OPTICAL DETECTION METHODS FOR MASS SPECTROMETRY OF MACROIONS

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Detection of macroions has been a challenge in the field of mass spectrometry. Conventional ionization-based detectors, relying on production and multiplication of secondary electrons, are restricted to detection for charged particles of $m/z < 1 \times 10^6$. While both energy-sensitive and charge-sensitive detectors have been developed recently to overcome the limitation, they are not yet in common use. Photon-sensitive detectors are suggested to be an alternative, with which detection of macroions (or charged particles) by either elastic light scattering (ELS) or laser-induced fluorescence (LIF) has been possible. In this article, we provide a critical review on the developments of novel optical detection methods for mass spectrometry of macroions, including both micron-sized and nano-sized synthetic polymers as well as high-mass biomolecules. Design and development of new spectrometers making possible observations of the mass spectra of macroions with sizes in the range of 10–10^3 nm or masses in the range of 1–10^6 MDa are illustrated. The potential and promise of this optical approach toward macroion detection with high efficiency are discussed in practical aspects. © 2004 Wiley Periodicals, Inc., Mass Spec Rev 23:443–465, 2004

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I. INTRODUCTION

Analysis of nanometer-sized particles is receiving ever-increasing attention recently in many fields of science and technology, ranging from physics through chemistry to biology and engineering. These particles (inorganic, organic, and biological) play important roles in nature as interstellar dust (Ehbrecht & Huisken, 1999), atmospheric aerosols (Reents et al., 1995; Kane, Oktem, & Johnston, 2001), viruses (Tito et al., 2000; Fuerstenau et al., 2001), and nanomaterials (Schaaff & Whetten, 2000; Khitrov & Strouse, 2003), etc. They differ from bulk materials in grain size, shape, and surface-to-volume ratio, which are the origins of their unique electrical, optical, thermodynamic,

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mechanical, chemical as well as biological properties. However, the small size of the nanostructures hampers the applications of well-established testing and measurement techniques for detailed analysis. This includes mass spectrometry, which is a useful diagnostic tool for nano-sized particles of various compositions, shapes, and dimensions.

Atmospheric aerosols are a citable example to show the importance of the mass analysis. The particles impact the earth's climate, air quality, and human health. To understand these impacts, it is vital to know the sizes, masses, and chemical compositions of these aerosols. The recent advances of single particle laser ablation time-of-flight (TOF) mass spectrometry have allowed real-time analysis of both sizes and chemical compositions of aerosol particles with diameter in the range of $d = 0.1–10$ μm (Noble & Prather, 2000; Buzorius et al., 2002). The analysis is accomplished by the use of a high power laser to vaporize and ionize the aerosol, followed by mass spectrometric measurement of the photoionized products in single particle events. The aerosol's mass (or density), on the other hand, can only be obtained indirectly by assuming the shape of the particle (Johnston, 2000). To provide more accurate information about the masses of atmospheric, environmental as well as biological aerosols (Sipin, Guazzotti, & Prather, 2003), development of new spectrometric methods for direct mass measurement is necessary and imperative.

Mass is a critical parameter for characterization and identification of biological molecules, complexes, assemblies, and particles. The advent of new ion sources, such as electrospray ionization (ESI) (Wong, Meng, & Fenn, 1988) and matrix-assisted laser desorption/ionization (MALDI) (Karas & Hillenkamp, 1988; Tanaka et al., 1988), has led to widespread applications of mass spectrometry in modern biomedical and biotechnology research (Dass, 2001). Very large biomolecules, including monoclonal human immunoglobulin IgM with $m = 980$ kDa (Nelson, Dogruel, & Williams, 1994) and DNA fragments containing 2,180 nucleotides (Berkenkamp, Kirpekar, & Hillenkamp, 1998), have been investigated with a MALDI-TOF-mass spectrometer (MS). For synthetic polymers (Nielen, 1999; Murgasova & Hercules, 2003), Schriemer & Li (1996) reported detection of doubly charged polystyrene ions with a mean molecular mass exceeding 1.5 MDa using the same type of instrument. This mass analysis range is comparable to that can be achieved by ESI, as first illustrated by Nohmi & Fenn (1992) and later by Smith et al. (1994) for polyethylene glycol ions carrying more than 4,000 charges. To go beyond $m/z = 1 \times 10^6$ (such as that of singly charged particles with sizes of $d > 10$ nm), the analysis is primarily limited by the detector, a problem similarly encountered in the earliest experiments of Dole et al. (1968) and Mack et al. (1970) in creating a molecular beam of macroions.

Electron multipliers of various types have received wide acceptance as the ion detectors in mass spectrometry over the past 30 years (Birkinshaw, 2002). The detectors are highly sensitive, robust, and easy to operate. They are the vital component of many detection systems and their performances are central to the measurement of mass spectra. However, being ionization-based (namely, relying on the production of secondary electrons or charges created in a semiconductor), they lack the detection sensitivity for high-mass ions because of the low velocity of the ions impinging on the detector elements (Beuhler & Friedman, 1980). For microchannel plates (MCP) as an example, the detection efficiency of the device for an ion with $m/z = 1 \times 10^4$ is approximately 80% at an impact energy of 20 keV, whereas it drops rapidly to ~3% at 5 keV (Gilmore & Seah, 2000; Fraser, 2002). A voltage as high as 1 MV has to be applied in order to accelerate a particle with $m/z = 1 \times 10^5$ to a velocity above the detection threshold ($\sim 1 \times 10^4$ m/sec) of MCP. Clearly, if larger or more complex biological systems (such as viruses and bacteria) are to be investigated, improvements in the efficiency of the ion detector are required (Siuzdak, 1994). Most recently, both energy-sensitive (or cryogenic) detectors (Frank et al., 1999; Kraus, 2002) and charge-sensitive (or inductive) detectors (Benner, 1997; Fuerstenau et al., 2001) have been developed and demonstrated that they are capable of detecting single, large biomolecular ions. Mass spectra of macroglobulin with masses up to 750 kDa can be obtained with MALDI-TOF-MS equipped with a semiconducting tunnel junction detector (Frank et al., 1999). Moreover, both the masses and charge states of ESI-generated intact viruses ($m > 1$ MDa) have been determined with use of a charge-sensitive TOF detection tube (Fuerstenau et al., 2001). Although substantial progresses have been made in these two directions, there remains much room for improvement of the performance of the detectors as well as the development of new ion detection methods (Cristoni & Bernardi, 2003).

Photon-sensitive (or laser-based) detectors are the third type of device with considerable potential for macroion detection. This type of detection, involving both elastic light scattering (ELS) and laser-induced fluorescence (LIF), is by no means new. Particular is the ELS, which has been utilized as a method for detection of macroions (more appropriately, charged particles) nearly a century ago by Millikan (1910) in his famous oil drop experiments. This detection method is still in use today for charged and neutral particles separated according to their electrical mobility (Kaufman, 1998) and aerodynamic size (Noble & Prather, 2000) in aerosol science. The LIF, on the other hand, is a routine technique in the study of atomic, molecular, and cluster ions in the spectroscopy and dynamics communities (Duncan, 2000). In contrast to ELS, the method has been rarely adopted to detect macroions for

mass spectrometry purpose. Since a large number of review articles on the single particle aerosol mass spectrometry based on aerodynamic sizing have been available in the literature (Suess & Prather, 1999; Noble & Prather, 2000; Sipin, Guazzotti, & Prather, 2003), they are not repeated here. In this article, we will focus on the application of the photon-sensitive detection methods to mass determination, rather than size determination, of nano-sized to micron-sized synthetic particles as well as high-mass biomolecules and biomolecular assemblies. Moreover, we will limit our discussion to the detection of macroions with $m/z > 1 \times 10^6$, a region relatively under-explored in the field of mass spectrometry. Since until very recently we have been almost the only group devoted to development of optical detection methods for mass spectrometry of macroions, this article is closer to a personal account than a review. In the following two sections, after a brief survey of both energy-sensitive and charge-sensitive detectors and an introduction of the optical detection method, applications of ELS and LIF to mass analysis of macroions leading to development of nanoparticle mass spectrometry are discussed.

II. MACROION DETECTION METHODS

A. Energy-Sensitive Detection

Utilization of energy-sensitive detectors for biopolymer mass spectrometry was first proposed by Twerenbold (1996). In contrast to the ionization-based detection, which depends on creation of secondary electrons from ion impact, the cryogenic detector is sensitive to the total energies carried by the incoming charged particles and hence is conceivably more sensitive to the ion impact from weakly ionizing, slow-moving molecules. Employing a liquid-He-cooled Sn-based semiconducting tunnel junction detector, Twerenbold et al. (1996) demonstrated the concept with lysozyme ($m = 14.3$ kDa) and other large biomolecules in a TOF-MS measurement. An Nb-based semiconducting tunnel junction detector was utilized almost at the same time by Frank et al. (1996) for high-efficiency detection of human serum albumin ($m = 66$ kDa). Single protein molecules with masses as high as 750 kDa can be detected with a signal-to-noise ratio of S/N $\approx$ 5/1 (Frank et al., 1999). Comparing the response to that of MCP indicated a much higher sensitivity per area of the detector element for the cryogenic detector at large m (Hilton et al., 1998). While the cryogenic detector can reach near 100% efficiency for the detection of macroions, the application requires the detector element to operate at very low temperatures (<2 K) and is disadvantageous in that the detector has a relatively long response time ($\sim$1 μsec) and a relatively small detection area (<1 mm^2) for TOF-MS measurements. Another impediment to the use of the cryogenic detector is that the detector element needs to be regenerated after long time accumulation of the non-volatile biomolecules on its surface. An in-depth review on the history of the development as well as the principle and operation of this novel detector for high-mass biomolecules has been given by Frank et al. (1999) and, more recently, by Kraus (2002).

B. Charge-Sensitive Detection

Charge-sensitive detection is the standard protocol in modern Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (Marshall, Hendrickson, & Jackson, 1998). Imaging currents are induced and detected when an ion package is in coherent orbiting motion between two parallel conductive plates. Unlike the electron multiplier, however, the detection does not require production of secondary electrons but rather the magnitude of the image currents is a function of ion density but is independent of mass. The fundamental detection limit of this method for singly charged ions is $\sim$100 in a 1 sec data acquisition period. Whereas the detection is not as sensitive as ion counting, it is a non-destructive method and has allowed observation of the mass spectra from sub-attomole protein samples (Valaskovic, Kelleher, & McLafferty, 1996) and enabled multiply charged polymeric ions to be characterized individually (Bruce et al., 1994; Cheng et al., 1994; Smith et al., 1994).

In view of the detection restriction of MCP and the high sensitivity of the image current method, Park & Callahan (1994) developed a three-layered detector sensitive to the charges of the investigated ions in a TOF measurement. A Faraday-type detector was developed independently by Imrie, Pentney, & Cottrell (1995) to provide reliable MALDI-TOF spectra of high-mass ions, including bovine insulin ($m = 5.7$ kDa), bovine serum albumin ($m = 66.4$ kDa), and fibrinogen L ($m = 330$ kDa). A sensitivity level comparable to that of MCP was established for large biomolecules such as immunoglobulin (IgG) monoclonal antibodies (Bahr et al., 1996). Further enhancement of the sensitivity with a multiple reflection scheme affords detection of a single 4.3-kilobase DNA ion carrying 75 charges in a gated linear electrostatic ion trap (Benner, 1997). However, to detect an ion ensemble with this novel device, synchronization of the ion motion to create a coherent ion packet inside the trap is required (Pedersen et al., 2001).

In development and application of the charge-sensitive technique to macroion detection, Benner and co-workers made notable contributions (Fuerstenau & Benner, 1995; Benner, 1997; Schultz, Hack, & Benner, 1998, 1999). In particular, the authors developed a non-destructive charge-sensitive tube, as originally invented

by Shelton, Hendricks, & Wuerker (1960) for multiply charged microparticles. The detector consists of an insulated drift tube mounted coaxially within a grounded shield with grids on either end. The passage of a charged particle through the detector induces a voltage proportional to the capacity of the system on the tube. The duration of this induced signal is equal to the TOF through the detector. Knowing the measured velocity and charge of one of these particles, together with measuring the voltage through which it has been accelerated, one can compute the mass of the particle. Utilization of this charge-sensing tube for macroion detection has been successfully demonstrated by Fuerstenau & Benner (1995) in simultaneous measurements of the charge numbers and masses of ESI-generated synthetic nanoparticles and DNA fragments individually. Application of the same technique to intact microorganisms has also enabled them to determine the absolute masses of both the spherical rice yellow mottle virus (RYMV) and the cylindrical tobacco mosaic virus (TMV). Their results are reproduced in Figure 1 (Siuzdak et al., 1996; Fuerstenau et al., 2001).

C. Photon-Sensitive Detection

Photon-sensitive detection can be utilized as a method for mass spectrometry of macroions as well (Cai et al., 2002a,b,c; Cai, Peng, & Chang, 2003; Peng et al., 2003). The method involves detection of ESL and LIF from micron-sized and nano-sized particles in the gas phase. Whereas the method differs from conventional ion detec-

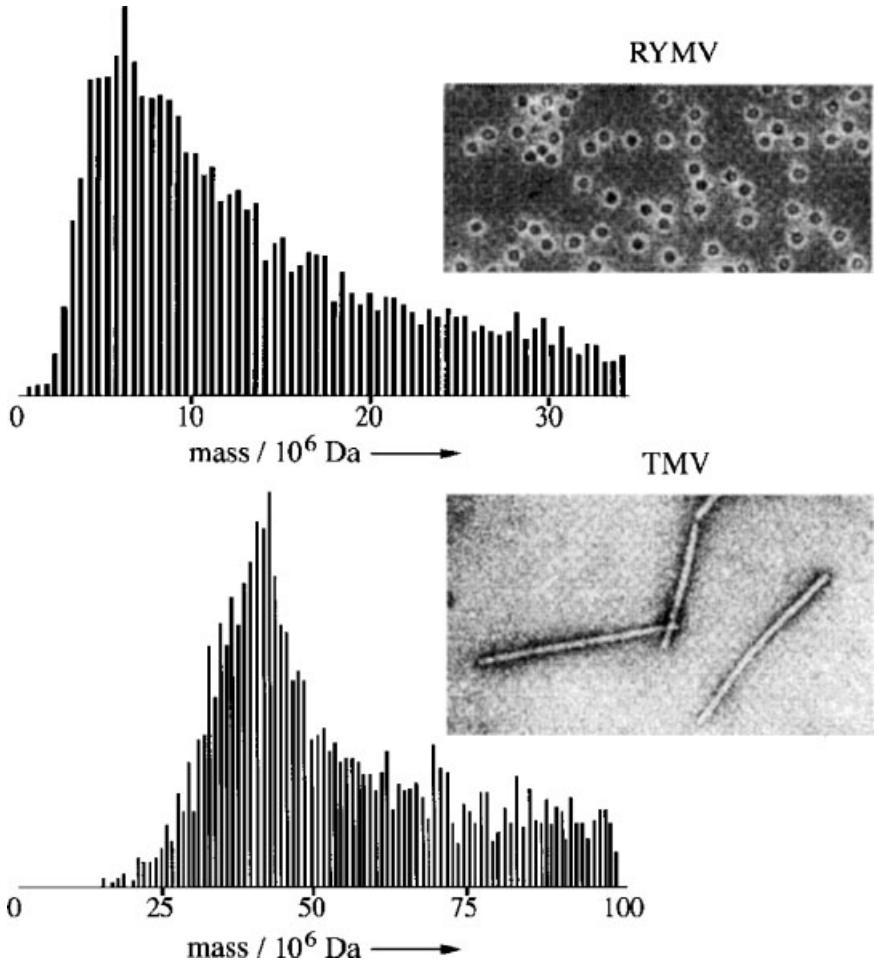

FIGURE 1. Mass spectra of the rice yellow mottle virus (RYMV, **top**) and the tobacco mosaic virus (TMV, **bottom**) analyzed with a charge-detection electrospray ionization (ESI)-time-of-flight mass spectrometer (MS). Inset, electron micrographs of icosahedral RYMV (diameter of 28.8 nm) and cylindrical TMV (~300 nm long and 17 nm in diameter). The known molecular masses of RYMV and TMV are 6.5 and 40.5 MDa, respectively. (Reproduced from Fuerstenau et al. (2001) with permission from Wiley-VCH, copyright 2001.)

tion in both principle and practice, it shares close similarities to those of the fluorescence imaging techniques commonly employed in condensed phases for detection of stained biomolecules in gels, microplates, and microfluidic channels after electrophoresis. One example for such detection is given by McClain et al. (2001), who demonstrated that both the light scattering and dye-labeling techniques are powerful means in revealing the molecular structure and compositions of bacteria on a microfabricated fluidic device. For *Escherichia coli* in particular, they showed that the coincidence detection rate between the LIF and ELS signals is more than 95%. The essential difference between these two methods is that it is very difficult to detect particles smaller than 100 nm with light scattering. The finding is in accordance with the conclusion reached by Hirschfeld, Block, & Mueller (1977) more than two decades ago in developing "virometry" with optical detection methods. The authors concluded therein that whereas the fluorescence signal levels are usually lower than those obtainable by light scattering, they change linearly with the fluorophore content, and cover a 10^2:1 dynamic range for known viruses. Scattering, on the other hand, goes with the inverse sixth power of the diameter, and has an instrumentally difficult 10^7:1 dynamic range. This flatter size dependence makes fluorescence the stronger signal for smaller viruses. The conclusions similarly apply to optical detection of charged viral particles suspended in the gas phase.

1. Elastic Light Scattering (ELS)

One of the famous examples in utilizing light scattering as a method to detect macroions (or, more appropriately, charged particles) can be attributed to Millikan (1910), who determined the elementary charge of an electron from close examination of the motions of the individual charged oil droplets with known masses between two electrostatic plates. Many experiments followed in an effort to measure the absolute mass of a single levitated particle based on the well-determined elementary charge using the Millikan condenser as an electrostatic picobalance (Davis, 1997). The Millikan condenser, however, is not ideal for levitating particles in free space over a long period of time. A better device to serve the purpose is the quadrupole ion trap (QIT), originally invented for the study of atomic and molecular ions (Paul, 1990). The capabilities of trapping and detection of a single charged particle as well as multiple particles in a QIT were first demonstrated by Wuerker, Shelton, & Langmuir (1959). With use of a carbon arc illuminator, light scattering from aluminum microparticles clearly revealed the particles' motions within the trap. Figure 2a and b show, respectively, a 2:1 Lissajous trajectory of a single trapped aluminum particle ($d \approx 20$ μm) and a crystal-like pattern of 32 aluminum particles observed by the authors. From the Lissajous trajectory, they determined the axial eigenfrequency (ω_z) for the single charged particle by applying a small ac voltage across the end-cap electrodes and visually observed when the particle's secular motion is in resonance with the applied signal. The mass-to-charge ratio (m/z) of the particle was then calculated from the expression (March & Hughes, 1989)

$$\frac{m}{z} = \frac{\sqrt{2}V_{ac}}{\omega_z r_0^2 \Omega}, \qquad (1)$$

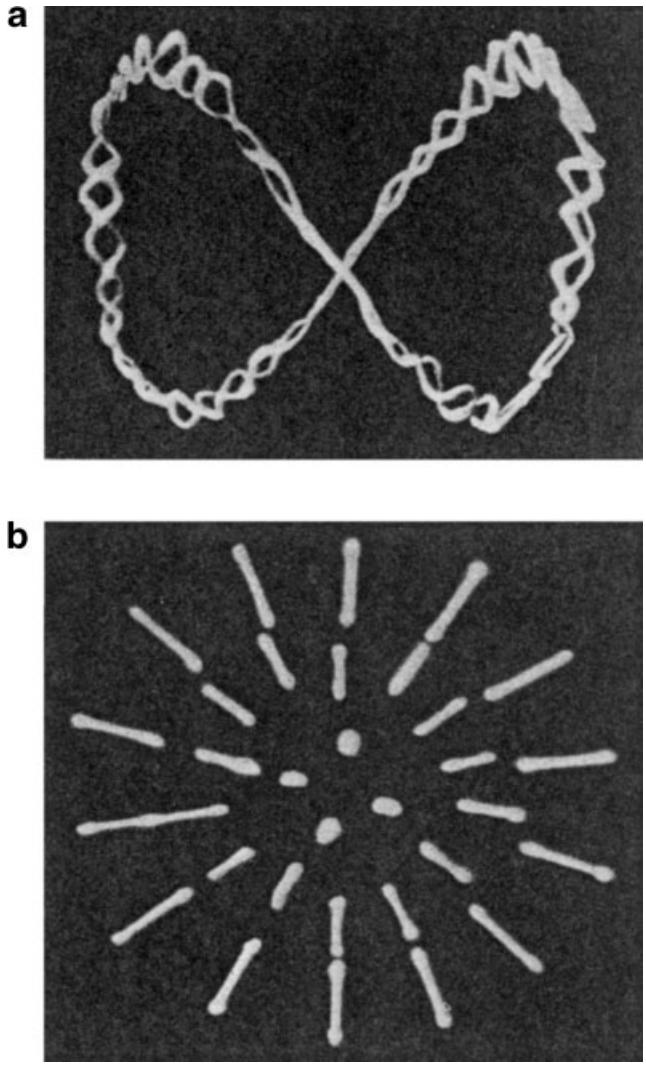

FIGURE 2. **a**: Photograph of a 2:1 Lissajous orbit in the r-z plane of a single charged aluminum microparticle (diameter ~20 μm). **b**: Crystal-like pattern of 32 condensed aluminum microparticles viewed in the r-θ plane. The frequency and voltage amplitude of the electrodynamic field used for the trapping were 200 Hz and 720×2 V (bipolar mode), respectively. (Adapted from Wuerker, Shelton, & Langmuir (1959) with permission from American Institute of Physics, copyright 1959.)

which is valid at the trap parameter $q_z \leq 0.4$, where

$$q_z = \frac{4ZeV_{ac}}{mr_0^2\Omega^2}, \tag{2}$$

Z is the charge number, e is the elementary charge, Ω is the angular frequency, V_{ac} is the voltage amplitude, and r_0 is the radius of the ring electrode. The precision of their measurements was on the order of 10^{-3}.

Gerlich and co-workers have recently addressed an interesting possibility of utilizing the QIT as a nanoparticle MS (Gerlich, Illemann, & Schlemmer, 2000; Schlemmer et al., 2001, 2003). A scheme for non-destructive high-resolution measurement and absolute mass determination of single charged particles was developed. The authors recorded and analyzed in detail the amplitude modulations of the ELS signals, from which the eigenfrequencies (and hence m/z) of the single trapped particles were deduced with high precision (better than 100 ppm) utilizing a fast Fourier transform (FFT) method. The absolute number of the charges was then determined by one electron differentials, following the procedures of Arnold (1979) and Philip, Gelbard, & Arnold (1983). Figure 3 shows the step charging of a single 500-nm SiO_2 sphere weighing 130 fg (corresponding to 10^{11} Da per particle) in a modified Paul trap as a result of electron bombardments. A mass measurement accuracy on the order of 10^{-4} was achieved in a 10 sec data collection period. On a longer time scale, the 10^{-6} range is accessible (Schlemmer et al., 2001). Similar conclusion was also reached by Hars & Tass (1995), who proposed to use a star branch counting method for high precision m/z measurements.

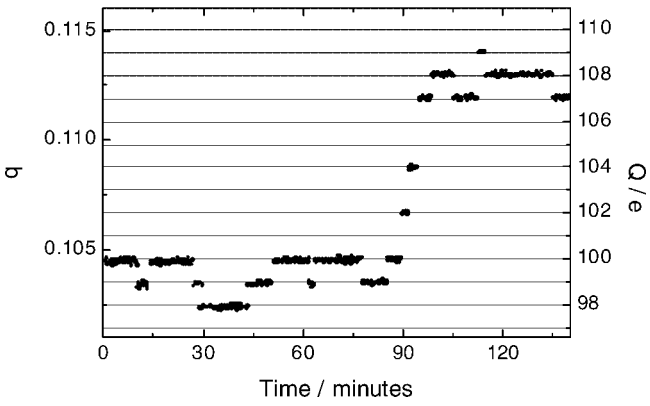

FIGURE 3. Step charging of a single 500-nm SiO_2 sphere during the online determination of its eigenfrequency. The charging current was so low that the emission of secondary electrons is separated as individual events with the charge differential $\Delta Q = (n-1)e$, where n is an integer and e is the elementary charge. The absolute number of the charges, $Z = Q/e$, was determined from the discrete $\Delta Q = -e$ events. (Adapted from Schlemmer et al. (2001) with permission from American Institute of Physics, copyright 2001.)

Inspired by the high-precision work of Gerlich and co-workers, Cai et al. (2002a,b) constructed a single particle ion trap MS equipped with a continuous-wave (CW) laser for particle illumination and a photomultiplier tube (PMT) for light detection. Different from the aforementioned methods, which all involve detection of a single particle inside the trap, particles with different m/z are ejected consecutively from the QIT and detected externally. The spectrometer so constructed combines the advantages of single particle detection and real-time monitoring capabilities and, hence, rapid and routine analysis of the particles is possible. However, when conducting the measurement, it faces the difficulty that the residence time of the individual particles within the laser excitation region is short, typically 100 μsec (Cai et al., 2002b). The time is ~4 orders of magnitude shorter than when they are confined in space for interrogation (typically 1 sec). An attempt to use this device to obtain the single particle mass spectra of polystyrene spheres with sizes of ~100 nm was unsuccessful; it suffered the difficulty of differentiating signals from backgrounds even at a very small forward scattering angle (Cai et al., 2002b). With use of an ellipsoidal reflector for light collection, Galli, Guazzotti, & Prather (2001) reported that the smallest particle they could detect with ELS is 80 nm. According to the Rayleigh–Debye theory, which is valid for homogeneous spheres with $a(n-1)/\lambda \ll 1$ under vacuum, the intensity of the scattered light collected at a forward scattering angle θ and a distance r is (Van de Hulst, 1957; Bohren & Huffman, 1983)

$$I = \frac{I_0 F(\theta, x, n)}{k^2 r^2} \tag{3}$$

where

$$F(\theta, x, n) = x^6 \left(\frac{n^2-1}{n^2+2}\right)^2 \cos^2\theta, \tag{4}$$

$x = 2\pi a/\lambda$, $k = 2\pi/\lambda$, λ and I_0 are the wavelength and intensity of the incident light, and a and n are the radius and refractive index of the particle, respectively. Since I is proportional to $a^6(n^2-1)^2/\lambda^4(n^2+2)^2$, to detect particles much smaller than the illuminator wavelength, one should go for a light source with a shorter wavelength and/or particles with a larger refractive index (Cai et al., 2002b).

An interesting device which makes possible ELS detection of nanometer-sized particles or even sub-nanometer-sized particles is known as the condensation particle counter (CPC) or the condensation nucleus counter (CNC). The device consists of a flow cloud chamber filled with saturating butanol vapor (Kaufman, 1998) or dibutyl

phthalate vapor (Gamero-Castaño & Fernández de la Mora, 2000). Nucleation occurs when charged particles pass through the chamber, forming micron-sized droplets to be detected by light scattering with an efficiency approaching unity. The CPC couples nicely with differential mobility analyzers (DMA), which separate particles according to their electrophoretic mobilities, and has been employed as a detector for ESI-generated protein and DNA macroions (Kaufman et al., 1996; Mouradian et al., 1997) as well as viral particles (Bacher et al., 2001). Additionally, it has been adopted to study the formation, size, and size distribution of clusters in the MALDI process, where nanometer-sized particles were generated from a matrix layer by laser ablation in ambient nitrogen at atmospheric pressure (Alves, Kalberer, & Zenobi, 2003). The typical electrophoretic mobility diameter spectra obtained with the DMA/CPC combination acting as a low resolution MS for viral particle analysis are shown in Figure 4 (Bacher et al., 2001).

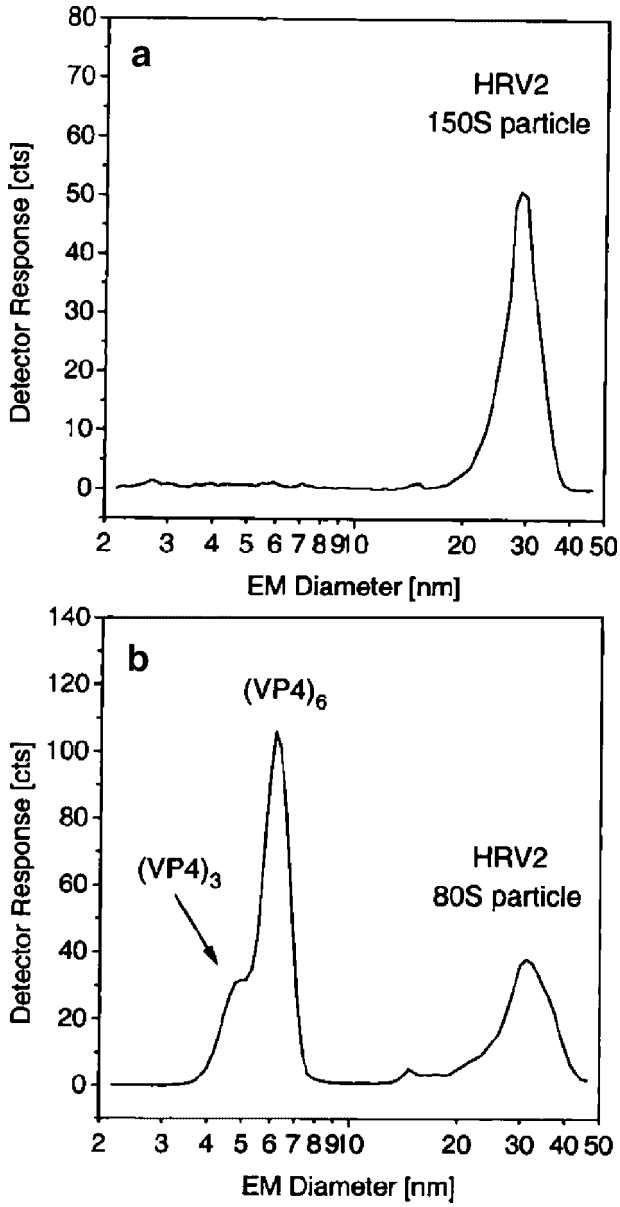

FIGURE 4. Electrophoretic mobility (EM) diameter spectra of intact human rhinovirus (HRV2) before (**a**) and after (**b**) heat degradation. The signals denoted $(VP4)_3$ and $(VP4)_6$ are non-specific oligomers of the viral capsid VP4 proteins that are released during the heat treatment. The molecular mass of the intact HRV2 virus was calculated to be more than 8 MDa based on the known building blocks and their sequences. (Reproduced from Bacher et al. (2001) with permission from John Wiley & Sons, Ltd., copyright 2001.)

2. *Laser-Induced Fluorescence (LIF)*

LIF is by far the most powerful means for the study of atomic and molecular ions confined in space. This is manifested in the detection of a single trapped ion by LIF in a Paul-like storage cell (Leibfried et al., 2003). Temperatures as low as 47 μK can be achieved for a single $^{198}Hg^+$ ion using sophisticated laser-cooling techniques (Diedrich et al., 1989). Interestingly, mass spectra of sympathetically cooled atomic ions can be acquired with LIF as well. A so-called "laser-cooled fluorescence mass spectrometry" has been developed by Baba & Waki (2001). By detecting LIF from laser-cooled Ba^+ ions in a tandem linear ion trap, the authors obtained the mass spectra of Xe isotopes from modulations of the observed fluorescence intensities with the supplemental ac frequency applied to the trap electrodes. Mass spectra of NH_4^+ and H_3O^+ are similarly obtainable from LIF detection of laser-cooled $^{24}Mg^+$ (Baba & Waki, 2002). Unfortunately, the mass spectral range of this method is quite limited, $m/z \approx 50-1{,}000$, as in the case of Ba^+.

Molecular ions have been detected directly with LIF in a linear electrodynamic trap (Welling, Thompson, & Walther, 1996; Welling et al., 1998) and a magnetic Penning trap (Wang, Hendrickson, & Marshall, 2001; Cage et al., 2002). Particularly, for molecular ions of fluorophores, Parks and co-workers observed LIF from protonated rhodamine 640 produced by ESI in a QIT (Khoury, Rodriguez-Cruz, & Parks, 2002). A total of ~1,000 ions in a laser probe volume were detected for the dye molecules under near background-free conditions. The high sensitivity of this method has enabled them to observe fluorescence resonance energy transfer (FRET) between two fluorophores attached to an oligonucleotide duplex at two different binding sites (Danell & Parks, 2003). Along with the observation of the isolated gas phase molecules, detection of a single rhodamine 6G embedded in a single

charged microdroplet levitated in a QIT has also been accomplished by Ramsey and co-workers (Whitten et al., 1991; Barnes et al., 1993). An S/N > 40 was readily achieved using CW laser excitation. Both the experiments demonstrate that LIF is a technique well suited for detection of fluorescent molecules or materials to be transported to the gas phase for close examination and, indeed, a fluorescence bio-aerosol MS has been developed very recently utilizing biomolecules such as tryptophan and riboflavin as the primary fluorophores to detect micron-sized particles (Pinnick et al., 1998; Pan et al., 2003c). For non-fluorescent materials, this optical approach is still applicable if the ions under investigation are first tagged with dye fluorophores and subsequently confined in an ion trap for repetitive LIF measurements.

Whereas fluorescent labeling is a troublesome technique, dye molecules have frequently served as a sensitive probe for specific analytical purposes (Haugland, 1996). For example, dye-labeled molecules have been exploited to study the spatial and temporal imaging of gas-phase protein and DNA molecules produced by matrix-assisted laser desorption (Heise & Yeung, 1994; Puretzky et al., 1999). Use of dye-labeling techniques has been shown to facilitate deduction of the protein structure information from mass spectrometry (Friess & Zenobi, 2001). Moreover, tagging of amino acids and peptides with near-UV absorbing chromophores has been undertaken to enhance the multiphoton ionization efficiency of biomolecules (Houston & Reilly, 2000). Finally, compositions of dye-labeled proteins that are extensively employed in molecular biology and environmental science have been analyzed with UV-visible spectroscopy in combination with MALDI-TOF-MS (Salih & Zenobi, 1998; Lu & Zenobi, 1999).

As a detection method for nano-sized materials and high-mass biomolecules, the benefit of dye-labeling for LIF detection is threefold. First, dye-labeling is in principle applicable to molecules and materials of any size and, therefore, the mass detection range of the LIF method is essentially limitless. Second, LIF is highly sensitive, allowing single dye or dye-labeled molecules to be detected (Moerner & Orrit, 1999). Therefore, it is an ideal method for detection of high-mass biomolecules and nano-sized bioparticles, which are typically low in ion density and can be fluorescently labeled readily. Third, the LIF selectively probes only sample molecules that are fluorescently labeled, unlike the ionization-based detectors which sense all ions. This, in effect, would reduce background signals arising from the cluster ions composed of matrix molecules and their fragment ions in the spectrum, a feature practically important in high-mass biomolecule mass spectrometry with MALDI as the ion source (Nelson, Dogruel, & Williams, 1994; Berkenkamp, Kirpekar, & Hillenkamp, 1998).

III. APPLICATIONS TO MICRON-SIZED AND NANO-SIZED PARTICLES

A. Ion Sources

MALDI and ESI are two soft ionization methods that have had major impact on the ability of using MS for the study of large biomolecules. First introduced to the scientific community nearly two decades ago (Yamashita & Fenn, 1984; Fenn et al., 1989, 1990), ESI has become such a versatile tool that it can transfer a wide range of non-volatile molecules (including both biological and synthetic polymers) from condensed phases to the gas phase without substantial decomposition. Whereas ESI is adopted more frequently than MALDI in analyzing large biomolecules because of its capability of creating multiply charged ions, it complicates the mass spectra, particularly, for macroions. For the MS2 virus ($d \approx 20$ nm) as an example, the number of charges that the particle can carry from ESI is more than 100. As a result, the peaks arising from the intact capsids of the viruses in the ESI-TOF spectrum are barely resolved and they can be identified only through careful charge state analysis (Tito et al., 2000). To simplify the spectra and facilitate subsequent mass analysis of mixtures, reduction of the charge states of the ESI-generated macroions is desired. Scalf et al. (1999) have suggested using radioactive ^{210}Po neutralizers to create bipolar ionizing gas within a collision cell to control the charge state, and successfully demonstrated that the charge distribution can be properly manipulated such that it principally consists of singly charged macroions (Scalf, Westphall, & Smith, 2000). Stephenson & McLuckey (1998) have also shown that gas-phase ion/ion reactions of the ESI-generated particles with ions of opposite polarity in either the source region or the ion trap region is an effective tactic to reduce the charge state to lower values (McLuckey & Stephenson, 1998). In this context, the ''photospray,'' based on atmospheric pressure photoionization (Robb, Covey, & Bruins, 2000) of carrier gases with a continuous Kr discharge lamp to produce macroions mainly in singly charged states through proton transfer reactions, is particularly suitable for such macroion studies.

Compared to ESI, MALDI is advantageous in producing macroions carrying fewer charges for simpler interpretation of the acquired mass spectra. Typically, only one or two charges are carried by the desorbed/ionized species (Hillenkamp & Karas, 2000; Karas & Kruger, 2003). It is an ideal method for bringing organic, inorganic, and biological macroions to the gas phase for high-precision mass measurement. However, the method is less gentle and hence less likely to be useful in the study of non-covalent macromolecular complexes and assemblies (such as viruses) under vacuum (Siuzdak, 1994). Other alternatives

to ESI as the macroion source may include the piezoelectric particle generator (Arnold & Folan, 1986), which is a droplet-on-demand device, allowing one single particle to be investigated at a time in a controlled manner. The sonic spray, which may be used over a wide range of buffers regardless of the conductivity of the solutions (Hirabayashi, Hirabayashi, & Koizumi, 1999), and the atmospheric pressure laser desorption/chemical ionization (Coon, McHale, & Harrison, 2002), which involves operation of MALDI in ambient air, are two additional methods of choice too.

B. Mass Analyzers

The mass analyzers in widespread use in current mass spectrometry of atomic, molecular, and cluster ions encompass quadrupole rods, ion traps, TOF tubes, ICR cells, magnetic sectors, etc. (McLuckey & Wells, 2001). Among them, TOF-MS is perhaps the most commonly used instrument in investigation of nano-sized materials and high-mass biomolecules. Examples of the former include thiolate-coated gold nanoclusters (Arnold & Reilly, 1998; Schaaff & Whetten, 2000), hydrogenated nanocrystalline Si (Ehbrecht & Huisken, 1999), CsI nanocrystals (Alvarez, Vezmar, & Whetten, 1998), and ZnS nanoparticles (Khitrov & Strouse, 2003), etc. The TOF-MS, in principle, has no mass analysis limits. However, in practice, both the mass resolution of the instrument and the sensitivity of the ionization-based detector become very poor when the m/z value of the sample ions approaches 1×10^6 (Nelson, Dogruel, & Williams, 1994; Schriemer & Li, 1996). To improve the efficiency of detecting larger particles, it requires the use of higher acceleration voltages (>30 kV) to increase the ion velocity. This, however, often leads to undesirable electric discharge of residual gas in the ion source.

The FTICR-MS suffers similar mass limitations for the detection of very-high-mass biomolecules (Russell, 1986; Kelleher et al., 1997; Marshall & Hendrickson, 2002). For a 7.0 T instrument, the theoretical upper mass limit for a singly charged room-temperature ion orbiting in a cubic cell of one-inch cross-sectional radius is ~5.89 MDa. This mass limit would be lowered to 274 kDa when a three-dimensional axial quadrupolar electrostatic trapping potential of $V_{\text{trap}} = 1$ V is applied to the ICR cell to facilitate the ion confinement (Marshall, Hendrickson, & Jackson, 1998). Whereas studies on the trapping, detection, and reaction of a single macroion in an ICR cell have shown that the spectrometer is capable of establishing a mass precision of 10^2 ppm for polyethylene glycol with $m > 5$ MDa, the ion has to carry ~5,000 charges in order to be detected (Smith et al., 1994). Furthermore, accurate mass determination can be made with a single ion approach, by which stepwise changes of the charge states of the individual ions via ion-molecule reactions within the cell can be positively identified (Bruce et al., 1994; Cheng et al., 1994). Chen et al. (1995) have extended the single-ion detection technique to simultaneously determine the charge numbers (from the image current density) and the mass-to-charge ratios (from the ion cyclotron frequency) for an ensemble of macroions. They were able to assess the molecular weight of coliphage T4 DNA with $m \approx 110$ MDa. Unfortunately, the error involved in the charge number assessment and therefore the mass determination is relatively large (~10%) because of the uncertainty about the size of the ion orbit in the ICR cell.

In line with the FTICR measurements, Fuerstenau & Benner (1995) measured simultaneously the masses and charge numbers of macroions using a charge induction tube acting both as a TOF mass analyzer and as a detector. For submicron-sized polystyrene spheres with an equivalent mean mass of 1×10^{10} Da, their results indicated that the average number of the charges carried by the ESI-generated particles was 2,500. Whereas the charge induction tube is well suited for the investigation of macroions with $m/z > 1 \times 10^6$, accuracy of the measurements was largely limited by the lack of a good control of particle velocity because of the gas flowing through the ESI source. Using a gated electrostatic ion trap, Benner (1997) measured the ESI-generated ions repetitively and managed to obtain a mass resolution of 25 for a 4.3-kilobase circular DNA molecule ($m \approx 2.88$ MDa and $z > 100$ charges). However, the measurement is expected to be difficult for ions with $m/z > 1 \times 10^6$ and having an aerodynamic velocity of ~300 m/sec (Sinha, 1984).

Since its invention in the 1960s (Paul, 1990), the QIT has been widely utilized as a device for mass determination and chemical reaction studies of ions of various kinds (March & Hughes, 1989; March & Todd, 1995; March, 2000; McLuckey et al., 2000). In most of the commercial QIT-MS available to date, a radio-frequency (RF) ac field is applied between the ring and end-cap electrodes, and the amplitude of the ac field is then ramped up to 15 kV to eject ions consecutively from the trap for mass analysis (Louris et al., 1987; Kaiser et al., 1991). The typical RF applied to the QIT operating in such a mass-selective axial instability mode is in the proximity of 1 MHz, which limits the mass analysis range of the spectrometer to $m < 10^3$ Da for singly charged species. Several techniques involving increasing the trap driving voltage, reducing the RF and the trap size, along with resonance ejection (March, 1992) have been implemented in an effort to extend the investigated m/z range beyond 1×10^5 (Reid et al., 2003).

In light of the importance of mass analysis of large biomolecular ions, such as those of non-covalent complexes and assemblies (Loo, 2000), an audio-frequency (AF) ion trap MS has been constructed by Cai et al.

(2002a,b,c). The construction combined the techniques developed in the pioneering work of Wuerker, Shelton, & Langmuir (1959) and Stafford (2002), with the trap operating in the mass-selective axial instability mode in the AF region. To obtain the spectrum, the amplitude of the ac field was swept continuously and the mass-selected trap-ejected particles were detected by light scattering at a small forward scattering angle with a photomultiplier. Employing this technique has allowed Chang and co-workers (Cai et al., 2002a,b,c) to extend the mass analysis range of the conventional QIT-MS to $m/z = 1 \times 10^9$. However, for nano-sized particles with masses in the range of $m = 10^6$–10^9 Da, the analysis is hampered by the lack of proper power amplifiers operating in the 1–10^2 kHz region.

A solution to this problem is to sweep the trap driving frequency at a constant voltage rather than to sweep the trap driving voltage at a constant frequency (Kofel et al., 1996; Schlunegger, Stoeckli, & Caprioli, 1999; Cai, Peng, & Chang, 2003; Peng et al., 2003). In analysis of high-mass ions (60–160 kDa) generated by MALDI in a QIT, Schlunegger, Stoeckli, & Caprioli (1999) adopted the frequency scan mode by sweeping $\Omega/2\pi$ from 30 to 10 kHz at $V_{ac} = 200 \times 2$ V and successfully obtained the mass spectrum of singly charged IgG at $m/z \approx 1.5 \times 10^5$. The key feature of this frequency scan mode is that it greatly widens the mass analysis range since according to Equation (2), a decrease of Ω by a factor of 10 is equivalent to an increase of V_{ac} by a factor of 100 when keeping q_z the same. For a singly charged sphere of $d = 10$–100 nm with unity density, the typical frequency scan range with the trap operating in the mass-selective axial instability mode is $\Omega/2\pi = 0.2$–30 kHz at $V_{ac} = 200$ V and $r_0 = 10$ mm.

The main difficulty in implementing the frequency scan mode in QIT-MS involves the electronics (Landais et al., 1998). Conventional RF power suppliers come with transformers, typically providing a secondary/primary voltage ratio of the order of 10^2:1 over a narrow frequency range. This scheme obviously cannot apply when conducting the frequency sweep. A direct power amplifier must be used; however, the highest voltage amplitude V_{ac} that can be attained is mainly limited by the breakdown potential of the solid state transistors used in the amplifier (Ting, 2001). Stacking the transistors in a cascade mode offers a solution to circumvent the problem. Another approach that might permit the frequency sweep over a wide range without the need of high-voltage power amplifiers is to use rectangular waves for ion trapping as well as mass analysis. Exploiting a square waveform generator and a high-voltage power switch to drive the ion trap, a "digital" QIT-MS technology has been developed by Ding et al. (2001) and Ding, Sudakov, & Kumashiro (2002).

An additional difficulty associated with the mass spectrometry of macroions with $m/z \approx 10^9$ is on how the MS can be calibrated. This appears to be an impediment to the measurement in such a high m/z domain since no standard references are available. Cai et al. (2002a,b) have developed a method to calibrate the AF QIT-MS based on a single particle approach. The authors determined the point of particle ejection, q_{eject} (theoretically, $q_{eject} = 0.908$), on the stability diagram of the AF ion trap using a single microparticle, similar to that performed for smaller molecular ions in an RF trap (Louris et al., 1987). The determination was made possible by measuring the radial secular frequencies of the particle inside the trap (Schlemmer et al., 2001), followed by monitoring the action of the particle ejection outside the trap via light scattering. An m/z accuracy approaching 0.1% is achievable after proper calibration of the AF QIT-MS (Cai et al., 2002a,b).

C. Detectors

1. ELS/Ellipsoidal Reflector

Figure 5 shows a schematic diagram of the MS, which is capable of conducting rapid and routine m/z analysis of single charged microparticles with optical detection methods (Cai et al., 2002a,b,c). The spectrometer so constructed (named single particle mass spectrometer, SPMS) involves integration of the ESI, QIT, and ELS technologies. The SPMS has three important characteristics. First, the ion trap is driven by an AF power amplifier optimized at 50–2,000 Hz. Second, collection of scattered light from a CW Ar ion laser (or a laser diode) is utilized as a particle detection method. Third, the individual peaks observed in the spectrum are derived from single particles, rather than an ion ensemble, thereby allowing high-resolution mass spectrometric measurements to be performed for micron-sized or even nano-sized particles.

Shown in Figure 6 is a typical single particle mass spectrum obtained from electrospray of a colloidal suspension (pH = 3.9) of amino-terminated polystyrene microspheres with $d = 0.91 \pm 0.022$ μm. The spectrum was acquired by trapping the particles at $V_{ac} = 420$ V, $\Omega/2\pi = 600$ Hz and a He buffer gas pressure of $p = 1$ mTorr, followed by ramping the operating voltage from 420 to 1,700 V for particle ejection. The individual peaks observed in the spectrum are the light bursts from the trap-ejected particles as they pass through the probe laser beam. From 100 repetitive measurements, the number of charges on each particle was found to distribute over 600–1,800 (Fig. 7b and c), deduced from the known mass of 4.1×10^{-13} g, diameter of 0.91 μm, and density of 1.05 g/cm^3 for the microspheres (Cai et al., 2002b). Particles with lower charge numbers ($Z < 600$) could also be identified when the trap operated at a lower frequency, $\Omega/2\pi = 400$ Hz (Fig. 7a). With use of the similar trapping and detection techniques, but replacing ESI by MALDI,

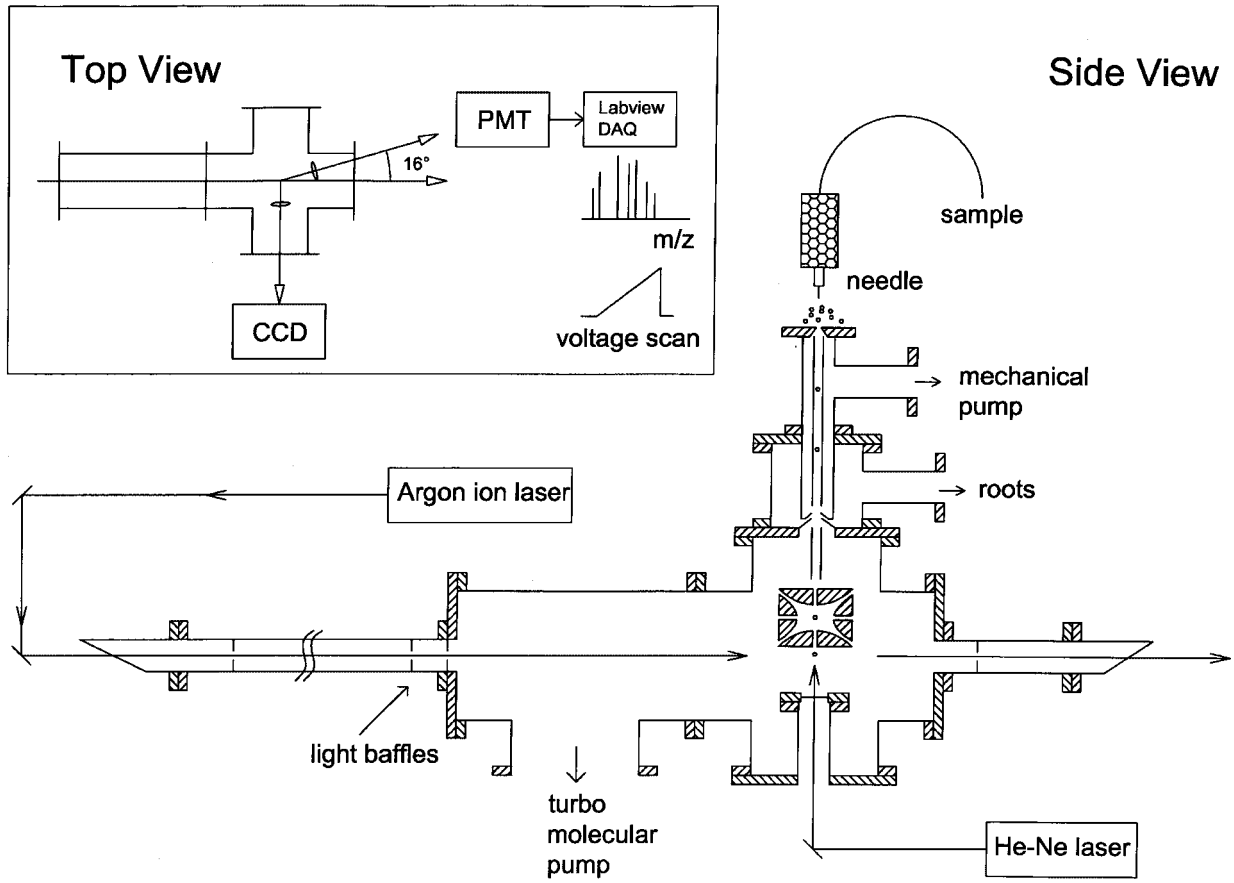

FIGURE 5. An audio-frequency ion trap MS equipped with an Ar ion laser for light scattering detection of mass-selected particles ejected from the trap. Note that two separate detection schemes are used to probe the individual particles inside and outside the quadrupole ion trap (QIT) simultaneously. (Reproduced from Cai et al. (2002b) with permission from American Chemical Society, copyright 2002.)

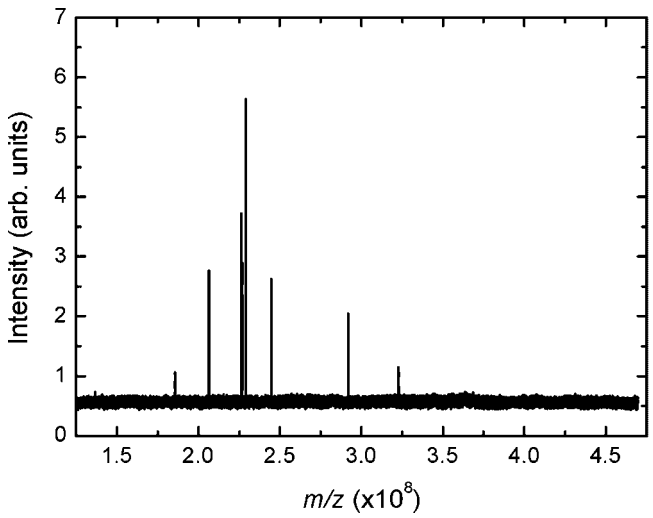

FIGURE 6. Single particle mass spectrum acquired for 0.91 μm particles with ESI as the ion source. (Reproduced from Cai et al. (2002b) with permission from American Chemical Society, copyright 2002.)

the mass spectrum of 1.10 ± 0.027 μm polystyrene microspheres was also obtainable at $\Omega/2\pi = 200$ Hz. The spectrum, as shown in Figure 8, covers the m/z range of $(1.5–5.0) \times 10^9$; it represents the first MALDI-QIT mass spectrum ever attained for synthetic polymers of any kinds with masses exceeding 10^{10} Da (Nielen, 1999; Cai et al., 2002c; Murgasova & Hercules, 2003). A comparison of Figures 6 and 8 clearly indicates that there are more MALDI-generated particles trapped than the ESI-generated particles in these two particular sets of measurement. Since the particles used in these two experiments are similar in size, the comparison further indicates that the average number of charges carried by the MALDI-generated particles is approximately one order of magnitude less than that resulted from ESI. However, to determine the absolute mass of each particle, reduction of the charge states to unity with a neutralization source prior to the m/z analysis is desired (Kaufman, 1998; McLuckey & Stephenson, 1998; Scalf et al., 1999).

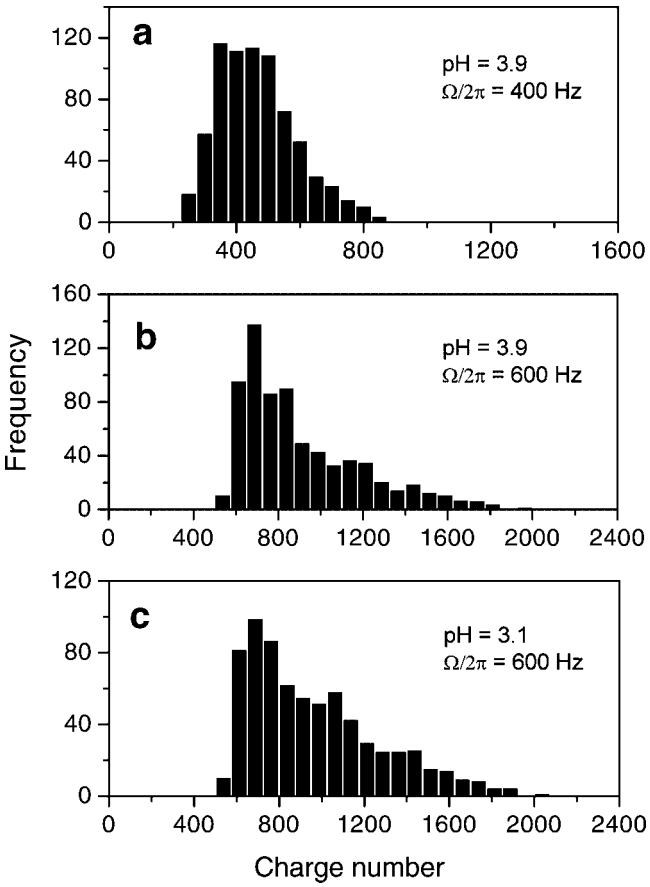

FIGURE 7. Dependence of the charge state distribution of ESI-generated polystyrene microspheres on trap driving frequency ($\Omega/2\pi$) and acidity (pH) of the sample solutions. The applied dc voltage between the needle and the capillary in the ESI source was +4 kV. (Reproduced from Cai et al. (2002b) with permission from American Chemical Society, copyright 2002.)

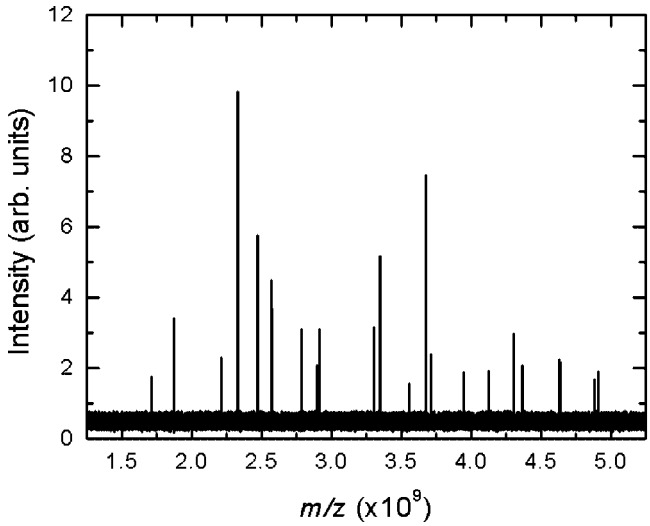

FIGURE 8. Single particle mass spectrum acquired for 1.10 μm particles with matrix-assisted laser desorption/ionization as the ion source. (Reproduced from Cai et al. (2002c) with permission from American Chemical Society, copyright 2002.)

The SPMS, in combination with a variety of existing techniques, can be applied to differentiating particles of different sizes, in addition to their masses. This, for example, can be accomplished by integrating two laser beams aligned perpendicular to each other into this spectrometer for aerodynamic sizing (Sinha, 1984; Noble & Prather, 2000). Alternatively, one can determine the size of the particle by analyzing the angle-resolved light scattering pattern with a high-resolution CCD camera according to the Mie theory (Barnes et al., 1997; Pan et al., 2003a). Moreover, attachment of an imaging current detector to measure the absolute charge numbers of the ESI-generated particles (Fuerstenau & Benner, 1995) after they pass through the illuminating laser beam is also a feasible approach.

Improvement of the sensitivity of the SPMS (Fig. 5) for nanoparticle analysis is possible by using an ellipsoidal reflector for light collection (Gard et al., 1997; Pan et al., 2003a). The improvement in the S/N level is twofolded. First, the ellipsoidal reflector collects over 40% of the ELS signals and, secondly, it images the scatted light into a small pinhole or an optical fiber, where it is detected by a PMT. A proper use of the pinhole can prevent most of the background scattered light from reaching the detector, thereby greatly enhancing the sensitivity of the ELS method. With this novel design, detection of a single particle as small as 80 nm with a diode-pumped Nd:YAG laser (532 nm) has been reported in the literature (Galli, Guazzotti, & Prather, 2001). Further reduction of the size detection limit is achievable using light with shorter wavelength such as the frequency-doubled Ar ion laser or the synchrotron radiation. Observation of the mass spectra from single bioparticles of sizes in the range of 50 nm is then practical. Future applications of this performance-improved SPMS may include identification and characterization of both micron-sized bioparticles, such as bacterial spores (Sinha et al., 1984; Gieray et al., 1997), and nano-sized bioparticles, such as bacteriophages/viruses (Siuzdak et al., 1996; Siuzdak, 1998; Fuerstenau et al., 2001), solely based upon their mass differences among different species (known as ''mass tagging'') without prior wet chemical treatment and cell digestion (Smith et al., 2001). Application of this technique to the

human genomic sequencing projects (Schultz, Hack, & Benner, 1998) is also appealing since the spectrometer is well suited for identification of chromosomal fragments with masses on the order of 10^{10} Da.

2. *LIF/Ion Trap*

On detecting nano-sized particles ejected from a QIT with LIF, the challenge lies in how to produce and collect enough photons from them. For the trap-ejected particle, the typical laser-sample interaction time is less than 100 μsec as it passes across a probe laser beam with a spot size of 3 mm (Cai et al., 2002b). The time obviously is too short to produce a sufficient amount of fluorescence for detection. To surmount this obstacle, a technology based on QITs has been developed (Zerega et al., 1999; Cai, Peng, & Chang, 2003; Peng et al., 2003). The apparatus, as shown in Figure 9a, consists of two traps in tandem, where the first trap (T1) serves as a trapping and mass-analyzing device, whereas the second trap (T2) serves to capture, slow down as well as concentrate the mass-selected particles ejected from the first trap. The LIF is then collected for particles damped to the center of the second trap by He buffer gas. In this operation, in order for the LIF/ion trap (LIF-IT) to actually function as a nanoparticle detector, undesired particle accumulation and mass discrimination must be eliminated. This is accomplished by switching off the power supplier of T2 for a few milliseconds after collection of each data point to empty the trap, along with the implementation of a dynamic trapping scheme for particle detection (Doroshenko & Cotter, 1997; Peng et al., 2003). Figure 9b shows the time sequences of the particle damping and dumping before and after the LIF detection to obtain the spectrum. Although the LIF-IT detector is slow (with a response on the time scale of milliseconds), it is not a serious impediment to the QIT-MS measurement. By utilizing this unique dual QIT arrangement and the frequency scan scheme, Chang and co-workers have been able to acquire the mass spectra of fluorescently labeled polystyrene nanoparticles with sizes comparable to those of viruses (Cai, Peng, & Chang, 2003; Peng et al., 2003).

A MALDI ion source was used to generate charged particles from samples containing fluorescein-labeled polystyrene spheres with sizes of 27 and 110 nm. In this experiment, a pulsed frequency-tripled Nd:YAG laser (355 nm) was sent through one of the holes on the ring electrode of T1 to irradiate the sample positioned on the opposite side for ion production (Fig. 9a). In order to achieve high trapping efficiency for the MALDI-generated particles, a He buffer gas pressure up to $p = 50$ mTorr was maintained in the chamber. Frequency scan, instead of voltage scan, was adopted to avoid arcing among the trap electrodes in the presence of such high-pressure He gas. An Ar ion laser (488 nm), focused to a spot size of ~200 μm at the trap center, selectively detected the particles ejected from T1 and captured by T2. Fluorescence from the trapped nanospheres was collected orthogonal to the probe laser beam through a 3.1-mm hole on the end-cap electrode and was guided to a thermoelectrically cooled PMT for photon counting.

Displayed in Figures 10 and 11 are the mass spectra derived respectively from a single scan and averaging of 10 scans for the 27 nm spheres containing 180 fluorescein equivalents per particle. Both the spectra were acquired by sweeping down the frequency of the first trap from $\Omega_1/2\pi = 6.0$ to 0.5 kHz at a constant voltage of $V_{ac,1} = 200$ V. The 27 nm fluorescent spheres have a mean mass of $m \approx 6.5$ MDa but with a mass spread of 4.0–9.8 MDa because of the size variation of ±4 nm. The statistically averaged mass spectrum plotted in Figure 11 shows a board feature centering on $m/z \approx 5 \times 10^6$, indicating that the mass spectrum is predominantly contributed by singly charged particles. In the same spectrum, features ascribable to doubly and triply charged particles can also be found at the lower m/z side, although they are much weaker in intensity.

Figure 12 displays the mass spectra of fluorescently labeled 110 nm particles from (a) a single scan and (b) averaging of 100 scans, acquired by sweeping down $\Omega_1/2\pi$ from 1.0 to 0.2 kHz at $V_{ac,1} = 200$ V to cover the m/z range of 48×10^6–$1{,}200 \times 10^6$. For these spheres, they have a mean mass of 440 MDa/particle and a mass distribution of 350–543 MDa/particle because of the size variation of ±8 nm. This suggests an m/z range of $(350–543) \times 10^6$ to $(58–91) \times 10^6$ for particles carrying 1–6 charges (Fig. 12b). It is likely that the sharp and well-separated features in Figure 12a are derived from the individual 110 nm fluorescently labeled spheres. The likelihood is very high because each one of them contains 7,400 fluorescein equivalents and should be detected readily (Peng et al., 2003).

The detection sensitivity of the dual QIT-MS depicted in Figure 9a may be enhanced tenfold by increasing the light collection efficiency using F/1 optics (Barnes et al., 1993). To enhance the sensitivity further, a more opened trapping device (Fig. 13) such as that developed by Schlemmer et al. (2001) can be exploited to minimize the level of the background scattered laser light. A spherical void electrodynamic levitator (Arnold & Folan, 1987; Arnold, 1991), in principle, can also be utilized to achieve near 100% of the LIF collection efficiency. With this improvement, a blue diode laser or a high-power light emitting diode (LED) may substitute the Ar ion laser as the light source (Wang & Morris, 2000; Pan et al., 2003b) to reduce both the cost and the dimension of the LIF-IT as a nanoparticle detector. Furthermore, by employing sample-

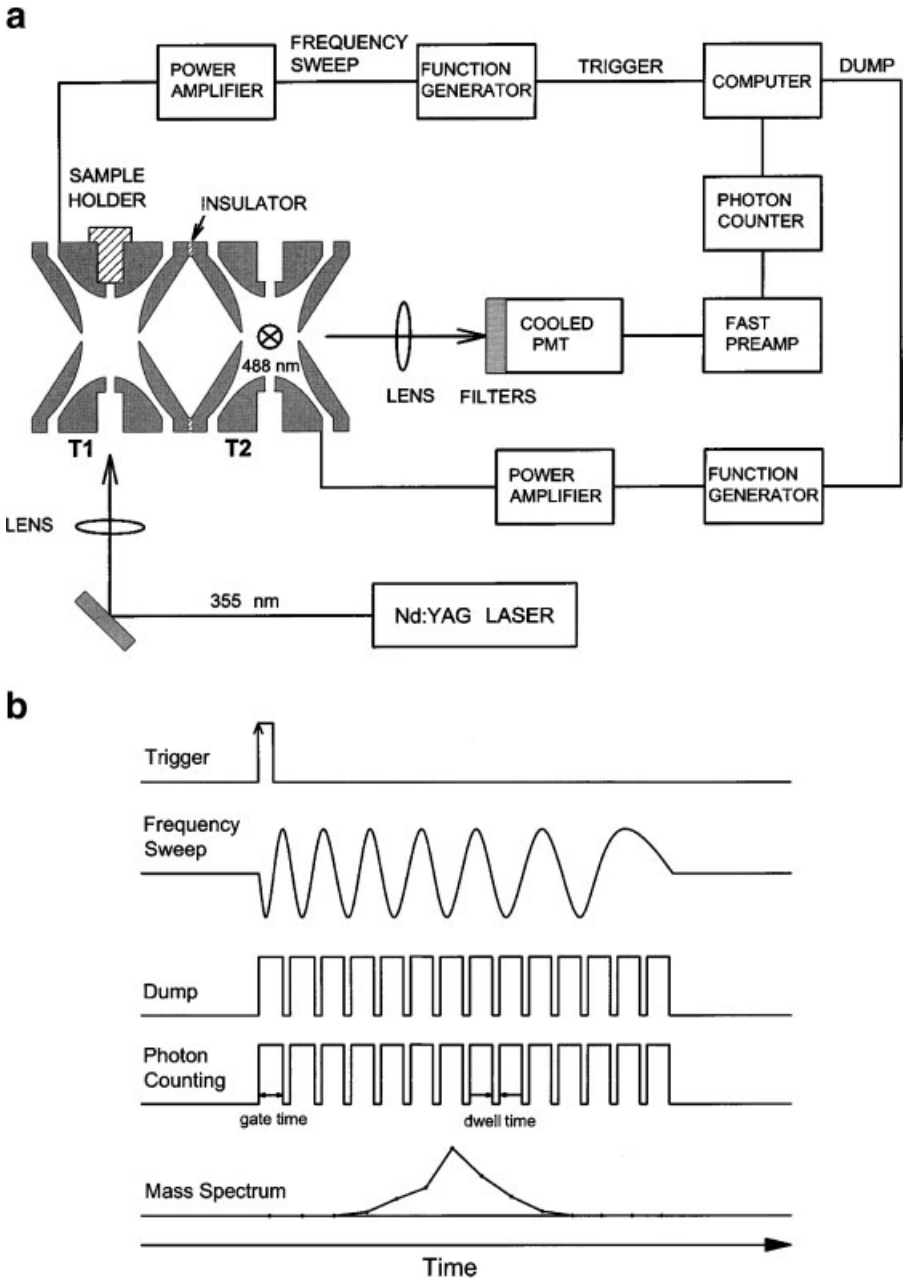

FIGURE 9. **a**: Schematic diagram of the dual QIT-MS. **b:** Time sequences of the frequency sweep, dynamic trapping, particle dumping, and fluorescence collection. (Reproduced from Cai, Peng, & Chang (2003) with permission from American Chemical Society, copyright 2003.)

specific dye-labeling techniques (Haugland, 1996), differentiation of particles of different origins is possible through multicolor fluorescence spectroscopy (Taylor, Fang, & Nie, 2000). This, in effect, would add a new dimension to mass spectral analysis of nanoparticles, which is expected to be an asset when the mass resolution of the spectrometer used is not sufficient to differentiate these complex particulates.

The LIF-IT detector may find useful applications in mass determination and, hence, the identification of biologically important particles such as viruses and other microorganisms without the need of proteolysis (Thomas,

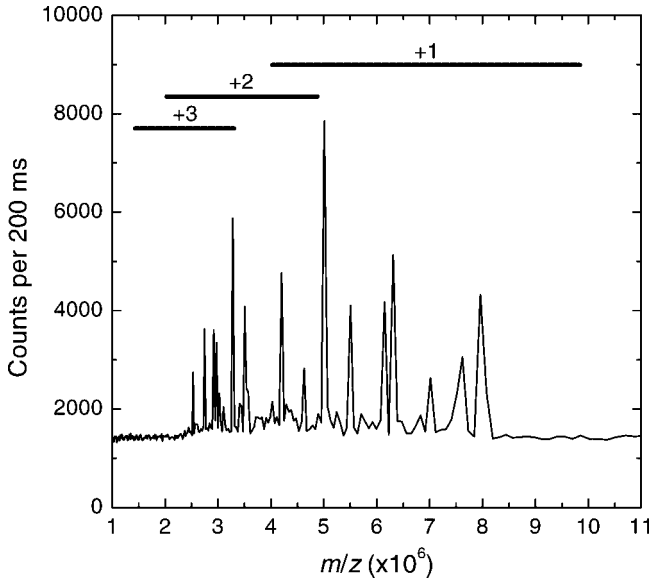

FIGURE 10. Single scan mass spectrum of 27 nm fluorescent polystyrene spheres. The spectrum was acquired by sweeping the ac frequency of T1 from 6.0 to 0.5 kHz at a constant ac voltage of 200 V. Sharp features observed for singly, doubly, and triply charged particles, are denoted by +1, +2, and +3, respectively. The horizontal bars indicate the m/z ranges of the individual charge states because of the large mass dispersion ($m = 4.0$–9.8 MDa) of the sample. (Reproduced from Cai, Peng, & Chang (2003) with permission from American Chemical Society, copyright 2003.)

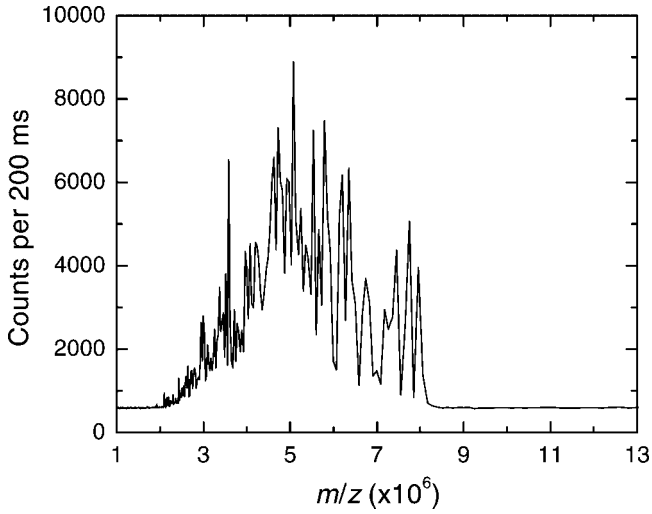

FIGURE 11. Mass spectrum of 27 nm fluorescent polystyrene spheres. The spectrum (average of 10 scans) was acquired under the conditions, $\Omega_1/2\pi = 6.0$ kHz $\rightarrow$ 0.5 kHz, $V_{ac,1} = 200$ V, $\Omega_2/2\pi = 18$ kHz $\rightarrow$ 1.5 kHz, $V_{ac,2} = 160$ V, in a dynamic trapping mode. (Reproduced from Peng et al. (2003) with permission from Elsevier, copyright 2003.)

Bakhtiar, & Siuzdak, 2000; Fenselau & Demirev, 2001; Lay, 2001; Ullom et al., 2001). The application appears practical since dye labeling has been a routine protocol in life science research. Furthermore, there exists a number of intrinsic biological fluorophores such as tryptophan and riboflavin for LIF detection (Pinnick et al., 1998; Pan et al., 2003c). Although the dye labeling is inconvenient from the viewpoint that one needs to quantify the extent of labeling prior to mass spectrometric analysis, the disadvantage can be minimized with the aid of optical spectroscopy (Haugland, 1996). Compared to the ESI-TOF-MS as conducted by Robinson and co-workers for large biomolecular complexes and assemblies (Rostom & Robinson, 1999; Rostom et al., 2000; Tito et al., 2000), the MALDI-LIF-IT-MS holds the promise that it does not require the particles to carry multiple charges for detection, a requirement often making spectral assignment indirect and difficult. The utility of this new mass spectrometric approach in biological applications has been demonstrated by Cai, Peng, & Chang (2003) for IgG (goat anti-mouse antibody, $m \approx 150$ kDa) fluorescently labeled with an average number of 6.2 Alexa Fluor 488 dye molecules ($m = 643$ Da). A typical mass spectrum derived from such an LIF measurement is shown in Figure 14.

Design and development of a mass analyzer better suited for the LIF-IT detector than the QIT is an important area for future work. Quadrupole MSs clearly are the first instrument of choice for this purpose and indeed the instruments have been employed by Siuzdak et al. (1996) and Siuzdak (1998) to select charged viral particles generated by ESI. Figure 15 shows the triple-quadrupole mass analyzer operating in a RF-only mode for separation/purification of the supramolecular complexes, RYMV and TMV. Unfortunately, the mass spectra of them could not be attained because these viruses are highly folded and their m/z distributions are far beyond the range that conventional voltage-scan quadrupole MSs can reach (typically $m/z < 4{,}000$). Whereas it is possible to extend the mass analysis range up to 45 kDa by reducing the quadrupole operating frequency from 1 MHz to 292 kHz (Beuhler & Friedman, 1982; Winger et al., 1993), a frequency as low as 50 kHz has to be applied in order to go beyond $m/z = 1 \times 10^6$. Operating the quadrupole rods in a frequency-scan mode (namely, the analyzer is scanned at both constant dc and constant ac voltages) offers a complementary alternative. It has been demonstrated by Landais et al. (1998) that scanning a quadrupole analyzer by varying the frequency at a constant amplitude would lead to a constant resolution over the entire mass range. Moreover, the mass resolution of the

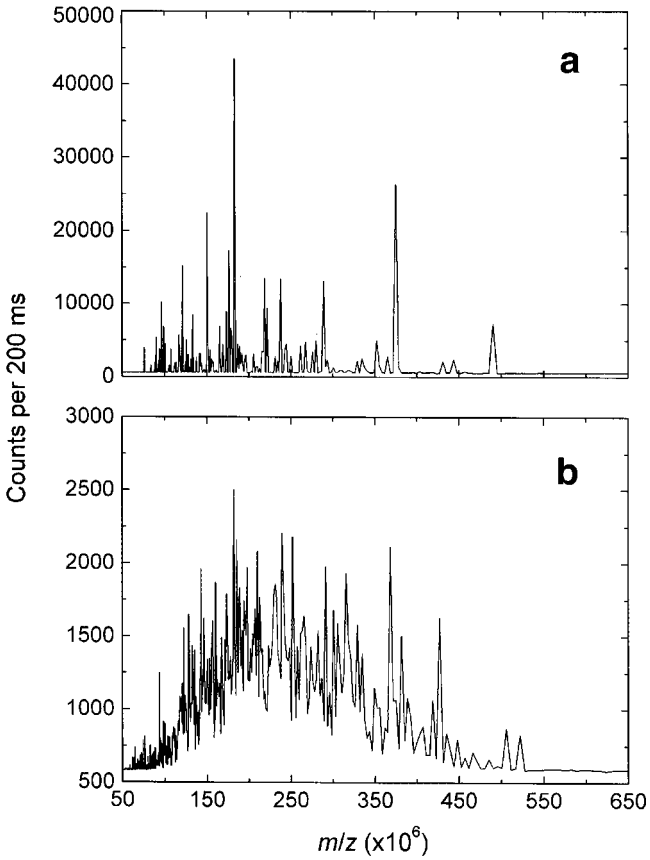

FIGURE 12. Mass spectra of 110 nm fluorescent polystyrene spheres, acquired in (**a**) a single scan and (**b**) 100 scans. The individual peaks observed in (a) are likely to derive from single particles because of the high content of the dye molecules. (Reproduced from Peng et al. (2003) with permission from Elsevier, copyright 2003.)

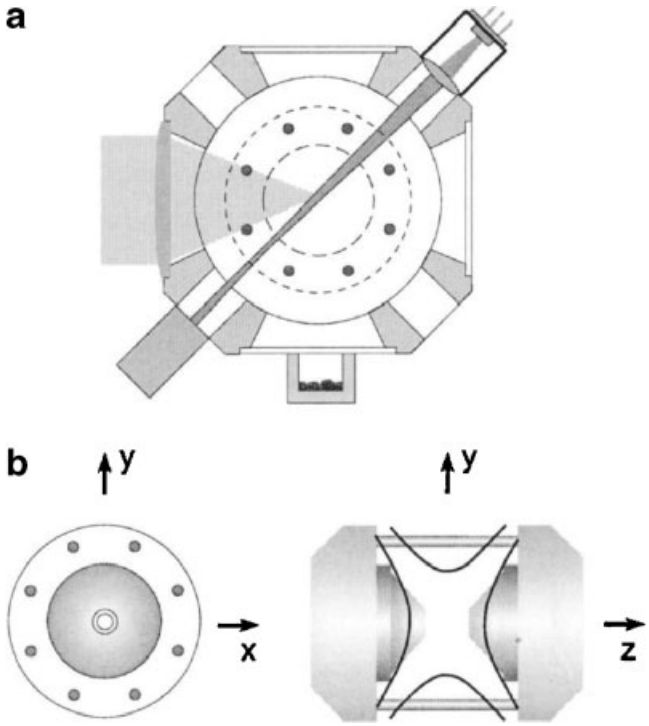

FIGURE 13. A modified QIT with large opening for light collection. Unlike the Paul trap with hyperbolic electrodes, the ring electrode of this trap is made of eight steering rods and the two end-caps are made of two conical electrodes. (Adapted from Schlemmer et al. (2001) with permission from American Institute of Physics, copyright 2001.)

analyzer would increase linearly with the applied ac voltage and is independent of its frequency. A mass resolution as high as 10^3 is expected to be achieved in the region $m/z > 1 \times 10^6$ when a frequency-variable power amplifier with a constant output voltage of 1,000 V is accessible. Such a frequency-scan quadrupole MS, equipped with the LIF-IT detector as illustrated in Figure 9, should be capable of producing reliable mass spectra for nano-sized materials and high-mass biomolecules carrying only few charges with m/z in the domain of 10^6–10^9.

IV. CONCLUSIONS AND OUTLOOKS

The optical (laser-based) detection approach with both ELS and LIF has been shown to be a viable alternative to the ionization-based detectors, charge-sensitive detectors, and energy-sensitive detectors for detection of macroions (or charged particles) in the gas phase. It provides an effective way of probing charged particles with sizes typical of $10–10^3$ nm or masses typical of $1–10^6$ MDa. The QIT has been chosen as the mass analyzer for these macroions because it is the only device known to date to be capable of conducting high precision mass measurements for single charged microparticles. With the QIT operating in the AF region and acting in either the voltage scan mode or the frequency scan mode, mass spectra of microparticles and nanoparticles of synthetic polymers and high-mass biomolecules have been obtained by Chang and co-workers (Cai et al., 2002a,b,c; Cai, Peng, & Chang, 2003; Peng et al., 2003). However, to obtain the mass spectra with high precision, further refinements of the detectors as well as developments of new mass spectrometric methods are required.

Table 1 summarizes the methods and parameters used to acquire the mass spectra of charged particles of different sizes (either $d > 100$ nm or $d < 100$ nm) as discussed in this article. Whereas the ELS method suffers the difficulty of detecting single charged particles with sizes smaller than

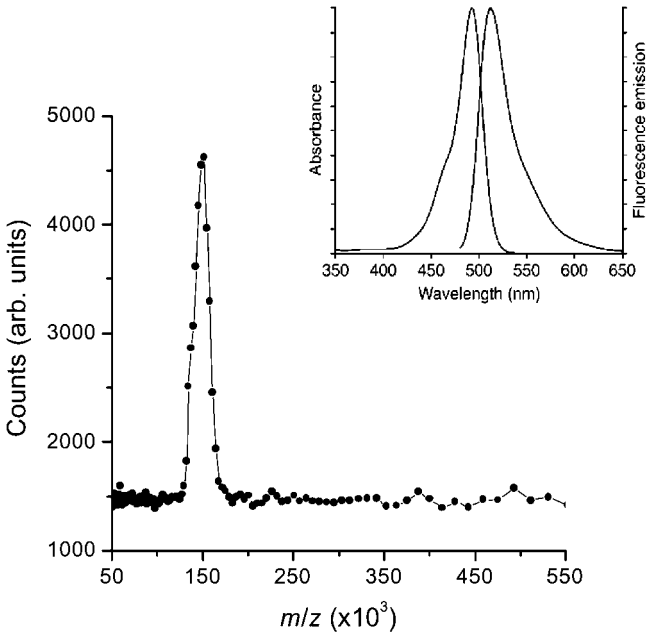

FIGURE 14. Mass spectrum of IgG fluorescently labeled with 6.2 Alexa Fluor 488 dye molecules. The spectrum (average of 10 scans) was acquired under the conditions, $\Omega_1/2\pi = 30$ kHz $\rightarrow$ 5 kHz, $V_{ac,1} = 200$ V, $\Omega_2/2\pi = 120$ kHz $\rightarrow$ 20 kHz, $V_{ac,2} = 160$ V, with the laser-induced fluorescence/ion trap operating in a dynamic trapping mode. The mass of each Alexa Fluor 488 is 643 Da, yielding a total mass of $\sim$150 kDa for the fluorescently labeled IgG. Inset, typical absorption and fluorescence spectra of Alexa Fluor 488 in solution.

100 nm, the LIF essentially has no size limitation. The success of the latter, however, strongly relies on how dye-labeling can be accomplished and how fluorescence can be collected from excited particles confined in a small laser probe volume. Hence, ELS still would be the method of choice in detecting particles larger than 100 nm. Although there remains much room for improvements for the performance of the detectors as well as the MSs, the future for the development of macroion mass spectrometry (particularly, nanoparticle mass spectrometry) is promising.

As a final remark, we would like to point out that laser desorption/ionization is a potentially useful method for detection of particles with sizes much smaller than 100 nm. This potential is shown in on-line aerosol analysis, where laser desorption/ionization has been used to detect particles that are smaller than optical detection allows, i.e., $d < 100$ nm (Reents et al., 1995; Carson, Johnston, & Wexler, 1997; Reents & Ge, 2000; Kane, Oktem, & Johnston, 2001; Reents & Schabel, 2001). In contrast to the LIF as illustrated in this article, no fluorescent labeling is required and, furthermore, masses of the resulting product ions can be identified with this technique. It is an emerging new approach toward mass spectrometry of nanoparticles and clearly desires exploration in future work.

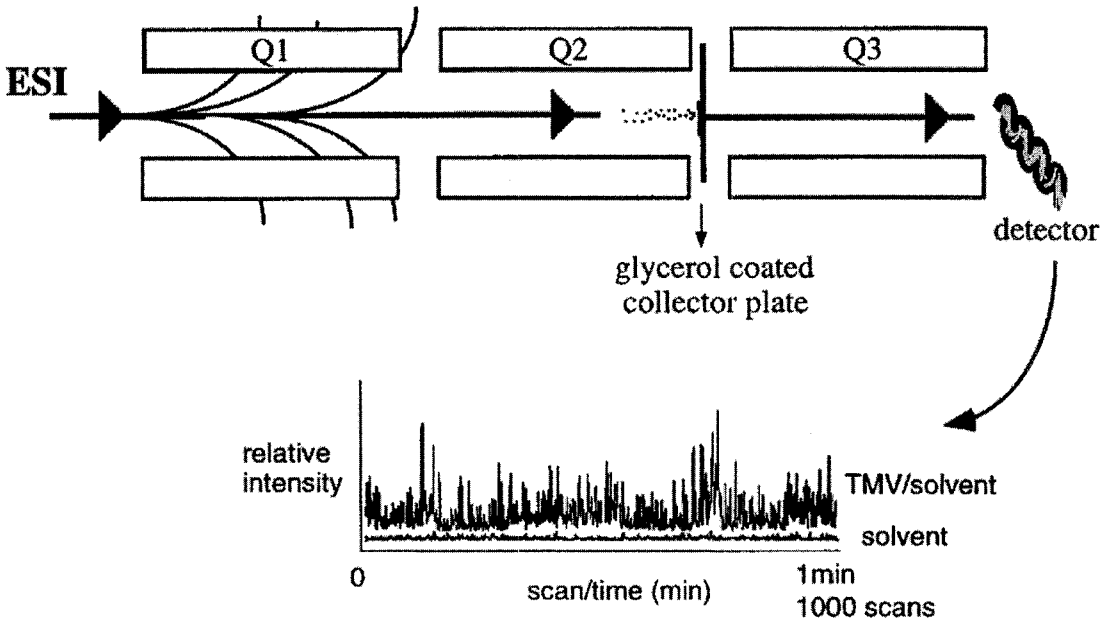

FIGURE 15. A modified triple-quadrupole MS for viral analysis. The viral ions were produced by ESI and they were selected by using a low accelerating voltage before $Q1$ so that only massive ions gain sufficient translational energies to pass through the quadrupole rods and reach the detector. The quadrupole mass analyzers were operated in an RF-only mode. (Reproduced from Siuzdak (1998) with permission from John Wiley & Sons, Ltd., copyright 1998.)

TABLE 1. Comparison of the parameters and methods typically used to obtain the ion trap mass spectra of macroions detected with optical methods for particles of different sizes

	Particle sizes	
Parameters/methods	$10–10^2$ nm	$10^2–10^3$ nm
Trap frequency ($\Omega/2\pi$)	$1–10^2$ kHz	0.1–1 kHz
Trap voltage (V_{ac})	200–400 V	200–2,000 V
Buffer gas pressure (p)	50 mTorr	1 mTorr
Ion source	Electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI)	ESI or MALDI
Spectrum acquisition	Frequency scan	Voltage scan
Detection	Laser-induced fluorescence (LIF)	Elastic light scattering (ELS)

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REFERENCES

Alvarez MM, Vezmar I, Whetten RL. 1998. On-line sampling and intact mass analysis of nanometer-size aerosols via time-of-flight high-mass spectrometry. J Aerosol Sci 29: 115–127.

Alves S, Kalberer M, Zenobi R. 2003. Direct detection of particles formed by laser ablation of matrices during matrix-assisted laser desorption/ionization. Rapid Commun Mass Spectrom 17:2034–2038.

Arnold S. 1979. Determination of particle mass and charge by one electron differentials. J Aerosol Sci 10:49–53.

Arnold S. 1991. A three-axis spherical void electrodynamic levitator trap for microparticle experiments. Rev Sci Instrum 62:3025–3028.

Arnold S, Folan LM. 1986. Fluorescence spectrometer for a single electrodynamically levitated microparticle. Rev Sci Instrum 57:2250–2253.

Arnold S, Folan LM. 1987. Spherical void electrodynamic levitator. Rev Sci Instrum 58:1732–1735.

Arnold RJ, Reilly JP. 1998. High-resolution time-of-flight mass spectra of alkanethiolate-coated gold nanocrystals. J Am Chem Soc 120:1528–1532.

Baba T, Waki I. 2001. Laser-cooled fluorescence mass spectrometry using laser-cooled barium ions in a tandem linear ion trap. J Appl Phys 89:4592–4598.

Baba T, Waki I. 2002. Chemical reaction of sympathetically laser-cooled molecular ions. J Chem Phys 116:1858–1861.

Bacher G, Szymanski WW, Kaufman SL, Zollner P, Blaas D, Allmaier G. 2001. Charge-reduced nano electrospray ionization combined with differential mobility analysis of peptides, proteins, glycoproteins, noncovalent protein complexes, and viruses. J Mass Spectrom 36:1038–1052.

Bahr U, Röhling U, Lautz C, Strupat K, Schürenberg M, Hillenkamp F. 1996. A charge detector for time-of-flight mass analysis of high mass ions produced by matrix-assisted laser desorption/ionization (MALDI). Int J Mass Spectrom Ion Processes 153:9–21.

Barnes MD, Ng KC, Whitten WB, Ramsey JM. 1993. Detection of single rhodamine 6G molecules in levitated microdroplets. Anal Chem 65:2360–2365.

Barnes MD, Lermer N, Whitten WB, Ramsey JM. 1997. A CCD based approach to high-precision size and refractive index determination of levitated microdroplets using Fraunhofer diffraction. Rev Sci Instrum 68:2287–2291.

Benner WH. 1997. A gated electrostatic ion trap to repetitiously measure the charge and m/z of large electrospray ions. Anal Chem 69:4162–4168.

Berkenkamp S, Kirpekar F, Hillenkamp F. 1998. Infrared MALDI mass spectrometry of large nucleic acids. Science 281:260–262.

Beuhler RJ, Friedman L. 1980. Threshold studies of secondary electron emission induced by macro-ion impact on solid surfaces. Nuclear Instrum Methods 170:309–315.

Beuhler RJ, Friedman L. 1982. A study of the formation of high molecular weight water cluster ions ($m/e < 59000$) in expansion of ionized gas mixtures. J Chem Phys 77:2549–2557.

Birkinshaw K. 2002. Preface/detectors and the measurement of mass spectra. Int J Mass Spectrom 215:vii–viii.

Bohren CF, Huffman DR. 1983. Absorption and scattering of light by small particles. New York: Wiley.

Bruce JE, Cheng X, Bakhtiar R, Wu Q, Hofstadler SA, Anderson GA, Smith RD. 1994. Trapping, detection, and mass measurement of individual ions in a Fourier transform ion cyclotron resonance mass spectrometer. J Am Chem Soc 116:7839–7847.

Buzorius G, Zelenyuk A, Brechtel F, Imre D. 2002. Simultaneous determination of individual ambient particle size, hygroscopicity, and composition. Geophys Res Lett 29:1974.

Cage B, McFarland MA, Hendrickson CL, Dalal NS, Marshall AG. 2002. Resolution of individual component fluorescence lifetimes from a mixture of trapped ions by laser-induced fluorescence/ion cyclotron resonance. J Phys Chem A 106: 10033–10036.

Cai Y, Peng W-P, Chang H-C. 2003. Ion trap mass spectrometry of fluorescently labeled nanoparticles. Anal Chem 75: 1805–1811.

Cai Y, Peng W-P, Kuo S-J, Chang H-C. 2002a. Calibration of an audio-frequency ion trap mass spectrometer. Int J Mass Spectrom 214:63–73.

Cai Y, Peng W-P, Kuo S-J, Lee YT, Chang H-C. 2002b. Single-particle mass spectrometry of polystyrene microspheres and diamond nanocrystals. Anal Chem 74:232–238.

Cai Y, Peng W-P, Kuo S-J, Sabu S, Han C-C, Chang H-C. 2002c. Optical detection and charge-state analysis of MALDI-generated particles with molecular masses larger than 5 MDa. Anal Chem 74:4434–4440.

Carson PG, Johnston MV, Wexler AS. 1997. Laser desorption/ionization of ultrafine aerosol particles. Rapid Commun Mass Spectrom 11:993–996.

Chen R, Cheng X, Mitchell DW, Hofstadler SA, Wu Q, Rockwood AL, Sherman MG, Smith RD. 1995. Trapping, detection, and mass determination of coliphage T4 DNA ions of 10^8 Da by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Anal Chem 67: 1159–1163.

Cheng X, Bakhtiar R, Van Orden S, Smith RD. 1994. Charge-state shifting of individual multiply-charged ions of bovine albumin dimer and molecular weight determination using an individual-ion approach. Anal Chem 66:2084–2087.

Coon JJ, McHale KJ, Harrison WW. 2002. Atmospheric pressure laser desorption/chemical ionization mass spectrometry: A new ionization method based on existing themes. Rapid Commun Mass Spectrom 16:681–685.

Cristoni S, Bernardi LR. 2003. Development of new methodologies for the mass spectrometry study of bioorganic macromolecules. Mass Spectrom Rev 22:369–406.

Danell AS, Parks JH. 2003. FRET measurements of trapped oligonucleotide duplexes. Int J Mass Spectrom 229:35–45.

Dass C. 2001. Principles and practices of biological mass spectrometry. New York: Wiley.

Davis EJ. 1997. A history of single aerosol particle levitation. Aerosol Sci Tech 26:212–254.

Diedrich F, Bergquist JC, Itano WM, Wineland DJ. 1989. Laser cooling to the zero-point energy of motion. Phys Rev Lett 62:403–406.

Ding L, Sudakov M, Kumashiro S. 2002. A simulation study of the digital ion trap mass spectrometer. Int J Mass Spectrom 221:117–138.

Ding L, Gelsthorpe A, Nuttall J, Kumashiro S. 2001. Rectangular wave quadrupole field and digital Q(IT)MS technology. Proceedings of the 49th ASMS Conference on Mass Spectrometry and Allied Topics, MPC80, Chicago, USA.

Dole M, Mach LL, Hines RL, Mobley RC, Ferguson LP, Alice MB. 1968. Molecular beams of macroions. J Chem Phys 49:2240–2249.

Doroshenko VM, Cotter RJ, 1997. Injection of externally generated ions into an increasing trapping field of a quadrupole ion trap mass spectrometer. J Mass Spectrom 31:602–615.

Duncan MA. 2000. Frontiers in the spectroscopy of mass-selected molecular ions. Int J Mass Spectrom 200:545–569.

Ehbrecht M, Huisken F. 1999. Gas-phase characterization of silicon nanoclusters produced by laser pyrolysis of silane. Phys Rev B 59:2975–2985.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1989. Electrospray ionization for mass spectrometry of large biomolecules. Sci 246:64–71.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1990. Electrospray ionization—Principles and practice. Mass Spectrum Rev 9:37–70.

Fenselau C, Demirev PA. 2001. Characterization of intact microorganisms by MALDI mass spectrometry. Mass Spectrom Rev 20:157–171.

Frank M, Mears CA, Labov SE, Benner WH, Horn D, Jaklevic JM, Barfknecht AT. 1996. High-efficiency detection of 66000 Da protein molecules using a cryogenic detector in a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. Rapid Commun Mass Spectrom 10:1946–1950.

Frank M, Labov SE, Westmacott G, Benner WH. 1999. Energy-sensitive cryogenic detectors for high-mass biomolecule mass spectrometry. Mass Spectrom Rev 18: 155–186.

Fraser GW. 2002. The ion detection efficiency of microchannel plates (MCPs). Int J Mass Spectrom 215:13–30.

Friess SD, Zenobi R. 2001. Protein structure information from mass spectrometry? Selective titration of arginine residues by sulfonates. J Am Soc Mass Spectrom 12:810–818.

Fuerstenau SD, Benner WH. 1995. Molecular weight determination of megadalton DNA electrospray ions using charge detection time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 9:1528–1538.

Fuerstenau SD, Benner WH, Thomas JJ, Brugidou C, Bothner B, Siuzdak G. 2001. Mass spectrometry of an intact virus. Angew Chem Int Ed 40:541–544.

Galli M, Guazzotti SA, Prather KA. 2001. Improved lower particle size limit for aerosol time-of-flight mass spectrometry. Aerosol Sci Tech 34:381–385.

Gamero-Castaño M, Fernández de la Mora J. 2000. A condensation nucleus counter (CNC) sensitive to singly charged sub-nanometer particles. J Aerosol Sci 31:757–772.

Gard E, Mayer JE, Morrical BD, Dienes T, Fergenson DP, Prather KA. 1997. Real-time analysis of individual atmospheric aerosol particles: Design and performance of a portable ATOFMS. Anal Chem 69:4083–4091.

Gerlich D, Illemann J, Schlemmer S. 2000. Experiments with trapped ions and nanoparticles. Molecular hydrogen in space. In: Combes F, Pineau des Forets G, editors. Stanford: Cambridge University Press.

Gieray RA, Reilly PTA, Yang M, Whitten WB, Ramsey JM. 1997. Real-time detection of individual airborne bacteria. J Microbiol Methods 29:191–199.

Gilmore IS, Seah MP. 2000. Ion detection efficiency in SIMS: Dependencies on energy, mass, and composition for microchannel plates used in mass spectrometry. Int J Mass Spectrom 202:217–229.

Hars G, Tass Z. 1995. Application of quadrupole ion trap for the accurate mass determination of submicron size charged particles. J Appl Phys 77:4245–4250.

Haugland RP. 1996. Handbook of fluorescent probes and research chemicals, 6th edn. Eugene: Molecular Probes.

Heise TW, Yeung ES. 1994. Spatial and temporal imaging of gas-phase protein and DNA produced by matrix-assisted laser desorption. Anal Chem 66:355–361.

Hillenkamp F, Karas M. 2000. Matrix-assisted laser desorption/ionisation, an experience. Int J Mass Spectrom 200:71–77.

Hilton GC, Martinis JM, Wollman DA, Irwin KD, Dulcie LL, Gerber D, Gillevet PM, Twerenbold D. 1998. Impact energy measurement in time-of-flight mass spectrometry with cryogenic microcalorimeters. Nature 391:672–675.

Hirabayashi Y, Hirabayashi A, Koizumi H. 1999. A sonic spray interface for capillary electrophoresis/mass spectrometry. Rapid Commun Mass Spectrom 13:712–715.

Hirschfeld T, Block MJ, Mueller W. 1977. Virometer: An optical instrument for visual observation, measurement, and classification of free viruses. J Histochem Cytochem 25:719–723.

Houston CT, Reilly JP. 2000. Photoionization studies of chromophore-labeled amino acids and peptides. J Phys Chem A 104:10383–10391.

Imrie DC, Pentney JM, Cottrell JS. 1995. A Faraday cup detector for high-mass ions in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 9:1293–1296.

Johnston MV. 2000. Sampling and analysis of individual particles by aerosol mass spectrometry. J Mass Spectrom 35:585–595.

Kaiser RE, Jr., Cooks RG, Stafford GC, Jr., Syka JEP, Hemberger PH. 1991. Operation of a quadrupole ion trap mass spectrometer to achieve high mass/charge ratios. Int J Mass Spectrom Ion Processes 106:79–115.

Kane DB, Oktem B, Johnston MV. 2001. Nanoparticle detection by aerosol mass spectrometry. Aerosol Sci Tech 34:520–527.

Karas M, Hillenkamp F. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 60:2299–2301.

Karas M, Kruger R. 2003. Ion formation in MALDI: The cluster ionization mechanism. Chem Rev 103:427–439.

Kaufman SL. 1998. Analysis of biomolecules using electrospray and nanoparticle methods: The gas-phase electrophoretic mobility molecular analyzer (GEMMA). J Aerosol Sci 29:537–552.

Kaufman SL, Skogen JW, Dorman FD, Zarrin F. 1996. Macromolecule analysis based on electrophoretic mobility in air: Globular proteins. Anal Chem 68:1895–1904.

Kelleher NL, Senko MW, Siegel MM, McLafferty FW. 1997. Unit resolution mass spectra of 112 kDa molecules with 3 Da accuracy. J Am Soc Mass Spectrom 8:380–383.

Khitrov GA, Strouse GF. 2003. ZnS nanomaterial characterization by MALDI-TOF mass spectrometry. J Am Chem Soc 125:10465–10469.

Khoury JT, Rodriguez-Cruz SE, Parks JH. 2002. Pulsed fluorescence measurements of trapped molecular ions with zero background detection. J Am Soc Mass Spectrom 13: 696–708.

Kofel P, Stöckli M, Krause J, Schlunegger UP. 1996. Matrix-assisted laser desorption/ionization using a new tandem quadrupole ion storage trap, time-of-flight mass spectrometer. Rapid Commun Mass Spectrom 10:658–662.

Kraus H. 2002. Cryogenic detectors and their application to mass spectrometry. Int J Mass Spectrom 215:45–58.

Landais B, Beaugrand C, Capron-Dukan L, Sablier M, Simonneau G, Rolando C. 1998. Varying the radio frequency: A new scanning mode for quadrupole analyzers. Rapid Commun Mass Spectrom 12:302–306.

Lay JO. 2001. MALDI-TOF mass spectrometry of bacteria. Mass Spectrom Rev 20:172–194.

Leibfried D, Blatt R, Monroe C, Wineland D. 2003. Quantum dynamics of single trapped ions. Rev Mod Phys 75:281–324.

Loo JA. 2000. Electrospray ionization mass spectrometry: A technology for studying noncovalent macromolecular complexes. Int J Mass Spectrom 200:175–186.

Louris JN, Cooks RG, Syka JEP, Kelley PE, Stafford GC, Jr., Todd JFJ. 1987. Instrumentation, applications, and energy deposition in quadrupole ion-trap tandem mass spectrometry. Anal Chem 59:1677–1685.

Lu JZ, Zenobi R. 1999. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identifying the composition of labeled proteins. Anal Biochem 269: 312–316.

Mack LL, Kralik P, Rheude A, Dole M. 1970. Molecular beams of macroions. II. J Chem Phys 52:4977–4986.

March RE. 1992. Ion trap mass spectrometry. Int J Mass Spectrom Ion Processes 118/119:71–135.

March RE. 2000. Quadrupole ion trap mass spectrometry: A view at the turn of the century. Int J Mass Spectrom 200: 285–312.

March RE, Hughes RJ. 1989. Quadrupole storage mass spectrometry. New York: Wiley.

March RE, Todd JFJ, editors. 1995. Practical aspects of ion trap mass spectrometry. Boca Raton: CRC Press.

Marshall AG, Hendrickson CL, Jackson GS. 1998. Fourier transform ion cyclotron resonance mass spectrometry: A primer. Mass Spectrum Rev 17:1–35.

Marshall AG, Hendrickson CL. 2002. Fourier transform ion cyclotron resonance detection: Principles and experimental configurations. Int J Mass Spectrom 215:59–75.

McClain MA, Culbertson CT, Jacobson SC, Ramsey JM. 2001. Flow cytometry of *Escherichia coli* on microfluidic devices. Anal Chem 73:5334–5338.

McLuckey SA, Stephenson JL, Jr. 1998. Ion/ion chemistry of high-mass multiply charged ions. Mass Spectrom Rev 17: 369–407.

McLuckey SA, Wells JM. 2001. Mass analysis at the advent of the 21st century. Chem Rev 101:571–606.

McLuckey SA, Wells JM, Stephenson JL, Jr., Goeringer DE. 2000. Novel quadrupole ion trap methods for characterizing the chemistry of gaseous macro-ions. Int J Mass Spectrom 200:137–161.

Millikan RA. 1910. A new modification of the cloud method of determining the elementary charge and the most probable of that charge. Phil Mag 19:209–228.

Moerner WE, Orrit M. 1999. Illuminating single molecules in condensed matter. Sci 283:1670–1676.

Mouradian S, Skogen JW, Dorman FD, Zarrin F, Kaufman SL, Smith LM. 1997. DNA analysis using an electrospray scanning mobility particle sizer. Anal Chem 69:919–925.

Murgasova R, Hercules DM. 2003. MALDI of synthetic polymers—An update. Int J Mass Spectrom 226:151–162.

Nelson RW, Dogruel D, Williams P. 1994. Mass determination of human immunoglobulin IgM using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. 8: 627–631.

Nielen MWF. 1999. Maldi time-of-flight mass spectrometry of synthetic polymers. Mass Spectrom Rev 18:309–344.

Noble CA, Prather KA. 2000. Real-time single particle mass spectrometry: A historical review of a quarter century of the chemical analysis of aerosols. Mass Spectrom Rev 19:248–274.

Nohmi T, Fenn JB. 1992. Electrospray mass spectrometry of poly(ethylene glycols) with molecular weights up to five million. J Am Chem Soc 114:3241–3246.

Pan YL, Aptowicz KB, Chang RK, Hart M, Eversole JD. 2003a. Characterizing and monitoring respiratory aerosols by light scattering. Opt Lett 28:589–591.

Pan YL, Boutou V, Chang RK, Ozden I, Davitt K, Nurmikko AV. 2003b. Application of light-emitting diodes for aerosol fluorescence detection. Opt Lett 28:1707–1709.

Pan YL, Hartings J, Pinnick RG, Hill SC, Halverson J, Chang RK. 2003c. Single-particle fluorescence spectrometer for ambient aerosols. Aerosol Sci Tech 37:628–639.

Park MA, Callahan JH. 1994. An inductive detector for time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 8:317–322.

Paul W. 1990. Electromagnetic traps for charged and neutral particles. Rev Mod Phys 62:531–540.

Pedersen HB, Strasser D, Ring S, Heber O, Rappaport ML, Rudich Y, Sagi I, Zajfman D. 2001. Ion motion synchronization in an ion-trap resonator. Phys Rev Lett 87:055001.

Peng W-P, Cai Y, Lee YT, Chang H-C. 2003. Laser-induced fluorescence/ion trap as a detector for mass spectrometric analysis of nanoparticles. Int J Mass Spectrom 229:67–76.

Philip MA, Gelbard F, Arnold S. 1983. An absolute method for aerosol particle mass and charge measurement. J Colloid Interface Sci 91:507–515.

Pinnick RG, Hill SC, Nachman P, Videen G, Chen G, Chang RK. 1998. Aerosol fluorescence spectrum analyzer for rapid measurement of single micrometer-sized airborne biological particles. Aerosol Sci Tech 28:95–104.

Puretzky AA, Geohegan DB, Hurst GB, Buchanan MV, Luk'yanchuk BS. 1999. Imaging of vapor plumes produced by matrix assisted laser desorption: A plume sharpening effect. Phys Rev Lett 83:444–447.

Reents WD, Jr., Ge ZZ. 2000. Simultaneous elemental composition and size distributions of submicron particles in real time using laser atomization/ionization mass spectrometry. Aerosol Sci Tech 33:122–134.

Reents WD, Jr., Schabel MJ. 2001. Measurement of individual particle atomic composition by aerosol mass spectrometry. Anal Chem 73:5403–5414.

Reents WD, Jr., Downey SW, Emerson AB, Mujsce AM, Muller AJ, Siconolfi DJ, Sinclair JD, Swanson AG. 1995. Single particle characterization by time-of-flight mass spectrometry. Aerosol Sci Tech 23:263–270.

Reid GE, Wells JM, Badman ER, McLuckey SA. 2003. Performance of a quadrupole ion trap mass spectrometer adapted for ion/ion reaction studies. Int J Mass Spectrom 222:243–258.

Robb DB, Covey TR, Bruins AP. 2000. Atmospheric pressure photoionization: An ionization method for liquid chromatography-mass spectrometry. Anal Chem 72:3653–3659.

Rostom AA, Robinson CV. 1999. Detection of the intact GroEL chaperonin assembly by mass spectrometry. J Am Chem Soc 121:4718–4719.

Rostom AA, Fucini P, Benjamin DR, Juenemann R, Nierhaus KH, Hartl FU, Dobson CM, Robinson CV. 2000. Detection and selective dissociation of intact ribosomes in a mass spectrometer. Proc Natl Acad Sci USA 97:5185–5190.

Russell DH. 1986. An evaluation of Fourier transform mass spectrometry for high mass applications. Mass Spectrom Rev 5:167–189.

Salih B, Zenobi R. 1998. MALDI mass spectrometry of dye–peptide and dye–protein complexes. Anal Chem 70:1536–1543.

Scalf M, Westphall MS, Smith LM. 2000. Charge reduction electrospray mass spectrometry. Anal Chem 72:52–60.

Scalf M, Westphall S, Krause J, Kaufman SL, Smith LM. 1999. Controlling charge states of large ions. Science 283: 194–197.

Schaaff TG, Whetten RL. 2000. Giant gold-glutathione cluster compounds: Intense optical activity in metal-based transitions. J Phys Chem B 104:2630–2641.

Schlemmer S, Illemann J, Wellert S, Gerlich D. 2001. Nondestructive high-resolution and absolute mass determination

of single charged particles in a three-dimensional quadrupole trap. J Appl Phys 90:5410–5418.

Schlemmer S, Wellert S, Windisch F, Grimm M, Barth S, Gerlich D. 2004. Interaction of electrons and molecules with a single trapped nanoparticle. Appl Phys A 728:629–636.

Schlunegger UP, Stoeckli M, Caprioli RM. 1999. Frequency scan for the analysis of high mass ions generated by matrix-assisted laser desorption/ionization in a Paul trap. Rapid Commun Mass Spectrom 13:1792–1796.

Schriemer DC, Li L. 1996. Detection of high molecular weight narrow polydisperse polymers up to 1.5 million daltons by MALDI mass spectrometry. Anal Chem 68:2721–2725.

Schultz JC, Hack CA, Benner WH. 1998. Mass determination of megadalton-DNA electrospray ions using charge detection mass spectrometry. J Am Soc Mass Spectrom 9:305–313.

Schultz JC, Hack CA, Benner WH. 1999. Polymerase chain reaction products analyzed by charge detection mass spectrometry. Rapid Commun Mass Spectrom 13:15–20.

Shelton H, Hendricks CD, Jr., Wuerker RF. 1960. Electrostatic acceleration of microparticles to hypervelocities. J Appl Phys 31:1243–1246.

Sinha MP. 1984. Laser-induced volatilization and ionization of microparticles. Rev Sci Instrum 55:886–891.

Sinha MP, Platz RM, Vilker VL, Friedlander SK. 1984. Analysis of individual biological particles by mass spectrometry. Int J Mass Spectrom Ion Processes 57:125–133.

Sipin MF, Guazzotti SA, Prather KA. 2003. Recent advances and some remaining challenges in analytical chemistry of the atmosphere. Anal Chem 75:2929–2940.

Siuzdak G. 1994. The emergence of mass spectrometry in biomedical research. Proc Natl Acad Sci USA 91:11290–11297.

Siuzdak G. 1998. Probing viruses with mass spectrometry. J Mass Spectrom 33:203–211.

Siuzdak G, Bothner B, Yeager M, Brugidou C, Fauquet CM, Hoey K, Chang CM. 1996. Mass spectrometry and viral analysis. Chem Biol 3:45–48.

Smith RD, Cheng X, Bruce JE, Hofstadler SA, Anderson GA. 1994. Trapping, detection, and reaction of very large single molecular ions by mass spectrometry. Nature 369: 137–139.

Smith RD, Paša-Tolić L, Lipton MS, Jensen PK, Anderson GA, Shen Y, Conrads TP, Udseth HR, Harkewicz R, Belov ME, Masselon C, Veenstra TD. 2001. Rapid quantitative measurements of proteomes by Fourier transform ion cyclotron resonance mass spectrometry. Electrophoresis 22:1652–1668.

Stafford GC, Jr. 2002. Ion trap mass spectrometry: A personal perspective. J Am Soc Mass Spectrom 13:589–596.

Stephenson JL, Jr., McLuckey SA. 1998. Charge manipulation for improved mass determination of high-mass species and mixture components by electrospray mass spectrometry. J Mass Spectrom 33:664–672.

Suess DT, Prather KA. 1999. Mass spectrometry of aerosols. Chem Rev 99:3007–3035.

Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. 1988. Protein and polymer analyses up to *z/m* 100,000 by laser ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrum 2:151–153.

Taylor JR, Fang MM, Nie S. 2000. Probing specific sequences on single DNA molecules with bioconjugated fluorescent nanoparticles. Anal Chem 72:1979–1986.

Thomas JJ, Bakhtiar R, Siuzdak G. 2000. Mass spectrometry in viral proteomics. Acc Chem Res 33:179–187.

Ting J. 2001. High-voltage current-feedback amplifier is speedy. EDN 46:136–137.

Tito MA, Tars K, Valegard K, Hajdu J, Robinson CV. 2000. Electrospray time-of-flight mass spectrometry of the intact MS2 virus capsid. J Am Chem Soc 122:3550–3551.

Twerenbold D. 1996. Biopolymer mass spectrometer with cryogenic particle detectors. Nuclear Instrum Methods A 370:253–255.

Twerenbold D, Vuilleumier JL, Gerber D, Tadsen A, van den Brandt B, Gillevet PM. 1996. Detection of single macromolecules using a cryogenic particle detector coupled to a biopolymer mass spectrometer. Appl Rev Lett 68:3503–3505.

Ullom JN, Frank M, Gard EE, Horn JM, Labov SE, Langry K, Magnotta F, Stanion KA, Hack CA, Benner WH. 2001. Discrimination between bacterial spore types using time-of-flight mass spectrometry and matrix-free infrared laser desorption and ionization. Anal Chem 73: 2331–2337.

Valaskovic GA, Kelleher NL, McLafferty FW. 1996. Attomole protein characterization by capillary electrophoresis–mass spectrometry. Science 273:1199–1202.

Van de Hulst HC. 1957. Light scattering by small particles. New York: Wiley.

Wang Y, Hendrickson CL, Marshall AG. 2001. Direct optical spectroscopy of gas-phase molecular ions trapped and mass-selected by ion cyclotron resonance: Laser-induced fluorescence excitation spectrum of hexafluorobenzene ($C_6F_6^+$). Chem Phys Lett 334:69–75.

Wang S-C, Morris MD. 2000. Plastic microchip electrophoresis with analyte velocity modulation. Application to fluorescence background rejection. Anal Chem 72:1448–1452.

Welling M, Thompson RI, Walther H. 1996. Photodissociation of MgC_{60}^+ complexes generated and stored in a linear ion trap. Chem Phys Lett 253:37–42.

Welling M, Schuessler HA, Thompson RI, Walther H. 1998. Ion/molecule reactions, mass spectrometry, and optical spectroscopy in a linear ion trap. Int J Mass Spectrom Ion Processes 172:95–114.

Whitten WB, Ramsey JM, Arnold S, Bronk BV. 1991. Single-molecule detection limits in levitated microdroplets. Anal Chem 63:1027–1031.

Winger BE, Light-Wahl KL, Loo RRO, Udseth HR, Smith RD. 1993. Observation and implications of high mass-to-charge ratio ions from electrospray ionization mass spectrometry. J Am Soc Mass Spectrom 4:536–545.

Wong SF, Meng CK, Fenn JB. 1988. Multiple charging in electrospray ionization of poly(ethylene glycols). J Phys Chem 92:546–550.

Wuerker RF, Shelton H, Langmuir RV. 1959. Electrodynamic containment of charged particles. J Appl Phys 30:342–349.

Yamashita M, Fenn JB. 1984. Electrospray ion source. Another variation on the free-jet theme. J Phys Chem 88: 4451–4459.

Zerega Y, Perrier P, Carette M, Brincourt G, Nguema T, Andre J. 1999. A dual quadrupole ion trap mass spectrometer. Int J Mass Spectrom 190/191:59–68.

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