on
said axis; and
ion optics switchable between a first mode in which
ions from the ion source are deflected from said axis and
guided to the reaction cell and product ions produced in the
30 reaction cell are guided back to said axis and to said
entrance aperture of the mass analyser, and a second mode in
which ions from the ion source are guided along said axis to

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the mass analyser for analysis without entering the reaction cell.

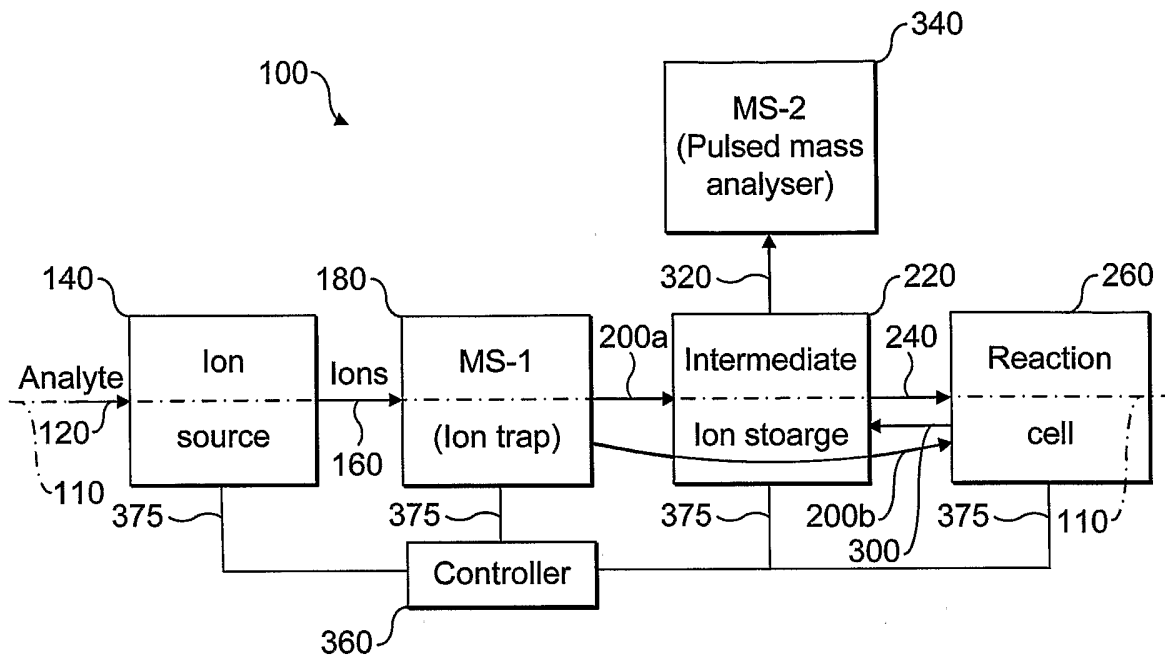


FIG. 1

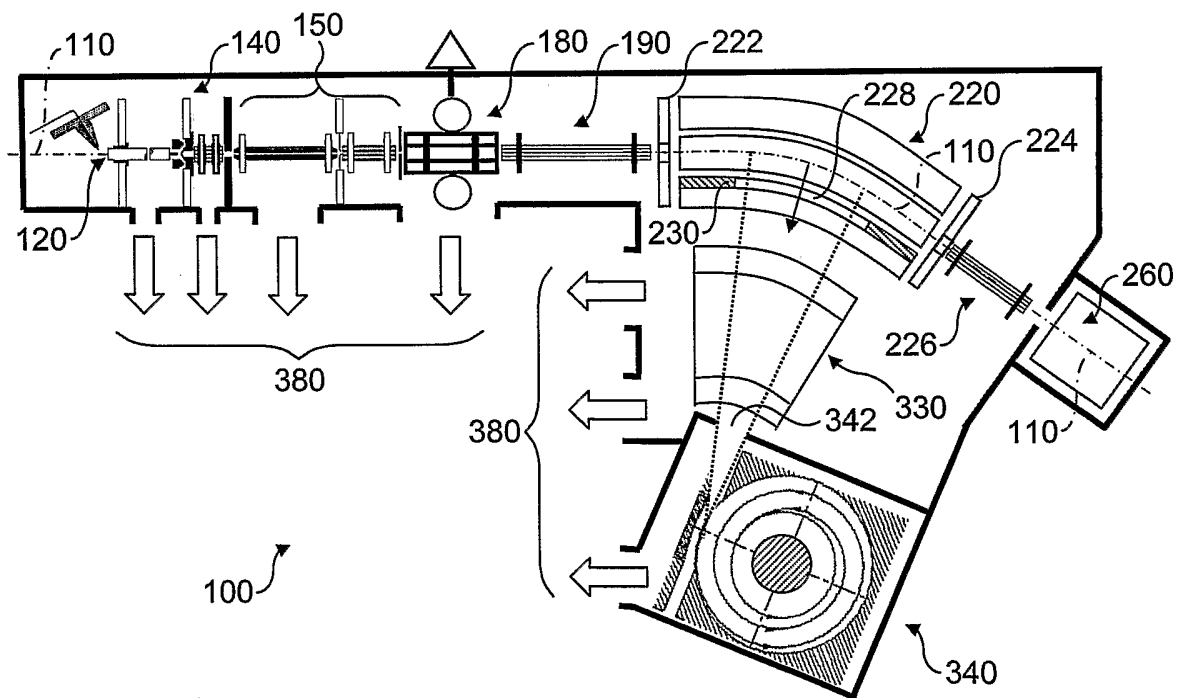


FIG. 2

2 / 2

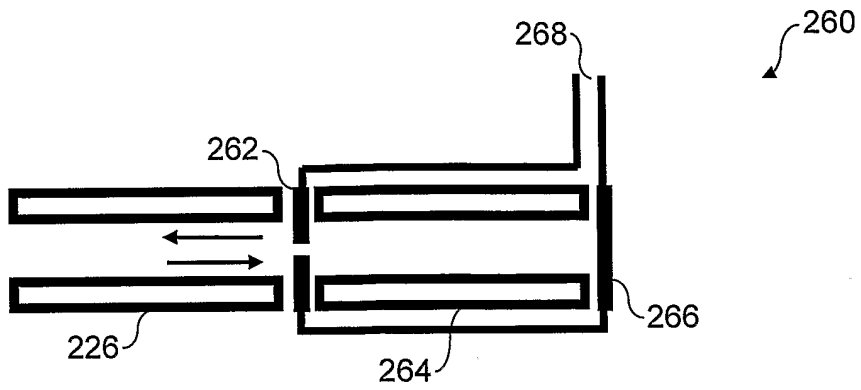


FIG. 3

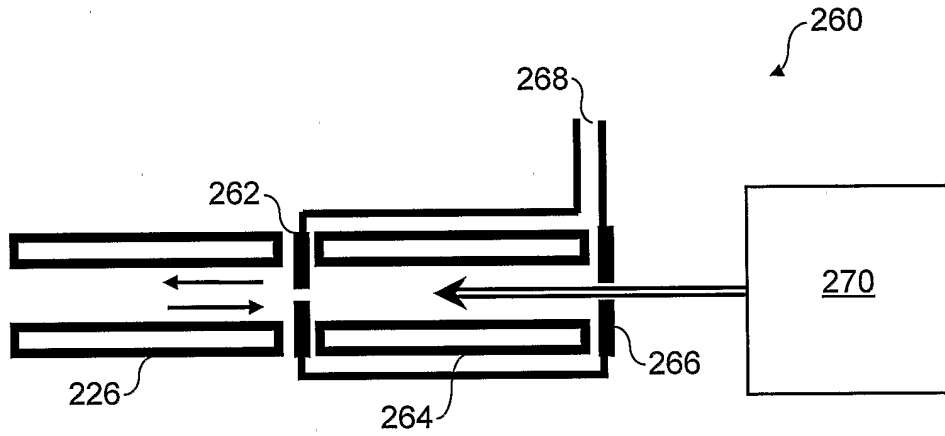


FIG. 4

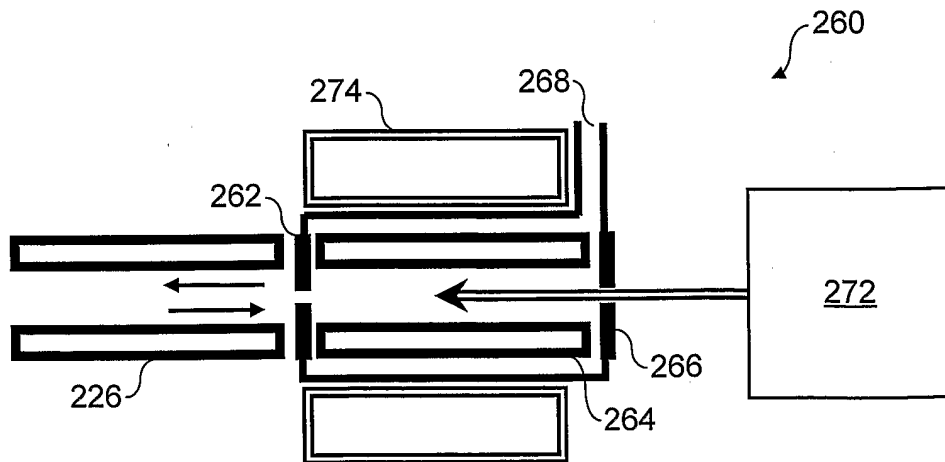


FIG. 5