Ion Trap Studies of Single Microparticles: Optical Resonances and Mass Spectrometry

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Abstract

Microparticle experiments conducted using a newly commissioned quadrupole ion trap (QIT) are reported. Single polystyrene microparticles are confined using three dimensional electrodynamic quadrupole fields and characterised by their fluorescence emission and secular frequency measurements. The advantages of this confinement technique are that single particle properties can be measured free from ensemble averaging effects and unperturbed by solvents and (or) substrates.

Fluorescence emission spectroscopy is used to investigate single fluorescent microspheres, with diameters ranging from 2 to 7 µm, contained within the QIT. As a result of optical microcavity effects, the emission spectra from single microspheres exhibit morphology dependent resonances (MDRs) that permit the determination of the particles’ size, shape and refractive index when compared to Mie Theory. Particles are trapped for periods extending to days, over which time small changes in their size and refractive index are detected and quantified. Non-degenerate azimuthal MDRs are observed for distorted microspheres. The splitting and shifting of the azimuthal resonances are modeled using a perturbation theory permitting the elucidation of the dimensions of the distorted microspheres.

The laser induced coalescence of trapped fluorescent polystyrene bispheres is investigated by monitoring MDRs in the emission spectra. Analysis of the MDRs using Mie theory allows determination of the radii and refractive index of the monomer units of the bisphere. Laser induced melting slowly transforms the bisphere into a single sphere whose volume is within 3% of the combined volume of the two initial spheres. The final stages of the structural transformation, where the particle has the shape of a slightly distorted sphere, are followed by analysing frequency shifts in the non-degenerate azimuthal MDRs. The morphological changes are facilitated by laser heating of the particle above the polystyrene glass transition temperature in accord with calculations estimating the particle’s temperature.

The mass spectrometric performance of the QIT developed in this work is cali-
brated by trapping single φ=2.02 µm (corresponding to a mass of 4.5×10^{−15} kg or 2.7×10^{12} Da) fluorescent polystyrene microspheres and measuring their secular oscillation frequencies and fluorescence spectra. A particle’s absolute mass and charge are found by measuring its secular oscillation frequencies within the QIT while initiating charge steps through the photo-ejection of electrons. The radius of the same microsphere is determined by analysing the MDR dominated fluorescence emission spectrum. The mass of the microsphere is calculated from the radius using the density of bulk polystyrene. For nine particles originating from the same stock sample, the masses obtained from the two methods agrees to within 3% with no systematic deviation. Analysis reveals that small uncertainties in the secular frequency measurements result in significant errors in the absolute charges and masses. Nevertheless, very good agreement between the average masses determined using the two techniques confirms that the value of the trap parameter (z₀) obtained from computer modeling is appropriate and that the effects of electrode misalignments are small.
Declaration

This is to certify that:

(i) the thesis comprises only my original work towards the PhD,

(ii) due acknowledgement has been made in the text to all other material used,

(iii) the thesis is less than 100,000 words in length, exclusive of tables, footnotes, bibliographies and appendices.

Adam J. Trevitt
Preface

The majority of the experimental work appearing in this thesis has either been published prior to submission, accepted for publication, or is soon to be submitted for publication.

In Section 4.3, the modeling and interpretation of the nondegenerate azimuthal optical resonances in the emission spectra of a spheroidal particle has been published in a paper entitled, “Observation of nondegenerate cavity modes for a distorted polystyrene microsphere.” A. J. Trevitt, P. J. Wearne, E. J. Bieske, and M. D. Schuder. Optics Letters 31, 2211 (2006). This paper is included as an appendix.


The work involving the observation of bispheres and tracking of shape transformations described in Section 4.4 is currently being prepared for submission in a paper entitled, “Laser induced coalescence of polystyrene micro-bispheres.” A. J Trevitt, P. J. Wearne, and E. J. Bieske.
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Chapter 1

Introduction

This thesis describes the development of a quadrupole ion trap (QIT) designed to levitate and confine single micrometer sized particles in a vacuum environment. Fluorophore labeled polystyrene microparticles (of diameters (φ) ranging from 2–7 µm) were successfully trapped using three dimensional electrodynamic quadrupolar fields and probed using laser fluorescence spectroscopy and QIT mass spectrometry. Analysis of optical resonance features contained in the single particle emission spectra permitted the determination of the particle’s size, shape and refractive index. Non-spherical particles including spheroids and bispheres were geometrically characterised by interpreting resonances in their optical emission spectra. In some cases, structural transformations of single particles were detected and quantified.

The mass spectrometric capabilities of the QIT were demonstrated in the absolute mass determination of single polystyrene microspheres with φ=2.02 µm. The absolute mass and charge of single microspheres were determined by measuring their secular oscillation frequencies within the QIT whilst initiating charge steps through photo-ejection of electrons. Using this technique, in conjunction with analysis of resonance features contained within the single particle fluorescence spectra, the mass spectrometric performance of the instrument was assayed and the trap calibrated.
1.1 Motivations for Microparticle Experiments

Particles of micrometer dimensions exist as atmospheric aerosols [1], interstellar dust [2], biological cells and bacteria [3]. Of particular topical interest is the role airborne microparticles (aerosols) play in the Earth’s climate, specifically their effect on rainfall, local temperature and air quality [4, 5]. Recently it has been revealed that atmospheric aerosols are of major importance in the Earth’s radiation budget, directly relating to the issue of global warming [6–8]. Anthropogenically generated aerosols, for example, can increase cloud cover by up to 5%, resulting in a substantial overall cooling of the Earth’s atmosphere [9].

There is increasing concern over the links between the accelerated production of anthropogenic aerosols and human health. Airborne particulate matter has been implicated in diseases of the respiratory and cardiovascular systems and blamed for increases in human allergies [5]. The rapid detection of potentially harmful microorganisms and bioparticles, specifically relating to bioterrorism, is another area of current societal interest [10]. Additionally, the characterisation of whole microorganisms and bioparticles has applications within the medical and pharmaceutical communities in aiding diagnosis and drug development [11].

Although it has become clear that the increasing presence of microparticulate matter has profound implications for the Earth and its inhabitants, much research is still required in order to characterise and understand the chemistry and physics of microparticle systems. The development of instrumentation to characterise and study the physical and chemical properties of single microparticles underlies this process.

Instruments designed to determine aerosol composition, particularly mass spectrometers, have contributed significantly to the understanding of the effect of aerosols on the Earth’s atmospheric chemistry and climate [12]. Although compositional information is crucial for aerosol characterisation, the size and shape have a dominant role in determining the scattering, absorption and extinction properties of microparticles [13]. Furthermore, the chemical and physical properties of
1.1. MOTIVATIONS FOR MICROPARTICLE EXPERIMENTS

Aerosols are often dependent on geometry. For example, according to Rosenfeld [14],

“\textit{The chemical composition of aerosol particles is much less important than their size in determining their ability to nucleate clouds, a result that will clarify aerosol effects on climate.}”

While extensive efforts have been directed at microparticle composition, there exists a need for instrumentation that accurately measures the mass, size, shape and refractive index of single microparticles.

Microparticles also exist in the interstellar medium (ISM) as cosmic dust where they contribute to the observed spectral properties of galaxies, molecular formation and the process of star formation [2]. As a consequence, extensive efforts have been invested in elucidating the chemical, physical and dynamical properties of microparticles in the ISM. The recently launched CASSINI space probe was equipped with a Cosmic Dust Analyser (CDA) to investigate dust grains in interplanetary space and in the jovian and saturnian systems [15]. One particular issue that has attracted substantial attention over the last 50 years is the presence of large quantities of molecular hydrogen in the ISM [16]. The mechanism of H$_2$ formation is thought to proceed via the recombination of H atoms adsorbed on interstellar dust grains [17]. To fully understand the processes involved in interstellar H$_2$ formation it is vital that the effects of grain size, shape and composition on the reaction are understood. Single particle levitation experiments under vacuum conditions offer an attractive prospect for studying surface mediated reactions like interstellar H$_2$ recombination, but substantial instrumental improvements are still required to yield useful results [18].

This present study is concerned with investigations of single microparticles confined within a three-dimensional electrodynamic field under vacuum conditions. Single particles are characterised using fluorescence and mass spectrometric techniques by their size, shape, mass and refractive index. The advantage of this approach is that single microparticles can be analysed free from environmental
perturbations such as solvents or substrates. Furthermore, as these are single microparticle experiments, the results are devoid of ensemble averaging.

1.2 Single Particle Fluorescence

Recent advances in single molecule spectroscopic techniques have permitted the study of molecular processes that would be otherwise masked by ensemble averaging [19, 20]. Similarly, spectroscopic investigations of single isolated microparticles have revealed spectral features that were unobservable in previous ensemble measurements [21, 22]. To illustrate this, Figure 1.1 shows two fluorescence emission spectra, one obtained from an aqueous colloidal solution of dye impregnated polystyrene microspheres (a) and the other from a single microsphere extracted from the bulk sample and confined in a QIT (b). The single particle spectrum contains fine structure, clearly not present in the ensemble bulk spectrum. These features are the result of optical cavity resonances called morphology dependent resonances (MDRs). As described in Chapter 4, MDRs provide information about the particle’s size, shape and refractive index. The MDR wavelengths are sensitive to the particle’s geometry and consequently the distribution of particle sizes in the colloidal sample results in a smooth ensemble spectrum with no sharp features (Figure 1.1a).

Interest in microcavity optical processes is not restricted to microparticle characterisation. Resonant recirculation within microcavities has practical uses in photonics and low-threshold lasers [23]. Moreover, the sensitivity of MDRs to surface perturbations makes microresonators suitable for a variety of sensory applications [24]. Already, microsphere based sensors have been developed for biological applications, including the label-free detection of proteins and DNA [25–28].

In this thesis, MDR dominated fluorescence spectroscopy is used to characterise single microparticles of various geometries. A brief historical overview regarding the MDRs of microparticles is given at the beginning of Chapter 4 followed by re-
1.2. SINGLE PARTICLE FLUORESCENCE

Figure 1.1: (a) Fluorescence emission spectra of aqueous dispersion of dye labeled polystyrene microspheres ($\phi \sim 2\mu m$) and (b) a single microsphere taken from this sample and trapped in the quadrupole ion trap developed in this work. The sharp peaks in (b) are due to optical cavity resonances.

Results concerning MDR dominated fluorescence spectra of microspheres, spheroids and bispheres. Analysis of the MDR wavelengths enables single fluorescently labeled polystyrene microspheres ($\phi = 2-7\mu m$) to be characterized by their size and refractive index. In some instances these properties are tracked for periods extending to days allowing small changes to be detected and measured. Fluorescence MDR emission spectra from spheroidal microparticles are also reported. These spectra contain additional MDR features due to the broken spherical symmetry. By implementing a perturbation theory, the dimensions of spheroidal particles are determined with nanometer accuracy. Laser-induced melting in fluorescently labeled polystyrene spheroids and bispheres is also described. By monitoring the MDRs in the emission spectra the structural transformation from spheroid or bisphere to sphere is surveyed and in some instances the dimensions of the intermediate structure is quantified.
One of the fundamental properties of a particle is its mass. For particles with diameters ranging from 10 nm to 100 µm mass determination can be problematic as they tend to have masses greater than the upper mass limits of conventional mass spectrometers (∼10⁻²¹ kg), yet less than the lower limits of mechanical weighing apparatuses (∼10⁻¹⁰ kg). At present, there exists no routine technique for accurate mass determination of single particles in this meso mass regime. Much of the recent mass spectrometric analysis of microparticle systems has been destructive in nature, directed at determining molecular and atomic composition rather than the mass of the particle itself. A typical destructive type analysis involves the laser ablation [29, 30] or thermal vaporisation [31] of single particles and the subsequent detection of atomic and molecular fragments using time-of-flight (TOF) mass spectrometry [32, 33]. In contrast, the work in this thesis is concerned with determining the masses of whole microparticles, non-destructively; an area of research that has attracted less attention.

Previous non-destructive mass measurements of whole microparticles have often involved incorporating optical detection and quadrupole ion trapping techniques [34]. Significant contributions to the development of single microparticle confinement and single particle QIT-MS are presented in Table 1.1. The first QITs were developed by Paul and coworkers in 1954 [35], and soon after microparticle confinement was demonstrated by Wuerker et al. [36]. In this pioneering work, one or more aluminium particles (ϕ∼20 µm) were confined within a QIT for time periods of up to days. They outlined the theory describing the motion of a charged particle in an oscillating three-dimensional quadrupolar field and pointed out that the secular frequencies of a trapped particle are proportional to its mass-to-charge ratio (m/Q). Experimentally, the secular frequency of a single particle was determined by tuning the frequency of an additional small AC voltage applied to the QIT electrodes whilst visually inspecting the particle. When the frequency of the

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*Wuerker reminisces about this early work in an article published 45 years later [37].
1.3. PARTICLE STORAGE AND MASS SPECTROMETRY

<table>
<thead>
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<th>Year</th>
<th>Milestone(s)</th>
<th>Ref.</th>
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<td>1954</td>
<td>Invention of the quadrupole ion trap (QIT)</td>
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<tr>
<td>1959</td>
<td>First demonstration of microparticle confinement in a QIT</td>
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<tr>
<td></td>
<td>Measurement of single particle secular frequencies</td>
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<td>1979</td>
<td>Electrodynamical balance (EDB), mass via charge stepping</td>
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<td>[39]</td>
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<tr>
<td>1980</td>
<td>EDB, simultaneous $m/Q$ and Mie scattering measurements</td>
<td>[40]</td>
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<td>[41]</td>
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<tr>
<td>1989</td>
<td>W. Paul and H. Dehmelt share the Nobel Prize in Physics “for the development of the ion trap technique”</td>
<td>[42]</td>
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<td>1995</td>
<td>Star-shape resonances trajectories, absolute mass determination</td>
<td>[43]</td>
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<td></td>
<td>Trapping of a cluster bacteria spores</td>
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<td>2001</td>
<td>Open electrode QIT, Fourier analysis of particle motion</td>
<td>[44]</td>
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<td></td>
<td>Absolute mass determination, secondary electron emission</td>
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<td>2002</td>
<td>Particle introduction techniques: ESI, MALDI, LIAD</td>
<td>[46]</td>
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<td>2003</td>
<td>Dual-QIT mass spectrometry with fluorescence detection</td>
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<td>2004</td>
<td>Single bioparticle QIT-MS ($E. coli$, red blood cells, bacteria)</td>
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</table>

Table 1.1: A summary of key milestones in single microparticle confinement and QIT mass spectrometry.

additional voltage differed from the particle’s secular frequency, beat frequencies were observed. These beat frequencies disappeared when the two frequencies were equal and at this point the oscillation amplitude markedly increased. Using this technique the secular frequencies were measured, permitting determination of the particle’s $m/Q$. The observation of particle motion, measurement of secular frequencies and exploration of conditions appropriate for storing charged particles all contributed to an early validation of the theory describing ion motion with a three-dimensional time-varying quadrupole field.

With few exceptions [54–56], electrodynamic fields were seldom used for single microparticle storage until the early 1980s when several groups began exploiting the QIT as an electrodynamic balance (EDB) for microparticle mass analysis [38–
In these experiments a DC potential was applied across vertically opposed hyperbolic electrodes in addition to the oscillating quadrupole field. The particle’s \( m/Q \) was deduced from the DC voltage required to compensate for gravity and balance the particle at the trap centre. The absolute charge and mass of the particle was subsequently determined by irradiating the particle with UV light, causing single electrons to be ejected. These charge changes required the particle to be rebalanced in a procedure reminiscent of the Millikan oil drop experiment [57]. Phillip et al. [39] reported a mass precision of \( \sim 4\% \) for 2.4 \( \mu \text{m} \) particles. The accuracy of the method was limited by uncertainties in the geometric term used to approximate the balancing electric field generated across the opposing hyperbolic electrodes.

Consecutive non-destructive and destructive mass spectrometric analysis of a single microparticle was demonstrated by Yang et al. [58]. In this experiment a single microparticle was confined within a QIT and the voltage required to compensate for gravity was used to ascertain the particle’s \( m/Q \). The absolute mass was estimated from the particle’s size by inspection with a telescope and knowledge of its density. A single laser pulse then irradiated the particle, ablating charged species, after which the QIT was switched to ion trapping mode. Trapped atomic and molecular ions were then mass analysed using mass selective ejection. Although this technique determined the mass and composition of a single microparticle, there appears to have been little subsequent work utilising the method. This is perhaps due to uncertainties in determining the \( m/Q \) using the electrodynamic balancing technique, and lower duty-cycles for single particle analysis compared with destructive TOF-MS based techniques.

Many different electrode configurations have been developed to levitate single microparticles, some inspired by the conventional Paul trap design [59, 60], as well as other arrangements including the spherical void trap [61], the cubic trap [62] and the double-ringed trap [63]. All these electrode variations have the capacity to levitate single particles for essentially unlimited time periods in controlled environments. Single particle confinement coupled with angular dependent Mie
scattering measurements is a particularly powerful combination for characterising single microparticles [64, 65] that has allowed researchers to observe phenomena such as phase transitions [66, 67] and morphological changes due to water exchange [68].

A recent notable study exploiting the advantages of single particle confinement involved the observation of *Rayleigh jets* emerging from levitated ethylene glycol droplets [69–71]. In these experiments a single charge microdroplet was held in an EDB and monitored using light scattering and high speed cameras. Over time the droplet was observed to become increasingly distorted, culminating in the emission of fine liquid jets from its surface, after which the droplet readopted a spherical shape (Figure 1.2). The observations were consistent with Rayleigh’s assertion that a liquid droplet is stable provided the Coulomb energy ($E_c$) is less than twice the droplet surface energy ($E_s$) [72]. By monitoring the light scattered from a levitated microdroplet, Leisner and coworkers tracked the shape distortions as the evaporation of neutral molecules increased $E_c$ [69]. As $E_c/2E_s$ approached 1, the quadrupole oscillation increased. After further evaporation, Coulomb instability caused the emission of highly charged liquid jets (Figure 1.2c) after which the droplet regained a spherical geometry, in accordance with the predictions of Rayleigh. These results, which validated the Rayleigh model for a charged droplet, have implications in technologies such as inkjet printing and electrospray ionisation.

The investigation of single microparticle motion in a QIT was revisited by Hars and Tass in 1995 [43]. They observed that single microparticles adopted stationary star-shaped trajectories when the driving frequency of the QIT corresponded to an integer multiple of the particle’s secular frequency. The number of points or “tips” of the star pattern was related to the $m/Q$ ratio of the particle. By changing the particle’s charge by known differentials the absolute charge and mass were determinable with a relative resolution of $10^{-3}$. Hars and Tass also reported the trapping of a cluster containing *bacillus* spores, one of the first demonstrations of trapping biologically relevant particles in a QIT [43].
Figure 1.2: Consecutive images of an evaporating levitated droplet approaching, then surpassing the Rayleigh limit (reproduced from Ref. [70]). The quadrupolar distortion increases as evaporation takes place (a-b). Eventually fine jets are emitted from the tips of the droplet (c-d) after which the droplet re-stabilises and resumes a spherical shape (e-f). The time lapse from (a) to (f) is 70 μs.

In 2001, a novel cage-like QIT electrode design was introduced by Schlemmer et al. [44], having the advantage of greater optical access to trapped particles while still generating an approximate quadrupole field near the trap’s centre. As outlined by Wuerker et al. [36], the secular frequencies of a trapped particle in a quadrupole field can be used to determine its $m/Q$. To measure these frequencies, Schlemmer et al. collected the light scattered from a single particle traversing in and out a laser beam directed through the trap’s center. The particle’s secular frequencies were extracted through Fourier analysis of the elastically scattered light signal. By tracking the secular frequencies and initiating single electron charge steps the absolute masses of single 500 nm SiO$_2$ particles were determined to a relative resolution of $10^{-4}$ [44]. Secondary electron emission from single SiO$_2$ particles was also investigated with this trap configuration [45]. Recently, a QIT of this design has been used to investigate the ionisation potentials of single nano and microparticles using synchrotron radiation [52, 53]. The research described in this thesis uses a QIT of the design introduced by Schlemmer et al., and is described in detail in Chapter 3.

In other recent studies Chang and coworkers have employed a more conventional
1.3. PARTICLE STORAGE AND MASS SPECTROMETRY

Paul trap design, to investigate polystyrene microparticles and diamond nanoparticles [47]. Charged particles were introduced into a QIT by electrospray ionisation (ESI) with ∼10 particles trapped simultaneously. Individual particles were detected as they were mass selectively ejected and crossed a continuous wave laser beam positioned outside of the trap. A mass spectrum of the trapped ensemble was generated by detecting light scattered from the particles as they were ejected and crossed the laser beam. This method was also used to investigate the performance of the QIT operating in mass selective ejection mode [73]. Non-ideal behaviour was attributed to electrode modifications and misalignments. Soon after, this mass selective ejection technique was extended to include a second trap in a tandem-QIT arrangement [49, 50]. In this configuration one trap confines a population of fluorophore-labeled particles (φ=27 nm) and sequentially mass-selectively ejects them into the second trap. The second trap confines the ejected particles, allowing for integration of the fluorescence signal, before dumping them in preparation for the next delivery. This technique is applicable to fluorescently labeled bio-particles and biomolecules. Chang and coworkers have also employed matrix-assisted laser desorption/ionisation (MALDI) for introducing fluorescently labeled particles into a QIT [48]. Using MALDI as a particle source rather than ESI greatly simplified particle introduction and sample preparation.

More recently, Chang and coworkers reported the single particle QIT-MS of single *E. coli* bacteria, introduced using MALDI, and mass analysed by exploiting the star trajectory method [51]. Single bacteria were detected in addition to aggregates containing as many as 15 *E. coli* units clustered together. To simplify particle introduction even further, a laser induced acoustic desorption (LIAD) method was recently developed, where particles are deposited on a silicon wafer. The backside of a silicon wafer was then irradiated with a laser pulse, generating an acoustic shock, resulting in liberation of charged particles from the front surface. This LIAD method was successfully implemented in the mass analysis of *E. coli* bacteria, red blood cells and vacinia virus particles with masses ranging from $1 \times 10^9$ to $1 \times 10^{14}$ Da (1.6 fg to 160 pg corresponding to a size range of 200 nm to 10 μm) [3].
Extraordinarily, single microparticle QIT-MS was able to differentiate between normal and anaemic red blood cells [3]. This work by Chang and coworkers has opened up a new field of mass spectrometry of biologically relevant particles.

The issue of mass calibration remains outstanding for microparticle QIT-MS due mainly to the absence of appropriate mass calibrants in this high mass regime. This is particularly significant as non-ideal or modified electrode geometries are used in practical microparticle QITs. Chapter 5 in this thesis addresses the issue of calibration in single microparticle QIT mass spectrometric experiments. Single fluorescent polystyrene microspheres are confined in a QIT and, using the secular frequency identification techniques pioneered by Wuerker et al. [36] and recently utilised by Schlemmer et al. [44], the absolute mass of each particle is obtained. Each microsphere is further characterised using MDR dominated fluorescence emission spectroscopy. Analysis of single particle emission spectra permits the size of the microspheres to be determined and their masses to be calculated. By comparing mass data obtained from each technique the performance of the QIT mass spectrometer commissioned in this work was calibrated.

1.4 Thesis Overview

The remainder of this thesis is organised in the following manner:

- Chapter 2 is comprised of two parts. The first describes the theory of QIT operation, with particular emphasis on how the frequency of motion of a single particle confined in a three-dimensional quadrupole field can be used to determine its $m/Q$ and ultimately its absolute mass and charge. The second part outlines the theory describing the interaction between electromagnetic radiation and single microparticles (Mie theory) focussing on how structures such as microspheres can act as optical cavities, dramatically affecting their spectroscopic properties.
• Chapter 3 details the experimental apparatus used in this work including the QIT and auxiliary instrumentation. Experimental methodologies are outlined including techniques used to generate and isolated single charged particles within the QIT.

• Chapter 4 describes size, shape and refractive index measurements for micrometer sized polystyrene spheres, spheroids and bispheres using MDR dominated fluorescence spectroscopy. New results concerning the modeling of optical resonances of axial-symmetric spheroids using a perturbation theory are described. Laser induced melting in non-spherical microstructures is reported and investigated.

• Chapter 5 is concerned with the mass spectrometry of single φ=2.02 μm fluorescent polystyrene microspheres with masses of ∼4.5×10^{-15} kg (2.7×10^{12} Da). A mass calibration procedure is described for single QIT-MS involving MDR dominated fluorescence spectroscopy and secular frequency measurement.
Chapter 2

Theoretical Background

2.1 Quadrupole Ion Trap Theory

A quadrupole ion trap (QIT) is a device capable of storing charged atoms, molecules and particles in an effective three dimensional electric potential well. The first QITs were developed by the group led by Wolfgang Paul at University of Bonn in the mid to late 1950’s [35]. The conventional design, which is often referred to as a Paul trap, consists of two opposing hyperbolically shaped “endcap” electrodes surrounded by a single “ring” electrode with a bi-hyperbolic cross-section. The electrode geometry has cylindrical symmetry about the z-axis (Figure 2.1). By applying a potential (V) to the endcap electrodes and maintaining the ring electrode at 0 V a saddle shaped potential surface is generated. When the applied potential is oscillated from +V to −V the saddle is inverted, resulting in a time-averaged three-dimensional potential minimum at the trap centre (Figure 2.2). The theory describing the motion of charged particles within a time-varying three-dimensional quadrupolar field is well developed and documented [74, 76]. In the following chapter only particular aspects of the theory of QITs are introduced with the main purpose being to reveal how the oscillation frequency of a charged

*Wolfgang Paul shared the 1989 Nobel Prize in Physics “for the development of the ion trap technique”.*
Figure 2.1: Cross section of a quadrupole ion trap (taken from Ref. [35]). This electrode configuration, commonly referred to as a Paul trap, is made up of two opposing hyperbolic endcap electrodes surrounded by a solid ring electrode with a bi-hyperbolic cross section. The electrode geometry has cylindrical symmetry about the $z$-axis.

A particle can be used to determine the particle’s mass-to-charge ratio ($m/Q$). The damping effect of a buffer gas on a trapped particle’s motion is also discussed.

### 2.1.1 Quadrupole Field Equations

The following description of the motion of a trapped charged particle in an ideal QIT follows closely the derivations given by March [75]. The generalised potential within a QIT is given in cylindrical coordinates $(r, z)$ by:

$$\Phi(r, z) = ar^2 - 2az^2 + c \quad (2.1)$$

Depending on the mode of operation, an equation for the electric potential can be obtained using the appropriate boundary conditions. Here the oscillating potential $\Phi_0$ is applied to the endcap electrodes and the ring electrode is held at ground, a configuration often referred to as single phase operation. The time varying
potential $\Phi_0$ is defined as:

$$\Phi_0 = U - V \cos \Omega t$$  \hspace{1cm} (2.2)$$

where $V$ and $\Omega$ are the amplitude and angular frequency of the oscillating potential respectively, and $U$ is the DC potential applied between the endcaps and the ring electrode. In this single phase operation the boundary conditions are:

$$\Phi(r_0, 0) = 0 = ar_0^2 + c$$  \hspace{1cm} (2.3)$$

for the potential at the ring, and

$$\Phi(0, z_0) = \Phi_0 = -2az_0^2 + c$$  \hspace{1cm} (2.4)$$

for the potential at the end caps. In an ideal QIT the distance between the two endcap electrodes is $2z_0$ and

$$r_0^2 = 2z_0^2$$  \hspace{1cm} (2.5)$$

Considering the two boundary conditions simultaneously results in $a=\Phi_0/4z_0^2$ and $c=\Phi/2$. Therefore, the potential within this QIT is:

$$\Phi(r, z) = \frac{-\Phi_0}{4z_0^2}(r^2 - 2z^2) + \frac{\Phi_0}{2}$$  \hspace{1cm} (2.6)$$
For a particle with mass $m$ and charge $Q$ within the quadrupole potential described by Eq. (2.6) the motions in the $r$ and $z$ directions are independent. The forces in the $r$ and $z$ directions ($F_r$ and $F_z$) are:

$$F_r = ma = m\frac{d^2r}{dt^2} = -Q\frac{\partial \Phi}{\partial r}$$

(2.7)

$$F_z = ma = m\frac{d^2z}{dt^2} = -Q\frac{\partial \Phi}{\partial z}$$

(2.8)

where $a$ is the acceleration of the particle. Using Eq. (2.6), Eq. (2.7) and Eq. (2.8) become:

$$F_r = ma = m\frac{d^2r}{dt^2} = \frac{Qr}{2z_0^2}$$

(2.9)

$$F_z = ma = m\frac{d^2z}{dt^2} = -\frac{Qz}{z_0^2}$$

(2.10)

Rearranging Eq. (2.9) and Eq. (2.10) and inserting Eq. (2.2) gives:

$$\frac{d^2r}{dt^2} - \frac{Q}{2mz_0^2}(U - V \cos \Omega t)r = 0$$

(2.11)

$$\frac{d^2z}{dt^2} + \frac{Q}{mz_0^2}(U - V \cos \Omega t)z = 0$$

(2.12)

These equations of motion resemble a Mathieu equation, which in its general form in some direction $u$ is:

$$\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0$$

(2.13)

To transform Eq. (2.11) and Eq. (2.12) so that they resemble Eq. (2.13) it is required that $\xi = \Omega t/2$ and

$$a_z = -2a_r = \frac{4QU}{mz_0^2}$$

(2.14)
2.1. QUADRUPOLE ION TRAP THEORY

\[ q_z = -2q_r = \frac{2QV}{mz_0^2\Omega^2} \]  

(2.15)

The two parameters \(a_z\) and \(q_z\) are known as stability parameters. Their values determine the stability of a particle whose motion is governed by Eq. (2.13). The solutions to the Mathieu equation in direction \(u\) are of the form [74]:

\[ u = \alpha' e^{\mu \xi} \sum_{n=-\infty}^{\infty} C_{2n} e^{2in\xi} + \alpha'' e^{-\mu \xi} \sum_{n=-\infty}^{\infty} C_{2n} e^{-2in\xi} \]  

(2.16)

where \(\alpha'\) and \(\alpha''\) are integration constants dependent on initial conditions. The \(C_{2n}\) and \(\mu\) terms depend on \(a_u\) and \(q_u\) but not on the initial conditions. Therefore, trapped particles with the same \((a_u, q_u)\) value will have the same periodic motion regardless of the initial phase of \(\Phi\). There are several “types” of solutions of the form Eq. (2.16) [74]. Here only the stable solutions will be discussed as they imply confinement of the particle within the QIT. Stable solutions occur only when \(\mu = i\beta\) is purely imaginary and \(\beta\) is non-integer.

Charged particles with stable solutions have periodic trajectories with a fundamental secular frequency \(\omega_0\) in addition to higher order frequencies \(\omega_n\) given by:

\[ \omega_n = (2n + \beta) \frac{\Omega}{2} \quad n = 0, 1, 2... \]  

(2.17)

The fundamental or secular frequencies in the \(z\) and \(r\) directions are:

\[ \omega_z = 2\omega_r = \beta_z \frac{\Omega}{2} \]  

(2.18)

By expressing \(\beta_z\) as a function of \(q_z\) the secular frequency can be related to the particle’s \(m/Q\) value. A good approximation for \(\beta_z\) for \(q_z < 0.7\) is [74]:

\[ \beta_z = \left[ a_z - \frac{(a_z - 1)q_z^2}{2(a_z - 1)^2 - q_z^2} - \frac{(5a_z + 7)q_z^4}{32(a_z - 1)^3(a_z - 4)} + \frac{(9a_z^2 + 58a_z + 29)q_z^6}{64(a_z - 1)^5(a_z - 4)(a_z - 9)} \right]^{1/2} \]  

(2.19)
Instead of Eq. (2.19), a simplified expression called the Dehmelt approximation is often used (considered valid for \( q_z \leq 0.4 \)), such that the relationship between \( \beta_z \) and \( q_z \) is approximated as \[ \beta_z = \sqrt{a_z + \frac{q_z^2}{2}} \tag{2.20} \]

The Dehmelt approximation implies that the particle behaves as an ideal three-dimensional harmonic oscillator such that the secular displacement \( Z \) in the \( z \) direction is:

\[
d\frac{d^2 Z}{dt^2} = -\omega_z^2 Z \tag{2.21}
\]

When \( U=0 \) (no DC potential difference between the ring and endcap electrodes), then \( a_z=0 \), so that the following useful relationships emerge:

\[
\omega_z = \frac{\beta_z \Omega}{2} \approx \frac{|q_z|}{2\sqrt{2}} = \frac{QV}{\sqrt{2} m\Omega z_0^2} \tag{2.22}
\]

Therefore the mass-to-charge ratio \((m/Q)\) can be determined from:

\[
\frac{m}{Q} = \frac{1}{\sqrt{2} \Omega z_0^2 \omega_z} \tag{2.23}
\]

where \( \omega_z = 2\omega_r \).

Although the Dehmelt approximation simplifies the analysis and permits the relationship between the trap parameters and the particle’s secular frequencies to be concisely expressed, the validity of this approximation for \( q_z \leq 0.4 \) warrants consideration. In Figure 2.3, \( \beta_z \) is plotted against \( q_z \) with and without the Dehmelt approximation Eq. (2.20). The inset in the figure illustrates that at \( q_z \sim 0.3 \) the Dehmelt approximation underestimates the value of \( \beta_z \) by \( \approx 2\% \). In this work the secular frequencies of trapped particles were measured with \( q_z \sim 0.3 \). In order to avoid any systematic errors in the calculation of \( m/Q \), Eq. (2.19) was used with
2.1. QUADRUPOLE ION TRAP THEORY

Figure 2.3: $\beta_z$ as a function of $q_z$ with (solid line) and without (dotted line) the Dehmelt approximation [Eq. (2.20) and Eq. (2.19) respectively]. The inset reveals that even at $q_z=0.3$ a significant ($\sim 2\%$) deviation exists.

\[ a_z=0 \text{ i.e.:} \]

\[ \beta_z = \sqrt{\frac{q_z^2}{2 - q_z^2} - \frac{7}{128} q_z^4 + \frac{29}{2304} q_z^6} \]  \hspace{1cm} (2.24)

2.1.2 Mass Selectivity

For trap parameters $V$ and $\Omega$, and with $U=0$ ($a_z=0$), only charged particles with $m/Q$ values corresponding to $q_z$ between 0 and 0.908 have stable solutions to Eq. (2.13) and remain confined in the QIT \[74, 75\]. Varying either $V$ or $\Omega$ can destabilise particles with particular $Q/m$ values and eject them from the trap. For example, an increase in $V$ will increase the $q_z$ value of the trapped particles having the effect of ejecting particles with the largest $Q/m$ (Figure 2.4).

Given appropriate operating parameters ($V$ and $\Omega$), particles with a range of $m/Q$ values can be confined within a QIT. Table 2.1 lists the driving frequencies required for stable confinement of particles ranging from a single polystyrene microsphere ($\phi=2 \mu m$) to a single calcium ion for a fixed voltage ($V=300$ V).
Figure 2.4: Assuming equal $Q$, particles of larger mass have lower $q_z$ values than smaller particles. Particles with $q_z>0.908$ are unable to be confined in the trap.

<table>
<thead>
<tr>
<th></th>
<th>$m$ (kg)</th>
<th>$Q/e$</th>
<th>$\Omega/2\pi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 μm polystyrene</td>
<td>4.5×10^{-15}</td>
<td>400</td>
<td>1 kHz</td>
</tr>
<tr>
<td>10 nm Au sphere</td>
<td>1.0×10^{-20}</td>
<td>10</td>
<td>90 kHz</td>
</tr>
<tr>
<td>$C_{60}$</td>
<td>1.2×10^{-24}</td>
<td>1</td>
<td>4 MHz</td>
</tr>
<tr>
<td>$^{40}\text{Ca}^+$</td>
<td>6.6×10^{-26}</td>
<td>1</td>
<td>8 MHz</td>
</tr>
</tbody>
</table>

Table 2.1: The approximate trap driving frequency ($\Omega/2\pi$) required for stable confinement ($q_z\sim0.3$) of various particles within a QIT. The values are appropriate for a QIT in single phase operation with $V=400\text{ V}$ and $z_0=5.9$.

### 2.1.3 Determining the sign of $Q$

When $U=0$ a QIT has no preference for trapping negative or positive particles with the same $m/Q$. In the case where a trapped particle is sufficiently large to be inspected visually, determining the sign of the particle’s charge can be achieved by applying a DC potential across the end cap electrodes and noting whether the particle is drawn towards the negative or positive electrode. If however, the particle is too small to be visually observed then deducing the sign of its charge can be problematic. When this is the case, the charge sign can be determined from the particle’s secular frequency in the following manner. As shown in Eq. (2.22), negative and positive particles with the same $Q/m$ oscillate with the same secular frequencies. However, when $U\neq0$, the secular frequencies of the trapped particle increase or decrease depending on the sign of $Q$. From Eq. (2.18) and Eq. (2.20):

$$\omega_u^2 = \left(a_u + \frac{q_u^2}{2}\right)\left(\frac{\Omega^2}{4}\right)$$

The sign of $a_u$ depends on both the sign of $Q$ and $U$ [Eq. (2.14)]. With $V$ and $\Omega$ held constant, the sign of $Q$ can be determined by applying a DC potential across
the ring and endcap electrodes ($U$) and monitoring the direction of the secular frequency shift. For example, a positive $U$ results in a positive shift in $\omega_z$ when $Q$ is positive and a negative shift when $Q$ is negative.

### 2.1.4 Gas Drag

Practical QITs are often operated using a background gas to dampen thermal motion of trapped species. This has been found to enhance their mass resolution [77]. In the case of microparticles, the presence of buffer gas has the potential to shift the secular frequencies by damping the particle’s periodic motion. The damping effect of a buffer gas has been investigated by Cai et al [73]. To account for aerodynamic drag on the particle’s motion an additional linear term ($b \frac{dz}{d\xi}$) is included in Eq. (2.13) which becomes:

$$\frac{d^2z}{d\xi^2} + b \frac{dz}{d\xi} + (a_z - 2q_z \cos 2\xi) z = 0 \quad (2.26)$$

The motion of the damped oscillator in the $z$ direction is described by:

$$z(t) = A_z e^{-kt} \cos(\omega_z t) \quad (2.27)$$

where $k = b\Omega/4$ and $\omega_z = \sqrt{(\omega_{0z}^2 - k^2)}$. The damping term $b$ for a microsphere is [73]:

$$b = \frac{8.6p}{\rho d \Omega} \sqrt{\frac{m_{gas}}{RT}} \quad (2.28)$$

where $p$ is the buffer gas pressure, $\rho$ is the density of the sphere with diameter $d$, and $m_{gas}$ and $T$ are the molar weight and temperature of the buffer gas. With $d=2 \mu m$, $\rho_0=1.05 \text{ g/cm}^2$ (for polystyrene), $p=0.001 \text{ Torr}$, $m_{gas}=4 \text{ g/mol}$ (for He), $T=300 \text{ K}$, and $\Omega/2\pi=300 \text{ Hz}$, corresponds to $b\approx0.0004$ and $k=0.2 \text{ Hz}$. Under these conditions shifting due to aerodynamic drag is on the order of $10^{-5} \text{ Hz}$ for a particle with $\omega_z/2\pi=20 \text{ Hz}$. In this work, single particle secular frequencies were measured at pressures of $\sim10^{-6} \text{ Torr}$ at fractional resolutions ($\Delta\omega/\omega$) on the order of $10^{-3}$, so that the effects of aerodynamic drag should be negligible.
CHAPTER 2. THEORETICAL BACKGROUND

2.2 Optical Properties of a Dielectric Microsphere

A complete analytical solution to Maxwell’s equations for the interaction between an electromagnetic wave and a dielectric sphere is known as Mie theory. The theory provides a description of many optical properties of dielectric microspheres including elastic scattering, absorption and extinction. In the following section the features of the Mie theory salient to this thesis are introduced and discussed. For a complete description of Mie theory, texts by van de Hulst and Bohren and Huffman are authoritative works.

2.2.1 Mie Theory

When describing the interaction between electromagnetic radiation and a sphere it is convenient to describe the system in terms of a unitless size parameter \( x \) defined as the ratio of the sphere circumference \( (2\pi a) \) and the vacuum wavelength of the incident radiation \( (\lambda) \):

\[
x = \frac{2\pi a}{\lambda}
\]  

(2.29)

When a plane wave of light is incident on a dielectric sphere with size parameter \( x \), the scattering efficiency, \( Q_{\text{sca}} \), is given by:

\[
Q_{\text{sca}} = \frac{2}{x^2} \sum_{l=1}^{\infty} (2l + 1) (a_l^2 + b_l^2)
\]

(2.30)

where the \( a_l \) and \( b_l \) terms, known as the scattering parameters, are:

\[
a_l = \frac{\psi_l(x)\psi'(m_\lambda x) - m_\lambda \psi_l(m_\lambda x)\psi'(x)}{\zeta_l(x)\psi'(m_\lambda x) - m_\lambda \psi_l(m_\lambda x)\zeta'(x)}
\]

(2.31)

\[
b_l = \frac{m_\lambda \psi''(x)\psi_l(m_\lambda x) - \psi_l(m_\lambda x)\psi''(x)}{m_\lambda \zeta_l(x)\psi_l'(m_\lambda x) - \psi_l(m_\lambda x)\zeta'(x)}
\]

(2.32)

Footnote: Mie Theory is named after Gustav Mie, one of the pioneers of the theory describing electromagnetic plane wave scattering by a dielectric sphere. His seminal paper was published in 1908 [78].
where \( m_\lambda \) is the refractive index of the sphere. The Riccatti-Bessel functions \( \psi_l(x) \), \( \chi_l(x) \), \( \zeta_l(x) \) are defined as:

\[
\psi_l(x) = \left( \frac{\pi x}{2} \right)^{1/2} J_{l+\frac{1}{2}}(x) \\
\chi_l(x) = \left( \frac{\pi x}{2} \right)^{1/2} N_{l+\frac{1}{2}}(x)
\]

and

\[
\zeta_l(x) = \psi_l(x) + i\chi_l(x)
\]

where \( J_{l+\frac{1}{2}}(x) \) and \( N_{l+\frac{1}{2}}(x) \) are the \( l+1/2 \) order Bessel and Neumann functions, respectively.\(^1\) In Eq. (2.31) and Eq. (2.32) the prime indicates a derivative with respect to the argument.

The \( Q_{sca} \) of a sphere can be thought of as a dimensionless measure of the sphere’s propensity to capture energy from a plane wave of incident light (wavelength \( \lambda \)) and redistribute that energy over all angles. Figure 2.5 is a plot of \( Q_{sca} \) versus size.

\(^1\)In practice, it is convenient to use the formalism of Probert-Jones for calculation of \( a_l \) and \( b_l \) as they are expressed as functions of \( \psi_l \) and \( \chi_l \) only [50].
Figure 2.6: $Q_{sca}$ as a function of wavelength for a sphere with $a=2\mu m$ and $m_\lambda=1.5$. Here, 595 nm corresponds to a size parameter ($x=2\pi a/\lambda$) of \(~21\).

A particularly notable feature is the presence of a ripple structure in $Q_{sca}$ that becomes sharper for larger values of $x$. Figure 2.6 plots $Q_{sca}$ as a function of wavelength for a dielectric sphere with a radius of 2 \(\mu m\).

### 2.2.2 Morphology Dependent Resonances (MDRs)

It is evident from Figure 2.5 and Figure 2.6 that certain ratios of the sphere’s circumference and the light’s wavelength are associated with a sharp increase in $Q_{sca}$. In geometrical terms, the existence of these spikes can be explained as follows. When light of wavelength $\lambda$ is incident on a dielectric sphere with radius $a$, a fraction of this light crosses the sphere’s boundary. Inside the sphere, light striking the boundary at angles greater than the critical angle will be total-internally reflected and skim around the interior of the sphere. If after a complete lap of the sphere the light returns in phase then a resonance or standing wave is formed as depicted in Figure 2.7. For large spheres ($a \gg \lambda$) the resonance condition is:

$$2\pi a \approx l\lambda$$  \hspace{1cm} (2.36)
where \( l \) is an integer. As the resonances are dependent on the size and shape of the particle they are termed morphology dependent resonances (MDRs) (also sometimes referred to as whispering gallery modes).

In terms of Mie theory, the MDRs correspond to the \( x \) values for which the denominators for either \( a_l \) and \( b_l \) approach zero, leading to a sharp rise in the value of \( Q_{\text{sca}} \). Transverse magnetic (TM) modes are associated with a dominating \( a_l \) term, and transverse electric (TE) modes with a dominating \( b_l \) term. The TM MDRs have no electric field intensity in the radial direction and the TE MDRs have no magnetic field intensity in the radial direction; they are perpendicular in polarisation. In addition to polarisation, each MDR is characterised by its mode order \( \nu \), corresponding to the number of electric field intensity maxima in the radial direction, and an angular mode number \( l \) which is half the number of electric field intensity maxima in the angular coordinate.\(^\dagger\) Each MDR can be uniquely designated as TP\(_\nu^l\), where P = E or M. To illustrate this, Figure 2.8 depicts the internal electric field magnitude for three different MDRs with \( \nu \)=1, 2 and 3, all with \( l \)=10. Figure 2.9 depicts angle-averaged cross sections of the electric field intensity as a function of radius for three MDRs with different \( \nu \) illustrating the difference in the electric field localisation in the radial direction between the different orders. The \( \nu \)=1 MDR is strongly localised near the surface. With increasing \( \nu \) the MDRs are increasingly delocalised, with the electric field penetrating deeper into the sphere. Also evident from Figure 2.9 is that the electromagnetic field ex-

\(^\dagger\)The microcavity notation used in this thesis conforms with that of Ching et al. in Ref. [81].
CHAPTER 2. THEORETICAL BACKGROUND

Figure 2.8: A representation of the internal electric field magnitude for the three MDRs all with the same $l$ but with different $\nu$.

Figure 2.9: Plots of the angle averaged internal field intensity of three different mode orders as a function of the normalised radius ($r/a$). $r/a=1$ corresponds to the sphere’s surface.
tends beyond the sphere’s boundary, emphasising that the geometrical description of MDRs eventually breaks down. The presence of this evanescent field implies that the MDRs are not entirely confined to the cavity. In fact the cavity is leaky, allowing light to couple in and eventually escape out. Confinement of the electric field and the prolonged photon storage time results in the enhancement of many optical processes including fluorescence, Stokes and anti-Stokes Raman scattering and lasing [22]. Previous experimental studies concerned with cavity enhanced optical phenomena of microparticles are discussed in the introduction in Chapter 4.

The MDRs of a spherical cavity are $2l + 1$ azimuthally degenerate, each with a distinct azimuthal mode number ($m$) where $m=l, l-1, ..., 0$. Photons associated with an MDR follow a path about a plane inclined at an angle $\theta$ where $\cos \theta = \cos(m/\sqrt{l(l+1)})$ as depicted in Figure 2.10. The consequences of breaking the spherical symmetry and subsequent frequency splitting of the azimuthal MDRs will be discussed later in Section 2.2.4.

Figure 2.10: Azimuthal MDRs have a circumferential path about a plane inclined at an angle $\theta$ to the equatorial plane, where $\cos \theta = (m/\sqrt{l(l+1)})$. 
2.2.3 Assignment of MDRs

The measured MDR frequencies of a sphere can be used to determine its radius \(a\) and refractive index \(m_\lambda\). This requires correctly assigning a mode order \(\nu\) and mode number \(l\) to each MDRs so that every measured MDR location uniquely corresponds to a zero of the denominator of either Eq. (2.31) or Eq. (2.32). In this work, the Probert-Jones [80] formalism for expressing the scattering parameters \(a_l\) and \(b_l\) is employed to simplify the task of accurately locating the MDR peak positions. New functions \(p_l\), \(q_l\), \(g_l\) and \(h_l\) are defined as:

\[
p_l = \psi_l(x)\psi'_l(mx) - m\psi_l(mx)\psi'_l(x) \tag{2.37}
\]

\[
q_l = \chi_l(x)\psi'_l(mx) - m\psi_l(mx)\chi'_l(x) \tag{2.38}
\]

such that

\[
a_l = \frac{p_l}{p_l + iq_l} \tag{2.39}
\]

and

\[
g_l = m\psi_l(x)\psi'_l(mx) - \psi_l(mx)\psi'_l(x) \tag{2.40}
\]

\[
h_l = m\chi_l(x)\psi'_l(mx) - \psi_l(mx)\chi'_l(x) \tag{2.41}
\]

so that

\[
b_l = \frac{g_l}{g_l + ih_l} \tag{2.42}
\]

Probert-Jones showed that the MDR peak positions occur when imaginary parts of either Eq. (2.39) or Eq. (2.42) are zero, i.e. when:

\[
q_l = \chi_l(x)\psi'_l(mx) - m\psi_l(mx)\chi'_l(x) = 0 \tag{2.43}
\]

\[
h_l = m\chi_l(x)\psi'_l(mx) - \psi_l(mx)\chi'_l(x) = 0 \tag{2.44}
\]

for TM and TE MDRs, respectively.

As there are many solutions to Eq. (2.43) and Eq. (2.44) it is of practical use
to be equipped with an approximate solution before seeking the exact solution. Fortunately, there are explicit asymptotic formulas for MDR locations developed. According to Lam et al. [82], the position of an MDR with mode order $\nu$ and mode number $l$ can be approximated by:

$$m_\lambda x = \eta + 2^{-1/3} \alpha_\nu \eta^{1/3} - \frac{P}{(m_\lambda^2 - 1)^{1/2}} + \left[ \frac{3}{10} 2^{-2/3} \right] \alpha_\nu^2 \eta^{-1/3}$$

$$- \frac{2^{-1/3} P(m_\lambda^2 - 2P^2/3)}{(m_\lambda^2 - 1)^{3/2}} \alpha_\nu \eta^{-2/3}$$

(2.45)

where

$$P = \begin{cases} 
\frac{m_\lambda}{1/m_\lambda} : & \text{for TE modes} \\
1/m_\lambda : & \text{for TM modes}
\end{cases}$$

(2.46)

$\eta = l + 1/2$ and $m_\lambda$ is the refractive index of the sphere. The $\alpha_\nu$ are roots of the Airy function $Ai(-z)$ (see Table 2.2). Using the asymptotic formula the approximate location for the MDR can be found for a given polarisation, $\nu$, $l$ and $m_\lambda$ and with this approximate solution constraints can be set for locating the root of the relevant characteristic equation [Eq. (2.43) or Eq. (2.44)].

<table>
<thead>
<tr>
<th>Mode order $\nu$</th>
<th>$\alpha_\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.338</td>
</tr>
<tr>
<td>2</td>
<td>4.088</td>
</tr>
<tr>
<td>3</td>
<td>5.521</td>
</tr>
<tr>
<td>4</td>
<td>6.787</td>
</tr>
<tr>
<td>5</td>
<td>7.944</td>
</tr>
</tbody>
</table>

Table 2.2: The first 5 roots ($\alpha_\nu$) of $Ai(-z)$ taken from Ref. [82].

In this work a typical analysis involved the assignment of a list of experimental MDR wavelengths from a single particle with the ultimate aim of determining the particle’s radius and refractive index. This was performed using a least squares minimisation algorithm similar to that reported by Eversole et al. [83] executed with the data analysis program proFit (QuantumSoft). The fitting routine sought
to minimize $\chi^2$, defined as:

$$\chi^2 = \sum_i (\lambda_{\text{theo}} - \lambda_{\text{exp}})^2$$  \hspace{1cm} (2.47)

where $\lambda_{\text{theo}}$ and $\lambda_{\text{exp}}$ are the computed and experimental measured MDR wavelengths. Variation of the refractive index with wavelength is accounted for by the Cauchy equation defined as $m_\lambda = A + B/\lambda^2$.

The fitting routine is executed as follows:

1. The experimentally measured MDR wavelengths are collated. Identification of patterns in the peak spacings allows MDRs of the same polarisation (TE or TM) and $\nu$ to be grouped and assigned, and initial guesses for $\nu$ and $l$ to be made. This process is aided by using approximations for the MDR locations Eq. (2.45) and separations [S2].

2. Main Loop:

   (a) MDR wavelengths are computed using the values of $\nu$ and $l$ for the respective polarisations (TE or TM). This is achieved initially using the asymptotic approximations for the MDR locations Eq. (2.45). Based on the approximate locations constraints are set for finding the analytic roots to either Eq. (2.43) for TM MDRs or Eq. (2.44) for TE MDRs.

   (b) The computed MDR locations are compared to the experimental values and $\chi^2$ is minimized by varying $a$, $A$ and $B$ using a Levenberg-Marquardt algorithm. This value of $\chi^2$ is recorded.

3. The MDR assignments in terms of polarisation, $\nu$ and $l$ are then adjusted manually and the main loop is recommenced. This process is repeated until the global minimum for $\chi^2$ is found and all theoretical and experimental MDR locations agree to within 0.1%.

Implementation of the algorithm is assisted by knowledge of the system under investigation. For example, previous knowledge of plausible refractive index values
and particle sizes narrow the range of possible $l$ values. Particle systems with $x \leq 18$ are usually simpler to characterise because they exhibit only $\nu=1$ MDRs with higher order resonances usually too broad to be resolved. In Section 4.2 the MDR fitting routine described above is used to fit the MDRs measured in the fluorescence emission spectra of single polystyrene spheres of $\phi \sim 2–7$ $\mu$m to extract radius and refractive index data.

Figure 2.11: $Q_{sca}$ as a function of wavelength showing the shift in the MDR location with change in refractive index with constant radius ($a=2000$ nm) (right) and a change in radius for $m_\lambda = 1.5$ (left).

The spectral locations of MDRs are dependent on the size and refractive index of the sphere. Small changes in these properties have significant spectral effects as illustrated in Figure (2.11) where shifts in the MDR locations are revealed for small changes in size and refractive index. An increase in radius incurs a red-shift in each MDR as in a larger cavity, for a given $l$, a longer wavelength is required. Increasing the refractive index also red-shifts the MDRs because the effective wavelength is shortened in a medium of higher refractive index. Therefore, the incident wavelength must be longer to sustain the resonance with the same $l$. In both cases in Figure (2.11) the perturbations are on the order of 0.1% and the resulting wavelength shift of the MDR is $\sim 0.3$ nm, a detectable shift using even a modest modern monochrometer.
2.2.4 Shape Distortions

When a sphere is distorted to a spheroid the $2l+1$ azimuthal degeneracy is removed. As a consequence, for a particular polarisation, $\nu$ and $l$ the system exhibits $(l+1)$ distinct azimuthal modes (labeled $m=l$, $l-1$,...0). Classically, the azimuthal modes of a spheroid propagate about a plane that is angled at $\theta$ relative to the plane of circular path where $\cos \theta \approx m/l$ for large $l$. The $m=l$ mode essentially propagates in the equatorial plane while the $m=0$ mode propagates in a plane containing the poles.

![Graphical representations of the azimuthal modes for $l=10$ and $\nu=1$.](image)

Figure 2.12: Graphical representations of the azimuthal modes for $l=10$ and $\nu=1$. In the spherical case (left) all the azimuthal modes are degenerate. For the prolate (middle) and oblate (right) spheroids the degeneracy is broken as each $m$ value corresponds to a different pathlength.

Examples of azimuthal non-degeneracy for prolate and oblate spheroids are shown in Figure 2.12 compared to the degenerate spherical case. In a prolate spheroid, the azimuthal modes circulate around great ellipses with the $m<l$ modes red-shifted compared to the circular equatorial mode ($m=l$). The converse is true for an oblate spheroid, as the longest path ($m=l$) is around the circular equator and the $m<l$ azimuthal modes are therefore blue-shifted.

The shifting and splitting of non-degenerate azimuthal MDRs can be quantitatively related to the distortion of the particle from sphericity using a perturbation formalism. Lai et al. [84] describe a sphere affected by an axially symmetric shape...
perturbation such that the radius is described by:

\[ r(\theta, \phi) = a + \Delta_L \sqrt{4\pi} Y_{L,0}(\theta, \phi) \]  

(2.48)

where \( \Delta_L \) is a weighting term for the \( Y_{L,0}(\theta, \phi) \) spherical harmonic. The shift in the frequency of \( m \)-modes (\( \delta \omega \)) compared to the frequency (\( \omega \)) for a sphere of equal volume with radius \( a \) is given as:

\[ \frac{\delta \omega}{\omega} = -\frac{\Delta_L}{a} F(L, l, m) \]  

(2.49)

where \( F(L, l, m) \) is defined as

\[ F(L, l, m) = A(L, l) f(L, l, m) \]  

(2.50)

with

\[ A(L, l) = \frac{2l + 1}{\sqrt{2L + 1}} \left[ C(llL; 000) \right]^2 \left[ 1 - \frac{L(L + 1)}{2l(l + 1)} \right] \]  

(2.51)

and

\[ f(L, l, m) = (-1)^m \frac{C(llL; m, -m, 0)}{C(llL; 000)} \]  

(2.52)

The \( C(j_1j_2j_3;m_1m_2m_3) \) are Clebsch-Gordan coefficients. Convenient recursion formulas for calculating \( A(L, l) \) and \( f(L, l, m) \) are given in Ref. [84].

This perturbation analysis is to first order a linear result [85], therefore additional spherical harmonic distortions can be added to model a more general axial-symmetric particle morphology:

\[ r(\theta, \phi) = a + \sqrt{4\pi} \sum_{k=1}^{\infty} \Delta_{2k} Y_{2k,0}(\theta, \phi) \]  

(2.53)

Note that for odd \( L \) distortions the function \( F(L, l, m) \) is zero and the \( m \)-modes remain degenerate [84].

The lowest order distortion to remove the \( m \) degeneracy is a quadrupole term
CHAPTER 2. THEORETICAL BACKGROUND

$Y_{2,0}(\theta, \phi)$, with

$$A(L = 2, l) = \frac{\sqrt{5}(l^2 + l - 3)}{(2l - 1)(2l + 3)}$$ (2.54)

and

$$f(L = 2, l, m) = 1 - \frac{3m^2}{l(l + 1)}$$ (2.55)

the resulting in $m$-mode shifts described by:

$$\frac{\delta \omega}{\omega} = -\frac{\Delta_2}{a} F(2, l, m) = -\frac{\Delta_2}{a} \frac{\sqrt{5}(l^2 + l - 3)}{(2l - 1)(2l + 3)} \left[ 1 - \frac{3m^2}{l(l + 1)} \right]$$ (2.56)

The $Y_{2,0}(\theta, \phi)$ distortion for $\frac{\Delta_2}{a} \ll 1$ results in an axial-symmetric spheroid and a shifting of the azimuthal mode frequencies that depends quadratically on $m$. Positive and negative values of $\Delta_2$ correspond to prolate and oblate spheroids, respectively. A cross section of the $Y_{2,0}(\theta, \phi)$ spherical harmonic is shown in Figure (2.13). Positive values of the spherical harmonic function (white areas) result in expansion along the $z$-axis and negative values (shaded grey) contribute to a symmetric contraction about the $x$-$y$ plane, the net effect being a prolate spheroid.

In the case of a negative $\Delta_2$, the action of the spherical harmonic perturbation is reversed, yielding an oblate spheroid. Also shown in Figure 2.13 is the $Y_{4,0}(\theta, \phi)$ spherical harmonic describing an octupolar distortion, the next highest order distortion to effect a shift of the azimuthal modes. In Section 4.3 non-degenerate azimuthal MDRs are resolved in the spectra of a distorted microparticle. By implementing the perturbation theory described here the dimensions of the particle are determined with nanometer precision by modeling the shifting and splitting in the non-degenerate azimuthal MDRs.

In the case where $l \gg 1$ then Eq. (2.56) can be approximated by [81]:

$$\frac{\delta \omega}{\omega} = -\frac{e}{6} \left[ 1 - \frac{3m^2}{l(l + 1)} \right]$$ (2.57)

with

$$e = 3\sqrt{\frac{5}{4}} \frac{\Delta_2}{a} = \frac{r_p - r_e}{a}$$ (2.58)
where \( r_p \) and \( r_e \) are the polar and equatorial radii, respectively. Eq. (2.57) has been used to quantify the distortion of moving fluorescent liquid droplets that undergo shape distortion due to aerodynamic drag [86–88]. In this thesis Eq. (2.57) was implemented to determine the distortion of a sphere at the final stages of laser-driven shape transformation (Section 4.4).

Figure 2.13: Cross section of the \( Y_{2,0} \) and \( Y_{4,0} \) spherical harmonic functions which correspond to quadrupolar and octupolar distortion, respectively. Positive (white) and negative (gray) regions of the functions are shown.
Chapter 3

Experimental Instrumentation and Methods

Considerable effort was directed at the construction, development and commissioning of a quadrupole ion trap (QIT) and auxiliary instrumentation. A feature of the QIT implemented in this work is its open electrode design that increases access to trapped particles for laser based interrogation techniques. Furthermore, the open design allows for increased solid angle detection for light scattering and fluorescence emission measurements. In what follows, the design of the QIT is detailed along with computer simulations modeling the electrical potential with the trap. This is followed by descriptions of the vacuum, laser, optics and signal processing systems. The experimental methods developed in the course of this research such as particle introduction, single particle isolation and the identification of small charge changes on highly charged particles are also outlined.

3.1 The Quadrupole Ion Trap

The quadrupole ion trap (QIT) utilised in this work has an electrode configuration which differs from that of an ideal quadrupole trap. It is designed to generate an approximate quadrupole potential about a small volume around the trap centre
while having the advantage of increased optical access to trapped species with its open electrode design. The conception and design of this QIT is described comprehensively in the PhD thesis of Illemann [89] and also by Schlemmer et al. [44].

The QIT electrodes and housing were fabricated by the School of Chemistry workshop at The University of Melbourne. The electrodes consist of two opposing conical endcap electrodes surrounded by a ring of eight rod electrodes as shown in Figure 3.1. The rod electrodes are 1.6 mm in diameter, and spaced evenly around a circle with radius 12.5 mm about the central symmetrical axis of the QIT. The distance between the opposing endcap electrodes is 6.6 mm. The endcap electrodes and rods were all electrically isolated using ceramic mounts and all electrodes were blackened using a colloidal graphite spray (Acheson).

### 3.1.1 Electric Field Simulations (SIMION)

As previously stated, the QIT electrodes are not of the ideal quadrupolar geometry. Therefore the $z_0$ parameter, which corresponds to half the distance between the endcap electrodes of an ideal QIT, cannot be directly measured but must be obtained by some other means. The ion optics simulation program SIMION 3D v7.0 was employed to model the potential distribution within the QIT. The electrode arrangement was constructed in SIMION using a resolution of 0.05 mm per graphical unit (0.05 mm/gu). In the simulation a positive 1 V potential was assigned to the endcap electrodes and all the 8 rod electrodes held at ground. An encompassing grounded cylinder (radius 14.85 mm) surrounded the 8 rod electrodes. The resulting equipotential field lines at the QIT centre are displayed in Figure 3.2. An array of 8000 potential data points with a grid spacing of 0.05 mm, corresponding to a $1 \text{mm} \times 1 \text{mm} \times 1 \text{mm}$ cube at the trap centre, was extracted from the SIMION simulation. The data was fitted to the following equation for a QIT operated in
3.1. THE QUADRUPOLE ION TRAP

Figure 3.1: The QIT used in this research. The QIT consists of two opposing conical endcap electrodes surrounded by eight rod electrodes that are electrically isolated using ceramic mounts. Shown is the QIT from the end-on (top-left), side-on (top-right), and three dimensional (bottom) perspectives.
Figure 3.2: Cutout image exported from simion 3D v7.0 revealing the characteristic quadrupole equipotential lines at the QIT centre.

single phase \[90\]:

\[
\frac{V(x, y, z)}{V_0} = C_0 + \frac{2z^2 - x^2 - y^2}{4z_0^2} + C_4 \frac{8z^4 - 24z^2(x^2 + y^2) + 3(x^2 + y^2)^2}{8z_0^4} \tag{3.1}
\]

Eq. (3.1) describes a static potential generated by an ideal QIT \([V(x, y, z)]\) normalised to the applied potential \((V_0)\) as the sum of a linear offset term \((C_0)\), a quadrupole component and an octupole component with a weighting coefficient of \(C_4\). In the fit \(V_0\) is fixed at 1 V and the best fit was achieved with the values shown in Table 3.1. The validity of the \(z_0\) term determined from the simion simulation is investigated in single particle mass calibration experiments described in Chapter 5.

<table>
<thead>
<tr>
<th>Term</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_0) (V)</td>
<td>1.0</td>
<td>fixed</td>
</tr>
<tr>
<td>(C_0)</td>
<td>0.830383</td>
<td>±2×10(^{-6})</td>
</tr>
<tr>
<td>(z_0) (mm)</td>
<td>5.889</td>
<td>±1×10(^{-3})</td>
</tr>
<tr>
<td>(C_4)</td>
<td>-0.043</td>
<td>±3×10(^{-3})</td>
</tr>
</tbody>
</table>

Table 3.1: Parameters obtained from a least squares fit of the \(V(x, y, z)\) data extracted from SIMION to Eq. (3.2).

Provided the amplitude \((z)\) of the particle’s motion is kept small \((z \ll z_0)\), the
octupole component in Eq. (3.1) is a very small fraction of the quadrupole component. This is illustrated in Figure 3.3 where the octupole term is expressed as a percentage of the quadrupole term as a function of distance in the $x$ direction. For example, if the oscillation amplitude of the particle is $200 \, \mu m$ in the $x$ direction then the octupole term is less than $0.01\%$ of the quadrupole term. Typically, in this work the amplitudes of trapped particles were maintained at $<200 \, \mu m$ for secular frequency measurements and therefore effect of the octupole term is ignored.

![Figure 3.3: The octupolar term as a percentage of the quadrupolar term [%\(\text{Oct/Quad}\)] calculated as a function of $x$ from Eq. (3.1) and the parameters in Table 3.1.](image)

The geometrical resolution of the electrode structure used in SIMION simulation has an effect on accuracy of the modeling. It is especially important to be mindful of this when dealing with curved electrodes. Forbes et al. [91] concluded that a resolution of $0.0444 \, mm/gu$ is sufficient for adequate modeling of QIT performance, while Appelhans and Dahl reported $0.0625 \, mm/gu$ as sufficient for accurate QIT modeling [92]. In both reported cases the ion traps had similar geometries ($z_0=7.07 \, mm$) to the one used in this work. The modeling performed in this work ($0.05 \, mm/gu$) was at the highest resolution achievable with the available version of SIMION, the limiting factors being the maximum array size permitted by the
3.2 Vacuum Chamber and Vacuum Systems

The QIT and its casing was mounted on three ruby spheres which provided a stable, electrically isolated platform. This mount was supported by an 8-inch UHV Conflat flange as shown in the photo in Figure 3.5. Vacuum compliant electrical feedthroughs were installed on this flange for supplying the electrodes with the required potentials. The flange with the mounted trap was designed so that the electrical feedthroughs and trap electrodes could be connected before insertion into the vacuum chamber. The trap was inserted within a custom built 6-way Conflat compatible UHV chamber with additional 2$\frac{3}{4}$-inch flange access ports depicted in Figure 3.4. A sample probe vacuum interlock was constructed on top of the 6-way chamber to allow sample probe tips to be introduced and removed without compromising the vacuum in the main chamber. The 6-way QIT UHV chamber was connected to a second 6-way UHV chamber, which supported a 170 L/s Turbo pump backed by a rotary pump. Pressures as low as 2 × 10$^{-7}$ Torr were obtainable. Inlet valves attached to this second chamber allowed for the controlled introduction of various gases (e.g. He and N$_2$). The pressure was monitored using thermocouple and ion gauges.

3.3 Driving Electronics

The driving power supply for the endcap electrodes, as depicted in Figure 3.6, consisted of a signal generator (Hameg HM8131-2) in conjunction with a wide-band amplifier (Krohn-Hite 7602M). The amplified output was stepped up using a ferrite core transformer yielding $V_{0-p}$ up to 2000 V. This AC voltage was monitored using a 100x high voltage probe (Tektronix P5100) connected to a digital...
Figure 3.4: Top and side views of the QIT UHV chamber.
Figure 3.5: Side-on photo of the QIT (within its casing), mounted on a stage and 8-inch Conflat vacuum flange.

Figure 3.6: Circuit diagram of the AC drive circuit.
multimeter (Keithley 2000) and a digital oscilloscope (Tektronix TDS 1012). To compensate for vertical displacement of charged particles due to gravity, a DC potential could be applied to the vertically opposing rod pairs using a dual tracking DC power supply (Topward 6603D). Typically the horizontally opposed rods pairs were electrically grounded as shown in Figure 3.6.

3.4 Laser Systems and Optical Setup

The laser excitation and optical detection arrangement for monitoring elastically scattered light is depicted in Figure 3.7. The gently focussed output of a continuous wave ($\lambda=532$ nm, 0.1–1 mW) Nd:YVO$_4$ laser (Coherent VERDI-V5) was aligned through the QIT centre via the holes in the endcap electrodes. Scattered/fluorescent light was collected by a 25 mm F/1 lens (L1) situated at right angles to the incident laser beam. The collected light passed through a window (W) in the vacuum chamber and struck a removable broadband dielectric mirror (rM) directing it through a 100 mm focusing lens (L2) onto a photomultiplier tube (PMT) (Hamamatsu H5784-02) via an adjustable spatial filter (A).

For fluorescence emission measurements, the mirror rM was removed allowing the light to be steered by two broadband dielectric mirrors (M) through a 150 mm focal length lens (L3) into a spectrometer (JY Triax 552). The focal length of L3 was chosen to match the numerical aperture (f/6.4) of the spectrometer. The spectrometer was of Czerny-Turner configuration with a 550 mm focal length and was equipped with a rotating turret armed with three different diffraction gratings (600, 1200 and 2400 g/mm). A liquid nitrogen cooled, front illuminated, open electrode, 1024x256 CCD detector (JY Symphony) was attached to the spectrometer and interfaced with a personal computer. The 600 g/mm grating was the most commonly used, giving a resolution of $\sim 0.05$ nm. The spectrometer was calibrated regularly using a HgAr lamp.

To excite particles labeled with blue absorbing fluorophores the beam from a tun-
Figure 3.7: A schematic overhead view of a typical optical detection configuration. The scattered light from the particle can be directed to a photomultiplier tube (PMT) or a spectrometer. See text for details.

Figure 3.8: The intensity of the scattered light detected at by PMT is modulated by the oscillating particle as it transverses in and out of the laser beam. Fourier analysis of the time domain signal permits extraction of the particle’s secular frequencies.
3.4. LASER SYSTEMS AND OPTICAL SETUP

A tunable (750-850 nm), passively mode locked, picosecond Ti:Sapphire laser (Tigerps, Time-Bandwidth) was externally frequency doubled using a $\beta$-barium borate (BBO) crystal (Kaston). The lasing wavelength was tuned by rotating an intracavity birefringent filter stack using a piezomotor. The piezomotor was controlled by RS-232 commands initiated using a custom written LABVIEW program. A small fraction of the laser output was collected and monitored using a compact fiber-optically coupled CCD spectrometer (HR 4000, Ocean Optics). The frequency doubled laser beam was gently focused and directed into the QIT through holes in the endcaps electrodes. In this configuration the 532 nm probe laser beam was aligned on the trapped particle orthogonal to both the Ti:Sapphire laser beam and the optical detection axis.

A CCD camera was used to inspect trapped particles using the arrangement shown in Figure 3.9. In the configuration where the probe laser was incident on a trapped particle at right angles to the QIT symmetrical axis, a lens of 150 mm focal length was used to couple light out through the hole in one of the endcap electrodes. A second lens ($f=1000$ mm), formed the image on a CCD camera which was connected to a viewing monitor.

![Figure 3.9: Optical setup for the CCD monitor camera.](image-url)
3.5 Signal Processing

As depicted in Figure 3.8 a single particle stored within the QIT scatters light as it traverses in and out of a laser beam directed through the trap centre. This elastically scattered light was detected by a PMT. The PMT signal was filtered using a low-pass Butterworth frequency filter (Krohn-Hite 3381) to remove any high frequency components and sent to a personal computer via a data acquisition PCI card (NI PCI-6024E). Custom LABVIEW programs were written to control the data acquisition and Fast Fourier Transform (FFT) the time domain signal, and to extract the particle’s secular frequency components [44].

Control over the number of data samples acquired (N) and the acquisition rate (samples per second, $N_S$) is vital for producing frequency spectra with the desired resolution. The resolution of the frequency spectrum ($\Delta \omega/2\pi$) can be calculated by:

$$\Delta \omega/2\pi = \frac{N_S}{N}$$ (3.2)

To ensure faithful sampling of the time domain signal it is a requirement that the acquisition rate is greater than twice the maximum frequency present in the signal (known as the Nyquist limit). In this work, for example, when measuring the secular frequency spectrum of a 2 $\mu$m polystyrene sphere oscillating with a radial secular frequency ($\omega_r/2\pi$) of $\sim$15 Hz, $N=40,000$ sample points were collected at a rate of $N_S= 200$ Hz with the low-pass Butterworth frequency filter cutoff set at 80 Hz. These parameters give a frequency resolution of $\Delta \omega/2\pi = 0.005$ Hz.

3.6 Charged Particle Sources

3.6.1 MALDI

Two particle sources, both involving laser desorption, were used in this work. The first method, a matrix-assisted laser desorption/ionisation (MALDI) process,
involved mixing an aqueous suspension of the particle’s of interest with an organic solution or matrix. Usually a 3-hydroxypicolinic acid (3-HPA) matrix was used as it has high absorption at 355 nm and has been used previously for desorbing polystyrene particles into a QIT [48]. To prepare a MALDI sample, 3–4 drops of an aqueous particle suspension were mixed with 3–4 drops of a saturated solution of 3-HPA in an acetonitrile/water mixture on a stainless steel sample tip. The sample was then dried under nitrogen and placed above the QIT situated in a vacuum chamber (see Figure 3.10). A single 7 ns focussed laser pulse, third harmonic ($\lambda=355$ nm) from a Q-switched Nd:YAG laser (Continuum Surelite), irradiated the sample, ejecting charged particles into the QIT. Buffer gas (He at 50-100 mTorr) was used to slow and damp the motion of desorbed particles, increasing the chances of successful confinement.

![Figure 3.10: Experimental configuration using the MALDI particle source and Nd:YVO$_4$ laser for fluorescence excitation.](image)

3.6.2 LIAD

The other particle introduction method used in this work was the recently reported laser induced acoustic desorption (LIAD) technique [3]. Preparing a LIAD sample involved placing 2–3 drops of a purified aqueous particle suspension on a 0.5 mm
thick Si wafer and drying it under nitrogen. The wafer was inserted above the trap within the vacuum housing (See Figure 3.11). A single focused (\(\lambda=532\) nm) laser pulse (\(\sim 40\) mJ), from a Q-switch Nd:YAG laser (Continuum Surelite), irradiated the wafer from behind initiating an acoustic shockwave causing charged particles to be desorbed from the Si wafer front surface. Helium buffer gas (50–100 mTorr) was used to slow the desorbed particles for more efficient trapping and to reduce the amplitude of trapped particles.

The LIAD method was found to have the following advantages compared to MALDI:

1. Simpler sample preparation with no organic matrix required.

2. No direct interaction between the sample and laser pulse, reducing photo-degradation of fragile samples.

3. Lower charge states. This is an advantage especially for large particles where, for higher charge states, it is increasingly difficult to resolve the single charge differentials required for absolute charge and mass determination.
4. Faster turn-around times. The LIAD method was less efficient than MALDI. This is an advantage as only one particle is required in the trap. Less time was spent ejecting unwanted particles from the trap.

5. For MALDI there is the possibility of producing pure matrix particles or particles contaminated with matrix material.\cite{93}

### 3.6.3 Single Particle Isolation

![Figure 3.12: Images of the static crystalline arrays of polystyrene microparticles observed from generated using the MALDI particle source.](image)

It was often the case that a swarm of $\lesssim 100$ particles were confined in the trap after a single laser pulse. Occasionally, interesting static patterns were observed as depicted in Figure\ref{fig:3.12}. These arrangements occur when the confining electrodynamic potential of the QIT is balanced by the electrostatic repulsion between neighbouring particles with like charge. These patterns were first reported by Wuerker\textit{ et al.} and described by them as ‘crystalline arrays’\cite{36}. Although visually interesting, only a single particle was usually desired within the QIT at the one time. By varying the trap voltage ($V$) and frequency ($\Omega$) systematically, particles were ejected until only a single particle was present.

Several methods were used to check that only one particle was confined in the QIT. Firstly, the presence of broadened or additional peaks in the FT spectrum usually indicated the presence of more than one particle. As well, inspection of the particle’s motion using the CCD camera was possible if the particles were sufficiently large. A lone trapped particle had a smooth continuous motion; any violent
jerking or rocking in the particle’s motion usually implied an intruding additional particle not illuminated by the probe laser. Once the unwanted particle(s) were ejected (by systematically varying $V$ and/or $\Omega$), the remaining particle adopted a smooth, periodic trajectory.

### 3.7 Charge Change and Absolute Charge Determination

The absolute charge $Q$ of a trapped particle with known $Q/m$ can be deduced by initiating charge changes of $\Delta Q$ and using the equation:

$$Q = \frac{\Delta Q \omega_i}{(\omega_f - \omega_i)}$$

where $\omega_i$ and $\omega_f$ are the initial and final particle oscillation frequencies after a charge change of $\Delta Q$. It is convenient to express $\Delta Q = Z e$ where $Z$ is the number of elementary charges and $e$ is the electron charge. By measuring the particle’s oscillation frequency during a series of charge changing events, a fundamental frequency step can be determined that corresponds to a $\pm e$ charge change. In this way, the absolute charge of the particle ($Q$) can be determined. This charge stepping method is similar to that reported by Philip et al. [39] and implemented recently by Schlemmer et al. [44].

Two techniques were employed to initiate charge change in single trapped particles, one based on electron impact and the other on photoionisation. These methods are described in the following sections.

#### 3.7.1 Electron Gun

An electron gun (RM Jordan C-950) was slightly modified and mounted on the QIT housing aimed at the trap centre. Electrons were emitted from a tungsten filament
maintained at a negative voltage relative to a grounded slit lens. Adjusting the current through the filament varied the emitted electron current while the voltage on the first slit lens controlled the energy of the emerging electrons. Electrons emerging from the first slit lens were focussed using a second split lens.

The electron gun was successfully used to change the charge of trapped polystyrene particles. These changes usually occurred via secondary electron emission, with the number of emitted secondary electrons difficult to control. The large variations in the number of emitted secondary electrons were likely the result of the distribution of energies of the electrons affecting the trapped particle. The electrons’ energy varies periodically with the potential at the trap centre. This problem could be addressed in the future with the appropriating time referenced gating of the electron gun emission with respect to the phase of the oscillating QIT potential.

3.7.2 Xe FlashLamp

To avoid the problems associated with the electron gun, a photon based ionisation source was employed. A UV-grade optical fiber was mounted on the trap housing and coupled, via a vacuum feedthrough, to an externally triggered Xe flashlamp (Ocean Optics PX-2, λ=220-750 nm). Photoionisation proved to be a more controllable method for inducing charge change on a trapped polystyrene microparticles. Two processes were probably responsible for charge changing events. First of all, the particles could lose electrons through direct photo-ejection caused by photons with λ<294 nm, whose energy exceeded the polystyrene work function (4.22 eV [94]). Alternatively, electrons photo-ejected from the metal electrodes may have been accelerated into the trap centre where they were either absorbed by the particle or induced secondary electron ejection. Often the flashlamp was fired >100 times before a change of one or several elementary charge units was observed.
3.7.3 Identifying Charge Changes

As mentioned previously, a change in charge state of a trapped particle invokes a shift in its secular frequencies. For highly charged particles however, it becomes a challenge to discern whether a small charge change has occurred. For example with \( Q = 500e \), a frequency resolution \( \geq 5 \) times better than \( \frac{1}{500} \omega_r \) was required to confidently resolve a charge step of \( \pm e \). For \( \omega_r/2\pi = 15 \) Hz this corresponds to a frequency resolution of 0.006 Hz; the data requiring over 160 seconds to acquire. After flashing the Xe lamp a 160 second acquisition period is necessary to decide if a charge change has occurred.

A simpler and faster technique to discern whether a charge step had occurred was to examine star trajectories present at certain trap drive frequencies. First reported by Hars and Tass [43], a single trapped particle adopts a stable star shaped trajectory when the drive frequency (\( \Omega \)) is equal to an integer multiple (\( n\omega \)) of the particle’s radial frequency. The number of branches in the star pattern is equal to \( n \). Shown in Figure 3.13 are two examples of star pattern trajectories.

![Figure 3.13: Star patterns observed view down the z-axis of the QIT. Stable star patterns occur when \( \Omega = n\omega_r \). Here images corresponding to \( n = 6 \) (left) and \( n = 7 \) (right) are shown.](image)

To detect small charge changes on highly charged particles (> 400e) a stable star pattern trajectory was established (with \( n=6 \) or 7) by carefully adjusting the drive frequency and monitoring the particle with a CCD camera imaging down the z-axis of the trap as depicted in Figure 3.9. When a stationary star trajectory was established, the Xe flashlamp was pulsed whilst watching the star trajectory on the
monitor. When a charge change occurred the star pattern began to rotate as the secular frequency of the particle was no longer precisely an integral fraction of the drive frequency. Subsequently, a secular frequency measurement was performed to determine the particle’s oscillation frequency. This technique was successful in initiating and identifying single electron differentials in particles with charges of up to 800\(e\).
Chapter 4

Single Particle Fluorescence

4.1 Introduction

This chapter is concerned with the emission spectra obtained from single fluorescent polystyrene microspheres, spheroids and bispheres confined within a quadrupole ion trap (QIT). As will be shown, the emission spectra from single microspheres are dominated by additional features that result from optical microcavity effects known as morphology dependent resonances (MDRs). The MDR dominated emission spectra of single fluorescent microspheres ($\phi=2–7 \mu m$) were recorded and analysed. Implementation of the MDR theory described in Section 2.2 allows determination of the particle’s size, shape and refractive index. As particle confinement within the QIT is essentially indefinite, these particle parameters can be studied for time periods extending to days. This is demonstrated through the tracking of a single $\sim7 \mu m$ polystyrene sphere over a 3 day period with nanometer scale deviations in the particle’s size detected and quantified.

Also reported in this chapter are studies involving the geometrical characterisation of non-spherical particles using MDR dominated emission spectroscopy. Single spheroidal particles were trapped and found to exhibit more complex MDR spectra than spherical particles. The additional features present in the spheroids’ emission spectra were analysed using a perturbation theory, ultimately revealing the particle
geometries. Furthermore, by collecting consecutive emission spectra over time it was observed that spheroidal particles progressively adopted a spherical geometry, in a structural transition consistent with laser-induced melting.

To confirm and study the occurrence of melting, single fluorescent polystyrene bispheres were confined in the QIT and subjected to prolonged periods of laser irradiation. By tracking the MDR dominated emission the clear geometrical transition from bisphere to single sphere was observed consonant with melting and a surface tension driven shape transformation. Supporting calculations are presented which predict that particle temperatures exceeding the glass-transition temperature ($T_g$) of polystyrene were accessible at the laser powers used in the work.

### 4.1.1 Previous studies

Optical resonances of single microspheres were first reported by Ashkin and Dziedzic in 1977 who observed that, at constant power, light of particular wavelengths imparted significantly higher radiation pressure on single optically levitated oil droplets [95]. This was attributed to resonant recirculation within the droplets in accordance with light scattering theory for microspheres (Mie Theory). It was realised that these optical resonances could be used to accurately determine many properties of a droplet including its size, refractive index and shape [97].

Soon after, Chang and coworkers reported resonance structure in the fluorescence emission spectra of single dye labeled polystyrene microspheres suspended in water [98]. This was the first demonstration that resonances could be excited from a source within a particle. It was understood that the boundaries of the microparticle where acting as walls of a three dimensional optical cavity. As a result, at particular resonating wavelengths sufficient optical feedback was present to enhance fluorescence and also non-linear processes such as lasing [99], stimulated Raman scattering [100] and multiple order Raman scattering [101]. These opti-

*Interestingly, optical resonance effects observed from doped millimeter sized spheres were reported in 1961. The authors report onset of “maser action” in the fluorescence emission [96].
4.1. INTRODUCTION

Cal resonances came to be known as morphology dependent resonances (MDRs) \cite{99}. The MDRs of a single microdroplet were used to investigate the physical and chemical properties of the droplet including evaporation/condensation rates \cite{102}, shape distortions \cite{103} and temperature \cite{104}.

Campillo and coworkers have reported many investigations into the optical properties of microdroplets emerging from a vibrating orifice aerosol generator (VOAG) \cite{105}. MDRs were recorded from the elastically scattered monochromatic light signal as a function of the droplet radius \cite{106}. They also probed fluorescently labeled microdroplets and reported that fluorophores embedded within a microsphere are prone to cavity quantum electrodynamic (QED) enhancement effects \cite{107, 108} that lead to low threshold lasing \cite{109, 110}. Their work into cavity mode assignments \cite{111}, in collaboration with Young and coworkers, culminated in a complete algorithm for analysis of the MDRs of a microsphere which yields the sphere’s radius and dispersion corrected refractive index \cite{83}.

The work presented herein is concerned with fluorescently labeled polystyrene microspheres. In this case, fluorescence enhancement at certain wavelengths is a result of coupling between the emitting fluorophores and the spherical cavity modes (MDRs) of the microsphere. Fermi’s golden rule implies that the probability of a transition is proportional to the density of final states. For an emitter within an optical cavity, the density of states is larger when the emission wavelength corresponds to a cavity mode, and consequently its emission rate is increased \cite{112, 113}. In spherical microcavities each MDR is $2l+1$ degenerate so for large $l$, substantial increases in emission transition rates are possible. It has been reported for Rhodamine 6G that at certain wavelengths the spontaneous emission decay rate is enhanced by 10 times within a 4 $\mu$m glycerol droplet compared to the bulk \cite{114}. In contrast, transitions that do not correspond to MDR wavelengths should be suppressed.

Polystyrene microspheres were chosen in these QIT studies as they are commercially available in a large range of sizes, are highly monodisperse, and have excellent
sphericity. Moreover, they are available already doped with a wide variety of fluoro-
rophores with the dopant molecules diffused throughout the volume of the sphere,
resulting in highly fluorescent particles. Polystyrene is also expected to be stable
under vacuum conditions, an assertion that was confirmed in this work. Addition-
ally, the emission spectra from single fluorescently doped polystyrene microspheres
should be dominated by MDRs [98]. The observation of MDR dominated emis-
sion from a trapped particle is confirmation that single particle confinement has
been achieved and the trapped particles were not agglomerates. Once confined in
the QIT, single particles can be spectroscopically interrogated without the prob-
lem of ensemble averaging. Furthermore, the particle properties are free from the
perturbative effects of solvents and substrates.

4.2 Spheres

This section details the characterisation of single fluorescently labeled polystyrene
microspheres (φ=2–7 µm) contained in a QIT. The emission from a single micro-
sphere was found to be dominated by peaks corresponding to MDRs not generally
present in the emission spectrum obtained from a bulk sample. The observation
of MDR dominated emission spectra was validation that single polystyrene mi-
croparticles can be trapped within a QIT. Once trapped, an analysis of the MDRs
in the spectra can be used to determine its radius (a) and refractive index (mλ). In
some instances these properties were monitored over several days to investigate the
temporal stability of polystyrene microspheres under vacuum conditions.

The fluorescence emission spectrum of a single fluorescent polystyrene microsphere
is shown in Figure 4.1 (solid line). The particle originated from an aqueous col-
loidal suspension of blue fluorophore impregnated polystyrene microspheres (Duke
Scientific Co., φ~2 µm) and was introduced into the QIT using LIAD (Section
3.6.2). Fluorophore excitation was achieved using the frequency doubled output
of a picosecond Ti:Sapphire laser (λ=402 nm). The emission profile is domi-
nated by sharp peaks that correspond to MDRs of the particle. For fluorophore
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Figure 4.1: MDR dominated fluorescence emission spectrum from a single dye labeled polystyrene microsphere (solid line), compared to the emission spectrum of the aqueous dispersion ensemble sample (dashed line). Also shown is the single sphere Mie scattering spectrum (dotted line) for the fitted parameters ($a=1012$ nm and $n_\lambda=1.548+10561 \, \text{nm}^2/\lambda^2$).

<table>
<thead>
<tr>
<th>$\lambda_{exp}$ (nm)</th>
<th>$\nu$</th>
<th>TE/TM</th>
<th>$l$</th>
<th>$\lambda_{theo}$ (nm)</th>
<th>$\lambda_{theo}-\lambda_{exp}$</th>
</tr>
</thead>
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<tr>
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<td>464.52</td>
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<td>478.71</td>
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<td>15</td>
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<td>1</td>
<td>14</td>
<td>563.37</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

Table 4.1: Fitted MDR peaks from Figure 4.1. Listed are the experimental MDR wavelengths ($\lambda_{exp}$), the assigned mode order $\nu$, polarisation (TE=1, TM=2) and mode number $l$. The theoretical MDR wavelengths $\lambda_{theo}$ and also the difference between the experimental and theoretical MDR wavelengths are also included.
CHAPTER 4. SINGLE PARTICLE FLUORESCENCE

Impregnated polystyrene microspheres there are emitters located throughout the sphere including deep near the sphere’s centre where cavity suppression is minimal \[112, 115\]. It is these fluorophores that contribute to the smooth background emission profile similar to that of the ensemble. The peaks in the fluorescence spectrum in Figure 4.1 are sharp and predominantly symmetric indicating that particle is highly spherical. For spheres of this size parameter \(x \sim 12.5\) all the MDRs observed are of mode order \(\nu = 1\). Using the algorithm described in Section 2.2.3 to fit the positions of the MDRs in Figure 4.1 the sphere was determined to have \(a = 1012 \pm 2\) nm and \(m_\lambda = 1.548 + 10561 \text{ nm}^2/\lambda^2\). The MDR assignments and computed locations are given in Table 4.1. The calculated scattering efficiency \(Q_{\text{sca}}\) spectrum for a sphere with \(a = 1012\) nm and \(m_\lambda = 1.548 + 10561 \text{ nm}^2/\lambda^2\) is also shown in Figure 4.1 determined using Eq. (2.30). Each MDR is labeled with its polarisation (TE or TM) and its mode number \(l\) (subscript). The measured fluorescence spectrum of an aqueous suspension of the polystyrene microspheres sample is also shown in Figure 4.1. The emission spectrum is smooth, broad, with no apparent resonant peaks. The inhomogeneous particle size distribution in the bulk colloidal sample leads to obscuration of the sharp MDR features associated with single particles.

The MDR fitting algorithm was also tested for larger spheres. In this case, fluorescent orange polystyrene microspheres (Bangs Laboratories \(\phi \sim 7\ \mu\text{m}\)) were trapped. The fluorophore excitation source was a CW Nd:YVO\(_4\) \((\lambda = 532.07\) nm) laser and the particles were introduced into the QIT using MALDI. A representative single particle emission spectrum is shown in Figure 4.2. For particles with this size parameter \(x \sim 25\), the spectral region contains MDRs with mode orders of \(\nu = 1, 2\) and 3. In this spectrum 65 peaks are unambiguously assigned with a mode order \(\nu\), mode number \(l\) and polarisation (TE/TM). The peaks are fitted to a sphere with \(a = 3589 \pm 5\) nm and \(m_\lambda = 1.545 + 9249 \text{ nm}^2/\lambda^2\). The MDR assignments, calculated wavelengths and the differences between experimental and theoretical peak wavelengths are given in Appendix A. The differences between the experimental and theoretical MDR positions \(\Delta\) are plotted as a function of wavelength in
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Figure 4.2: Single particle emission spectrum of a fluorescent polystyrene microsphere. The MDR peaks were fitted to a sphere with $a = 3589 \pm 5$ nm and $m_\lambda = 1.545 + 9249 \text{nm}^2/\lambda^2$. The fitted MDR wavelengths, assignments and comparison between experimental and theoretical locations are given in Appendix A.

Figure 4.3: The difference between the experimental and fitted MDR wavelengths ($\Delta$) for the MDRs from Figure 4.2 (MDR wavelengths are listed in Appendix A) plotted as a function of wavelength. No significant systematic error in the data is apparent. The different mode orders are plotted in the colours as indicated by the legend.
Figure 4.3, revealing that there is no obvious systematic trend in the errors.

To investigate temporal stability, single polystyrene microspheres were trapped and monitored over periods extending to days. Figure 4.4 depicts the fluorescence emission spectra obtained from the same trapped microsphere at one-day intervals, over three days. The MDR wavelengths for each of the three spectra in Figure 4.4 were fitted to obtain the data displayed in Table 4.2. Figure 4.5 is a portion of spectrum A from Figure 4.4 showing the MDR assignments for each peak in this region.

Over the three-day period there was a systematic blue-shift in the MDR wavelengths (Figure 4.4). The fitted data in Table 4.2 reveals that the microsphere’s radius increases over this time interval while there is decrease in the refractive index. It is plausible that the changes in size and refractive index are due to the slow evaporation of aqueous solvent molecules within the microsphere resulting in a small but measurable expansion of the sphere and a concurrent decrease in refractive index. Another possible explanation is that the measured size increase was due to deposition of a lower refractive index material over the 3 days e.g. water or pump oil.

Also noticeable in Figure 4.4 is the change in relative peak intensities of MDRs associated with different mode order. The inset in Figure 4.4 shows a group of three MDRs, each with different mode order, where the change in relative peaks heights is apparent. The variation in the relative peak intensities is explicable by considering the excitation or input resonance in each instance. Using the fitted parameters in Table 4.2, the nearest MDR to the excitation wavelength (λ=532.07

<table>
<thead>
<tr>
<th>Day (Spectrum)</th>
<th>(a) (nm)</th>
<th>(m_\lambda) at 532 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A)</td>
<td>3397 ±6</td>
<td>1.585 ±0.015</td>
</tr>
<tr>
<td>2 (B)</td>
<td>3405 ±5</td>
<td>1.578 ±0.014</td>
</tr>
<tr>
<td>3 (C)</td>
<td>3430 ±7</td>
<td>1.566 ±0.019</td>
</tr>
</tbody>
</table>

Table 4.2: Fitted radius \((a)\) and refractive index \((m_\lambda)\) data using the MDR positions for the three spectra in Figure 4.4. Note that the refractive indices are corrected for dispersion.
Figure 4.4: Fluorescence emission spectra of a polystyrene microparticle taken over 3 days. Inset: a group of three MDRs with different mode orders showing the systematic shift of the MDRs and also the variation in relative intensities.
nm) is calculated for each day A–C. In the case corresponding to spectrum A, the nearest input MDR is TE$_{57}^{1}$ at 532.3 nm. For B the input resonance is also closest to TE$_{57}^{1}$ at 531.5 nm. However, for C the input wavelength is close to both TE$_{57}^{1}$ at 531.2 nm and TE$_{47}^{3}$ at 532.8 nm. Accordingly, for spectra A and B the first order resonances are the most intense, but in C the third order resonances are of comparable intensities to the first order resonances. The input coupling efficiency is maximised when the input wavelength overlaps well with an MDR of the cavity. In the case of spectrum A the overlap is good, resulting in strong output MDR intensities. In the case of spectrum C, the input wavelength is further from a cavity resonance and therefore the output MDRs are weaker compared to the broad baseline emission profile of the fluorophore. This correlation between the laser wavelength and the strength of the observed MDRs is consistent with similar observations for dye labeled microdroplets [116].
4.3 Spheroids and Shape Distortion

Over the course of this work it was found that several particles exhibited more complex MDR spectra indicating non-spherical geometries. Analysis revealed that axial-symmetric particles, specifically prolate spheroids, were responsible for these spectra. In this section, resolved non-degenerate azimuthal MDRs are reported in the emission spectra of a single polystyrene microparticle. The azimuthal modes are assigned \((m=0,...,l)\) and the spectra found to be consistent with the particle having a prolate spheroid geometry. In the spheroidal case, azimuthal MDRs are non-degenerate causing them to split and shift in wavelength to an extent that depends on the degree of distortion. Measurement of the shifting and splitting of spectrally resolved azimuthal modes provided an opportunity to test current theoretical descriptions of distorted microsphere MDRs. It is described below how an existing perturbation theory [84] can be extended to model the wavelengths of non-degenerate azimuthal modes from a particle, which enables determination of the particle’s dimensions. Intriguingly, the particle’s shape changed slowly over the course of the measurement and eventually becomes spherical, indicative of laser-induced melting. By monitoring the MDRs these morphological changes can be followed and quantified.

A limited number of single microparticle shape deformation studies have been reported. One interesting study involved the observation of Rayleigh jets from evaporating levitated ethylene glycol droplets (described in Section 1.3). Dynamic quadrupolar shape distortions were detected by measuring elastic light scatter over time and using a high-speed camera [69,71]. Other work by Arnold and coworkers investigated the induced shape distortion in levitated droplets by superimposing an additional time-varying electric field to a QIT arrangement [117]. The additional time-varying field was driven at a much higher frequency (3-16 kHz) compared to the trapping field (60 Hz). The high frequency field was found to have a significant effect on a droplet scattering spectrum whereby the MDRs diminished in intensity and split. These observations were attributed to dynamically driven quadrupolar
shape distortions caused by electromechanical stress at the droplet surface caused by the additional electric field.

Non-degenerate azimuthal MDRs have been investigated in flowing microdroplet experiments. High resolution stimulated Raman scattering (SRS) spectra of flowing ethanol microdroplets revealed additional peaks that were found to be consistent with non-degenerate azimuthal MDRs \[86\]. Wavelength variations in the lasing emission along the rim of single flowing microdroplets, measured as a function of distance from the droplet equator, were also explained in terms of broken azimuthal degeneracy \[118\]. In flowing microdroplet systems, inertial effects cause the droplets to distort to an oblate spheroid geometry and as a consequence, the azimuthal MDRs have different circumferential pathlengths and resonate at different wavelengths. Azimuthally non-degenerate MDRs were also found to be temporally distinguishable, as shown by the temporal precession of the SRS signal from ethanol microdroplets \[87, 88\].

In the aforementioned microdroplet experiments, the degree of distortion was described by the parameter \(e\), defined as \(e = (r_p - r_e)/a\), where \(r_p\) and \(r_e\) are the polar and equatorial radii, respectively, and \(a\) is the radius of a sphere with equal volume. The ability to determine the spheroid’s geometry was restricted in these instances as the precise volume of the droplet, \(i.e.\) the value of \(a\), was not known. In the fluorescence spectra reported in this section, non-degenerate azimuthal MDRs are spectrally resolved and assigned for polystyrene microspheroids. By tracking the MDRs over time what appeared to be laser-driven melting caused a shape change with the spheroid ultimately adopting a spherical geometry. In its spherical form, the size \((a)\) and volume of the particle can be accurately determined and as a result, the shape and geometric parameters \((r_p\) and \(r_e)\) of the particle in its spheroidal forms are extracted with nanometer accuracy.

Single, fluorophore labeled polystyrene microspheres (Sigma-Aldrich, nominal diameter 2.16 µm) were introduced into the QIT using the MALDI technique. Approximately 100 W/cm\(^2\) from a continuous wave Nd:YVO\(_4\) laser (\(\lambda=532\) nm) irradiated
Figure 4.6: Fluorescence spectra from a single polystyrene particle taken at ~5 minute intervals (A-D). Spectral changes are due to the particle’s relaxation from spheroidal to spherical shape. At bottom (E) is the predicted scattering spectrum for a sphere with $a=1363$ nm and $m_\lambda=1.549+10230\ \text{nm}^2/\lambda^2$. 
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The trapped particle. Figure 4.6 shows consecutive emission spectra for a particle under continuous laser irradiation recorded at \( \sim 5 \) minute intervals. The emission profile changes significantly over 20 minutes. Initially the spectrum displays several broad peaks tailing off to the blue (spectrum A). After some time, small resolvable peaks are apparent to the blue of TE_{20} and TE_{19} peaks (spectra B and C). These features are attributable to non-degenerate azimuthal modes associated with a non-spherical particle. After 20 minutes, the emission spectrum became that of a spherical particle and the MDRs are modeled and labeled accordingly. The calculated Mie scattering spectrum (Figure 4.6E) was generated assuming a particle \( a=1363 \text{ nm} \) and \( m_\lambda=1.549+10230 \text{ nm}^2/\lambda^2 \).

The non-degenerate azimuthal MDRs are clearly resolved in traces B and C (Figure 4.7) and have wavelengths consistent with the particle being a prolate spheroid. For a given \( l \) the different possible \( m \)-modes have different effective pathlengths and thus have different resonant wavelengths as outlined in Section 2.2.4. The \( m=l \) mode, approximately confined around the spheroid’s circular equator, has the shortest wavelength, while the \( m=0 \) mode confined to an ellipse passing through the poles has the longest wavelength (Figure 4.8). The separation between the \( m \)-modes decreases as \( m \rightarrow 0 \). The dimensions of the spheroids can be obtained by analysing the shifting of the \( m \)-modes using the perturbation theory of Lai et al. [84].

It was outlined in Section 2.2.4 how the splitting and shifting of non-degenerate azimuthal MDRs can be quantitatively related to the distortion of the particle using a perturbation formalism. It is evident from Figure 4.9 that the relative \( m \)-mode displacements \( (\delta \omega/\omega) \) in spectra B and C display an almost quadratic dependence on \( m \) as predicted by Eq. (2.56) assuming the particle has a quadrupolar distortion. However a small additional quartic term is necessary to fit the data. By extending Eq. (2.53) to include the \( Y_{4,0}(\theta, \phi) \) axial-symmetric octupolar distortion the radius is described by:

\[
r(\theta, \phi) = a_0 + \Delta_2\sqrt{4\pi}Y_{2,0}(\theta, \phi) + \Delta_4\sqrt{4\pi}Y_{4,0}(\theta, \phi) \tag{4.1}
\]
Figure 4.7: Expanded sections of spectra B and C from Figure 4.6. The non-degenerate azimuthal MDRs are labeled. The $m=l$ mode has shortest wavelength and the $m=0$ mode longest, consistent with the particle being a prolate spheroid.

Figure 4.8: Depiction of the different azimuthal MDRs for a prolate spheroid. The spectrum is the TE$_{19}$ MDR from Figure 4.6C. The aspect ratio of the spheroid is exaggerated for clarity.
Figure 4.9: Measured fractional frequency shifts for the TE$_{20}$ and TE$_{19}$ $m$-modes in spectra B and C (points). The lines represent frequency shifts calculated using Eqn. 4.2 and the fitted $\Delta_2$ and $\Delta_4$ values given in Table 4.3.

Table 4.3: Fitted values of quadrupole ($\Delta_2$) and octupole ($\Delta_4$) distortion parameters (in nm) for TE$_{19}$ and TE$_{20}$ $m$-modes in spectra B and C. Also listed are the spheroid’s polar and equatorial radii ($r_p$ and $r_e$ in nm).
and the azimuthal mode shifting described by:

\[ \frac{\delta \omega}{\omega} = -\frac{\Delta_2}{a} A(L = 2, l) f(L = 2, l, m) - \frac{\Delta_4}{a} A(L = 4, l) f(L = 4, l, m) \]  \hspace{1cm} (4.2)

The \( A(L = 2, l) \) and \( f(L = 2, l, m) \) terms were calculated in Section 2.2.4 [Eq. (2.54) and Eq. (2.55)]. For \( L = 4 \)

\[ A(L = 4, l) = \frac{27(l^2 + l - 10)(l + 2)(l - 1)}{4(2l - 1)(2l + 5)(4l^2 - 9)} \]  \hspace{1cm} (4.3)

and

\[ f(L = 4, l, m) = 1 - \frac{5(6l^2 + 6l - 5)m^2}{3l(l^3 + 2l^2 - l - 2)} + \frac{35m^4}{3l(l^3 + 2l^2 - l - 2)} \]  \hspace{1cm} (4.4)

With this additional octupolar distortion term the frequency shifts for the non-degenerate modes have a quadratic and quartic dependence on \( m \). The \( m \)-mode frequencies in the TE\(_{20}\) and TE\(_{19}\) regions of spectra B and C were fitted to Eq. (4.2) yielding the \( \Delta_2 \) and \( \Delta_4 \) values (Table 4.3). The value of \( a_0 \) in Eq. (4.1) was constrained by assuming constant particle volume during the measurements.

With the introduction of the additional octupole term the fitted and experimental \( m \)-mode displacements are in very good agreement (Figure 4.9).

The evolving emission spectra in Figure 4.6 are consistent with a slow change in the particle’s shape from prolate spheroid to sphere. The particle’s morphological change, presumably driven by surface tension, suggests that the particle’s temperature exceeded the polystyrene glass transition temperature \( (T_g) \), which for bulk material is \( \sim 373K \) [119]. The fitted radius of the ultimate sphere in Figure 4.6 (1363 nm) is somewhat larger than the manufacturer’s quoted radius for the polystyrene spheres (1080 nm). Tellingly, a sphere with radius 1361 nm has the same volume as two spheres of radius 1080 nm. Therefore it seems likely that the emission spectra in Figure 2 (A-D) represent the signature of the final stages of two smaller spheres fusing into one.
4.4 Bispheres

In order to explore and confirm the occurrence of laser-induced melting in single trapped polystyrene microparticles, several bisphere systems were investigated using MDR dominated emission spectroscopy. Using the same techniques for fitting the MDR locations of single spheres as in Section 4.2, the radii of each of the monomer units within each bisphere were obtained. Under prolonged periods of laser irradiation the monomer units of the bisphere appeared to melt and slowly coalesce forming a single sphere. The MDRs of the particle are monitored throughout the transition.

The coagulation of optically manipulated liquid droplets has been studied by Reid and coworkers using cavity enhanced Raman scattering (CERS) [120–122]. The CERS spectrum of each droplet was tracked as the two droplets were brought into contact and coalesced to form one larger droplet. As the fusion in these instances was rapid, no information was gained about the morphology of the intermediate droplet. In the current work, the slow, laser induced coagulation of fluorescently labeled polystyrene bispheres confined in a quadrupole ion trap (QIT) is reported. By following the fluorescence MDRs the progressive transformation from a bisphere to a larger, single sphere is investigated.

Single spheres with size parameter $x > 1$ scatter light predominately in the forward and backward directions [13]. For single bispheres it follows that the orientation of the bisphere with respect to the excitation light has a pronounced effect on its optical properties due to the optical interaction between the spherical monomer units. When an incoming plane of light is directed along the common axis of the bisphere (endfire incidence), monomer-monomer optical cross-coupling is maximized. In this instance the scattering properties of the bisphere are complicated by additional MDRs arising from inter-sphere coupling. Inter-sphere coupling diminishes sharply when the angle of incidence of the incoming light deviates by only a few degrees from endfire incidence. Light incident at 90 degrees to the common axis of the bisphere (broadside incidence) results in minimal cross-coupling between the
monomer units and they are essentially optically non-interacting. This difference in endfire and broadside illumination has been investigated through scattering calculations by Fuller \[123\] and experimentally verified \[124, 125\]. Fuller concluded that if the orientation of a bisphere with respect to the incident light is random, then any inter-sphere optical interaction between the monomers is washed out and no significant broadening or shifting of the respective monomer MDRs will be observed \[123\]. It is expected that in the current work a bisphere contained in the QIT would tumble many times during the fluorescence integration interval (\(~10–40\) s) and that the fluorescence emission spectrum should be dominated by MDRs of the individual monomer units.

In the current work, polystyrene microspheres were introduced into the QIT using LIAD. Occasionally single bisphere particles were observed. The emission spectrum of a single bisphere is shown in Figure 4.10A. The spectrum of a bisphere in this size regime is distinguished by the appearance of broad peaks which appear on closer inspection to be resolved doublets. Analysis reveals that the spectrum is a composite of the spectra for two monomer sphere having slightly different radii. Shown in Figure 4.10B–E are subsequent fluorescence emission spectra of the same bisphere taken after consecutive 30 minute intervals of CW laser irradiation (\(\lambda=532\) nm at \(~160\) W/cm\(^2\)). The changing fluorescence profile is consistent with melting of the bisphere, destroying the spherical degeneracy of both monomers. As a consequence, the MDRs broaden (spectrum B) and become more complex as the two monomer units gradually fuse together (spectra C and D). There is some structure present in spectra C and D but the corresponding geometries are difficult to deduce. The final spectrum E shows clear sharp MDRs characteristic of a larger single sphere.

The MDRs in Figure 4.10A were found to be consistent with a bisphere comprised of monomer units with \(a=1010 \pm 4\) nm and 1013 \(\pm 4\) nm, with \(m_\lambda=1.546+11860\) nm\(^2/\lambda^2\) and 1.545+11910 nm\(^2/\lambda^2\), respectively. The experimental MDR dominated fluorescence spectrum and fitted Mie scattering spectra for each monomer are shown together in Figure 4.11A. Weak features not predicted by either of the
Figure 4.10: The fluorescence emission spectrum of a bisphere system (A). B–E are the emission spectra after consecutive 30 minute periods of cw laser irradiation at 160 W/cm². The spectral evolution from A to E is consistent with a shape transformation from bisphere to a larger single sphere.
Figure 4.11: Fluorescence emission spectra of a dye labeled polystyrene bisphere (A) with fitted Mie scattering spectra for each monomer with radii of 1010 nm (blue) and 1013 nm (red). Weak bisphere modes are also identifiable (*). After laser induced melting, a single sphere is formed (B) with a fitted radius $a = 1262$ nm.

Calculated spectra shown in Figure 4.11A (denoted by *) are likely bisphere cross coupling modes. For all the resolvable doublets in Figure 4.11A the peak with the shorter wavelength, which corresponds to the smaller of the bisphere monomers, is more intense. This intensity difference is explained by considering the strength of the input resonance. The smaller and larger monomer units have a $\text{TE}_{121}$ MDR at 401.9 nm and 402.7 nm, respectively. The smaller monomer is closer to resonance with the excitation light ($\lambda = 402.0$ nm) and therefore has more intense output MDRs.

After laser induced melting was completed the MDR positions in Figure 4.11E were fitted to a sphere of $a = 1262 \pm 6$ nm and $m_\lambda = 1.556 + 11060 \text{ nm}^2/\lambda^2$ as shown in Figure 4.11B. The difference in the corresponding final volume compared to the sum of the fitted volumes of the monomers in the bisphere is 3%, which falls within
the range expected from the uncertainties in the radii.

The final stages of coalescence are captured in better detail for a second bi-
sphere (Figure 4.12). The MDRs of the bisphere before laser melting (Figure
4.12A) were fitted to give monomer radii of 1006 ± 4 nm and 1009 ± 3 nm, with
$m_\lambda=1.551+12400 \text{ nm}^2/\lambda^2$ and $1.555+11760 \text{ nm}^2/\lambda^2$, respectively. Spectra B–E
show the emission spectra of the bisphere after it was irradiated with a CW laser
($\lambda=532 \text{ nm at } \sim 270 \text{ W/cm}^2$) for consecutive 1 minute periods. The bottom trace
F is the predicted Mie scattering spectrum for a single sphere with a fitted ra-
dius of 1268 ± 4 nm with $m_\lambda=1.550+10960 \text{ nm}^2/\lambda^2$. In this case the difference in
volume of the bisphere and the final sphere is good to 0.4%.

\begin{table}[h]
\centering
\begin{tabular}{lcc}
\hline
Spectrum & $e \times 10^{-2}$ & $r_e-r_p$ (nm) \\
\hline
B & 1.63 ±0.14 & 20.7 ±0.3 \\
C & 0.87 ±0.11 & 11.1 ±0.5 \\
D & 0.54 ±0.18 & 6.8 ±0.6 \\
\hline
\end{tabular}
\caption{Data obtained using (2.57) and the MDR peaks present in Figure
4.7B-D. The data are averaged over the four peaks. $e=(r_p-r_e)/a$ where $r_p$
and $r_e$ are the polar and equatorial radii, respectively.}
\end{table}

The MDRs in spectra B–D trail to the blue and exhibit a systematic shift in the
peak maximum compared to the MDRs of the final sphere (E). This is expected
for non-degenerate azimuthal MDRs of a prolate spheroidal geometry. After addi-
tional laser irradiation, surface tension drives the spheroid to a sphere (spectrum
E). Each peak maximum in spectra B–D corresponds to the $m=0$ MDR, which for
a prolate spheroid is the most red-shifted of the non-degenerate azimuthal modes
for a given $l$. The MDR peak locations in B–D relative to the spherical case E are
analysed using Eq. (2.57) to give the polar and equatorial radii. Calculated data
averaged over the four peaks, are given in Table (4.4). The differences in $r_p$
and $r_e$ are on the order of tens of nanometers for micron-sized particles, demonstrating
the sensitivity of MDRs to small shape distortions.
Figure 4.12: Fluorescence MDR emission of a bisphere (A) and subsequent spectra acquired after laser irradiation of $\sim 270 \text{ W/cm}^2$ for 1 minute intervals (B–E). The bottom spectrum F is the Mie scattering spectrum fitted to a single sphere with $a=1268 \text{ nm}$ and $m_\lambda=1.550+10960 \text{ nm}^2/\lambda^2$. In this case, the final stages of melting are evident (B–E) were the trend towards sharper MDRs is consistent with the improving sphericity of the structure. The shape distortions in B–D are quantified and the data listed in Table 4.4.
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4.5 Laser Induced Particle Melting

Laser induced melting was observed for polystyrene spheroids and bispheres therefore it is appropriate to discuss the energy balance to assess whether the polystyrene glass transition temperature ($T_g$) is accessible. The temperature of a trapped particle ($T$) can be estimated by balancing the rate of heating from laser absorption ($P_{abs}$) with the cooling due to buffer gas collisions ($P_{buffer}$) and blackbody emission ($P_{black}$) [45, 89]:

$$P_{abs} = P_{buffer} + P_{black} \quad (4.5)$$

These rates are defined as follows. The rate of heating of the particle due to photon absorption from the CW Nd:YVO$_4$ laser can be calculated from [13]:

$$P_{abs} = Q_{abs}(\lambda)I_{laser}a^2 \quad (4.6)$$

where $I_{laser}$ is the laser power density, and $Q_{abs}(532 \text{ nm})$ is the particle’s absorption efficiency at the wavelength of the excitation laser (532 nm). Polystyrene microspheres have an imaginary refractive index of about $5 \times 10^{-4}$ at 532 nm [126], which for $a \sim 1.01 \mu\text{m}$, corresponds to $Q_{abs}(532 \text{ nm}) \gtrsim 0.01$.

The rate of cooling due to monatomic buffer gas collisions with the particle surface, assuming a thermal accommodation coefficient of 1 $^\dagger$ can be estimated by integrating the energy transfer for each collision over the Maxwell-Boltzmann distribution for incoming and outgoing encounters [89, 129]. The energy flux from the buffer gas to the particle is:

$$P_{in} = 4\pi a^2 \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{0}^{\infty} v_x N_{dens} \frac{1}{2} m (v_x^2 + v_y^2 + v_z^2) \left(\frac{m_{gas}}{2\pi kT_{gas}}\right)^{3/2} e^{-m_{gas}(v_x^2+v_y^2+v_z^2)/2kT_{gas}} dv_x dv_y dv_z \quad (4.7)$$

$^\dagger$Calculated using the FORTRAN Mie scattering code of Wiscombe [127].

The thermal accommodation coefficient ($\alpha$) is the fraction of heat transferred between the colliding gas atom and the surface. Making $\alpha=1$ assumes that the gas atoms leave with the same temperature as the particle after the collision i.e. maximised buffer gas cooling [128].
and the energy flux from the particle surface is:

\[
P_{\text{out}} = 4\pi a^2 \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \int_{0}^{\infty} v_x N_{\text{dens}} \frac{1}{2} m(v_x^2 + v_y^2 + v_z^2) \\
\left( \frac{m_{\text{gas}}}{2\pi kT} \right)^{3/2} e^{-m_{\text{gas}}(v_x^2 + v_y^2 + v_z^2)/2kT} dv_x dv_y dv_z
\]

(4.8)

where \( T_{\text{gas}} \) is the temperature of the buffer gas (mass \( m_{\text{gas}} \)), \( k \) is the Boltzmann constant, and \( N_{\text{dens}} \) is the number density of gas particles. By integrating Eq. (4.7) and Eq. (4.8), the total energy flux is given by:

\[
P_{\text{buffer}} = P_{\text{in}} - P_{\text{out}} = 4\pi a^2 N_{\text{dens}} \sqrt{\frac{2kT_{\text{gas}}}{\pi m_{\text{gas}}}} k(T_{\text{gas}} - T)
\]

(4.9)

with \( N_{\text{dens}} = p/kT_{\text{gas}} \), where \( p \) is the gas pressure.

The rate of cooling due to blackbody emission for a sphere is [130]:

\[
P_{\text{black}} = 4\pi a^2 \int_{0}^{\infty} \epsilon(\lambda) P_{\text{Planck}}(T) d\lambda
\]

(4.10)

where \( \epsilon(\lambda) \) is the emissivity, which is equal to \( Q_{\text{abs}}(\lambda) \) [130], and \( P_{\text{Planck}}(T) \) is the Planck distribution. Integrating \( Q_{\text{abs}}(\lambda) \) over the blackbody emission range requires the knowledge of the material’s complex refractive index over these wavelengths (\( \sim 4-10 \mu m \)). For polystyrene these data could not be found in the literature. Therefore, a range of \( Q_{\text{abs}}(\lambda) \) values were used in the calculations to explore its effect on the final particle temperature (outlined below). With \( Q_{\text{abs}}(\lambda) \) constant, \( P_{\text{black}} \) is calculated using the Stefan-Boltzmann law:

\[
P_{\text{black}} = 4\pi a^2 Q_{\text{abs}} \sigma_{\text{sb}}(T^4 - T_{\text{wall}}^4)
\]

(4.11)

and \( \sigma_{\text{sb}} \) is the Stefan-Boltzmann constant and \( T_{\text{wall}} \) is the temperature of the surrounding chamber walls.

In the experiments reported in this chapter, helium was used as the buffer gas with \( p = 50 \) mTorr, and \( T_{\text{gas}} = T_{\text{wall}} = 295K \). The actual photon density flux on the particle
at the trap centre is difficult to ascertain, so a conservatively low estimate was used. For laser photon densities ($\sim 135$ W/cm$^2$) irradiating a polystyrene particle of radius $a=1.01$ µm, equilibrium temperatures were calculated over a range of $Q_{abs}$ for blackbody emission and are shown in Table 4.5. These estimates suggest that at the laser powers used in this work, temperatures above the $T_g$ (373K) for polystyrene are accessible and therefore non-spherical polystyrene microstructures can be driven by surface tension to adopt a spherical geometry.

<table>
<thead>
<tr>
<th>Blackbody $Q_{abs}$</th>
<th>Temperature ($T$)/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>430</td>
</tr>
<tr>
<td>0.5</td>
<td>460</td>
</tr>
<tr>
<td>0.1</td>
<td>510</td>
</tr>
<tr>
<td>0.0</td>
<td>535</td>
</tr>
</tbody>
</table>

Table 4.5: Particle temperature ($T$) estimates using Eq. (4.5) and varying the blackbody $Q_{abs}$ value. Note that the glass transition temperature for polystyrene ($T_g$) is 373K.

4.6 Summary

The outcomes of the work described in the chapter can be summarised as follows:

- Single polystyrene particles ($\phi=2-7$ µm) were introduced using laser desorption techniques (MALDI and LIAD) and trapped in a QIT.
- Fluorescent polystyrene microspheres were sized with nanometer accuracy by analysis of their MDR dominated emission spectra.
- Monitoring MDRs of a single particle permits the detection of small changes in size, shape and refractive index. Once confined in the QIT, a particle’s size and refractive index were tracked over long time periods (days).
- Non-degenerate azimuthal MDRs were resolved. Analysis of the splitting and shifting of these peaks revealed shape distortions on the nanometer scale. The geometries of axial-symmetric particles can be described as the sum of
4.6. SUMMARY

spherical harmonics and the wavelengths of non-degenerate azimuthal MDRs calculated using perturbation theory.

- Laser radiation was shown to induce melting of trapped particles changing their morphology, improving sphericity. In the case of bispheres, laser melting instigates coalescence of the monomer units to one larger sphere, a process that can tracked by monitoring the MDRs.
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Chapter 5

Single Microparticle Mass Spectrometry

5.1 Introduction

Accurate mass determination for particles in the 0.01-100 µm size regime is desirable to characterise atmospheric aerosols, viruses, bacteria and advanced particulate materials. However, particles in this size range are generally too massive for conventional mass spectrometers yet too small for conventional mass balances. Quadrupole ion traps (QITs) operated at audio frequencies coupled with optical detection techniques offer an alternative means to mass analyse species in this size regime [34]. At present, an outstanding issue for single microparticle QIT-MS is the lack of an appropriate mass calibration procedure, a problem due in part to the difficulty in obtaining calibrant particles with well defined masses. In principle, the mass to charge ratio \( \frac{m}{Q} \) of a charged particle contained in an ideal Paul trap with infinite hyperbolic electrodes can be ascertained from its secular oscillation frequencies, once the electrode spacing is known [36, 44]. However, practical QIT mass spectrometers invariably have non-ideal geometries and the electrodes are often modified to accommodate laser beams and detection optics. Mechanical imperfections and electrode misalignments can also affect QIT performance. As a
consequence, the accuracy of particle mass spectrometric measurements is usually limited by uncertainties in the QIT geometry.

A recently reported QIT-MS calibration procedure by Peng et al. [46] involved the mass analysis of single 0.895 µm and 0.269 µm diameter polystyrene microparticles originating from highly monodisperse stock samples supplied by NIST. The method entailed visually monitoring star oscillation trajectories and initiating charge steps using electron impact to obtain the absolute particle mass. The measured masses of the 0.895 µm particles were found to be in excellent accord with size and density data specified by the supplier, but a small discrepancy was found in the case of the 0.269 µm particles. One disadvantage of the method is that it relies on size measurements made by the particle manufacturer. A more desirable situation would be to ascertain both the size and mass of the same particle in situ. Davis and Ray achieved this for a 2 µm diameter microparticle contained in an electrodynamic balance (EDB) [40]. The particle’s size was obtained from analysis of the angle dependent elastic light scattering, and its mass from the magnitude of the electric field required to balance the particle at the EDB centre. Radii found using the two methods agreed to within 20%. Recently, Davis and coworkers [41] improved this procedure for larger microspheres (φ=21 µm) with radii determined using EDB, aerodynamic drag and light scattering measurements agreeing to within 3.9%.

This chapter describes the use of MDR enhanced fluorescence spectra from single, φ=2.02 µm fluorescent polystyrene microparticles (m=4.5×10⁻¹⁵ kg, 2.7×10¹² Da) to calibrate a QIT for mass determination. Analysis of the morphology dependent resonances (MDRs) of a single microsphere allowed the particle’s size and refractive index to be determined accurately (as outlined in Section 2.2). The mass of the microsphere was then calculated using the bulk polystyrene density. The m/Q of the same microparticle was ascertained from the measurement of its secular frequency in the trap. By initiating single electron charge steps the particle’s absolute mass was determined [44]. This procedure was performed for 9 particles originating from the same stock sample. The correlation between the masses
5.2 EXPERIMENTAL METHODS

obtained from the MDR analysis and secular frequency technique along with the mass precision and accuracy of the measurements is discussed, and improvements to the procedure are suggested.

5.2 Experimental methods

Fluorophore labeled polystyrene microspheres of nominal diameter of $\phi \sim 2 \mu \text{m}$ were obtained as an aqueous suspension (Duke Scientific Co.). Each particle contains fluorescent dye molecules (MW=200-300 Da) incorporated into the polystyrene matrix. The manufacturer’s quoted density for the particles is 1.05 g/cm$^3$ corresponding to density of bulk polystyrene. Particles from the stock, when analysed using a transmission electron microscope (TEM), revealed a packing structure consistent with a narrow particle size distribution and excellent sphericity (Figure 5.1). Size measurements of 16 particles using the TEM images yielded an average radius of 1002 ±15 nm.

Particles were introduced into the QIT using the LIAD technique (Section 3.6.2). For fluorophore excitation the particles were irradiated by the frequency doubled output ($\lambda = 402$ nm) from a picosecond Ti:Sapphire laser. For the secular frequency measurements the gently focussed output of a continuous wave Nd:YVO$_4$ laser ($\lambda = 532$ nm, ~1 mW) was aligned through the QIT centre perpendicular to the Ti:Sapphire laser beam (as depicted in Figure 3.11 in Section 3.6.2).

For the particles used in this work ($m \sim 4.5 \times 10^{-15}$ kg), consideration must be given to the gravitational force which perturbs the particle’s doubly degenerate radial secular oscillation ($\omega_r$), splitting it into vertical ($\omega_y$) and horizontal ($\omega_x$) components. To compensate for gravity and maintain the particle at the trap centre, a DC potential was applied symmetrically between the vertically opposed pairs of rod electrodes. It has been shown that provided the particle is balanced at the trap centre, the secular frequencies correspond to those described by Eqs. (2.15), (2.18) and (2.24) [89]. As a consequence of the probe laser orientation only
Figure 5.1: TEM micrographs of \( \sim 2 \, \mu m \) polystyrene microparticles (Duke Scientific Co.) used in the QIT mass spectrometry studies.
\( \omega_x \) and \( \omega_z \) were measurable. In this work, the \( m/Q \) of a particle was determined from \( \omega_x \) using an 8th order polynomial obtained by inverting the relation between \( q_z \) and \( \beta_z \) [Eq. (2.24)]. During a frequency measurement the particle’s oscillation amplitude was monitored using a CCD camera and maintained at 150 ±20 µm. Each secular frequency measurement entailed the collection of 40,000 sample points at a collection rate of 200 Hz corresponding to a frequency resolution of 0.005 Hz.

## 5.3 Results and Discussion

The MDR dominated fluorescence emission from a single polystyrene microsphere is shown in Figure 5.2a. The MDR peaks are sharp and symmetric indicating that the particle has high sphericity [131]. Furthermore, the MDR spacing reveals that the particle is a single sphere rather than a bisphere or agglomerate. The particle’s radius and refractive index were determined from an analysis of the MDR wavelengths and found to be consistent with a sphere of radius \( a = 1010 \pm 1 \) nm and refractive index \( m_\lambda = 1.551 + 11430 \, \text{nm}^2/\lambda^2 \). The calculated Mie scattering spectrum for a sphere with the fitted parameters is also shown in Figure 5.2a where the good match between theory and experiment is clearly apparent. Assuming a density of 1.05 g/cm\(^3\) (corresponding to the bulk polystyrene density), the particle is calculated to have a mass of \( m_{\text{MDR}} = 4.54 \times 10^{-15} \) kg. Note that polystyrene has been shown to have a density close to that of the bulk for structures down to nanometer scales [132].

The \( m/Q \) of the same microsphere was determined by measuring its horizontal secular frequency (\( \omega_x \)) and using Eqs. (2.15), (2.18) and (2.24). A representative Fourier transform (FT) spectrum is shown in Figure 5.3 where \( \omega_x \) is identified. The temporal stability of the particle’s secular frequency was investigated by tracking \( \omega_x \) over a 9 hour period. Figure 5.3 reveals that the secular frequency remains constant over this time interval to within the frequency resolution of the measurement (<100 ppm).
Figure 5.2: (a) Fluorescence emission from a single microsphere (upper) and calculated scattering spectrum (lower) for the fitted parameters of $a=1010 \pm 1$ nm and $m_\lambda=1.551+11430$ nm$^2$/λ$^2$. (b) A frequency spectrum of the particle’s motion with trap drive parameters $V_{0-p}=1444$, $\Omega/2\pi=400$ Hz. The strong peak corresponds to the secular frequency in the $x$ direction ($\omega_x$) and the smaller peak to its harmonic ($2\omega_x$).
The particle’s absolute charge and mass were ascertained by initiating charge steps using the Xe flashlamp. In the current case, $\omega_x$ was measured for eight different charge states (Figure 5.4a). Determination of the particle’s absolute mass ($m_{FT}$) and charge ($Q=Ze$, where $Z$ is an integer) was achieved by a procedure similar to that reported by Peng et al. [46] and Tito et al. [133]. The first step in this procedure required a series of charge assignments ($Z_i$) be made for the eight $Q/m$ states. This was done by assigning a $Z$ value to the first $Q/m$ state and using the ratios of $Q/m$ values to make $Z$ assignments for the other states. Next, the corresponding masses for the eight $Q/m$ states were calculated, along with the average mass and its standard deviation. This procedure was repeated, varying the initial integer charge state assignment ($Z_1$) from 1 to 2000, to ascertain the set of charge states that yielded the average mass with the lowest standard deviation. The outcome is conveniently represented as a plot of standard deviation ($\sigma=\Delta m_{FT}/m_{FT}$) versus $m_{FT}$, in which the mass corresponding to the most appropriate charge state assignments can be identified (Figure 5.4b). The inset in Figure 5.4b shows the five points about the minimum, the charge state assignments of which are listed in Table 5.1. The minimum in Figure 5.4b corresponds to an initial charge number
Z=405 and \( m_{FT} = 4.72 \times 10^{-15} \) kg, which is \(~4\%\) larger than \( m_{MDR} = 4.54 \times 10^{-15} \) kg.

<table>
<thead>
<tr>
<th>Label</th>
<th>( Z_1 )</th>
<th>( Z_2 )</th>
<th>( Z_3 )</th>
<th>( Z_4 )</th>
<th>( Z_5 )</th>
<th>( Z_6 )</th>
<th>( Z_7 )</th>
<th>( Z_8 )</th>
<th>( m_{FT} \ [10^{-15} \text{kg}] )</th>
<th>( \sigma \ [10^{-4}] )</th>
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Table 5.1: The eight assigned charge states \( (Z_i) \) and corresponding average mass \( (m_{FT}) \) and standard deviation \( (\sigma) \) for the points labeled (I–V) in the inset of Figure 5.4.

Nine microspheres from the same stock sample were investigated using the procedure outlined above to determine a series of \( m_{FT} \) and \( m_{MDR} \) values. From the fluorescence spectra it was found that the particles’ radii ranged from 1002.7 to 1013.8 nm, with an average value \( \bar{a} = 1011.1 \pm 2.2 \) nm in good agreement with the TEM size measurements (1002 ±15 nm). The standard deviation of \( a \) (\( \approx 0.2\% \)) is considerably less than the manufacturer’s claimed tolerance (1.5%). The average radius (\( \bar{a} \)) for the 9 particles corresponds to an average mass \( \bar{m}_{MDR} = 4.55 \pm 0.03 \times 10^{-15} \) kg which compares very well with the average mass determined from the \( \omega_x \) and charge stepping measurements (\( m_{FT} = 4.54 \pm 0.12 \times 10^{-15} \) kg).

The \( m_{MDR} \) values are plotted against the \( m_{FT} \) values for the 9 particles in Figure 5.5. Fitting the data to a straight line yields \( m_{MDR} = (1.002 \pm 0.010) \times m_{FT} \). The good correlation between \( m_{MDR} \) and \( m_{FT} \) confirms that the trap parameter \((z_0=5.89 \text{ mm})\) determined from the SIMION simulations described in Section 3.1.1 is appropriate and that any effect of electrode misalignment is small.

There is the possibility that the density of the polystyrene microsphere differs slightly from the bulk value because of the incorporation of the dye molecules. If this was the case, the \( m_{MDR} \) values would be systematically too high or too low. However, one might expect that significant changes in the density would be reflected in changes in the material’s refractive index. The average refractive index for the 9 particles determined from the MDR analysis is 1.583 ±0.006 at 589 nm,
Figure 5.4: (a) A plot of a single particle’s oscillation frequency. Frequency steps correspond to charge changes initiated by the Xe flashlamp. The right-hand axis is the absolute charge state ($Z$) for each frequency step. The trapping parameters ($V_0-p=750$ at $\Omega/2\pi=330$ Hz) are kept constant throughout the measurement. Also shown (b) is a plot of the standard deviation ($\sigma$) against mean mass corresponding to a range of charge assignments. The lowest $\sigma$ value corresponds to $m_{FT}=4.72 \times 10^{-15}$ kg. The charge assignments (I–V) in the inset are listed in Table 5.1.
very close to the reported refractive index of polystyrene (1.59 at 589 nm) [134]. This suggests that, despite being impregnated with fluorescent molecules, the density of the microspheres is very similar to that of bulk polystyrene. Furthermore, no correlation is observed between either $m_{FT}$ or $m_{MDR}$ and a particle’s refractive index, which, if present, would imply particle-to-particle density variations.

It is apparent from Figure [5.5] that the range of the $m_{FT}$ data is $\approx 4$ times larger than that of the $m_{MDR}$ data. The range of the $m_{FT}$ data primarily reflects the uncertainty in the $m_{FT}$ measurements rather than a spread in particle sizes otherwise the $m_{MDR}$ and $m_{FT}$ values would have a similar distributions.

The main random error in $m_{FT}$ is likely to arise from the uncertainty in the absolute charge state assignment which originates from the uncertainty in the $Q/m$ values. The precision of the $Q/m$ determination is in turn governed by errors in $\omega_x$, $\Omega$ and $V$ [see Eq. (2.4)] which have estimated relative errors of the order of $3.3 \times 10^{-4}$, $1 \times 10^{-5}$ and $1 \times 10^{-3}$, respectively.
Because $\Omega$ and $V$ are fixed during a charge stepping experiment, it is likely that the $Q$ and $m_{FT}$ determinations are affected mainly by random errors in $\omega_x$ due to the finite resolution of the frequency measurements. To explore the relationship between errors in $\omega_x$ and the errors in the charge ($Q$) and mass ($m_{FT}$) values, we ran a series of simulations on model data for a 2.02 $\mu$m polystyrene sphere.

To begin, the particle was assumed to have undergone a series of 7 charge steps corresponding to charge numbers ($Z_j$) of 450, 453, 454, 456, 457, 458, 461, and 463, which are typical values for the $\phi=2.02$ $\mu$m particles characterised in this work. The corresponding $\omega_x$ values were calculated using Eqs. (2.15), (2.18) and (2.24). These $\omega_x$ values were then varied randomly over a range determined by an assumed relative error ($\Delta \omega_x/\omega_x$). The charge and mass assignment procedure outlined above was then performed using the noisy $\omega_x$ values to recover the particle’s mass ($m_{FT}$) and charge states $Z_j'$ which, because of the introduced random errors, did not necessarily correspond with the initial assumed charges. The simulation procedure is represented as follows:

$$
\begin{bmatrix}
Z_1 \\
\vdots \\
Z_7
\end{bmatrix}
\rightarrow
\begin{bmatrix}
(\omega_x)_1 + \delta_1 \\
\vdots \\
(\omega_x)_7 + \delta_7
\end{bmatrix}
\rightarrow
\begin{bmatrix}
Z'_1 \\
\vdots \\
Z'_7
\end{bmatrix}
$$

where $\delta_j=R(\Delta \omega_x/\omega_x) \times (\omega_x)_j$ and $R$ is a random number between -1 and 1.

This procedure was repeated 1500 times to gain a statistically significant estimate of the uncertainty in the charge and mass. The results of the simulations are displayed in Figure 5.6 where the relative uncertainty in the charge state assignment ($\Delta Z/Z$) is plotted against the relative error in $\omega_x$ ($\Delta \omega_x/\omega_x$). It is apparent that relatively small fractional errors in $\omega_x$ lead to much larger relative errors in the absolute charge number ($Z$) and consequently in the absolute mass ($m_{FT}$). It is evident from the simulations that errors in $\omega_x$ on the order of the experimental frequency resolution (0.005 Hz, corresponding to a $\Delta \omega_x/\omega_x=3.3 \times 10^{-4}$), lead to uncertainties in the charge and mass of 1-2%, comparable with the observed 2.6%
spread in the $m_{FT}$ values for the 9 particles.

The fluctuations in $\Omega$ and $V$ during a series of secular frequency measurements should be much less compared to their absolute uncertainties (and to the uncertainty in $\omega_x$) and therefore are not expected to contribute significantly to the precision of $Q$. They may however, have a small effect on the overall accuracy of $m_{FT}$. The presence of fluctuating surface charges on the electrodes may affect the measured $\omega_x$ values, thereby influencing the accuracy and precision of the $m_{FT}$ determinations. In this regard it is worth recalling that the charge state changes were effected by UV light from a pulsed Xe lamp, a fraction of which would have impinged on the electrode surfaces. While it is difficult to quantify the surface charge effects, they may account for part of the 2.6% spread in the $m_{FT}$ data for the nine particles.

![Figure 5.6](image_url)

**Figure 5.6:** Relative error in the charge state assignment ($\Delta Z/Z$) plotted against the relative error in the secular frequency measurement. The histogram shows the distribution of charge states ($Z'$) arising from a relative error in $\omega_x$ of $3.3 \times 10^{-4}$.
5.4 Future Considerations

There are several improvements that could be made to the mass calibration procedure. Primarily, the $m/Q$ determinations would be more accurate if the size of the polystyrene microspheres was reduced so that they held less charge. In this case loss or gain of a single electron would lead to a larger change in secular frequency ($\Delta \omega_x$). To investigate this further, the charge assignment simulation described above was performed for a $\phi=1.5 \mu m$ polystyrene sphere holding just over half the number of charges typically observed for the $2.02 \mu m$ particles in this work (the particle’s charge was scaled with surface area). For 7 charge states ranging from 250–262 and with $\Delta \omega_x/\omega_x = 3.3 \times 10^{-4}$, $\Delta Z/Z$ was found to be reduced by about a third ($7.1 \times 10^{-3}$) compared to the $\phi=2.02 \mu m$ case ($1.10 \times 10^{-2}$).

A consequence of reducing the sphere size is a decrease in the MDR density in the visible fluorophore emission range, which in turn reduces the accuracy of the mass determined from the MDR analysis. To overcome this, a fluorophore could be chosen that emits in the near UV (300–400 nm). As illustrated in Figure 5.7, where the elastic scattering spectrum is plotted for a $\phi=1.5 \mu m$ polystyrene sphere, there are sufficient sharp MDR peaks in the 300–400 nm range to enable an accurate size determination. The onset of absorption for polystyrene is $\sim 280$ nm [135, 136], so that to investigate even smaller particles with shorter MDR wavelengths, another material would need to be used (e.g. SiO$_2$). As an alternative to fluorescence emission, MDR wavelengths could also be measured from an elastic light scattering spectrum acquired using a suitable tunable light source (e.g. synchrotron radiation).

A further issue when reducing the particle size, pertains to the collection of sufficient light at the CCD camera to monitor the particle’s oscillation amplitude. In the current arrangement the CCD camera collects light at 90 degrees with respect to the excitation laser beam. Significant improvements in detection efficiency would be realised if the collection angle was reduced to 45 degrees, with the light collected between two rod electrodes. Figure 5.8 plots the calculated scattering...
Figure 5.7: Computed elastic scattering spectrum for a polystyrene sphere with $\phi=1.5$ $\mu$m.

Efficiency ($Q_{sca}$) as a function of angle for polystyrene spheres with $\phi=1.5$ and 2.0 $\mu$m. A larger detection solid angle is also available between the rod electrodes (as represented by the bars in Figure 5.8) than through the endcap electrode (as in the current arrangement).

5.5 Conclusion

This chapter described a mass calibration method for a single particle QIT mass spectrometer. The procedure combines MDR enhanced fluorescence spectroscopy with single particle frequency measurements and charge stepping. For the QIT developed in this work, average masses obtained for nine polystyrene particles using each technique are in excellent agreement ($m_{MDR}=4.55 \pm 0.03 \times 10^{-15}$ kg and $m_{FT}=4.54 \pm 0.12 \times 10^{-15}$ kg). This suggests that the QIT geometrical parameter ($z_0=5.89$ mm) obtained by the computer simulation described in Section 3.1.1 is appropriate. The analysis reveals that quite large errors (2.6%) in the charge assignment and therefore the mass determination result from relatively modest fractional errors in the secular frequency measurements. Nevertheless, the analysis validates the mass spectrometric performance of the QIT instrument developed in

*Angular dependent $Q_{sca}$ data were calculated using the FORTRAN Mie scattering code of Wiscombe [127].
Figure 5.8: Angle dependent light scattering efficiency for polystyrene spheres with $\phi = 1.5$ and 2.0 $\mu$m. The bars represent the collection angles available for detection in the current setup and in a suggested future arrangement.
this work.
Bibliography


Appendix A

Measured MDR peak wavelengths ($\lambda_{\text{exp}}$) from Figure 4.2 along with the assignments of mode order ($\nu$), polarisation (TE=1,TM=2) and mode number ($l$). Calculated MDR wavelengths for fitted microsphere parameters of $a=3589$ and $m=1.545+9249\text{nm}^2/\lambda^2$ are also listed ($\lambda_{\text{theo}}$) in addition to the difference between the measured and calculated MDR wavelengths ($\Delta$).

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Observation of nondegenerate cavity modes for a distorted polystyrene microsphere

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Nondegenerate azimuthal morphology-dependent resonances are observed for a distorted, fluorescently labeled polystyrene microsphere levitated in a quadrupole ion trap. Modeling the individual resonances by using perturbation theory allows a determination of quadrupole and octupole distortion parameters. The particle's shape changes slowly over the course of the measurement and eventually becomes spherical. The morphological changes are facilitated by laser heating of the particle above the polystyrene glass transition temperature. We demonstrate a method of transforming a trapped particle to a sphere and rendering its azimuthal modes degenerate. © 2006 Optical Society of America

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It is well known that a microsphere can act as a three-dimensional optical cavity exhibiting morphology dependent resonances (MDRs) or whispering gallery modes.1-4 The MDRs of a dielectric sphere, which are distinguished by polarization (TE or TM), mode order \( \nu \), and angular momentum number \( \ell \), have \( 2\ell + 1 \) azimuthal degeneracy. The degeneracy is removed when the sphere is distorted to an ellipsoid so that for a given polarization, \( \nu \), and \( \ell \), the system exhibits \( (\ell + 1) \) distinct azimuthal modes (labeled \( m = \ell, \ell - 1, \ldots, 0 \)). Optical effects resulting from shape distortion have been reported for droplets generated by a vibrating orifice where periodic distortions from sphericity occur on millisecond time scales.5,6 In this Letter, we study the slow morphological changes of a single fluorescent polystyrene microparticle confined in a quadrupole ion trap (QIT). The shape parameters for the particle are extracted by modeling the splitting and shifting of nondegenerate azimuthal MDRs by using the perturbation theory developed by Lai et al.7

We report here a case in which nondegenerate azimuthal MDRs are spectrally resolved and assigned, thus providing an opportunity to test and apply current theoretical descriptions of distorted microsphere MDRs.8 Furthermore, this work demonstrates that laser-induced heating can transform a trapped polystyrene particle into a sphere and render its azimuthal modes degenerate. The QIT used in this study (shown in Fig. 1) is similar to that reported by Schlemmer et al.8 and was operated in single-phase mode at 250 Hz frequency and 740 V amplitude. Fluorophore labeled polystyrene microspheres (Sigma-Aldrich, nominal diameter 2.16 \( \mu \)m) were introduced into the QIT by using matrix-assisted laser desorption–ionization (MALDI), with 3-hydroxypicolinic acid (3-HPA) as the absorbing matrix. To prepare a MALDI sample, three to four drops of an aqueous suspension of the polystyrene particles were mixed with three to four drops of a saturated solution of 3-HPA in an acetonitrile–water mixture on a stainless steel sample tip. The sample was then dried under nitrogen and placed above the QIT situated in a vacuum chamber. A single 7 ns laser pulse, third harmonic (\( \lambda = 355 \) nm) from a Q switched Nd:YAG laser, irradiated the sample, ejecting charged particles into the QIT. Usually the particles carried 1000–1400 elementary positive charges. By systematically varying the trap driving voltage and frequency, the particles were ejected until only one remained. By using He buffer gas (~100 mTorr) we damped the remaining particle's motion so that its oscillation amplitude was <0.1 mm. Approximately 2 mW from a continuous wave 532 nm Nd:YVO4 laser irradiated the trapped particle. Scattered or fluorescent light was collected perpendicular to the incident laser beam by using a 25 mm diameter F/1 lens and directed through an edge filter to remove 532 nm light and then into a spectrometer (with a resolution ~0.03 nm) equipped with a CCD detector interfaced to a personal computer.

The fluorescence emission spectrum of most trapped particles was dominated by the MDR's characteristic of a dielectric sphere and could be modeled using Mie theory yielding the particle's radius \( R \), and refractive index \( n_0 \).9 However, several particles exhibited more complex spectra indicating distortions from sphericity. Moreover, the particles appeared to exhibit a higher degree of morphological change than had previously been observed for charged microdroplets.10

![Fig. 1. View of the experimental configuration.](image-url)
peared to gradually relax to a spherical form. For example, Fig. 2 shows the consecutive spectra for a particle at \( t = 0 \) min intervals. Spectral changes are due to the particle's relaxation from spheroidal to spherical shape. E is the predicted scattering spectrum for a sphere with \( a_0 = 1363 \text{ nm} \) and \( m_1 = 1.549 + 10.230 \text{ nm}^2/\lambda^2 \).

To derive information on the particle's shape we follow Lai et al., who developed expressions for the MDR frequencies of a distorted dielectric sphere. They consider a sphere affected by an axially symmetric shape perturbation such that the radius is

\[
r(\theta, \phi) = a + \Delta r(\theta, \phi) = a + \Delta L \sqrt{4\pi} Y_{L,0}(\theta, \phi),
\]

where \( \Delta \) is a weighting term for the \( Y_{L,0}(\theta, \phi) \) spherical harmonic. The shift of the \( m \) modes (\( \delta \omega \)) with the same \( l \) relative to that of a sphere with radius \( a \) is given as

\[
\frac{\delta \omega}{\omega} = -\frac{\Delta \omega}{a} F(L, l, m),
\]

where \( F(L, l, m) \) is defined as

\[
F(L, l, m) = A(L, l)f(m),
\]

with

\[
A(L, l) = \frac{2l + 1}{\sqrt{2L + 1}} \frac{C(\ll L; 000)}{L(L + 1)} \left[ 1 - \frac{L(L + 1)}{2l(l + 1)} \right],
\]

\[
f(m) = (-1)^m \frac{C(\ll L; m, -m, 0)}{C(\ll L; 000)}.
\]

The \( C(j_{2/3}; m, m, m) \) are Clebsch–Gordan coefficients.

For odd \( L \) distortions, \( F(L, l, m) \) is zero and the \( m \) modes remain degenerate. The lowest-order perturbation in \( m \) to remove the degeneracy is a quadrupole distortion \( Y_{2,0}(\theta, \phi) \), which for \( l \geq 1 \) results in \( m \)-mode shifts described by

\[
\delta \omega(m) = \frac{\lambda^2}{4\pi r^2} \Delta \omega(L, l) \left[ 1 - \frac{L(L + 1)}{2l(l + 1)} \right].
\]

The \( \Delta \omega(L, l) \) coefficients are given in Table 1.

Fig. 2. A–D, Fluorescence spectra from a single polystyrene particle taken at \( t = 0 \) min intervals. Spectral changes are due to the particle's relaxation from spheroidal to spherical shape. E is the predicted scattering spectrum for a sphere with \( a_0 = 1363 \text{ nm} \) and \( m_1 = 1.549 + 10.230 \text{ nm}^2/\lambda^2 \).

Fig. 3. Expanded sections of spectra B and C from Fig. 1. Nondegenerate azimuthal MDRs clearly resolved in traces B and C (Fig. 3) have wavelengths consistent with the particle's being a prolate spheroid.

Fig. 4. Measured fractional frequency shifts for the TE_{20} and TE_{19} m modes in spectra B and C (points). Lines represent frequency shifts calculated by using Eq. (8) and the fitted \( \Delta_2 \) and \( \Delta_4 \) values in Table 1.
heat input due to laser light absorption and heat loss due to black body radiation and buffer gas cooling. The final radius of the sphere in Fig. 2(D) (1363 nm) is somewhat larger than the manufacturer’s quoted radius for the polystyrene spheres (1080 nm). Tellingly, a sphere with a radius of 1361 nm has the same volume as two spheres with a radius of 1080 nm. Therefore it seems likely that the emission spectra in Figs. 2(A)–2(D) are the signatures of the final stages of two smaller spheres fusing into one.

In summary, we have shown that it is possible to laser melt an isolated microparticle suspended in a QIT, transforming it into a sphere, and rendering its azimuthal modes degenerate. The quantitative details of the spheroidal particle’s shape have been extracted by assigning and modeling the spectrally resolved nondegenerate azimuthal modes.

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### References

Calibration of a quadrupole ion trap for particle mass spectrometry

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Abstract

A quadrupole ion trap (QIT) is calibrated for microparticle mass spectrometry by confining $\phi = 2.02 \mu m$ dye-labeled polystyrene microspheres and measuring their secular oscillation frequencies and fluorescence spectra. A particle’s absolute mass and charge are found by measuring its secular oscillation frequencies within the QIT while initiating charge steps through photo-ejection of electrons. The radius of the same microsphere is determined by analyzing the fluorescence emission spectrum, which is dominated by optical cavity resonances, employing Mie theory. The mass of the microsphere is calculated from the radius using the density of bulk polystyrene. For nine particles originating from the same stock sample, the masses obtained from the two methods agree to within 3% with no systematic deviation. Nevertheless, excellent agreement between the average masses determined using the two techniques confirm that the value of the trap parameter ($z_0$) obtained from computer modeling is appropriate and that effects of electrode misalignments are small.

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Keywords: Quadrupole ion trap; Microparticle; Optical resonance; Mie scattering

1. Introduction

Accurate mass determination for particles in the 10–10,000 nm size regime is desirable to characterize atmospheric aerosols, viruses, bacteria and advanced particulate materials. However, particles in this size range are usually too large for conventional mass spectrometers yet too small for conventional mass balances. There have been several efforts towards developing techniques for the non-destructive mass spectrometric characterization of single particles, particularly in quadrupole ion traps (QITs) [1].

The foundations of single microparticle QIT mass spectrometry (QIT-MS) were established by Wuerker et al. who demonstrated that the mass-to-charge ratio ($m/Q$) of a single trapped particle can be determined from its secular oscillation frequencies in a QIT [2]. Subsequently, several groups have applied this strategy to single microparticle mass measurements [3–6], culminating in the work of Peng et al. who determined the masses of individual biological microparticles including *Escherichia coli* and human red blood cells [7,8].

One outstanding problem for single microparticle QIT-MS involves mass calibration of the device for operation in the high mass regime. In principle, once the electrode spacing is known, the mass-to-charge ratio of a charged particle in an ideal Paul trap with infinite hyperbolic electrodes can be ascertained from its secular oscillation frequencies. However, practical traps have electrodes that are non-ideal and which may be misaligned. As pointed out by Schlemmer et al. [5] electrode imperfections and misalignments on the order of tens of microns can lead to systematic errors in $m/Q$ measurements in the percent range.

A recently reported calibration procedure involved mass analysis of single 0.895 $\mu m$ diameter polystyrene microparticles originating from a highly monodisperse stock sample [9]. The method entailed visually monitoring star oscillation trajectories and initiating charge steps using electron impact to obtain the particle’s absolute charge and mass. Measured particle masses agreed very well with masses deduced from size and density data specified by the particles’ supplier. One disadvantage of the method is that it relies on size data from the manufacturer. A more desirable procedure would involve ascertaining the size and mass of the same particle in situ. This was attempted some time ago by Davis and Ray [10], who measured the size and mass for a single microsphere ($\phi = 2 \mu m$) using an electrodynamic...
balance (EDB). The particle’s size was obtained from analysis of the angle dependent elastic light scattering and its mass from the electric field required to balance the particle at the EDB centre. Radii deduced using the two methods agreed to within 20%. Recently, Zheng et al. improved this procedure for larger microspheres (\( \phi = 21 \mu m \)) in an EDB, with radii determined through spring point, aerodynamic drag and light scattering measurements agreeing to within 3.9% [11].

In the current work, we characterize single, \( \phi = 2.02 \mu m \) dye-labeled polystyrene microparticles (4.5 \( \times 10^{-15} \) kg, 2.7 \( \times 10^{12} \) Da) by obtaining their cavity enhanced fluorescence spectra, and by measuring their secular oscillation frequencies in the QIT. Analysis of optical morphology dependent resonances (MDRs) of a single microsphere allowed the particle’s radius to be determined, and thenceforth for its mass to be ascertained [12]. The \( m/Q \) of the same microparticle was measured from observation of its secular frequency in the trap. In turn, by initiating single electron charge steps the particle’s absolute charge and mass were determined. Following a discussion of both techniques, we present data for nine particles, and finally discuss sources of error in the mass determinations.

2. Experimental methods

2.1. Materials

The fluorescent polystyrene microparticles used in this study were commercially obtained as an aqueous suspension (Duke Scientific, nominal diameter \( \sim 2 \mu m \)). Each particle contains fluorescent dye molecules (MW = 200–300 Da) incorporated into the polystyrene matrix. The particles have a broad absorption peaking at 412 nm and an emission envelope extending over 440–550 nm with broad peaks at 445 and 473 nm. The manufacturer’s quoted density for the particles is 1.05 g/cm\(^3\), which corresponds to the density of bulk polystyrene.

2.2. The quadrupole ion trap

The quadrupole ion trap (QIT) used in this work follows the design of Schlemmer et al. [5] consisting of two opposing conical “endcap” electrodes surrounded by eight rod electrodes, which replace the ring electrode of a conventional Paul trap (see Fig. 1). This electrode geometry generates an approximate quadrupole potential in a small volume around the trap centre while having the advantage of excellent optical access to the trapped particle [13]. The QIT was housed in a UHV chamber (Fig. 2) evacuated by a 170 L/s turbo pump. The secular frequencies of a single particle were measured at a pressure of \( \sim 10^{-6} \) Torr. Fluorescence spectra were obtained in the presence of helium buffer gas (50 mTorr) to reduce the particle’s oscillation amplitude.

2.3. Theoretical background

The equations describing the stability and motion of a charged particle in a quadrupole field are well known [14]. Here, we introduce only the equations relevant for determining the \( m/Q \) of a trapped particle from its frequency of motion [2,5]. The stability parameters in the axial (\( z \)) and radial (\( r \)) directions for a QIT operated in single phase (oscillating potential applied to endcap electrodes and ring electrode held at ground) are:

\[
q_z = -2q_r = \frac{2QV}{m_z^2\Omega^2},
\]

where \( V \) is the amplitude of the oscillating voltage applied to the QIT endcap electrodes at an angular frequency of \( \Omega \). In an ideal QIT, \( z_0 \) is half the distance between the endcap electrodes. The potential distribution within the QIT was modeled using the simulation program SIMION 3D v7.0 (Idaho National Laboratories) with a resolution of 0.05 mm/gu over a 1 mm\(^3\) cube. The quadrupole field near the trap centre was found to be consistent with \( z_0 = 5.89 \) mm.

A particle confined in an ideal Paul trap has secular frequencies in the radial (\( \omega_r \)) and axial (\( \omega_z \)) directions of:

\[
\omega_z = 2\omega_r = \frac{\beta_z \Omega}{2}
\]

where, for \( q_z < 0.7 \), the dependence of \( \beta_z \) on \( q_z \) is adequately approximated by

\[
\beta_z = \left( \frac{q_z^2}{2 - q_z^2} - \frac{7}{128} q_z^4 + \frac{29}{2304} q_z^6 \right)^{1/2}
\]

For the secular frequency measurements, \( q_z \) was maintained at \( \sim 0.3 \) by choosing appropriate values for \( V \) and \( \Omega \) (\( V \approx 1500 \) V and \( \Omega/2\pi \approx 350 \) Hz). The \( m/Q \) value is obtained from \( \omega_r \) through the inverse relation between \( q_z \) and \( \beta_z \) which was approximated as an 8th order polynomial.

The gravitational force perturbs the particle’s motion splitting the doubly degenerate radial secular oscillation (\( \omega_r \)) into vertical (\( \omega_y \)) and horizontal (\( \omega_x \)) components. To compensate for gravity and maintain the particle at the trap centre, a dc potential was applied between the vertically opposed pairs of rod electrodes. It has been shown that provided the particle is balanced at the trap center the secular frequencies correspond to those described by Eqs. (1)–(3) [5,13]. Note that as a consequence of the probe laser orientation only \( \omega_x \) and \( \omega_z \) were measured in this work (see Fig. 1).
2.4. Optical set-up

For the secular frequency measurements, the gently focussed output of a continuous wave Nd:YVO₄ laser (λ = 532 nm, Coherent VERDI-V5) was aligned through the QIT centre (Fig. 1). Elastically scattered 532 nm light was collected by a 25 mm F/1 lens and directed to a photomultiplier tube (PMT) (Hamamatsu HS784-02) through a spatial filter. The signal from the PMT was filtered using a low-pass Butterworth frequency filter (Krohn-Hite 3381) to remove high frequency components. The filtered signal was sent to a personal computer via a data acquisition card (NI PCI-6024E). The time domain signal was Fourier transformed (FT) to ascertain the particle’s secular oscillation frequencies. During a frequency measurement, the particle was monitored using a video camera and its oscillation amplitude was maintained at 150 ± 20 μm. Each frequency measurement entailed collection of 40,000 sample points at a collection rate of 200 Hz, corresponding to a frequency resolution of 0.005 Hz.

For fluorescence measurements, the particles were irradiated by the λ = 402 nm frequency doubled output from a picosecond Ti:Sapphire laser (Tiger-ps, Time-Bandwidth). The light was directed through the QIT via holes in the endcap electrodes (Fig. 1). Fluorescence from the particle was collected by a 25 mm F/1 lens and directed into a spectrometer (JY Triax 552) with a liquid nitrogen cooled CCD detector (JY Symphony) and 600 g/mm grating. The spectral resolution was ~0.05 nm. The spectrometer was regularly calibrated using a HgAr lamp.

2.5. Particle injection and single particle isolation

Polystyrene particles were introduced into the QIT using laser induced acoustic desorption (LIAD) [8,9]. Preparing a LIAD sample involved placing two to three drops of a purified aqueous particle suspension on a 0.5 mm thick Si wafer which was then dried under nitrogen. The wafer was held above the QIT by a stainless steel rod that was inserted into the vacuum chamber through a vacuum interlock (Fig. 1). A single focussed 7 ns laser pulse (λ = 532 nm, ~40 mJ) from a Q-switch Nd:YAG laser irradiated the Si wafer from behind causing charged particles to be desorbed from the front surface. Helium buffer gas (50 mTorr) slowed the desorbed particles so that they could be trapped efficiently.

Several methods were used to check that only one particle was confined in the QIT. The presence of broadened or additional peaks in the FT spectrum usually indicated the presence of more than one particle. Additionally, when inspected with a video camera, a lone particle had a smooth periodic trajectory, whereas irregularities in the particle’s motion usually implied presence of one or more additional intruding particles (not necessarily illuminated by the probe laser). Once unwanted particles were ejected (by systematically varying V and/or Ω), the remaining particle adopted a smooth, periodic trajectory.

2.6. Charge stepping

The absolute charge and mass of each particle were determined by measuring ωₓ for a number of different charge states which could be converted to m/Q values using Eqs. (1)–(3).

Charge stepping of single polystyrene microparticles was accomplished using the output from a pulsed Xe flashlamp (Ocean Optics PX-2, λ = 220–750 nm) transmitted to the trap through a UV-grade optical fibre. Two processes were presumably responsible for charge changing events. First of all, the particles could lose electrons through direct photo-ejection caused by photons with λ < 294 nm, whose energy exceeded the polystyrene work function (4.22 eV [15]). Alternatively, electrons photo-ejected from the metal electrodes may have been accelerated into the trap centre where they were either absorbed by the particle or induced secondary electron ejection. Often the flashlamp was fired >100 times before a change of one or several elementary charge units was observed.

For highly charged particles, it was difficult to discern quickly whether a small charge change had occurred after irradiation. A simple and rapid technique was to view star trajectories which are present at certain trap drive frequencies [4,7]. A single trapped particle adopts a stable star trajectory in the x−y plane with n branches when the drive frequency (Ω) is equal to an integer multiple of the particle’s radial frequency (nω₀). Two examples of star trajectories are shown in Fig. 3. Usually, to detect small charge changes on highly charged microparticles (Q > 400e), a stable star pattern trajectory was established (with n = 6 or 7) by carefully adjusting the drive frequency while viewing the particle using a video camera. Once a stationary star trajectory was established, the Xe flashlamp was
pulsed. When a charge step occurred, the star pattern began to rotate slowly because the drive frequency was no longer an integer multiple of the particle’s secular frequency. Subsequently, the particle’s new secular frequency was measured.

3. Results and discussion

The MDR dominated fluorescence emission from a single polystyrene microsphere is shown in Fig. 4. The MDR peaks are sharp and symmetric indicating that the particle has high sphericity [16]. Furthermore, the MDR spacing reveals that the particle is a single sphere rather than a bisphere or agglomerate. The particle’s radius and refractive index were determined from an analysis of the MDR wavelengths [12] and found to be consistent with a sphere of radius $a = 1010 \pm 1$ nm and refractive index $m_{\lambda} = 1.551 + 11430 \text{nm}^2/\lambda^2$. The calculated Mie scattering spectrum for a sphere with the fitted parameters is also shown in Fig. 4. Clearly, the match between theory and experiment is excellent. Using the bulk polystyrene density (1.05 g/cm$^3$), the particle was calculated to have a mass $m_{\text{MDR}} = 4.54 \times 10^{-15}$ kg. Note that polystyrene has been shown to have a density close to that of the bulk for structures down to nanometer scales [17].

The $m/Q$ of the same microsphere was determined by measuring its horizontal secular frequency ($\omega_x$) and using Eqs. (1)–(3). A typical FT spectrum is shown in Fig. 5, where $\omega_x$ is identified. The particle’s absolute charge and mass were ascertained by initiating charge steps using the Xe flashlamp. In this case, $\omega_x$ for the particle was measured for eight charge states (Fig. 6a).

Determination of a particle’s absolute mass ($m_{\text{FT}}$) and charge ($Q = Ze$, where $Z$ is an integer) was achieved by the procedure described in references [9] and [18]. In the first step, a series of charge assignments ($Z_1$) were made for the eight $Q/m$ states. This was done by assigning a $Z$ value to the first $Q/m$ state and using the ratio of $Q/m$ values to make $Z$ assignments for the other states. Next, the corresponding masses for the eight $Q/m$ states were calculated, along with the average mass and its standard deviation. This procedure was repeated by varying the initial integer charge state assignment $Z_1$ from 1 to 2000 to ascertain the set of charge states that yielded the average mass with the lowest standard deviation. The outcome is conveniently represented as a plot of standard deviation ($\sigma$) versus $m_{\text{FT}}$, in which the mass corresponding to the best charge state assignments can be identified (Fig. 6b). The result for the data shown in Fig. 6 is $m_{\text{FT}} = 4.72 \times 10^{-15}$ kg, which is $\sim$4% larger than $m_{\text{MDR}} = 4.54 \times 10^{-15}$ kg.

Nine microspheres from the same stock sample were investigated using the procedure outlined above to determine a series of $m_{\text{FT}}$ and $m_{\text{MDR}}$ values. From the fluorescence spectra, it was found that the particles’ radii varied from 1002.7 to 1013.8 nm with an average value $\bar{a} = 1011.1 \pm 2.2$ nm. The standard deviation of $a$ ($\sim$0.2%) is considerably less than the manufacturer’s specified tolerance (1.5%). The value of $\bar{a}$ corresponds to an average mass $\bar{m}_{\text{MDR}} = 4.55 \pm 0.03 \times 10^{-15}$ kg, which compares very well with the average mass determined.
from the \( Q/m \) and charge stepping measurements \((\bar{m}_{\text{FT}} = 4.54 \pm 0.12 \times 10^{-15} \text{ kg})\).

The \( m_{\text{MDR}} \) values are plotted against the \( m_{\text{FT}} \) values for the nine particles in Fig. 7. Fitting the data to a straight line yields \( m_{\text{MDR}} = (1.002 \pm 0.0010) \times m_{\text{FT}} \). The excellent correlation between \( m_{\text{FT}} \) and \( m_{\text{MDR}} \) suggests that the trap parameter \( z_0 = 5.89 \text{ nm} \) determined from the computer simulations is indeed appropriate, and that the effect of any electrode misalignment is small.

There is the possibility that the density of the polystyrene sphere differs slightly from the bulk value because of the incorporation of the dye molecules. If this was the case, the \( m_{\text{MDR}} \) values would be systematically too high or too low. However, one might expect that significant changes in the density would be reflected in changes in the material’s refractive index. The average refractive index value determined from the Mie analysis for the nine particles is \( 1.583 \pm 0.006 \) at 589 nm, very close to the reported refractive index of polystyrene (1.59 at 589 nm; Ref. [191]), suggesting that, despite being impregnated with fluorescent molecules, the density of the microspheres is very similar to that of bulk polystyrene. Furthermore, we observe no correlation between \( m_{\text{FT}} \) or \( m_{\text{MDR}} \) and the refractive index values, which, if present, would imply particle-to-particle density variations.

It is apparent from Fig. 7 that the range of the \( m_{\text{FT}} \) data is \( \sim 4 \) times larger than that of the \( m_{\text{MDR}} \) data. Obviously, the \( m_{\text{FT}} \) spread primarily reflects the uncertainty in the \( m_{\text{FT}} \) measurements rather than resulting from a range of particle sizes, otherwise \( m_{\text{MDR}} \) and \( m_{\text{FT}} \) would have similar spreads.

The main random error in \( m_{\text{FT}} \) is likely to arise from the uncertainty in the absolute charge state assignment which originates from the uncertainty in the \( Q/m \) values. The precision of the \( Q/m \) determination is governed by errors in \( \omega_x, \Omega \) and \( V \) (see Eq. (1)). We estimate that the relative errors in \( \omega_x, \Omega \) and \( V \) are of the order of \( 3.3 \times 10^{-4} \), \( 1 \times 10^{-5} \) and \( 1 \times 10^{-3} \), respectively.

Because \( \Omega \) and \( V \) are fixed during a charge stepping experiment, it is likely that the \( Q \) and \( m_{\text{FT}} \) determinations are affected mainly by random errors in the \( \omega_x \) measurements. To systematically explore the relationship between errors in \( \omega_x \) and in the resulting charge \( Q \) and mass \( m_{\text{FT}} \), we ran a series of simulations on model data for a 2.02 \( \mu \text{m} \) polystyrene sphere. To begin with, the particle was assumed to have undergone a series of seven charge steps corresponding to charge numbers of 450, 453, 454, 456, 457, 458, 461 and 463, which are typical \( Z \) values for the particles characterized in this work. The corresponding \( \omega_x \) values were calculated using Eqs. (1)–(3). These \( \omega_x \) values were then varied randomly over a range determined by an assumed relative error \( (\Delta \omega_x/\omega_x) \). The charge and mass assignment procedure outlined above was then performed using the noisy \( \omega_x \) values to recover the particle’s mass and charge states which, because of the introduced random errors, did not necessarily correspond with the initial assumed charges. For each assumed relative error in \( \omega_x \), this process was repeated 1500 times to gain a statistically significant estimate of the uncertainty in the \( Z \) and \( Q \) and mass \( (m_{\text{FT}})\).

The results of the simulations are displayed in Fig. 8, where the relative uncertainty in the charge state assignment \( (\Delta Z/Z) \) is plotted against the relative error in \( \omega_x \). It is evident that relatively small fractional errors in \( \omega_x \) lead to much larger relative errors in the absolute charge number \( (Z) \) and consequently in the absolute mass \( (m_{\text{FT}}) \).

It is clear from the simulations that, for example, errors in the \( \omega_x \) frequency measurements on the order of the experimental frequency resolution (0.005 Hz, corresponding to a fractional error of \( 3.3 \times 10^{-4} \)), leads to uncertainties in the charge and mass of 1–2\%, comparable with the observed 2.6\% spread in the \( m_{\text{FT}} \).
values for the nine particles. The fluctuations in \( Q \) and \( V \) during a series of secular frequency measurements should be much less than their absolute uncertainties (and to the uncertainty in \( \omega_x \)) and therefore are not expected to contribute significantly to the precision of \( Q \). They may, however, have a small effect on the overall accuracy of \( m_{\text{FT}} \). The presence of surface charges on the electrodes may also have an effect on the measured \( \omega_x \) values, thereby influencing the accuracy and precision of the \( m_{\text{FT}} \) determinations. In this regard, it is worth recalling that the charge state changes were effected by UV light from a pulsed Xe lamp, a fraction of which would have inevitably impinged on the electrode surfaces. While it is difficult to quantify the surface charge effects, they may account for part of the 2.6% spread in the \( m_{\text{FT}} \) data for the nine particles.

There are several improvements that could be made in the mass calibration procedure, particularly relating to the determination of the particles’ absolute mass and charge from measurements of their secular frequencies in the trap. Most significantly, the \( m/Q \) determinations would be more accurate for smaller particles with lower charges for which an incremental charge change would lead to a larger frequency shift. The difficulty is that a smaller particle would exhibit fewer MDR’s in the range of the dye emission profile, reducing the accuracy of the radius determined from the Mie analysis. However, this limitation could be overcome by using UV light to irradiate particles doped with appropriate fluorophores and monitoring emission in the 300–400 nm range.

4. Summary

A calibration procedure for a microparticle QIT-MS is described. The procedure combines MDR enhanced fluorescence spectroscopy with single particle frequency measurements and charge stepping. The average masses obtained for nine particles using each technique are in excellent agreement with the expected results.

\[
\langle m_{\text{MDR}} \rangle = 4.55 \pm 0.03 \times 10^{-15} \text{ kg and } \langle m_{\text{FT}} \rangle = 4.54 \pm 0.12 \times 10^{-15} \text{ kg}.
\]

This suggests that the trap geometrical parameter \( z_0 = 5.89 \text{ mm} \) obtained by computer simulation is appropriate. The large relative errors of the absolute charges and masses determined from secular frequency measurements could be reduced in the future by studying smaller particles.

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