LASER-BASED DIAGNOSTIC TECHNIQUES IN SINGLE PARTICLE ANALYSIS: APPLICATIONS TO AMBIENT AEROSOL CHARACTERIZATION AND CANCER CELL DETECTION

By

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To my husband, Chris; and my daughter, Quin.
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<td>Atomic absorption spectroscopy</td>
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<td>AES</td>
<td>Atomic emission spectroscopy</td>
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<td>AFS</td>
<td>Atomic fluorescence spectroscopy</td>
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<td>CCD</td>
<td>Charge coupled device</td>
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<td>CEC</td>
<td>Circulating epithelial cell</td>
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<td>CHC</td>
<td>Chlorinated hydrocarbon</td>
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<td>CTC</td>
<td>Circulating tumor cell</td>
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<td>DMPS</td>
<td>Differential mobility particle sizer</td>
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<td>EEP</td>
<td>Ethyl-3-ethoxypropionate</td>
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<td>FCAS</td>
<td>Fluorescence-activated cell sorting</td>
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<td>FCM</td>
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<td>HEPA</td>
<td>High efficiency particle air</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>ICP-AES</td>
<td>Inductively-coupled plasma atomic emission spectroscopy</td>
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<td>LA-ICP-MS</td>
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<td>LA-ICP-OED</td>
<td>Laser ablation inductively-coupled plasma optical emission spectrometry</td>
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<td>LIBS</td>
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<td>Laser-induced plasma</td>
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<td>LTE</td>
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<td>LMMS</td>
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<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
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<td>MD</td>
<td>Molecular dynamics</td>
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<td>Abbreviation</td>
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<td>NAD(P)H</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
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<td>OTC</td>
<td>Occult tumor cell</td>
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<td>PDA</td>
<td>Photodiode array</td>
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<td>PE</td>
<td>Prompt emission</td>
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<td>PF/FD</td>
<td>Photofragmentation/fragment detection</td>
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<td>PI</td>
<td>Photoionization</td>
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<tr>
<td>PLD</td>
<td>Pulsed laser deposition</td>
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<td>PMT</td>
<td>Photomultiplier tube</td>
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<tr>
<td>PPI</td>
<td>Prompt photoionization</td>
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<tr>
<td>REIS</td>
<td>Rare event imaging system</td>
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<tr>
<td>RTE</td>
<td>Radiative transfer equation</td>
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<tr>
<td>SE</td>
<td>Stimulated emission</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<td>VOC</td>
<td>Volatile organic compound</td>
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Up until relatively recently, most analysis techniques have relied on obtaining the bulk characteristics of a system of interest, metaphorically focusing on understanding the forest with little concern for individual trees. Increasingly it has become apparent that in many applications such an approach is inadequate, as the entire system may be more accurately characterized by a small portion of its components, even to the level of single particles, whose relative importance to the whole may be grossly underestimated by statistical averaging with bulk properties. The detection limits required for such an analysis combined with the frequent need for real-time, in situ data makes optical techniques uniquely suited for many of these applications.

In the present study, three spectroscopic methodologies are investigated for the analysis of single particles entrained in fluids. In the first component, a laser-induced plasma interacting with a single aerosol particle is numerically modeled to consider the effects of finite particle evaporation and diffusion rates on measured parameters. Although these processes have previously been considered to occur instantaneously for applications with Laser-Induced Breakdown Spectroscopy (LIBS) and therefore not influence the observed signal, this investigation shows that the local properties surrounding the aerosol particle-derived analyte vary from the bulk condition and thereby emphasizes the need for care to be taken in data
acquisition timing and interpretation. In particular, finite scales of heat and mass transfer directly impact the analyte response. In the second investigation, Laser Photofragmentation/Fragment Detection (PF/FD) is considered as an alternative method of aerosol analysis with particular emphasis on application to studying organic species and heterogeneous aerosol chemistry. PF/FD is experimentally shown to be capable of detecting both sodium aerosols and a representative vaporous solvent (ethyl-3-ethoxypropionate, EEP) as well as characterizing their interaction. In addition, oxygen is found to be a quenching species for the signal emitted from the vaporous EEP, underlying the importance of considering the carrier gas composition when using this analysis technique. Finally, flow cytometry (FCM) is investigated for potential application to the detection of circulating tumor cells (CTCs), namely, rogue cells present within the bloodstream of cancer or pre-cancer patients. As part of this investigation, an experimental test rig was constructed and demonstrated proof-of-concept detection of test particles in deionized water solution. Measurements of particles in a static environment confirmed that the variation in signal as a result of spatial variation within the sample tube was negligible, as was the effect of containing the particles within suspension. The optimal concentration of particles for detection by the system was statistically calculated along with the variation in percentage of particles illuminated as a function of both laser frequency and volumetric flow rate and compared to experimental results.
CHAPTER 1
INTRODUCTION

Overview

Do the needs of the many outweigh the needs of the few, or the one? While this question may be debated by philosophers and Trekkies alike for generations to come, in many scientific fields it is becoming clear that although valuable information can be gained by measuring bulk characteristics, it is equally important to quantify and understand the components making up the whole, sometimes down to the level of a single particle. Optical analysis tools based on laser diagnostics are uniquely suited to provide this knowledge: lasers can be focused down to a small sampling volume; analysis is rapid (in many cases real time) allowing for a particle-by-particle analysis of large systems that would be impractical to process using other means; and although the results are highly specific and accurate they can be applied to a broad range of analytes in solid, liquid, and gaseous form. The focus of this thesis will be on the use of three forms of laser-based diagnostics (laser-induced breakdown spectroscopy, photofragmentation/fragment detection, and flow cytometry) in single-particle analysis with application to aerosol systems and particles in suspension, including the detection of cancer-indicative circulating tumor cells and ambient air particulate matter. In this introduction to the research, a brief description of the use of lasers in analytical chemistry is provided along with a background into aerosols and circulating tumor cells and the motivation for study. A more detailed description of each of the three techniques is also provided along with a review of the relevant literature in each field and the objectives of the present work.

Use of Lasers in Atomic Spectroscopy

Each of the three techniques discussed in this thesis fall under the broad category of atomic/molecular spectroscopy, a series of analysis tools based on how matter responds when
subjected to incident electromagnetic radiation (Sneddon et al., 1997). The three subsets of atomic/molecular spectroscopy are:

- Atomic Absorption Spectrometry (AAS)
- Atomic Emission Spectrometry (AES)
- Atomic Fluorescence Spectrometry (AFS)

In AAS, light of a particular wavelength ($\lambda$) is imposed upon some species of interest. The wavelength of light is chosen such that it will excite the ground state atoms to a higher energy level, thus causing the intensity of the incident light to be attenuated. The amount of light absorbed by the sample will vary based on the Einstein probability of absorption and the energy difference between the ground and upper energy state (characteristics of the particular species and the incident wavelength of light) and the number of atoms per volume through which the light travels. By measuring the light incident upon and transmitted through a sample volume, the concentration of a species of interest can be determined (Sneddon et al., 1997). Although AAS has many useful applications, it is not included as part of this study and is not further discussed herein.

Whereas AAS relies upon the excitation of species to higher energy levels for analysis, AES is based upon the opposite process, the emission of light from atoms excited to a higher energy state as they transition back to the ground state. In the case of AES, the energy provided to excite the atoms to upper levels is not wavelength specific, with sources including flames, arcs, sparks, and various types of plasmas including inductively coupled plasmas, direct current plasma, and microwave plasmas (Sneddon et al., 1997). Laser-induced breakdown spectroscopy (LIBS), one of the analysis tools used in this study, is a method of AES where the plasma is created with a highly focused energetic laser beam; the details of LIBS are discussed in a later section. Although there are several photofragmentation/fragment detection (PF/FD) schemes,
the type of analysis used in the present study wherein excess laser energy results in prompt emission of radiation from resultant fragments is also a form of emission spectroscopy.

The last category of atomic spectroscopy, AFS, is similar to AAS in that the excitation source for the atoms or molecules is light of a particular wavelength chosen to lie specifically within an absorption band of the species of interest. Once excited, the species may subsequently undergo deexcitation via fluorescence in which light of a characteristic wavelength for the species is emitted. In AFS, this emitted light is measured allowing identification of the species of interest within the unknown target sample (Sneddon et al., 1997). Flow cytometry (FCM), the final laser-based diagnostic considered in this study, is a form of AFS. Although this technique will be described later in this thesis, flow cytometry is in broad terms the application of AFS in the biological and medical community for the detection of specific types of cells or organisms within fluid suspension (such as bloodborne species), with the targeted samples either possessing natural absorption/emission bands accessible by available lasers or, more commonly, with the targeted samples marked with a fluorescent dye for detection (Carter and Meyer, 1994).

**Aerosols: Characteristics and Motivations for Study**

The definition of an aerosol is solid or liquid particulate matter suspended within a gas; although technically “aerosol” refers to the entire system (particles and gas), common use of the term has made “aerosol” synonymous with the particulate matter itself (Suess and Prather, 1999). Because of the inherent negative effect that an overabundance of aerosols can have on the human population, the history of observation and study of particulate matter in the atmosphere is very long: ancient Romans noted and complained of the air quality in their city, and as early as 1273 government regulation to limit particulate matter was instituted when coal burning was banned in London to reduce air pollution (Spurny, 1999). Over the years, increasing amounts of anthropogenic aerosols combined with focused population centers within cities have made the
problem of particulate pollution more serious, with some of the most catastrophic events occurring in this century. In the London smog episode of 1952, over 4000 people died, and although technology at the time prohibited the exact identification of the pollutant responsible, the correlation between deaths and concentration of particulate matter in the air is qualitatively strong (Figure 1-1).

Particulate matter within the atmosphere comes from a variety of sources, both natural and anthropogenic (Table 1-1). Although the vast majority of aerosols come from natural sources and therefore are key to the understanding of the role of particulate matter in environmental processes such as radiative forcing, anthropogenic sources of aerosols tend to be focused in large population centers where local effects on human health and environment are felt the strongest, such as in the 1952 London smog event.

Although aerosols can have a very direct effect on humans through respiratory effects and air quality concerns, they also have a large impact on the environment and energy budget of the planet itself. Because the size of many particles is approximately equal to the wavelength of visible light, Mie scattering results in radiation from the sun being reflected back into space resulting in an overall cooling of the Earth’s surface in a phenomena known as the Whitehouse Effect (Finlayson-Pitts and Pitts, 2000). In the case of larger aerosols such as those originating from volcanic eruptions, longer wavelength radiation can be backscattered to the Earth’s surface, in this case having a localized warming effect (Finlayson-Pitts and Pitts, 2000). In addition to scattering effects, aerosol particles can have a direct impact on the radiation budget of the Earth via absorption. Carbonaceous particles absorb incident solar radiation and convert it to heat, and dust particles absorb infrared radiation coming off the planet’s surface before it can escape into space, both of which result in overall heating of the atmosphere (Finlayson-Pitts and Pitts, 2000).
Because of the various heating and cooling effects aerosols can have on the radiation budget, the overall effect for any given aerosol in any given location on Earth is a product of its individual properties (Figure 1-2).

In addition to the direct effects aerosol particles have on the radiation budget and atmospheric composition of the Earth, several indirect effects are also observed. Small particles act as cloud condensation nuclei and can also effect cloud lifetime; like aerosols, clouds can result in either shortwave radiation being reflected back into space or longwave radiation being trapped within the atmosphere (Finlayson-Pitts and Pitts, 2000). In addition to cloud formation, many aerosol particles are involved in heterogenous reactions within the atmosphere. In this process, gases adsorb onto the solid or liquid surfaces and undergo complex reactions either with the aerosol species themselves or with other gaseous species with the particulate matter acting as a reaction site and in some cases a catalyst (Ravishankara, 1999). Although these processes are poorly understood and the effects on the atmosphere are difficult to quantify at the present time, research indicates these processes may have a significant effect on local atmospheric chemistry (Ravishankara, 1999; Oh and Andino, 2002). NaCl in particular, the aerosol of interest in the present study, has been shown to participate in heterogenous reactions with atmospheric species including HNO₃, H₂SO₄, and ClONO₂ to produce gaseous hydrochloric acid and chlorine gas (Hemminger, 1999).

Given the large effect aerosols have on both the environment and human health, it is vital for techniques to be developed that can categorize both physical parameters such as size along with chemical composition. The two diagnostics considered for application to aerosol analysis within this study, LIBS and PF/FD, allow for rapid chemical analysis of single particles and
provide information that can be used in conjunction with bulk analysis techniques for more complete characterization of aerosol samples (De Bock and Van Grieken, 1999).

**Circulating Tumor Cells and Diagnosis/Prognosis of Cancer**

Cancer is a broad category of diseases wherein abnormal cells begin to grow within some portion of the body, often rapidly multiplying and spreading throughout the patient (American Cancer Society, 2007). Although certain behaviors such as smoking and a sedentary lifestyle as well as infections such as hepatitis B and the human immunodeficiency virus (HIV) can increase the risk of developing certain types of cancer, anyone of any age can be stricken with some form of the disease. As of 2003, 10.5 million Americans were alive who had been affected by cancer at some point in their lives, and it was projected that in 2007 close to 1.5 million new cases would be diagnosed; close to 600,000 people are expected to die from the disease in the same year (American Cancer Society, 2007). Because of the widespread and catastrophic nature of this illness, it is imperative for advances to be made in the diagnosis and treatment of cancer.

Although the number of cancer cases is on the rise, the survival rate for the disease has improved in recent years, up from approximately 51% in the period of 1975-1977 to 66% for the period of 1996-2002 (American Cancer Society, 2007). One of the principle reasons behind this relatively dramatic improvement in prognosis is the advancement of early detection methods that allow many forms of cancer to be caught and brought into remission while treatment methods are most effective (American Cancer Society, 2007B). Mortality rates for colon, rectal, uterine, and breast cancer, all of which currently have clinical early diagnosis screens, have shown some of the largest decline in recent years, and in the case of some cervical and colorectal cancers the identification of precancerous abnormalities can result in effective treatment to eliminate cancer from developing (American Cancer Society, 2007B).
One of the more recent developments in early cancer detection has been in identifying and characterizing circulating tumor cells (CTCs), also referred to as circulating epithelial cells (CECs) or occult tumor cells (OTCs). CTCs develop within solid cancers as epithelial (skin) cells and enter the bloodstream of the patient where they can be distinguished from normal blood cells by characteristic surface proteins (Beckman, 2006). In addition to their potential for use in diagnostics, CTCs are also suspected as the vehicle of cancer metastasizing in other areas of the body, one of the primary causes of cancer mortality (Pachmann et al., 2005). By identifying and quantifying these cells within the bloodstream of patients already diagnosed with cancer the effectiveness of the current treatment can be assessed and improvements can be made to better the prognosis for the individual (Ghossein and Rosai, 1996). Research has also found that CTCs are present within the bloodstream of patients with cancer in remission, and it is hypothesized that increased knowledge of these cells will allow for enhanced understanding of the mechanisms of cancer dormancy (Meng et al., 2004).

The problem in detecting CTCs lies in their very low concentration compared to normal cells even within a cancer-stricken patient. For example, any given individual may have as few as a thousand circulating within their bloodstream (Beckman, 2006). Because CTCs are so diluted with normal cells, methods of detection must be both very sensitive and extremely accurate to avoid false positives. Initial attempts to detect CTCs were very successful in that they were detected in as many as 96% of cancer patients; however, these results were soon discounted when it was discovered that circulating marrow elements (normal components in human blood) were also being detected by the early techniques (Ghossein and Rosai, 1995). It has only been the advancement of methods capable of single particle (e.g., single cell) analysis
such as flow cytometry that make CTC detection a viable candidate for diagnosis and prognosis of cancer.

**Numerical Modeling of Laser Induced Breakdown Spectroscopy (LIBS) Plasma**

Laser-induced breakdown spectroscopy (LIBS) is an elemental composition analysis that has been used to study solid, liquid, and gaseous analytes (Radziemski and Cremers, 1989; Winefordner et al., 2004), and has more recently been examined with the goal of understanding analyte interactions for aerosol systems (Hohreiter and Hahn, 2006; Diwakar et al., 2007; Lithgow and Buckley, 2005; Gornushkin et al., 2004; Hahn and Lunden, 2000). In the present study, a numerical simulation of a LIBS plasma interacting with an aerosol particle will be developed with the specific goal of understanding the effect finite evaporation and diffusion times have on the resultant analytical signal. An overview of the fundamentals of LIBS is provided as well as a survey of relevant research into modeling efforts in the field.

**Fundamentals of LIBS**

As previously mentioned, LIBS is a form of atomic emission spectroscopy, a broad category of analysis tools that rely on the unique optical features individual elements emit when decaying from an excited state (Cremers and Radziemski, 2006; Radziemski, 2002; Winefordner et al., 2004). In all forms of AES, an analyte is vaporized, atomized, and generally ionized, yielding a mix of neutral atoms and ions with which an energy source stimulates the resultant species to an unstable excited state. As excited species decay back to their ground states they emit light of a characteristic wavelength that can be used qualitatively to determine the elemental composition of the original sample, and when intensity is calibrated to molar concentration or mass the signal can also quantitatively resolve the initial analyte composition. In the LIBS technique specifically, a pulsed laser focused down to a small target volume causes the formation of a high-temperature (tens of 1000’s of degrees) plasma and thereby acts as a single source for
excitation as well as vaporization/atomization/ionization (Cremers and Radziemski, 2006). In this case, the early emission from the plasma is white light (i.e. broadband continuum) spectral radiation caused by the recombination of free electrons and ions along with bremsstrahlung radiation; however, over time the continuum emission fades allowing for individual atomic spectral features corresponding to constituent species to be discerned.

A basic experimental set-up for LIBS requires a laser as the energy source, focusing/collection optics, and a detection device (Figure 1-3). The only requirement of the laser itself is that it is capable of producing sufficient power during pulsed operation to generate the plasma itself; the most commonly used laser for this purpose is a solid state laser such as an Nd:YAG (Lee et al., 1997). In order to achieve the necessary power, a lens is used to focus the laser beam down to a small volume in which plasma formation occurs. Emitted light is directed back toward some type of detection device, with options including a simple camera, photomultiplier tube (PMT), photodiode array (PDA), or charge transfer device such as a charge-coupled device (CCD). Because the LIBS signal is time variant, a gating mechanism is required to obtain a signal integrated over a specific length of time at an optimal delay interval (Lee et al., 1997; Cremers and Radziemski, 2006).

Like all forms of AES, LIBS presents advantages over chemical digestion forms of species analysis in that it can distinguish all elements and can detect multiple elements in the same sample simultaneously (Cremers and Radziemski, 2006). In addition, LIBS goes beyond other AES techniques in that analysis is performed in real time with virtually no sample preparation (in many cases in situ), it has high sensitivity, and is applicable to all phases of matter. Because of these unique advantages, LIBS has been applied over an ever widening range of applications, including hazardous emissions detection, environmental monitoring, sorting of industrial
materials, and aerosol analysis (Radziemski, 2002). LIBS has even been used to clean historical documents while monitoring composition to ensure no damage to the underlying fibers (Kaminska et al., 2007).

Despite the positives, however, LIBS is not a flawless technique, with one key concern being matrix effects the plasma may have on the emitted signal. Although for quantitative analysis of a given species the resultant signal must be independent of additional elements within the plasma, temperature, and so forth, this situation is not the case within all LIBS plasmas and care must be taken to insure robust conditions. The nature of LIBS also presents a possible disadvantage when analyzing large samples; the focused laser provides a point source analysis of the target’s surface, therefore non-homogeneities within the bulk of the material may be difficult if not impossible to detect (Cremers and Radziemski, 2006). The point-source nature of LIBS becomes an advantage, however, in the case of aerosol analysis. Assuming complete vaporization of the target particle, sample non-homogeneities do not present a concern in this case, and quantitative analysis of particle ensembles as well as individual aerosol particles has been shown to be possible (Hahn and Lunden, 2000).

**Modeling of Laser-Induced Plasmas (LIP)**

Although empirically LIBS analysis of materials in solid and aerosol systems has been quite effective, the theoretical understanding of the governing physics is incomplete. Portions of the fundamental processes of solid/aerosol LIBS such as laser ablation and plasma formation are shared with several practical applications including micromachining, pulsed laser deposition (PLD), and laser surgery, as well as with analytical techniques such as matrix-assisted laser desorption/ionization (MALDI), laser microprobe mass spectrometry (LMMS), and inductively coupled plasma mass spectrometry and optical emission spectrometry (LA-ICP-MS and LA-ICP-OED) (Zhigilei, 2003; Capitelli et al., 2004; Bogaerts et al., 2003). The large body of modeling
work into laser-induced plasmas (LIP) in related fields contains highly relevant insight into the physics of LIBS (Capitelli et al., 2000).

Several steps occur during the formation of a LIP from a solid surface, including (Bogaerts et al., 2003; Capitelli et al., 2004):

- Absorption of incident laser energy by the analyte, resulting in ablation of material,
- Formation of a plume of vaporized material which expands outward into the surrounding background gas and may ionize to form a high density/high temperature plasma,
- Interaction of the plasma with the surrounding gas through heat and mass transfer leading to eventual cooling, species recombination, and the dissipation of the plasma/plume.

Each of these phases is highly complex and not completely understood, therefore virtually all modeling efforts (including the present study) are contained to explaining only a portion of the LIP stages, focusing on one or more of the above described processes. A review of the current body of work is provided with the aim of giving an overview of the broad methodologies and assumptions used in theoretical explanation of laser-induced plasmas to put the present work in context, and is not intended to be an exhaustive survey of all relevant research. As previously mentioned, LIP fundamentals cross a tremendous number of diagnostic techniques and applications and a complete description of all related work is beyond the scope of this thesis.

**Laser ablation of the analyte material**

The physics of laser ablation depends on properties of the incident laser (wavelength, irradiance, pulse length), the ambient environment (gas composition and pressure), and the target material (composition, particle size in the case of aerosols). Models have been developed for various combinations of operating conditions, in general relying upon a macroscopic description of the laser/solid interaction based on the heat conduction equation (Bogaerts et al., 2003). Ho et al. (1995) developed this type of model to describe the ablation of various metals using a ns-pulsed ultraviolet (UV) laser. The incident laser beam was modeled as a circular spot on the
analyte surface with a simplified triangular profile of irradiation with time over the course of each pulse. The formed plume/plasma were assumed to be optically thin, thereby neglecting any energy loss to the plasma and any thermal effects a temperature rise therein would have on the analyte. The reflectivity and absorptivity of the solid govern both the penetration depth and the energy absorbed by the surface; the calculated penetration depth is shallow enough (~10 nm) compared to the modeled beam diameter (mm) that a one-dimensional heat conduction equation could be used to describe heat transfer within the material. An enthalpy formulation (which does not account for the moving phase boundary explicitly) accounted for material phase change. Mass, momentum, and energy conservation were used to model the liquid/vapor transition region as a Knudsen discontinuity layer assuming surface vaporization only, and the resulting vapor was treated as an ideal gas. Using this simplified model, Ho et al. were able to develop the rate of surface temperature increase, maximum surface temperature, melting depth, and ablation depth as a function of laser fluence. They were also able to determine order of magnitude approximations of the vapor speed at the Knudsen layer surface and the vapor/temperature variation within the discontinuity, as well as qualitatively describe the velocity field including the formation of supersonic speeds and shock wave discontinuities.

Although a heat conduction model such as the one previously described is sufficient in the case of ns-lasers incident on metal surfaces when the short energy relaxation time of the material ($10^{-13}$ s) relative to the pulse allows for the conversion of optical energy to heat to be assumed to be instantaneous, this simplification is not valid for ps- and fs-pulsed lasers or for materials with longer energy relaxation times (Bogaerts et al., 2003) or very strong absorption (Zhigilei, 2003). In these cases, a much more computationally expensive microscopic scale model based on the
Boltzmann transport equation (describing electron transport and electron-lattice interactions), a molecular dynamics simulation, or a hybridization of the two is required.

Zeifman et al. (2002) distinguished between two regimes of laser removal of organic solids as determined through molecular dynamics (MD) modeling. If the laser fluence is low enough, the system is in the “desorption” regime and the macroscopic thermal model is a reasonable (computationally simpler) approximation. At some higher fluence, however, an “ablation” threshold is crossed and complexities such as explosive boiling and hydrodynamic motion develop and invalidate the thermal approximation. In their model, they used an MD simulation to describe laser ablation of organic solids based on the breathing sphere model. In this method, every molecule is approximated as a single particle with properties characteristic of an organic material. An extra internal degree of freedom (“breathing mode”) is given to each particle to simulate the process of photon driven molecular excitation and subsequent vibrational relaxation, allowing one to effectively model through control of the characteristic frequency of this mode the conversion of energy absorbed to the translational and internal motion of surrounding molecules. Laser irradiance enters the system as kinetic energy equivalent to the energy of each incident photon being imparted to randomly chosen molecules. Using this type of model, Zeifman et al. were able to capture the ejection of clusters of molecules from non-thermal processes that cannot be modeled using the macroscopic heat conduction approach. Although developed in this case for application to laser ablation of solids, the ability to model these processes is particularly relevant to the development of a complete model of LIBS analysis of aerosols. A variety of materials and particle sizes are analyzed using the LIBS technique, and many may be prone to fragment and explode in non-thermal processes within the incident laser beam (Lushnikov and Negin, 1992).
Expansion of the material plume and plasma formation

Once analyte material has been ablated, the resulting vapor plume (which may ionize and form a plasma) begins to expand outward. Because experimental evidence has shown that in the case of LIBS aerosol analysis spatial variations in analyte concentration exist at the time scales of optical sampling (Simpson et al., 2007; Hohreiter and Hahn, 2006; Diwakar et al., 2007), modeling the temporal progression of this plume is fundamental to a complete understanding of LIBS.

In general, the progression of material from an ablated surface is modeled using one of three broad approaches: development of an analytical solution, use of a fluid dynamic model, or Monte Carlo simulation (Capitelli et al., 2004). Unfortunately most models for plume development describe expansion into a vacuum, an unrealistic environment for many typical LIBS applications (Bogaerts, 2003); however, some work has been done to determine the effects of higher pressure on plume/plasma expansion.

The analytical models fall into several categories. In a complicated simulation of ablation in vacuum, high vapor densities result in the formation of a Knudsen layer through which particles must pass before entering unsteady adiabatic expansion (Capitelli et al., 2004). A more simplified approach to modeling plume expansion in a vacuum can be taken if the density of the vapor is assumed to be low enough that no Knudsen layer forms and particles move directly into free expansion (Capitelli et al., 2004; Arnold et al., 1999). Within an ambient gas, the surrounding pressure may be high enough that an alternative approach of a point-blast model may be realistically applied; however, at moderate pressures an initial stage of free expansion may be followed by the development of internal and external shock waves and explosive expansion (Arnold et al., 1999). An alternative approach was proposed by Arnold et al. (1999), who considered the expansion of a plume of laser-ablated material into ambient pressure by
developing an analytical solution to the transition from free molecular expansion to point-blast regime. The boundary layer of the vapor and the ambient gas is taken to be an impenetrable piston, thus there is no mixing between the two regions; a spherical 1-D geometry is assumed with only radial dependence. Conservation laws for energy, mass, and momentum are developed from the gas dynamic equations using assumed thermodynamic variable profiles. Material reflected inward off the boundary forms a shock wave propagating back toward the ablating surface, whereas the compression of the ambient gas by the expanding plume results in the generation of an outward propagating shock wave. The ability of this model to capture shock behavior is significant, particularly considering the requirements of a LIBS model where shock waves are experimentally observed.

A second approach used in simulating plasma expansion is a fluid dynamic model based on some simplified form of the compressible Navier-Stokes equations for a multi-species gas, often the Euler equations (Capitelli et al., 2004; Casavola et al., 2003). These models have the advantage of providing spatial and temporal resolution of macroscopic parameters such as velocity, density, pressure, and temperature. In order to more fully understand the complicated evolution of the plasma, however, further assumptions are required, and a variety of simplifying strategies have been employed by different researchers.

In order to gain more accurate insight into chemical composition, Colonna et al. (2001) improved upon basic hydrodynamics models that assumed ablated material was chemically inert by coupling one-dimensional time dependent Euler equations with a chemical equilibrium model. Initial results qualitatively compared well with experimental data; however, the authors postulated that deviations were due both to uncertainties in the initial condition parameters input into the model and that the fundamental assumption of local thermodynamic equilibrium (LTE)
is not correct at all times and under all conditions. In order to improve upon this model, the authors coupled their hydrodynamic model with chemical kinetics equations with finite reaction rates and varied initial condition parameters to determine the effect on model output (Casavola et al., 2003).

Many hydrodynamic models assume an optically thin plasma, e.g., no radiation is absorbed or emitted by the system and interaction with the laser and the environment does not need to be considered when applying Euler’s equations. In order to investigate the validity of this assumption Ho et al. (1996) utilized a two-dimensional radiation transport model to calculate a radiation source term for inclusion in the energy balance of the system. The vapor phase was taken as an ideal gas and LTE was assumed so that the Saha equation could be used to calculate the concentration of ionized and neutral particles, a requirement in the calculation of the spectral absorption coefficient. From their work they concluded that significant absorption of laser energy occurs at times within the plasma, to the extreme of the vapor layer being virtually opaque by the end of the laser pulse.

It is important to note, however, that experimental evidence indicates that LTE conditions may not exist at all times of plasma evolution. To investigate the LTE assumption, De Giacomo et al. (2001) developed a state-to-state radiative model to interpret experimental electron number density and atomic/ionic state distribution function data obtained via optical emission spectroscopy in a LIP. They found that at very short times (5-10 ns) LTE conditions exist within the plasma, but that at times of 30-180 ns rapid plasma expansion causes a decrease in electron number density and a corresponding increase in radiative processes such as spontaneous emission causing deviation from LTE. At longer times out to a delay of 300 ns (the limit of their experiment) they found re-absorption and stimulated emission played a larger role, canceling the
radiative effects leading to deviations from LTE and restoring ionic/atomic temperature
equilibrium. The validity of the LTE assumption in LIBS applications in particular is, however,
still a matter of debate. Capitelli et al. (2000) summarize several experimental and theoretical
investigations of LIBS plasmas assuming both equilibrium and non-equilibrium conditions and
conclude that although temperatures and free electron densities typically observed in LIBS
should satisfy LTE conditions the characteristic time scale of radiative processes and the time
required to obtain pressure equilibrium during rapid plasma expansion may create non-LTE
conditions.

Although there is much knowledge to be gained from hydrodynamic models of
plasma/plume expansion, gas-dynamic models assume that ablated particles follow a Maxwellian
velocity distribution and undergo frequent collisions; therefore, this type of model may fail in
less dense plumes or those with strong density gradients (Capitelli et al., 2004; Itina et al., 2003).
Furthermore, a continuous hydrodynamics model does not capture well the diffusion of ablated
material within the ambient gas. In answer to these shortcomings, other researchers have taken
the alternate approach of particle modeling using Direct Monte Carlo simulation. Using this
approach, Itina et al. (1997) were able to model on a particle level the interaction of ablated
material with surrounding gas molecules and determine that momentum transfer can result in
ablated particles recoiling back to the surface and in some cases re-condensing. Because of the
inability of Monte Carlo simulation to handle dense flows, the authors further expanded their
work to develop a hybrid model (Itina et al., 2003). In this case, a hydrodynamics model was
used during the initial phase of plasma expansion with the output fed to a Direct Monte Carlo
simulation during later times when particle concentration is low in order to capture mass
diffusion and heat exchange within the plume. Although this method did allow for simulation of
the interaction of ablated material with the ambient gas and captured the formation of a shock wave, heat and mass exchange between the plasma and the environment was neglected.

**Plasma decay through interaction with the environment**

Each of the described models contributes in some way to the overall understanding of laser-induced plasmas; however, much of the focus is on the interaction of the laser with the analyte material and plume dynamics during time scales concurrent with the laser pulse. Although understanding these processes is key to unraveling the fundamental physics, the time scales of measurement of LIBS as well as the related analytical technique of LA-ICP-MS require simulations be extended to after the termination of the laser pulse when the plasma has begun to cool and the continuum emission subsides enough for spectral characteristics to be distinguished for species identification. In addition, it is critical for a complete LIBS model to simulate the radiative emission of a plasma given that the analytical analysis relies upon optical data for species identification and quantification.

To meet the above criterion of LIBS, Gornushkin et al. (2001) developed a semi-empirical model for a LIP designed to simulate the radiative emission that would be observed from a plasma with a given temperature distribution and species composition. An approximate solution to the radiative transfer equation (RTE) valid for non-resonant atomic transitions was obtained allowing calculation of both continuum and spectral emission for a two-species plasma, assuming the plasma met LTE conditions, contained static chemical composition, and considering only the first ionization state of each species. The model is unique from previous work in that in calculating spectral intensity the re-absorption of emitted radiation by the plasma itself was taken into account as opposed to assuming it to be optically thin. The model was limited, however, in that it relied heavily on approximations for physical parameters based on
experimentally observed data, including temperature profile characteristics, plasma expansion speed, stoichiometry of the component species, and the total number of atoms within the plume.

The same researchers have gone on to expand the model by simulating temperature, plume expansion, and velocity fields through the cooling phase of the plasma by solving mass, momentum, and energy conservation equations, hence the model’s reliance on empirical data is thereby reduced to only the initial conditions of temperature profile and composition (Gornushkin et al., 2004). LTE and static composition is still assumed. The model was further enhanced to solve the RTE for the general case and thus account for both resonant and non-resonant atomic transitions, to include multiple ionization of component species, to account for instrument parameters in experimental data used for comparison, and to allow the inverse problem to be solved whereby the physical parameters of temperature and free electron density of a plasma could be determined given experimentally measured optical values (Gornushkin et al., 2005). Verification of model results with experimental data from LIBS applied to a solid metal surface showed good comparison, although some deviations were observed, particularly for doubly ionized species. However, the model does fail to account for some physically observed occurrences such as shock waves.

**Challenges Specific to Modeling LIBS for Aerosol Analysis**

In addition to the fundamental understanding of laser-induced plasmas (LIP) required for the theoretical characterization of LIBS, unique challenges present themselves in regard to modeling the technique in the analysis of aerosols. Energy from the incident laser pulse must vaporize the particles within the target sample volume and subsequently atomize and ionize the resultant gaseous species in order for a signal to be observed; aerosol particles may undergo surface melting and vaporization due to heating or may first fractionate and explode (Lushnikov and Negin, 1993). If any of these processes is incomplete, quantitative correlation of the
resultant signal to the initial concentration of analyte is impossible. In addition to original particle size and composition the processes of vaporization, atomization, and ionization also depend upon the temperature and free electron density of the plasma itself (Diwakar et al., 2007). These properties exhibit spatial gradients within the plasma volume as well as variation with time over the plasma lifetime, meaning that the location of an aerosol particle within the sample space will determine local condition (Simpson et al., 2007). As a further complication, although bulk plasma properties are not effected by the presence of particles within the target volume, it has been determined that the aerosol itself may impact the surrounding local conditions by absorbing energy during vaporization (Hohreiter and Hahn, 2006; Diwakar et al., 2007).

One of the primary concerns of both empirical and theoretical studies of LIBS for aerosol analysis is ensuring accurate correlation of a measured signal to the original concentration of a target element within the particle stream. For a quantitative LIBS signal to be robust, matrix effects must be negligible, meaning that the acquired signal is a function only of analyte concentration and is not altered by changes in plasma condition or the presence of other species (Hohreiter and Hahn, 2006). In order to account for matrix effects, aerosol signals may be calibrated with the use of the ion/neutral emission ratio for a given element; specifically, the relative concentrations of ions and neutrals and subsequently the ratio of emitted intensity is a function of plasma temperature and free electron density in accordance with the Saha equation if LTE conditions exist (Radziemski and Cremers, 1989). In general it is assumed for model simplification that the time scales of the particle processes of melting, evaporation, dissociation, and atomic diffusion throughout the plasma volume are short enough to be neglected, therefore, analyte species concentration can be assumed uniform and the measured intensity values as described above are a function of bulk plasma condition (Hohreiter and Hahn, 2006; Gornushkin
et al., 2004). Recent experimental evidence, however, has shown that dissociation of particles occurs on finite time scales relative to the delay times at which LIBS intensity data are typically recorded, meaning that spatial inhomogeneities exist during the measurement period and the ratio of analyte signals, including ions-to-neutrals, is therefore a function of localized temperature and free electron density (Hohreiter and Hahn, 2006; Diwakar et al., 2007; Lithgow and Buckley, 2005).

Plasma matrix effects are also a concern in a related analytical technique, namely inductively-coupled plasma atomic emission spectroscopy (ICP-AES) as well as laser ablation inductively-coupled plasma mass spectroscopy (LA-ICP-MS) (Mermet, 1998; Lehn et al., 2002; Guillong and Gunther, 2002; Bian et al., 2006). To better understand and control such effects, Tognoni et al. (2007) have developed an alternative diagnostic to ascertain the robustness of the analytic plasma. By taking the ratio between the ion/neutral ratios of two different species, the dependence on free electron density is removed, and the metric becomes a function only of plasma temperature. Inversely, using the measured ion/neutral intensity values for two species, the temperature can be calculated (Tognani et al., 2007). Within this model, local thermal equilibrium (LTE) is assumed, and the plasma temperature is assumed to be uniform. As was previously discussed, however, particles in a laser-induced plasma dissociate and analyte species diffuse through the plasma volume at finite time scales; subsequently, various concentrations of each species are present at differing temperatures as the plasma cools non-uniformly.

**Objectives of the Present Work**

In the present study, a numerical model will be used to develop the temporal evolution of the temperature profile and distribution of analyte species throughout the plasma volume using finite time scales for particle evaporation/dissociation and atomic diffusion. A parametric study on the effect of various initial conditions such as temperature profile and particle
size/composition will be performed along with an investigation of model simplifications such as
the assumption of single ionization of species and neglecting the effect of re-absorption of
radiation by the plasma. These effects will be explored in terms of the evolution of the
temperature and species concentration fields. In addition, applicability of the Tognoni ratio as a
diagnostic for aerosol LIBS analysis will be assessed. In particular, the limitations of the single
ionization state assumption is assessed at the relatively high plasma temperatures characteristic
of laser-induced plasmas, notably at early times.

**Laser Photofragmentation/Fragment Detection (PF/FD) of Aerosol Particles**

Laser Photofragmentation/Fragment Detection (PF/FD) is another spectroscopic detection
scheme used to evaluate the chemical composition of both gaseous and particulate analyte
species. Because of the ability to simultaneously analyze both phases, PF/FD is uniquely suited
for the study of aerosol systems where the particles themselves, the carrier gas, and the
interaction between the component species within both are of research interest. In the present
study, PF/FD will be applied to an aerosol system consisting of sodium chloride (NaCl) particles
suspended in nitrogen/oxygen mixtures of various composition percentages along with vaporous
ethyl-3 ethoxypropionate (EEP), a known environmental contaminant representative of industrial
solvents and unburned hydrocarbons. An overview of the fundamentals of PF/FD is provided
along with a survey of research into applying PF/FD for the quantification of hydrocarbons and
halogen species in both gaseous and aerosol form.

**Fundamentals of Photofragmentation/Fragment Detection**

In the PF/FD technique, a pulsed laser is used to photolyze analyte species thereby
breaking them down into component fragments (Rodgers et al., 1980; Simeonsson and Sausa,
1996; Simeonsson and Sausa, 1998). The wavelength of the incident laser must overlap with an
absorption feature of the target molecule and its energy after focusing must be sufficient to
dissociate the target molecule, but unlike in the case of LIBS there is insufficient energy to cause plasma formation nor to completely atomize all molecules within the target volume (Simeonsson and Sausa, 1996; Rodgers et al., 1980). This characteristic allows some molecular information (i.e. structure) to be retained and facilitates the identification of large, complicated species (such as organic hydrocarbons) for which basic atomic data may be insufficient for robust identification (Monterola, 2007).

Once target molecules have been photolyzed, the resultant fragments are detected using one of several optical techniques, including (Simeonsson and Sausa, 1998):

- Laser-induced fluorescence (LIF)
- Photoionization (PI)
- Laser ionization recombination emission (LIRE)
- Stimulated emission (SE)
- Prompt photoionization or prompt emission (PPI or PE)

In case of the first four detection techniques, a second laser pulse is required to cause optical emission by the photofragments and thus allow their detection, although depending on the absorption characteristics of these molecules the wavelength of this pulse may be coincident with the photofragmenting wavelength and thus the same laser can be used for both processes. In the case of prompt emission, however, sufficient excess energy exists within the molecular fragments to leave them in an excited state which decays and spontaneously emits light characteristic of the species (Simeonsson and Sausa, 1998). In the present study PF/FD with prompt emission is used as the analysis technique. In simple molecules with bond energies sufficiently low enough to allow dissociation, excess energy is stored in the form of exciting one or more electrons to a higher electronic state, which subsequently emits light at a wavelength unique to that molecule or atom for the transition back to a lower or ground state (Figure 1-3). In the case of NaCl, one of the target species of the present study, it is the sodium atom that is left
in an excited state and subsequently emits light at the characteristic wavelengths of 589.0 nm and 589.6 nm (Oldenborg and Baugheum, 1986). For more complex molecules (Figure 1-3), the parent molecule may fragment into smaller molecules rather than atomic species, with excess energy absorbed into one or more of the remaining bonds which subsequently emits light at a wavelength characteristic of the overall molecular structure. In the case of EEP, both CH and C2 molecules are observed following photofragmentation with a 193 nm excimer laser.

Like most laser spectrometric techniques (including the aforementioned LIBS technology), PF/FD offers significant advantages over traditional off-line methods of chemical analysis in that it provides nearly instantaneous results for even trace amounts of analyte species with a high degree of species differentiation (Simeonsson and Sausa, 1996; Simeonsson and Sausa, 1998). PF/FD is particularly useful for analyzing molecules unsuited for detection with other, direct methods of spectroscopic analysis; for example, PF/FD is applicable to molecules with weak optical transitions or broad spectral features when probed using direction detection methods such as laser induced fluorescence (LIF), photoionization, and absorption (Simeonsson and Sausa, 1996; Simeonsson and Sausa, 1998). Although the analyte itself may contain poorly defined spectral features, the photofragmentation products are smaller molecules or atoms generally more easily identified and as such can be used to reconstruct the parent species.

Previous Applications of PF/FD

PF/FD has been applied to analyzing a wide variety of different systems, including for gas phase analysis monitoring of NaCl in coal plant exhaust to reduce fouling (Chadwick et al., 1995; Chadwick et al., 1997), quantification of lead (in both particulate and vaporous form) as an environmental pollutant (Buckley et al., 2002), detection of the photochemical smog/acid rain contributing species NO and NO2 (Simeonsson et al., 1999), and analysis of atmospheric ammonia (Halpern et al., 1979; Buckley et al., 1998; Haak and Stuhl, 1984; Schendel et al.,
Photofragmentation has also been used for the detection and quantification of many types of particulate species, such as the air pollutants ammonium nitrate and ammonium sulfate (Damm et al., 2001), carbonaceous particles and soot generated by combustion (Damm et al., 2001; Stipe et al., 2005), and a variety of sodium containing aerosol particles (Hidalgo Núñez et al., 2000; Hidalgo Núñez and Omenetto, 2001; Choi et al., 2005).

A review of various research efforts into the use of PF/FD to analyze both solid and particulate matter is provided, with specific focus on studies aimed at detecting and quantifying species related to the molecules of interest in the present study, sodium chloride and EEP (a complex hydrocarbon).

**Analysis of gaseous species**

Use of the PF/FD technique for practical application of vaporous alkali analysis was carried out by Oldenborg and Baugheum (1986). The authors note that UV irradiation of alkalis and subsequent detection of emitted fluorescence is one of the oldest types of PF/FD, with results reported within the literature as early as the 1920’s. Alkali species are found in many veins of coal deposits, particularly domestic sources, and when vaporized in the power generation process they can cause extensive corrosion to turbine blades and components when present in even trace amounts. The authors investigated the use of a 193 nm excimer laser (the same type laser used in the present study) for prompt emission PF/FD analysis of a variety of alkali species, including NaCl, NaOH, NaO, Na₂O, Na₂SO₄, KCl, KOH, KO, K₂O, KO₂, and K₂SO₄. They determined that the different emission wavelengths of species with different alkali components (e.g., Na vs. K) are easily distinguished by the characteristic emission wavelengths (589 nm for Na*, 766 nm for K*), but that by varying the wavelength of the fragmenting laser the different dissociation threshold energies for each molecule can be employed to distinguish between molecules with the same alkali but a different anion. The authors further determined that the wavelength of the
fragmenting laser must be less than that corresponding to the threshold energy of photodissociation of a given molecule for the process to be temperature independent; at wavelength longer than the threshold value some photodissociation may still occur but with a strong temperature dependence.

Chadwick et al. (1995) further considered the use of PF/FD for the detection and quantification of vaporous sodium species. The authors generated gaseous mixtures designed to emulate the exhaust stream of a coal plant and introduced known quantities of NaCl and NaOH. A 193 nm excimer laser was then used to photofragment the sodium species, which retained enough energy to be in the $3^2P$ excited state and subsequently fluoresce at the characteristic wavelengths of 589 and 589.6 nm. Although the emission of these wavelengths unequivocally identifies the presence of sodium, the authors make use of the molecular dissociation process inherent in PF/FD to further distinguish between the two target species. Whereas the photodissociation of NaCl requires a threshold energy of 145.9 kcal/mol (corresponding to an incident fragmenting wavelength of less than 195.9 nm), the photodissociation of NaOH requires 130.0 kcal/mol (219.8 nm). 193 nm incident light has enough excess energy to leave sodium from both molecules in the $3^2P$ state, but sodium fragments from NaOH may also be left in the $3^2D$, $4^2P$, and $4^2S$ electronic states which fluoresce at their own unique wavelengths. By monitoring the wavelength corresponding to the $3^2D \rightarrow 3^2P$ transition at 819 nm the authors were able to discern the presence and concentration of NaOH, and subsequently by assuming all of the sodium in the sample is coming from one of the two target species, the NaOH concentration is subtracted from the total concentration of sodium to determine the concentration of NaCl specifically. The authors are able to determine the presence of NaCl down to a detection limit of 0.1 ppb, with an upper limit of linear response of intensity to concentration at 20 ppm, above
which radiative trapping begins to inhibit the signal. It is noted within the work that quenching by other species within the gas stream can be significant, which the authors correct for by calibrating the intensity to concentration signal with a carrier gas with the same concentration of non-sodium species as the testing sample. In a later paper (Chadwick and Morrison, 1995) the same authors go on to use Monte Carlo simulation to model the trapping/quenching phenomena and are thus able to make theoretical predictions regarding fluorescence intensity under different conditions without the need to calibrate for each case specifically.

The application of PF-FD in the analysis of complex hydrocarbons was investigated by McEnally et al. (1994), in this case focusing on the detection of the environmentally detrimental chlorinated hydrocarbons (CHCs). In this case, a 193-nm pulsed excimer laser was used to photofragment the molecules and a second tunable dye laser (wavelength range of 270-280 nm) was used to excite the resulting C-Cl fragments. By probing a variety of electronic transitions by varying the wavelength of the incident laser, the researchers were able to detect the presence of CHCs down to a detection limit of 5 ppb. The technique was found to be able to determine the presence of the C-Cl fragment from a variety of CHC sources, including chloromethanes, chloroethanes, and chloroethylene.

**Analysis of particulate species**

Just as in the case of LIBS, use of PF/FD for aerosol analysis presents unique challenges in that the laser must first evaporate and vaporize the target molecule (or fraction thereof) before component species can be detected (Stipe et al., 2005; Hidalgo Núñez et al., 2000). Although PF/FD work into this area is more limited, studies do exist into using the technique to study aerosols systems, including those containing hydrocarbon and sodium species.

Hidalgo Núñez et al. (2000) analyzed both sulfuric acid and sodium containing aerosols along with the interaction between the two using PF/FD (along with LIBS). Sulfuric acid
particles were grown by homogeneous nucleation of vapor generated off a concentrated sulfuric acid pool with the concentration controlled by subsequently removing a fraction of the resultant aerosol with a high-efficiency particle air (HEPA) filter. Sodium chloride aerosol samples were created by employing an ultrasonic nebulizer containing a solution of NaCl particles using air doped with sulfuric acid particles in the method described above as the carrier gas. This combination of particles will result in the reaction of NaCl and sulfuric acid to form Na₂SO₄, with the gas stream also containing some unreacted NaCl particles. Based on the wavelength required to photodissociate the target species and excite the resultant Na atoms into an excited state (NaCl, $\lambda = 196$ nm; Na₂SO₄, $\lambda = 179$ nm), only the unreacted NaCl particles will result in the emission of the signature 589.0/589.6 nm light thus enabling the concentration of this species to be determined. By measuring the loss in concentration as measured with PF/FD for NaCl aerosol streams after interaction with the sulfuric acid aerosol, the researchers were further able to indirectly measure the concentration of Na₂SO₄ particles. Using a differential mobility particle sizer (DMPS) to determine both concentration and surface area of the NaCl aerosol yielded a linear relationship between the fractional loss of NaCl signal and the number of surface NaCl molecules, indicating a preliminary finding that the reaction of sulfuric acid with NaCl occurs on the aerosol surface.

In order to extend the results of the previous study, Hidalgo Núñez and Omenetto (2001) went on to investigate the use of PF/FD for detection of NaCl, NaOH, and Na₂SO₄ aerosols independently of interaction with other species, with a specific research interest of determining some of the effects a particulate analyte would have on the measured signal. For this study, aerosols of each target species were created by nebulization of solution. By comparing the PF/FD signal with the sodium emission observed from a purely thermal process of using a flame
to vaporize the aerosol species the researchers were able to determine that PF/FD is highly efficient in melting and evaporating the target particles. In addition, the results of Chadwick et al. (1995) in finding radiative trapping to dampen the observed signal of NaCl at high concentrations in the vapor phase were replicated in this case for the species in particulate form. Finally, with the use of time resolved observation of the sodium signal from different aerosol species, the authors determined that the lag time to reach peak intensity differs for various parent molecules.

Although all of the aforementioned work has focused on detecting either the gaseous or particulate component of an aerosol species, it is possible to measure both phases within the same system simultaneously using PF/FD. Damm et al. (2001) did just that in studying the carbonaceous species present in combustion exhaust; in this case, particulate carbon is found within a gas stream containing among other species vapor-phase hydrocarbons. In this study, PF/FD analysis using a 193 nm excimer laser is used with carbonaceous particles detected via observation of excited atomic carbon emission at 248 nm and gaseous hydrocarbons (or those condensed on the particle surface) detected by observation of CH and C2 fluorescence at 431 and 468 nm. To quantify the effects the presence of vaporous hydrocarbons would have on the particulate signal, separate experiments were performed by first analyzing the particulate matter alone by removing the gaseous hydrocarbons using a denuder and then by removing the particulate matter via filtration to analyze the vapor species alone. The CH and C2 fluorescence signals were found to decrease when particulate matter was filtered out of the gas stream, indicating that either a signal was coming from the particles themselves or that gaseous hydrocarbons were condensing on the particles and thus were removed from the stream during the filtration process. Given that no CH/C2 signal was observed when the denuder (capable of
removing condensed and well as vaporous hydrocarbons) was employed, it was concluded that loss due to condensed vapors on removed particles was to blame. It was further determined by analysis of the carbon fragment emission that the fluorescence yield for particulate sources of this signal is up to four times greater in magnitude than for gas phase hydrocarbons typically found in exhaust stream. This finding has practical implications in the specific use of PF/FD for analysis of combustion exhaust in that only in systems with a low gaseous hydrocarbon output such as diesel engines can the particulate matter be effectively measured without first removing the vaporous hydrocarbons.

**Objectives of the Present Work**

In the present study, the applicability of PF/FD for the detection of solid sodium chloride (NaCl, the primary aerosol species originating as sea salt) and gaseous ethyl-3-ethoxypropianate (EEP, a complex hydrocarbon present in paint thinners and other industrial solvents) will be investigated. In the case of NaCl, PF/FD as a detection scheme has been previously examined; however, as previously outlined most studies focus on vapor phase analysis whereas in the current work this species will be analyzed in particulate form. In the case of EEP, no previous studies into the effectiveness of PF/FD as a detection scheme exist; the present work will focus on the applicability of the technique in detecting this complex hydrocarbon species. In order to investigate the possibility of oxygen as a quenching species, studies will be carried out for both analytes in nitrogen/oxygen mixtures ranging from pure nitrogen to a mixture with the approximate nitrogen/oxygen ratio found in air (4:1). In addition to the quantified emission intensity measurements used for species detection, time resolved data to determine the delay in onset of fluorescence and fluorescence lifetime will be carried out to determine the relative characteristics for each of the two species. Potential pathways of photofragmentation will be
proposed for EEP based on the results of these studies and the applicability of PF/FD to NaCl in particulate form will be analyzed and compared with the previous body of knowledge.

**Single Particle Fluorescence for Cancer Detection**

The specificity and low detection limits of laser induced fluorescence make it well suited for single particle measurements, both for analysis of aerosol species and for quantifying particulate matter in suspension. The practice of using fluorescence for the detection and analysis of cells in solution (often *in vivo* such as in the blood stream) is referred to as flow cytometry (FCM); in the present study, the use of FCM for analysis of particles in suspension will be investigated with the specific goal of better quantifying the technology for use in detecting circulating tumor cells. A review of the fundamental theory of laser induced fluorescence as well as flow cytometry is provided along with an overview of advances in the use of laser induced fluorescence for single particle detection of bioaerosols and biological cells. In addition, the body of previous work into flow cytometry for detection of CTCs is reviewed along with a brief outline of the goals of the present study.

**Fundamental Theory: Laser Induced Fluorescence and Flow Cytometry (FCM)**

As previously mentioned, laser induced fluorescence relies on exciting a species of interest with a wavelength of light within an absorption band of the target atom or molecule and subsequently measuring the light emitted to quantify the target species as it drops back to the ground state. When incident energy is absorbed, it may result in transition from the ground electronic state (*S₀*, Figure 1-5) to one of several excited electronic states (for example *S₁*, *S₂*). Within each electronic energy level there are multiple vibrational energy levels, and incident energy may excite the atom into any one of these states (Lakowicz, 1999). In general, atoms will relax to the lowest vibrational level of an electronic state quickly via internal conversion, a non-fluorescent process. From the lowest vibrational state of the excited electronic state the particle
may release energy via fluorescence to return to the ground state; the light emitted in this process is what is measured and quantified in fluorescence spectroscopy. Because of the process of internal conversion, the emitted light is of a longer wavelength (e.g., lower energy) than the excitation light, which allows the two to be segregated from each other with the use of optical filters thereby reducing interference of the measured signal by the incident light (Ormerod, 1994).

Fluorescence, however, is not the only process by which excited atoms can return to the ground state (Lakowicz, 1999). In some species, molecules may also undergo a process called intersystem crossing which eventually results in decay through phosphorescent emission; because this process is not of interest in the present study it will not be discussed further. In addition to intersystem crossing, there are other methods that an excited atom may lose energy and return to the ground state without fluorescing. This decrease in fluorescence can occur due to a variety of processes which collectively are known as quenching, and include collisions with other molecules as well as static quenching through the formation of nonfluorescent complexes with other species; the other molecule in each of these processes which results in a decrease in emitted intensity is referred to as the quencher (Lakowicz, 1999). Note that quenching processes may also decrease the signal observed in prompt emission PF/FD when other species interact with the excited atoms/molecules before emission occurs. Because the presence of quenchers makes correlation of fluorescence intensity with target species concentration a function of the composition of the system, their presence and effects must be understood to allow quantitative analysis of fluorescence as well as PF/FD data.

Flow cytometry (FCM) is the application of fluorescence spectroscopy to detecting and quantifying biological molecules (for example single cells) in suspension within a fluid stream
(O’Connor et al., 2001; Ormerod, 1994). Although there are some molecules of interest that inherently fluoresce, in general fluorescent dyes/probes are used which selectively attach themselves to the cells of interest and thus allow their quantification (Weiss, 1999; O’Connor et al., 2001). There are numerous applications of FCM in the biomedical community, with a large body of work in using FCM for diagnostic purposes; other applications include DNA sequencing, quantification of enzymes, diffusion analysis, toxicology studies, plant physiology, and more (Weiss, 1999; O’Connor et al., 2001; Moerner, 2007).

FCM offers many advantages over other analysis methods for biological systems. Because the technique is based on single particle analysis, extremely low concentrations of analyte may be detected that would otherwise be missed through the use of ensemble averaging (Edel and de Mello, 2003). Single particle analysis also allows for the time resolved study of fluctuating systems, for example to examine the reaction pathways of specific molecules (Weiss, 1999). Because the technique allows for cells to remain in suspension, FCM can be used to study cells in vivo and in vitro, thus allowing the complex interaction between biomolecules to be examined within their native environment (Moerner, 2007). The non-destructive nature of FCM allows for the design of systems incorporating fluorescence detection and identification with a physical sorting scheme so that molecules of interest can be isolated for further types of chemical analysis (O’Connor et al., 2001).

Despite its advantages, there are some limitations to FCM and care must be taken in the design of analysis systems based on this technique. Because the concentrations of the species of interest are very low and single particles are being detected, it is important for as much of the carrier fluid to pass within the probe volume of the laser as possible to avoid missed particles (Dittrich and Manz, 2005). The signal emitted from a single particle is very small, therefore it is
relatively difficult to isolate light emitted from species of interest from background light coming from a variety of sources including Raman and Rayleigh scattering, fluorescence emitted from the slide or tube used to contain the analyte material, and from dark current within the detector (Edel and de Mello, 2003; Weiss, 1999). Several strategies are typically employed to improve the signal-to-noise ratio, including the use of a small excitation volume (via physical containment and/or detection through pinhole observation), high efficiency collection optics, detectors with high quantum efficiency and as low a dark noise as possible, and the reduction of background fluorescence via prebleaching of the solution to remove solvent impurities, time-gated detection, and the use of high optical grade containment materials to reduce background fluorescence (Edel and de Mello, 2003; Weiss, 1999).

**Single Particle Fluorescence: Applications to Biological Molecules**

Fluorescence spectroscopy has been used for single particle detection, quantification, and analysis of biological molecules in both aerosol form and for particles in suspension (flow cytometry). A review is provided of the advancement of research for molecules in both types of systems with a specific focus on the use of flow cytometry for the detection of circulating tumor cells.

**Detection of bioaerosols**

Although biological aerosols exist in relatively low concentrations within the Earth’s atmosphere compared to other types of particulate matter, they are responsible for a wide range of diseases effecting plants, animals, and humans (Pinnick et al., 1998; Seaver et al, 1999). Because there are so few molecules of interest to sample and in many cases harmful biological species are closely related to benign ones, detection strategies must have very low limits of detection and provide a high degree of specificity, analogous to the requirements of flow
cytometry for cancer detection. The following is an overview of research into applying single particle fluorescence spectroscopy for identifying and quantifying biological aerosols.

A prototype instrument for the detection of bioaerosols using both aerodynamic sizing of the particles and fluorescence spectroscopy was proposed by Hairston et al. (1997). Because marking of bioaerosols with fluorescent dyes is impractical, these researchers (and most others in the field of aerosol detection) rely on intrinsic fluorophores present in bioaerosols for identification of the target analyte. In this case, the authors detect the presence of reduced nicotinamide adenine dinucleotide phosphate [NAD(P)H], a chemical found within viable bacterial spores, by excitation with 325 nm light and subsequent detection of emitted fluorescence in the characteristic wavelength range of 420-580 nm. By combining measurements of aerodynamic size and the identification of NAD(P)H, the researchers are able to determine if the spores of interest, in this case *Bacillus subtilis* var. *niger*, are distinguishable from similarly sized non-biological aerosols. The prototype instrument identified the fluorescence signal of interest within 17% of nebulized spore samples compared to only 3% of control particles of the same size. Given that the estimated viability of *Bacillus* based on previous studies is ~12%, this detection percentage indicates promise that in addition to distinguishing biological aerosols from non-biological ones the system can also determine the viability of the bacterial spores.

Although identifying aerosols as biological in origin or not is significant, from a practical standpoint of health concerns it is also imperative to be able to distinguish between species to determine if a particular sample poses health or environmental risk. To this end Seaver et al. (1999) developed a prototype instrument also based on dual detection of aerodynamic size and fluorescence measurements and determined its effectiveness in distinguishing between select
bioaerosols. In this case, a 780-nm diode laser was used as a scattering source for particle size detection which in turn triggered the firing of a 266-nm UV laser as a fluorescence excitation source. This wavelength of radiation induces fluorescence from several molecules found in biological species, including the amino acids tryptophan, tyrosine, and phenylalanine along with DNA. In both laboratory and field trials the authors tested the instrument with samples of the bacterial endospore *Bacillus subtilis* along with the vegetative bacterial cells *Erwinia herbicola* and *Escherichia coli*. Although all three species fluoresce under 266-nm light and scatter 780-nm light, the size difference between the particles and differing relative concentrations of the fluorescing molecules make it possible to distinguish between the species by combining the size and fluorescence data (Figure 1-6). The authors note that there are some limitations to the system, most notably that the wide range of bioaerosols makes identification in this manner impossible without *a priori* knowledge of the species present within the system of interest. In addition, even in the case of a limited system of two species size/fluorescent intensity for each may not be different enough to be distinguishable in all combinations of species. Despite these limitations, however, the authors demonstrated that on a limited and specific basis fluorescence combined with aerodynamic size can distinguish between some different bioaerosols of interest.

In order to refine the chemical analysis of bioaerosols to allow species distinction, researchers based out of Yale University and the U.S. Army Research Laboratory have investigated the possibility of capturing fluorescence spectra (as opposed to simple intensity measurements) from individual bioaerosol particles (Pan et al., 2003; Pinnick et al., 1998; Nachman et al., 1996). In the original prototype, a continuous wave 488-nm argon laser was used as the excitation source with a conditional sampling strategy wherein spectra were recorded only when the emitted intensity crossed a certain threshold value indicating the presence of a
particle in the beam (Nachman et al., 1996). Although single particle fluorescence was detected, the signal was relatively weak, therefore the system was improved by using the 488-nm laser as a trigger to fire a pulsed 266-nm UV laser; in addition to the pulsed laser providing more power, the absorption cross section of the molecules of interest (amino acids, NADH, and flavin compounds) is larger at the shorter wavelength (Pinnick et al, 1998). Individual spectra recorded with the system were clearly unique based on the source of particles introduced to the instrument (Figure 1-7); however, the wide range of possible bioaerosols with similar concentrations of the molecules detected by fluorescence still precludes this type of system from identifying an unknown aerosol specimen. Once again, however, promise is shown in at least determining the presence of bioaerosols and distinguishing them from non-biological molecules.

One area of interest with relevance to the present study is comparing laser-induced fluorescence techniques when applied to bioaerosols with fluorescence detection schemes applied to particles in suspension. In order to compare the signals obtained from species in the two media, Faris et al. (1997) measured the absolute fluorescence cross sections for two types of bacterial spores, *Bacillus subtilis* and *Bacillus cereus*, in both wet and dry aerosol form and while in liquid suspension. Excitation wavelengths were varied from between 228-nm and 303-nm, with the researchers determining optimal excitation for both aerosols and particles in suspension within the range of 270-nm and 280-nm. The researchers determined that although qualitatively the shape of the fluorescent cross section as a function of emission wavelength for a given species is similar for spores in aqueous solution or in wet or dry aerosol form, there is a significant increase in fluorescence for both wet aerosols and particles in suspension over the same species in dry aerosol form. The key result of this study in terms of implications to the present research is that although particles in suspension may emit fluorescence of a different
magnitude than those in dry aerosol form the general qualitative behavior is the same, thus advancements in fluorescence technology for aerosol detection has implications for flow cytometry and vice versa.

**Detection of circulating tumor cells**

A review of previous research applying single particle fluorescence analysis for the quantification of circulating tumor cells (CTCs) is provided. Included are both laboratory studies into the feasibility of the technique and clinical studies of the effectiveness of CTCs detected with fluorescence techniques in diagnosed cancer patients and quantifying their prognosis for survival.

As early as 1994 studies were undertaken by Gross et al. into the use of fluorescence for the detection of cancer cells in both blood and bone marrow. Patients preparing to undergo high-dose chemotherapy often have blood stem cells and bone marrow obtained before the treatment begins which can later be transplanted back to the same patient following the procedure to reduce the risk of transplant rejection. Cancer cells within the specimen are purged prior to infusion back to the patient, therefore it is vital for techniques to exist which can identify very small (10^-6 to 10^-7) concentrations of tumor cells amidst the surrounding tissue to prevent re-introduction of cancer to the treated individual. Gross et al. tested the viability of using a multi-dye technique for fluorescence detection of a known breast carcinoma line (BT-20) *in vivo* in both blood and bone marrow samples isolated onto glass slides. Cells were dyed with a mixture of three anti-cytokeratin antibodies designed to affix themselves to the type of cancer cells used within the study; each of the three antibodies was identified with a different dye color. In addition, non-cancerous leukocytes, platelets, and erythroid cells were tagged with a fourth color of dye with the aim of using this color as exclusionary. With this methodology, Gross et al. were able to accurately identify rare cells at a concentration of as low as 10^-7. This research
demonstrated both the ability to identify CTCs with flow cytometry as well as the value of an exclusionary dye affixed to non-cancerous tissue in improving accuracy and specificity of the technique.

Kraeft et al. (2000) advanced from experimental investigation of the theory of CTC detection to developing the Rare Event Imaging System (REIS), a fluorescence based device designed for the detection of CTCs at very low frequencies within blood and bone marrow. Although more rapid than traditional microscopic identification of cancer cells, this technique still relies on samples isolated onto slides and does not allow real-time detection or in vitro analysis. In this study, blood was taken from patents diagnosed with breast or small lung cancer in addition to the use of isolated breast and lung cancer cell lines as reference samples. Initially, cancer cells were labeled solely with fluorescently labeled autocytkeratin antibodies; however, this technique resulted in identification of cancer cells within 17% of control blood samples from healthy individuals. The methodology was improved by adding fluorescently dyed epithelial cell molecules (adheres to cancer cells’ surfaces), eliminating all false positives. The authors were able to detect CTCs using this dual wavelength technique at a frequency as low as a single cancer cell per one million healthy mononuclear cells and further reiterated the importance of a multi-labeling strategy in robust identification of circulating tumor cells.

The same authors went on to perform a quantitative comparison of REIS to manual microscopic detection of fluorescently dyed CTCs in blood samples drawn from cancer patients (Kraeft et al., 2004). In this study, samples were drawn from both healthy individuals and those diagnosed with breast cancer. Specimens were analyzed both with the automated REIS fluorescence-based instrumentation and by multiple observers using the traditional manual method for the detection of CTCs. In this study, no false positives were observed for the REIS
system and it identified 14 out of 35 positive slides which manual screening did not originally identify as cancer-positive. The authors demonstrated in this study that in addition to being faster than manual microscopy for the identification of tumor cells REIS also has higher sensitivity of detection.

In an effort to reduce the time scale of sampling in fluorescence detection of CTCs, Hseih et al. (2006) developed a fiber-optic array technology for the detection of fluorescence emission from tagged cancer cells. Like the aforementioned work, this technique relies on dying the cancer cells with multiple markers and immobilizing the suspension onto a glass substrate prior to detection. Although not real time, the increased probe area of this system allows for a scanning speed of up to 500 times the rate of manual fluorescence microscopy detection of CTCs. In addition, the technique has high accuracy and specificity, identifying CTCs in 12 of 14 breast cancer patients while detecting no cancerous cells within the 10 healthy control individuals. The authors note that although their system is not real time, studying static samples on glass slides does allow for identified cancer cells to be isolated for further study.

In a clinical investigation of the use of CTC detection for prognosis of cancer patients Garcia et al. (2007) used flow cytometry fluorescence-activated cell sorting (FACS) to quantify the presence of CTCs in individuals treated for prostrate cancer. Samples were taken from 41 individuals diagnosed with metastatic hormone-refractory prostrate cancer both prior to starting and throughout the course of chemotherapy treatment. In the FCAS technique, cancer cells are marked with two fluorescence dye markers; the suspension is analyzed while contained within an analysis tube. The concentration of tumor cells within the patients was tracked following each cycle of chemotherapy to determine the progression of treatment, and in addition the concentration of CTCs was correlated with overall survival time. Within the study, it was
determined that all but four of the 41 patients had detectable levels of CTCs and that over the course of chemotherapy the concentration of CTCs dropped in tandem with a decrease in prostate-specific antigen, a previously identified prognostic factor for survival of patients with this type of cancer. It was further determined that an increased concentration of CTCs independently correlated with a reduced survival time (Figure 1-8). This study demonstrates the clinical value of fluorescence based detection of CTCs in the tracking of cancer patients undergoing chemotherapy and in overall prognosis for survival.

**Objectives of the Present Work**

In the present study, groundwork into the use of flow cytometry for the quantification of single particles in suspension will be carried out. A dynamic test bed is designed and constructed for analysis of particles in a moving fluid stream, thus allowing the possibility of real-time detection and opening the door for more rapid *in vivo* and *in vitro* detection of CTCs in the cancer detection application. Complimentary static experiments are used to verify that no change in signal is observed with spatial variation of the particle within the sample chamber and to determine that water as a carrier fluid had no quenching effect on the signal observed from the fluorescent beads used to test the system. Statistical investigations into the effect of concentration of particles, laser firing frequency, and flow rate on the percentage of particles that could be detected are undertaken and experimentally validated to optimize the system.
Table 1-1. Estimate of the annual emissions of particulate matter from various sources.

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount, Tg/yr [10^6 metric tons/yr]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Soil dust</td>
<td>1000-3000</td>
</tr>
<tr>
<td>Sea salt</td>
<td>1000-10000</td>
</tr>
<tr>
<td>Botanical debris</td>
<td>26-80</td>
</tr>
<tr>
<td>Volcanic dust</td>
<td>4-10000</td>
</tr>
<tr>
<td>Forest fires</td>
<td>3-150</td>
</tr>
<tr>
<td>Gas-to-particle conversion</td>
<td>100-260</td>
</tr>
<tr>
<td>Photochemical</td>
<td>40-200</td>
</tr>
<tr>
<td>All Natural Sources</td>
<td>2200-24000</td>
</tr>
<tr>
<td>Direct emissions</td>
<td>50-160</td>
</tr>
<tr>
<td>Gas-to-particle conversion</td>
<td>260-460</td>
</tr>
<tr>
<td>Photochemical</td>
<td>5-25</td>
</tr>
<tr>
<td>All Anthropogenic Sources</td>
<td>320-640</td>
</tr>
</tbody>
</table>

Data from Hinds, 1999.
Figure 1-1. Daily death rate in London during the 1952 smog event along with the concentration of particulate matter and SO$_2$ (Finlayson-Pitts and Pitts, 2000).
Figure 1-2. Absorption and scattering of radiation by dust particles at various latitudes along with the total net effect on the local atmosphere (Finlayson-Pitts and Pitts, 2000).

Figure 1-3. Basic diagram of a LIBS system, where L = laser, M = mirror, LP = laser pulse, CL = lens, P = plasma, T = target, FOC = fiber optic cable, S = spectrograph, AD = array detector, GE = gating electronics, and C = computer (Cremers and Radziemski, 2006).
Figure 1-4. Diagram of photofragmentation. In the upper case, a simple two component molecule is fragmented with one component species left in an excited state which decays to emit light of a characteristic wavelength for that element. In the lower case, photofragmentation results in the formation of one atom and one molecule with the wavelength of the emitted light corresponding to the excess energy stored in the remaining bond.

Figure 1-5. Diagram showing the absorption of incident radiation by an atom and the possible mechanisms via which it may return to the ground state. This type of plot is typically referred to as a Jablonski diagram (Lakowicz, 1999).
Figure 1-6. Plot of fluorescence induced from 266-nm light vs. scattered 780-nm light demonstrating the unique combination of characteristics for three biological species (Seaver et al., 1999).
Figure 1-7. Fluorescence spectra recorded from individual bioaerosols of different composition (Pinnick et al, 1998).

Figure 1-8. Mortality rate of prostate cancer patients sorted by detected concentration of circulating tumor cells (Garcia et al., 2007).
CHAPTER 2
NUMERICAL SIMULATION OF LASER-INDUCED BREAKDOWN SPECTROSCOPY: MODELING OF AEROSOL ANALYSIS WITH FINITE DIFFUSION AND VAPORIZATION EFFECTS

Introduction to Model Philosophy

The emphasis of the current laser-induced plasma work is on the role of analyte transport, finite-scale diffusion, and temperature non-uniformity induced by particle-derived atoms. Therefore, a hydrodynamic simplification was employed (i.e. fixed volume) to reduce computational time and enable a parametric study covering the significant temporal computational domain that is necessary to capture the relevant time-scales. Such a calculation was not feasible for the current scope of work (i.e. computationally expensive) using a fully-coupled radiative, conductive, diffusive, and hydrodynamic model. For all calculations, the initial plasma dynamic is neglected, and the plasma volume is assumed to start \( t = 0 \) with a simplified initial temperature profile consisting of either a uniform temperature or a parabolic profile peaking at the plasma center and decreasing to a minimum at the plasma edge.

A particle consisting of magnesium and cadmium is introduced at the center of the plasma at \( t = 0 \). The mass of the particle is allowed to dissociate (mass vaporization per time) linearly. In reality, the mass dissociation rate will vary with surface area and plasma temperature causing a non-linear introduction of mass; however, for the present study a linear rate was taken as an approximation. After dissociation from the particle, gaseous atoms diffuse throughout the plasma using either an empirically based or theoretically calculated diffusion coefficient. Using the Saha equation and Boltzmann relation, the ion/neutral ratio for each species is spatially resolved and the emitted intensity is then integrated over the plasma volume to simulate measured values of individual ion/neutral ratio for each species. Using a method proposed by Tognoni et al. (2007), a temperature (assumed representative of the average plasma) is then
back-calculated from “measured” intensity, thus obtaining a single calculated value for each time step that would be indicative of the value determined from an actual experimental measurement, as recently implemented (Diwakar et al., 2007). Comparison of the modeled temperature fields to the simulated analyte-based temperature values will offer additional insight into the role of local plasma conditions on quantitative analyte measurements, as well as offer new perspectives as to the role of finite-scale dissociation and diffusion for aerosol particle-derived analytes to complement recent experimental efforts (Hohreiter and Hahn, 2006; Diwakar et al., 2007).

Method of Numerical Simulation

During the initial development of the model, certain simplifications regarding the calculation of the diffusion coefficient for each species, the initial temperature profile, the evaporation time rate of the particle, and the ionization state of the plasma ions were made; in addition, the plasma was assumed to be optically thin. Under these assumptions, a parameteric study was performed determining the effect of particle size, relative species concentration in the original aerosol particle, and mass diffusion outer boundary condition on model results. Although these results are qualitatively valid, the model was improved to incorporate a more realistic approximation for the diffusion coefficient and to account for second as well as first ionization states of each species. The most realistic of the considered boundary conditions for mass diffusion (diffusion into zero concentration) was assumed. Using this expanded model construction, a parametric study into the effect of initial temperature profile, evaporation time scale, and the inclusion of second ionization states was performed along with an expansion of the original study into the effect of particle size on model output. The validity of assuming an optically thin plasma was also investigated.
**Initial Model Construction**

The plasma volume was modeled as a 3-mm diameter radially symmetric sphere of the carrier gas argon, with the size chosen based on empirical observations of typical LIBS plasmas (Hohreiter and Hahn, 2006). The temperature and species concentrations throughout the volume were modeled using explicit finite difference schemes with a fixed grid system (Figure 2-1; Incropera and DeWitt, 2002; Ozisik, 1993). Computational restraints limited the radial divisions to 100, with a node spacing of \( dr = 15 \, \mu m \). For stability the time step was set at \( dt = 0.1 \, ns \) with results saved every 10 ns to limit memory requirements. The model was run for a total of 30 \( \mu s \), chosen as a realistic time scale over which robust intensity measurements can be obtained from LIBS plasmas (Hohreiter and Hahn, 2006).

Emission from an aerosol particle only occurs as the mass dissociates and diffuses, processes that have been experimentally observed to occur on finite time scales. In this case, the particle was assumed to not directly interact with the incident laser beam, therefore the processes of light absorption could be ignored and only thermal interactions with the plasma were considered (Lushnikov and Negin, 1993). To simulate this process, an aerosol particle of diameter \( d_p \) (taken for two cases, 100 nm and 250 nm) with some mass fraction of magnesium and cadmium (\( y_{mg} \) and \( y_{cd} \), taken in one case as 0.5/0.5 and another as 0.75/0.25) was placed within the central volume element and assumed to linearly melt/evaporate over the course of 15 \( \mu s \) and subsequently diffuse according to Fick’s Law throughout the plasma volume. The diffusion coefficient (D) was allowed to vary with temperature (Equation 2-1).

\[
D(T) = D_{ref} \left( \frac{T}{T_{ref}} \right)^{3/2}
\]  

(2-1)
The time scale of dissociation and the reference value of the diffusion coefficient ($D_{\text{ref}} = 0.04 \text{ m}^2/\text{s} @ T_{\text{ref}} = 15000 \text{ K}$) were taken from empirical observation (Hohreiter and Hahn, 2006). The two species were modeled separately, although in this case the same diffusion coefficient and dissociation time scale are used for each. The central node boundary condition is therefore a combination of a zero inward flux condition (symmetry), diffusion in the outward radial direction, and a generation term for each species $i$ over the first $15 \mu$s (taken as zero afterward; Equation 2-2).

$$\dot{n}_i = \left[ \frac{y_i \cdot \rho_p \cdot \pi \cdot d^3_p}{15 \epsilon - 6} \right] \cdot \frac{1}{MW_i} \cdot N_A$$

(2-2)

The bracketed term is the mass generation rate ($\dot{n}_i$) for each species, determined from the particle density $\rho_p$ calculated from a weighted average of the densities of Mg and Cd; $N_A$ is Avagrado’s number ($6.02e23 \#/\text{mol}$) and MW is molecular weight. The model was run twice using two different outer boundary conditions to determine the effect of varying this condition; in the first, mass was allowed to diffuse out into zero concentration, and in the second, zero flux was allowed across the boundary. The initial concentration of both species throughout the control volume was taken as zero. A baseline case considered was uniform concentration of component species over all time and volume elements, simulating the assumption of instantaneous diffusion of species relative to measurement time. Table 2-1 summarizes the conditions for the different cases considered.

The temperature field was calculated by developing an explicit finite difference scheme from conservation of energy in each volume element over the same grid used for the concentration fields. The carrier gas was taken as pure argon, with the specific heat and thermal conductivity allowed to vary with temperature (lagged from the previous time step). Thermal
conductivity and specific heat were calculated as a function of temperature using piecewise linear fits to experimentally measured values for argon (Atsuchi et al., 2006; Maouhoub, 2000). The density of argon was also varied with a time lag dependence on temperature using an empirically determined result (Fujisaki, 2002). The outer boundary lost energy through radiative transfer to an environment at \( T_E = 293 \) K, whereas the boundary condition at the inner node was taken as insulated across \( n_1 \) (radial symmetry) combined with a loss term due to energy taken in the process of melting/evaporating the particle. This loss term was a constant over the first 15 \( \mu s \) and zero for subsequent times, and was calculated from the latent heat of vaporization \( (\Delta H_{\text{vap}}) \) and latent heat of fusion \( (\Delta H_{\text{fus}}) \) for each species (Equation 2-3).

\[
\dot{E}_{\text{gen}} = - \sum_{i=Mg,Cd} \dot{m}_i \cdot \frac{1}{MW_i} \cdot (\Delta H_{\text{vap},i} + \Delta H_{\text{fus},i})
\]  

(2-3)

In general, it was determined that given the small amount of mass generated and the relatively large volume of the central element compared to particle size the effect on central node temperature was minimal; however, tests over a smaller volume indicated that if computational power permitted smaller steps in space and time, an appreciable effect on local temperature could occur as has been seen experimentally around aerosol particles (Diwakar et al., 2007). As a simplified initial condition, the temperature throughout the plasma was taken to be uniform at an empirically determined reasonable temperature for a LIBS plasma following termination of the laser pulse, \( T_0 = 15000 \) K (Hohreiter and Hahn, 2006).

Once the temperature and concentration fields were developed, the ion/neutral concentrations were determined by simultaneously solving the Saha equations for each species (cadmium, magnesium, and the carrier gas, argon). The Saha equation (Radziemski and Cremers, 1989) relates the concentration of ions (I) to neutrals (II) for a given species with the temperature and free electron density (Equation 2-4, example given for magnesium).
\[
\frac{n_e \cdot M_{g}^{\text{II}}}{M_{g}^{\text{I}}} = 2 \cdot \frac{U_g^{\text{II}}(T)}{U_g^{\text{I}}(T)} \cdot \left[ \frac{2\pi m_e k_b T}{\hbar^2} \right] \cdot \exp \left( \frac{-E_{\text{ion}}}{k_b T} \right) = K_{g}(T)
\] (2-4)

In Equation 2-4, \(M_g\) is the number density of magnesium, \(U\) is the electronic partition function (itself a function of temperature), \(m_e\) is the mass of an electron, \(k_b\) and \(h\) are Boltzmann’s and Planck’s constant, and \(E_{\text{ion}}\) is the ionization potential for the species. Note that the ionization energy defect (lowering) due to the local effect of surrounding electrons is neglected due to an insignificant effect on the calculation in this case. The electronic partition function was calculated as a function of temperature using parameterized relations for each species (Irwin, 1981); ionization energies were obtained from the NIST Spectroscopic Handbook (Sansonetti et al., 2007). Note that for a given species, the right hand side of the equation is a function only of temperature, labeled as \(K\) above, which can be calculated at each volume element for each time from the temperature field. We consider only the first ionization of each species, therefore the total number density of any given species (calculated for each aerosol species as described above and from the density as a function of temperature for argon) is the sum of the ions and neutrals for each species (Equation 2-5, 2-6, and 2-7).

\[
M_{g}^{\text{TOT}} = M_{g}^{\text{I}} + M_{g}^{\text{II}}
\] (2-5)

\[
C_{d}^{\text{TOT}} = C_{d}^{\text{I}} + C_{d}^{\text{II}}
\] (2-6)

\[
A_{r}^{\text{TOT}} = A_{r}^{\text{I}} + A_{r}^{\text{II}}
\] (2-7)

The free electron density is related to the number concentration of the ions (Equation 2-8).

\[
n_e = M_{g}^{\text{II}} + C_{d}^{\text{II}} + A_{r}^{\text{II}}
\] (2-8)

Given that the total number concentration of each species and temperature within every volume element is known, the three Saha equations (one for each species), three conservation equations, and the free electron density equation can be solved simultaneously to obtain the
seven unknowns: ion and neutral concentration of each of the three species and free electron density.

After the number concentration of ions and neutrals are known, the emitted intensity for the transition of a species \( s \) in excited state \( i \) to ground state \( j \) can be calculated (Equation 2-9; Tognoni et al., 2007).

\[
\tilde{I}_{ij}^s = F \exp(\lambda) \cdot A_{ij} n_i^s(T, n_e)
\]  

(2-9)

In this equation, \( F \exp(\lambda) \) is an lumped parameter of experimental efficiency (taken as unity in all cases for our purposes), \( A_{ij} \) is the transition probability, and \( n_i^s \) is the number density of the excited state. The number density of the excited state can be related to the total number concentration of a given ion or neutral via the Boltzmann relation (Equation 2-10).

\[
n_i^s = \frac{n_o g_i \exp\left(-\frac{E_i}{k_B T}\right)}{U^s(T)}
\]  

(2-10)

Substituting this result into the intensity equation gives intensity as a function of known parameters (Equation 2-11).

\[
\tilde{I}_{ij}^s = F \exp(\lambda) \cdot A_{ij} \cdot \frac{n_o g_i \exp\left(-\frac{E_i}{k_B T}\right)}{U^s(T)}
\]  

(2-11)

In this equation \( n_o \) is the total concentration of the ion or neutral species (determined above) and \( g_i \) and \( E_i \) are the degeneracy and energy state of the upper level. Transition probabilities, degeneracies, and energy levels were all obtained from the NIST handbook (Sansoneetti et al., 2007). The transitions of interest for each species were chosen based on common lines used in obtaining ion/neutral ratios, the wavelengths of which were: Mg\(_I^I\) = 285.2 nm; Mg\(_II^I\) = 280.3 nm; Cd\(_I^I\) = 228.8 nm; and Cd\(_II^I\) = 226.5 nm. Note that all of the lines of
interest are in the same range of wavelengths, improving the likelihood that the experimental
measurement efficiencies ($F_{\text{exp}}$, above) are close in value (Diwakar et al., 2007; Tognoni et al., 2007).

Once the intensity distribution for each species is known, the values from each node $n$
from innermost (1) to outermost (M) can be used to determine the total (e.g., measured) intensity
at each time step ($\tau$; Equation 2-12).

$$\tilde{I}_{\text{TOT}}(\tau) = \frac{\pi}{6} dr^3 \cdot I^s(1, \tau) + \sum_{n=2}^{M} 4\pi dr^3 (n - 1)^2 I^s(n, \tau)$$  \hspace{1cm} (2-12)

It is important to note that the calculated value is spatially integrated and assumes an
optically thin plasma (e.g., no emitted light is reabsorbed). The ion/neutral ratio is then
calculated as the ratio of measured intensities, e.g. for magnesium (Equation 2-13).

$$R_{\text{Mg}}^{II}(\tau) = \frac{I_{\text{TOT}}^{II}(\tau)}{I_{\text{TOT}}^{I}(\tau)}$$  \hspace{1cm} (2-13)

The Tognoni ratio is then taken as the ratio of this value for each of the two species
(Equation 2-14).

$$R_{\text{Togn}}^{\tau}(\tau) = \frac{R_{\text{Mg}}^{II}(\tau)}{R_{\text{Cd}}^{II}(\tau)}$$  \hspace{1cm} (2-14)

For the complete development of the relationship of this ratio to temperature the reader is
referred to Tognoni et al. (2007); however, essentially the Saha/Boltmann/conservation equations
for each species are combined to remove electron density dependence and yield temperature as a
function only of the ratio given above and known values (Equation 2-15).

$$k_{B}T(\tau) = \ln \left[ \frac{\left( E_{i}^{\text{II}} + E_{i\text{ou}} \right)_{\text{Cd}} - \left( E_{i}^{\text{II}} + E_{i\text{ou}} \right)_{\text{Mg}}}{\left( F_{\text{exp}}(\lambda_{\text{II}}) \right)_{\text{Cd}} - \left( F_{\text{exp}}(\lambda_{\text{i}}) \right)_{\text{Mg}} \cdot \left( \frac{A_{i}^{\text{II}} g_{i}^{\text{II}}}{A_{i}^{\text{I}} g_{i}^{\text{I}}}_{\text{Cd}} \cdot \left( \frac{A_{i}^{\text{II}} g_{i}^{\text{II}}}{A_{i}^{\text{I}} g_{i}^{\text{I}}}_{\text{Mg}} \right) \cdot R_{\text{Togn}}^{\tau}(\tau) \right)} \right]$$  \hspace{1cm} (2-15)
This calculated value was compared to the simulated temperature distribution at each time step to determine how the Tognoni temperature calculation would analyze a plasma with varying temperature and concentration throughout the volume. For comparison, at each time step $\tau$ a volume-weighted temperature was calculated from the simulated temperature field (Equation 2-16).

\[
T_{VOL}(\tau) = \frac{\frac{\pi}{6} \cdot dr^3 \cdot T(1, \tau) + \sum_{n=2}^{M} 4\pi dr^3 (n-1)^2 T(n, \tau)}{\frac{4}{3} \pi R^3}
\]  

(2-16)

where $R$ is the radius of the plasma volume.

**Expanded Model Construction**

The overall layout of the plasma and the finite difference scheme used in the initial model construction were retained in the expanded model, with the plasma again being modeled as a 3-mm diameter, radially-symmetric sphere of argon divided into 100 radial divisions of $dr = 15 \mu m$. The model again ran over 30 $\mu$s with a time step of $dt = 0.1$ ns with results saved every 10 ns.

The differences between the expanded model and the initial model are:

- Outer boundary condition in the mass diffusion equation (assumed in this case to diffuse to an environment at zero concentration, the most realistic boundary from the previous parametric study)
- Expansion of the particle size range considered
- Parametric study of evaporation time scale
- Method of calculation of diffusion coefficient
- Assumed initial temperature profile
- Consideration of the effects of an optically thick plasma
- Inclusion of second as well as first ionization states
For the aerosol particle diameter ($d_p$), the baseline case was again taken as 100 nm and in a parametric comparison as 250 nm, 1000 nm, and 2500 nm with a mass fraction of 50% magnesium and 50% cadmium ($y_{Mg} = 0.5$, $y_{Cd} = 0.5$). In addition to the original time scale of 15 ns, an evaporation time of 150 ns (e.g., essentially instantaneous regarding LIBS-based analysis times) was also considered.

Instead of using an empirical approximation for the diffusion coefficient based solely on temperature, binary diffusion coefficients for each species (i.e. neglecting the presence of the small amount of the other particle-derived analyte) within the argon carrier gas were calculated theoretically using Chapman-Enskog theory for binary mixtures of gases at low to moderate pressures as given in Equation 2-17 (Turns, 2000).

$$D_{AB} = \frac{3}{16} \left( \frac{4\pi k_B T}{p MW_{AB}} \right)^{1/2} \frac{\pi \sigma_{AB}^3 \Omega_D}{f_D}$$  \hspace{1cm} (2-17)

In Equation 2-17, $A$ denotes argon and $B$ is either magnesium or cadmium. Additional parameters are defined as: $k_B$ is the Boltzmann constant, $T$ is plasma temperature [K], $p$ is pressure [Pa], $R_u$ is the universal gas constant, $MW_{AB}$ is the harmonic mean of the molecular weights of species A and B, $\sigma_{AB}$ is the arithmetic mean of the hard sphere collision diameters of A and B, $f_D$ is a theoretical correction factor assumed to be unity, and $\Omega_D$ is a calculated dimensionless parameter (Equation 2-18; Turns, 2000).

$$\Omega_D = \frac{A}{(T^*)^B} + \frac{C}{\exp(D \cdot T^*)} + \frac{E}{\exp(F \cdot T^*)} + \frac{G}{\exp(H \cdot T^*)}.$$  \hspace{1cm} (2-18)

The constants in Equation 2-18 are: $A = 1.06036$; $B = 0.15610$; $C = 0.19300$; $D = 0.47635$; $E = 1.03587$; $F = 1.52996$; $G = 1.76474$; and $H = 3.89411$. $T^*$ is the non-dimensionalized temperature using the Lennard-Jones energy ($\epsilon$) of each species A and B (Equation 2-19).
\[ T^* = \frac{k_B T}{(\varepsilon_A \varepsilon_B)^{1/2}}. \]  

Values for the hard sphere collision diameters and Lennard-Jones energies for each species were obtained from Svehla (1962). The values of the diffusion coefficient calculated in this manner for the overall temperature range of 3200 K to 25000 K observed in the model ranged from 0.001 – 0.033 m²/s for magnesium in argon, and from 0.001 – 0.027 m²/s for cadmium in argon. This range is slightly lower than the range of 0.017 – 0.063 m²/s reported based on experimental observation of calcium diffusing in an air plasma (Hohreiter and Hahn, 2006); however, given the different species involved, these calculations are considered quite reasonable in view of their overlap.

For initial condition, the temperature throughout the plasma was taken to be parabolic, ranging from a temperature minimum at the plasma edge of 15000 K to a maximum at the central node of 25000 K. These values are considered reasonable for a laser-induced plasma following initial expansion and are a more realistic approximation of the temperature profile than the uniform temperature value assumed in the initial model construction.

In this simplified heat transfer model, the assumption is made that the plasma does not act as a participating medium and cannot emit or absorb radiation at the interior nodes. To assess the validity of this assumption, the radiative energy lost at each point in space and time in the plasma was also calculated using the line of sight analysis as described in Gornushkin et al. (2004). In this model, the energy lost per unit volume of plasma per unit time (\( q_{\text{rad}} \)) is given by integrating the following over solid angle (\( \Omega \)) and frequency (\( \nu \); Equation 2-20).

\[ q_{\text{rad}} = \int d\nu \int k_\nu'(I_\nu^b - I_\nu) d\Omega. \]  

(2-20)
In Equation 2-20 \( I_\nu^b \) is the blackbody spectral radiation, \( I_\nu \) is the spectral radiance at any given point in the plasma, and \( \kappa_\nu \) is the frequency dependent absorption coefficient. Because it is not possible to know the absorption coefficient at all frequencies for all points in the plasma, an approximation is made (Equation 2-21) using the Planck mean absorption coefficient (\( \kappa_{mean}^\Sigma \)), which accounts for free-bound and free-free transitions but which neglects bound-bound transitions (Gornushkin et al., 2004).

\[
\kappa_{mean}^\Sigma = \left( \frac{128}{27} k_B \right)^{1/2} \left( \frac{\pi}{m_e} \right)^{3/2} \frac{z^2 e^6 G n_e}{h \sigma c^3} \frac{1}{T^{5/2}} \sum_i \sum_j n_i^{(j)}. \tag{2-21}
\]

In Equation 2-21, \( m_e \) and \( e \) represent the mass and charge of an electron, \( z \) is the charge of the ion (\( z = 1 \) for single ions), \( G \) is the Gaunt factor (approximated as 1 in our case), \( h \) is Planck’s constant, \( \sigma \) is the Stefan-Boltzmann constant, \( c \) is the speed of light, \( n_e \) is the free electron density, and \( n_i^{(j)} \) is the concentration of species \( j \) (argon, cadmium, or magnesium) in ionization state \( i \) (\( i = 1 \) or 2). Neglecting bound-bound transitions is justified in this case because the primary radiative energy loss from the plasma occurs early on when temperatures are at their highest and the emitted radiation is mostly broad band (Gornushkin et al., 2004).

This radiative energy loss value was included in the conduction model as a heat sink. This computationally expensive additional calculation was performed over for the first 1 \( \mu \)s of the model, and the temperature results were compared to those obtained without including internal radiative energy loss. Over this time period, the maximum temperature difference between the two modeling approaches throughout the plasma volume was found to be less than 10 K. Given that the temperature of the plasma was in the range of 5000-25000 K, such a difference was considered negligible, and the internal radiative energy loss of the plasma was neglected in the model. This result may also indicate that energy transfer within the plasma volume is primarily
governed by heat conduction due to temperature gradients caused by radiative heat loss from the boundary. The resulting savings in computational time was many orders of magnitude on the hardware used, a Dell Precision 690 workstation.

In the initial model construction, only the first ionization state for atoms was considered in calculating the ion/neutral concentration fields. At high temperatures, this assumption may not be valid; therefore, in the expanded model construction second ionization states were also considered. In addition to the equation relating the relative concentration of ion/neutral atoms (Equation 2-14), a Saha equation (Equation 2-22) also exists relating the relative concentration of singly ionized atoms to doubly ionized ones (Gornushkin et al., 2004).

\[
\frac{n_e \cdot Mg_{III}}{Mg_{II}} = 2 \cdot \frac{U_{III}(T)}{U_{II}(T)} \cdot \left[ \frac{2\pi m_e k_h T}{\hbar^2} \right] \cdot \exp \left( -\frac{E_{ion}^{III}}{k_h T} \right) = K_{Mg}^{III}(T) \tag{2-22}
\]

The ionization energy defect is again neglected. Since ionization states higher than two are not included, the total number density of any given species (calculated for each aerosol-derived species as described above, and from the density as a function of temperature for argon) is now calculated for each species (Equations 2-23, 2-24, and 2-25).

\[
Mg^{TOT} = Mg^{I} + Mg^{II} + Mg^{III} \tag{2-23}
\]
\[
Cd^{TOT} = Cd^{I} + Cd^{II} + Cd^{III} \tag{2-24}
\]
\[
Ar^{TOT} = Ar^{I} + Ar^{II} + Ar^{III} \tag{2-25}
\]

The free electron density is related to the number concentration of the ions (Equation 2-26).

\[
n_e = Mg^{II} + Cd^{II} + Ar^{II} + 2 \cdot Mg^{III} + 2 \cdot Cd^{III} + 2 \cdot Ar^{III} \tag{2-26}
\]

Given that the total number density of each species and temperature within every volume element are known, the six Saha equations (one for each species at each of the two ionization
states), three conservation equations, and the free electron density equation can be solved simultaneously to obtain the ten unknowns: singly ionized, doubly ionized, and neutral particle concentration for each of the three species, and free electron density.

After the number densities of ions and neutrals are known, the spatially resolved emitted intensity for each species is calculated as described for the initial model construction and integrated over the plasma volume to obtain a single intensity measurement for each spectral line as a function of time. The ion/neutral ratios and Tognoni ratio is also calculated as previously described. It should be noted that in the Tognoni analysis only the first ionization state is considered, therefore second ionization states may introduce error into the use of this diagnostic to determining plasma temperatures, an effect that can be investigated with the expanded model construction.

**Initial Model Results**

**Effect of Finite Evaporation and Diffusion Time Scales**

Because the melting/evaporation time scales and diffusion coefficients for both magnesium and cadmium are taken to be identical in the initial model construction, the evolution of the concentration fields for each species in each of the five examined cases is qualitatively identical, with only the magnitude of the concentration varying due to the different molecular weights and the initial mass fraction and total particle diameter for each case. Furthermore, the three cases with the identical boundary condition of flux to zero concentration also display qualitatively identical behaviors therefore only cases 1 (uniform concentration of both species over time), 2 (flux to zero concentration), and 3 (no diffusion out) are considered for comparison. The evolution of Cd concentration with time can be compared for cases 2 and 3 along with the constant concentration condition (Figure 2-2), and the total mass within the plasma volume as a function of time can be considered (Figure 2-3).
Due to the injection of mass into the central volume element for cases 2 and 3 there is a strong concentration gradient over the first 15 μs. This mass diffuses outward, and eventually reaches the plasma boundary; in the case of the flux to zero concentration, mass begins to exit the plasma space, whereas for no loss the concentration field begins to level out and eventually approaches the equivalent mass constant concentration condition. Note that the concentration axis of Figure 2-2 is on a log scale, and that the concentration in the outermost volume elements is several orders of magnitude less than the central concentration for both diffusion cases during the period of mass injection in the central volume element. From Figure 2-3, it can be seen that over time in the mass loss case approximately 70% of the total mass is lost to the boundary. Although a flux out of the plasma volume is the most realistic boundary condition, it should be noted that flux to zero concentration is an oversimplification, as species concentrations will start to rise outside of the plasma (particularly given that aerosol LIBS is typically performed in enclosed chambers); therefore, mass loss from the volume would be expected to be less than the modeled case. In addition, the time scale of melting and evaporation could only be roughly estimated from empirical observation, therefore if these processes occur at slower rates than the model predicts the concentration gradient during the mass release period would be less steep, resulting in reduced diffusion. It has been empirically observed (Hohreiter and Hahn, 2006) that in the case of a calcium particle, the observed mass appears to just reach the edge of the plasma volume at 30 μs; the errors described above could account for the more significant mass loss seen in the model, although it is also difficult to tell empirically exactly when diffusion out of the volume starts to occur.

Temperature Profile

Because the temperature field is developed independently of the concentration fields, the results for all 5 cases are identical (Figure 2-4). As time progresses, radiative heat loss to the
boundary results in a radial temperature gradient with the temperature drop reaching the plasma center at around 10 μs. Because the parabolic initial temperature profile is fundamentally more accurate than the uniform temperature profile assumed in the initial model construction, a more thorough discussion of the model output is included with the expanded model results.

**Free Electron Density**

The evolution of free electron density ($n_e$) with time can be compared for cases 1-3 (Figure 2-5, a-c). Note that even on a log scale the cases are virtually identical for radial position less than 1 mm despite the relatively high concentration of aerosol mass in that region for cases 2 and 3, indicating that it is the ionization of the carrier gas argon that is the primary controlling factor for free electron density. To examine this hypothesis further, the percentage difference in free electron density of cases 1 and 3 vs. temperature is considered (Figure 2-5, d). It is clear in this plot that the influence of varying aerosol species concentration on free electron density is only significant at temperatures less than 5000 K. This is consistent with both experimental observation and other modeling results that show the relationship between $n_e$ and $T$ for temperatures below 7000 K remains unchanged in an argon plasma with the addition of other elements to the matrix (Atsuhi et al., 2006). Although the temperatures at which we see deviations are lower than in other studies, one must keep in mind that we are comparing the change in free electron density between two different concentrations of the same aerosol species, as opposed to comparing a pure argon plasma with a multi-element plasma; however the temperatures at which we see deviation are reasonable when compared to the other observations.

**Temperature Prediction Using Dual-Species Ion/Neutral Ratio**

The Tognoni predicted temperature as a function of time can be considered for cases 1-3 (Figure 2-6); also shown is the volume integrated temperature at each time and the modeled temperature in the outermost volume element, the central volume element, and a volume element
halfway in between. It was determined that changing the initial particle mass or relative concentrations of magnesium and cadmium (cases 4 and 5) had no effect whatsoever on the temperature calculations, therefore the results are the same as for case 2 (diffusion out into zero concentration).

For the cases of timed mass release within the central volume element, the Tognoni temperature is shown to initially model the central volume element temperature. As time progresses and the aerosol mass begins to diffuse out into areas of lower temperature, the Tognoni measured temperature drops as well, with deviations between the case of diffusion to zero concentration and no diffusion out occurring as soon as mass begins to diffuse out of the plasma volume. As the concentration field for the no flux out case approaches a uniform concentration, the measured temperature begins to approach the condition of a uniform and constant concentration. For this case, the Tognoni temperature only predicts the plasma temperature during the initial (uniform temperature) time step and subsequently is an average value over the volume.

It is interesting to note that even with a uniform and constant concentration the Tognoni metric does not reproduce the volume-weighted temperature profile, with a temperature variation of up to 2000 K at the longest time interval. This deviation is unsurprising, as the Tognoni metric is developed to produce a single temperature value from a single pair of ion/neutral ratios. If the intensity from each volume element in the model is independently used to obtain a local Tognoni ratio and subsequently converted to a local temperature, this calculated value is identical to the modeled local temperature. Experimentally, however, spatially resolving the emitted intensity from each species is difficult (requiring multiple intensity measurements and the use of Abel inversion for calculation of the plasma field), and is rarely undertaken. Because
of this limitation, the Tognoni metric can only accurately quantify localized temperature around 
a particle while the bulk of the mass is confined to a region of uniform local temperature. As the 
temperature variations becomes larger in the regions containing aerosol species, the deviation 
between the Tognoni temperature and a volume weighted temperature becomes more significant, 
as is shown in the model as increased deviation as a function of time as the plasma edge cools 
and the gradient in the volume increases.

**Expanded Model Results**

**Effect of Finite Evaporation and Diffusion Time Scales**

In addition to the 15-µs evaporation time based on experimental data from an evaporating 
calcium particle assumed in the initial model construction (Hohreiter and Hahn 2006), the case 
of 150-ns evaporation time was also considered. The latter evaporation scale is representative of 
a case where the aerosol particle is assumed to vaporize essentially instantaneously, but in which 
subsequent diffusion still occurs on a finite time scale.

At short time scales (< 0.1 µs) both cadmium and magnesium are contained within a radius 
of 0.6 mm, even at the shorter evaporation time of 150 ns (Figures 2-7 and 2-8). Following 
about 1 µs after breakdown, particle-derived mass has begun to reach the outermost edge of the 
plasma volume and starts to diffuse out into the surrounding environment. By 30 µs, the species 
profile has begun to level out, yielding a more uniform distribution of the analyte species within 
the plasma volume. Such a condition is approaching the ideal situation of uniformly dispersed 
analyte in an unperturbed plasma, although this ideal is not truly reached by 30 µs. It is also 
interesting to note that even with an evaporation time scale of 150 ns (two orders of magnitude 
less than the empirically observed time scale for calcium particles) a gradient in concentration of 
ten orders of magnitude from the center of the plasma to the edge can still be observed at a time 
delay of 1 µs.
To illustrate the changes within the plasma volume as a function of time, the concentrations at three radial locations for cadmium for both the 15-µs and 150-ns evaporation times are compared (Figure 2-9); similar behavior was observed for magnesium. The most significant difference in profile between the two evaporation times is observed within the central node, as one would expect. Infusion of gaseous mass through evaporation of the solid particle offsets the loss from the node by diffusion to the rest of the plasma, resulting in an increase in gaseous mass over the course of the evaporation time followed by a rapid decline due to diffusion after the particle is completely vaporized. At both the midpoint to the plasma edge and the outermost node, mass rapidly increases over the first 10-15 µs due to diffusion, with the rate of increase tapering off at longer time scales as a more uniform profile develops and diffusion thereby slows due to the reduction in species gradient.

**Free Electron Density**

The evolution of free electron density over time for three radial locations (center node, midpoint to plasma edge, and outermost node) along with a volume weighted average value is shown in Figure 2-10; note that the plot for the outermost node is on a log scale, in comparison to a linear scale for the other two locations and the volume weighted average. Because the expanded model includes seconds ionizations, values are not identical to the results obtained in the initial model construction; however, the relatively low concentration of aerosol species results in the bulk free electron density being primarily controlled by the concentration of argon (itself a function of temperature through the density calculation) and the temperature of the plasma. As the plasma cools, the overall bulk concentration (as shown in the volume weighted average) decreases as heat is lost from the plasma edge and the entire volume begins to cool. At the central node and at a point halfway to the plasma edge, there are slightly variations in free electron density due to changes in concentration of the aerosol species; however, a much more
significant change of several orders of magnitude is observed at the outermost node where the
temperature change with time is largest due to radiative heat transfer to the surrounding
environment.

Temperature Profile

The evolution with time of the temperature at three points within the plasma along with
the volume weighted average temperature is shown in Figure 2-11. At short times, heat is lost
very rapidly through radiative transfer with the environment, resulting in a drop in temperature
of the outermost node from the initial temperature of 15000 K to 5000 K within 5 µs. Beyond
the initial rapid loss in temperature, a more gradual heat loss is observed as heat is conducted
away from the core of the plasma and radiated from the outer edge, with the temperature at 30 µs
falling to about 3300 K. At both r = 0.75 mm and at the plasma center where heat loss is
governed by conduction within, a more gradual drop in temperature is observed, with the central
node reaching a final value of about 13800 K and the midpoint radius reaching 12800 K after 30
µs. The overall volume weighted temperature for the entire plasma volume drops from
approximately 19200 K at the initial time to approximately 9800 K at 30 µs.

One of the motivating factors of the current modeling effort was determining possible
effects the evaporation/dissociation of the aerosol solid might have on the local plasma
temperature. To investigate this effect, the parametric study performed in the initial model
construction was expanded to consider aerosol particle diameters of 100, 250, 1000, and 2500
nm; the central (particle containing) node temperature for each of these cases is shown in Figure
2-12. A local depression in temperature due to heat consumed by evaporation and dissociation
processes of the particle can be observed in all cases, with the effect increasing for larger particle
sizes. Between the minimum and maximum particle size modeled (d_p = 100 nm, d_p = 2500 nm)
there is a difference of up to 100 K during the period of particle evaporation, with conduction
rapidly causing convergence of the two profiles after this 15 µs period. Although this scale of
temperature depression is modest compared to the observed temperatures at this node of 14000-
25000 K, it is important to keep in mind that computational limitations restricted the radial step
size to 15 µm, therefore the full effect of particle vaporization on local temperature may be
masked by the relatively large volume (i.e. large heat capacity) of the central node within this
model. In a recent paper, the role of local plasma perturbations about aerosol particles was
experimentally investigated and revealed data consistent with local temperature suppression
(Diwakur et al., 2007). Such a concept is corroborated by the current study, although the
magnitude is different due to the differing local volumes.

**Temperature Prediction Using Dual-Species Ion/Neutral Ratio**

By calculating the emitted intensity for two magnesium lines (Mg\textsubscript{I} = 285.2 nm; Mg\textsubscript{II} =
280.3 nm) and two cadmium lines (Cd\textsubscript{I} = 228.8 nm; Cd\textsubscript{II} = 226.5 nm) the ion/neutral ratio for
each species along with the dual-species ratio of ion/neutral ratios, as proposed by Tognoni et al.
for plasma temperature evaluation, was readily calculated. Using this value, the temperature as
predicted by this approach for the modeled plasma can be determined and compared to the
temperature at each time step. In addition to the calculations made using both first and second
ionization, the expanded model was also run assuming only first ionization states for each of the
species and the dual-species ion/neutral ratio temperature prediction was calculated for
comparison to isolate the effects the single ionization simplification (assumed in the Tognoni
model) has on predicted temperature independently of other model modifications. As a further
study, the model was run using the same diffusion coefficient for both species, calculated as the
average value of the theoretical diffusion coefficient calculated for each species. As can be seen
in Figure 2-13, at very short time scales (< 0.5 µs) the predicted temperature in all cases
reasonably approximates the model temperature of the central node, e.g., the local temperature
surrounding the particle. As time progresses, however, the predicted temperature does not capture the behavior of either the central node or the volume weighted average temperature, the same results as found using the initial model construction. Using an identical diffusion coefficient for both species results in under-prediction of central node temperature, undoubtedly because mass has begun to diffuse out of the central node and the analyte species are encountering a varying temperature field. Note, however, that the predicted temperature is not a good approximation for the volume weighted temperature field, indicating that the analyte species are still encountering local plasma conditions that vary on average from the bulk plasma state, thereby supporting the concept of a local plasma environment.

The dual-species ratio calculation results in an over-prediction of temperature when the diffusion coefficient is allowed to vary with species, with calculated values higher than the maximum temperature within the model. Although this effect is observed in the expanded model even when only first ionization of species is considered, the over-prediction is magnified when considering both first and second ionization of species, resulting in a calculated temperature of up to 4000 K greater than the central node temperature at the same point in time. This error is introduced because a required assumption of the dual-species ion/neutral ratio for temperature calculation is that both species are at the same local plasma condition of both temperature and free electron density in order to be able to use the dual ratio to resolve both parameters. In this case, the varying diffusion rates cause the individual species to have different concentration fields across gradients in both temperature and free electron density, resulting in an under-defined problem of trying to resolve two different temperature and free electron densities with one ion/neutral ratio at each set of conditions. This effect is in addition to the issue realized in the case of identical diffusion coefficients for each species, in which the system of equations
would be well defined at any given point in space (allowing temperature and free electron
density calculation), but with the experimental limitation of only being able to measure a single
intensity integrated over all points at any given instant in time. Such an integrated measurement
results in an average temperature prediction that is indicative of neither the local conditions
around the analyte nor the bulk plasma.

**Lewis Number Calculations**

One of the principle findings of this numerical study is that the finite time scales of
particle evaporation/dissociation and subsequent mass diffusion from an aerosol particle results
in local plasma conditions surrounding the analyte that vary from the bulk (i.e. volume averaged)
plasma condition. To further investigate this point, the Lewis number \( Le = \frac{\alpha}{D} \), where \( \alpha \) is the
thermal diffusivity and \( D \) is the mass diffusivity) was calculated at all points in time and space
using the average diffusion coefficient for magnesium and cadmium, and the thermal diffusivity
\( (\alpha = k/\rho c) \) calculated from the plasma condition at each node. Also calculated was a volume
weighted average Lewis number at each instant in time (Figure 2-14). Calculated values range
from 0.67 – 3.63 for magnesium and from 0.83-4.48 for cadmium. The result is that at all points
in time the Lewis number is on the order of unity. This agrees well with earlier estimates
recently reported (Diwakur et al., 2007). This finding indicates that the time scales of heat and
mass diffusion are comparable, meaning that analyte species will encounter temperature
gradients while diffusing through the plasma volume, and that an assumption of instantaneous
diffusion throughout a uniform plasma at bulk conditions is not valid. Accordingly, a near unity
Lewis number is indicative of finite diffusion of heat into the region of particle dissociation,
which also suggests initial suppression of local plasma temperature as energy that is utilized for
particle dissociation can only be replaced over a finite time scale.
Discussion and Conclusions

A simplified model of the temperature and concentration fields of an aerosol LIBS plasma was developed using an explicit finite difference scheme assuming an initial uniform temperature profile and using an empirical approximation for the binary diffusion coefficient; an expanded model was also constructed assuming an initial parabolic temperature profile and calculating a species-specific theoretical binary diffusion coefficient. The temperature model results qualitatively match what is observed experimentally, with radiative heat loss occurring at the boundary and temperature loss propagating inward to the center of the plasma. Concentration fields for particle species also qualitatively match empirical observations of aerosol particles melting/evaporating and diffusing throughout the plasma volume on finite time scales, phenomena typically neglected in aerosol LIBS models. Through the use of the Saha equation and Boltzmann relation, the ion/neutral ratio for each species as well as the coupled Tognoni ratio was calculated, and the temperature predictions of the Tognoni technique based on experimentally measurable parameters were determined.

One of the primary goals of the present study was examining the effects of finite evaporation and diffusion time scales on aerosol LIBS analysis. Based on a simplified numerical simulation of an aerosol particle evaporating and dissociating on a prescribed and finite time scale, and diffusing through the plasma volume using both empirically and theoretically calculated diffusion coefficients, it is shown that the time scales of these processes result in analyte species encountering local conditions which vary from those in the bulk plasma. This conclusion is further supported by Lewis number calculations for the expanded model, found to be approximately unity for both modeled species, indicating that the time scales of mass and thermal diffusion are comparable and gradients of concentration distribution of aerosol species within the plasma cannot be neglected. An example of the possible effect this finding can have
on LIBS-based analysis of aerosol particles is shown through the application of the dual-species ion/neutral (e.g. Tognoni) ratio for temperature calculation. Such a measure at very short time scales may accurately resolve local plasma conditions surrounding the aerosol particle, but provide increasingly reduced accuracy at longer time scales as aerosol mass diffuses across temperature and free electron variations within the plasma. This effect is observed even when only first ionization states are considered (an assumption of the dual-species ratio calculation) and the diffusion coefficient is non-species specific; however, including higher ionization states and allowing the diffusion coefficient to vary with species further magnifies the inaccuracies.

Fundamentally, the implication is that care must be taken for quantitative analysis of aerosol systems via LIBS and other plasma based diagnostic tools, given that the assumptions of analyte response being tied to the bulk plasma conditions may in fact be challenged by the concept of localized plasma conditions. Such an effect may play a role in explaining the size-dependency of carbon-based particles as was recently noted (Hohreiter and Hahn, 2005), and may indicate that despite the greater difficulty in acquiring spatially resolved intensity measurements such data may be required for accurate analysis of the analyte. These results may also have fundamental implications for LIBS analysis of solid, liquid, and gas systems, where local effects on the analyte signal may again play a significant role as opposed to the influence of the bulk plasma. Proper design of analysis schemes, including careful attention to the temporal evolution of the analyte signal and careful matching of analyte and calibration sources is necessary for quantitative analysis.
Table 2-1. Summary of test cases for the initial LIBS model

<table>
<thead>
<tr>
<th>Case</th>
<th>$d_p$ (nm)</th>
<th>% Mg/% Cd</th>
<th>Boundary Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>50/50</td>
<td>(Uniform concentration, all times)</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>50/50</td>
<td>Flux to zero concentration</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>50/50</td>
<td>No diffusion out</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>50/50</td>
<td>Flux to zero concentration</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>75/25</td>
<td>Flux to zero concentration</td>
</tr>
</tbody>
</table>
Figure 2-1. Finite difference grid system for plasma volume.

Figure 2-2. Cadmium concentration as a function of radial position for several time steps for (a) Case 2: diffusion into zero concentration and (b) Case 3: no mass loss from plasma volume. The red line in each plot shows Case 1: uniform concentration of equivalent initial particle mass over all time.
Figure 2-3. Total mass within the plasma volume for Cases 1 (constant/uniform concentration), 2 (Diffusion out into zero concentration), and 3 (no mass loss).

Figure 2-4. Temperature evolution using the simplified model construction with uniform initial temperature.
Figure 2-5. Evolution with time of free electron density for (a) Case 1: Constant/uniform concentration (b) Case 2: Diffusion to zero concentration (c) Case 3: No diffusion out of plasma volume. (d) Gives the difference in free electron density between cases 1 and 3 versus temperature.

Figure 2-6. Plasma temperature as a function of time for (a) the actual finite difference model results and (b) the Tognoni prediction under various conditions compared to volume weighted temperature.
Figure 2-7. Concentration of magnesium as a function of radial position for select time steps (as noted) and assuming linear particle evaporation/dissociation over 15 μs and 0.15 μs.
Figure 2-8. Concentration of cadmium as a function of radial position for select time steps (as noted) and assuming linear particle evaporation/dissociation over $15 \mu s$ and $0.15 \mu s$. 
Figure 2-9. Temporal evolution of the concentration of magnesium at the central node, halfway to the plasma boundary, and at the plasma edge, assuming linear particle evaporation/dissociation over 15 μs and 0.15 μs.
Figure 2-10. Volume-weighted average free electron density of the plasma as a function of time, along with the free electron density at three select locations (central node, halfway to the plasma boundary, and at the plasma edge) within the plasma volume.
Figure 2-11. Volume-weighted average plasma temperature as a function of time, along with the plasma temperature at three select locations (central node, halfway to the plasma boundary, and at the plasma edge) within the plasma volume.
Figure 2-12. Plasma temperature as a function of time within the central (particle containing) node of the plasma for four values of initial aerosol particle diameter.
Figure 2-13. Volume-weighted plasma temperature calculated using the Tognoni et al. (2007) dual species ion/neutral ratio for various conditions: (1) assuming only first ionization states with species dependent diffusion coefficient, (2) using the first and second ionization states with species dependent diffusion coefficient, (3) using the first and second ionization states but assuming a species independent diffusion coefficient. In addition, the true volume-weighted average plasma temperature and central node temperature of the model are included.
Figure 2-14. Lewis number calculated from the thermal diffusivity of the plasma and the average diffusion coefficient for both magnesium and cadmium as a function of radial position for select time steps.
CHAPTER 3
LASER PHOTOFRAGMENTATION FOR DETECTION OF SODIUM AEROSOLS AND ETHYL-3-ETHOXYPROPIONATE

Laser Photofragmentation/Fragment Detection (PF/FD) has shown promise for the qualitative and quantitative analysis of species unsuitable for detection with direct spectroscopic techniques such as laser induced fluorescence; however, much of the body of previous research has focused on its application to gas-phase alkali species, with only limited application to either complex hydrocarbon species or solid phase analytes. The goal of the current work is the investigation into PF/FD as a monitoring and detection strategy for both atmospheric aerosols and noxious hydrocarbon vapors, with a further investigation of the technique as a method of studying heterogeneous chemistry.

In the present study the analysis of particulate matter will be considered for the case of sodium aerosols generated from varying concentrations of NaCl, NaOH, and Na2SO4 in deionized water solutions, along with an aerosol generated from nebulization of solutions diluted from an ICP-grade analytical standard of Na in 2% HNO3. The technique will also be tested for the detection of a representative solvent, in this case gaseous ethyl-3-ethoxypropionate (EEP), a volatile organic compound (VOC) pollutant found in paint thinner and used in particularly high volume for the stripping of aircraft paint by the U.S. Air Force (Swenson et al., 1998). In addition to studying the alkalis and VOC separately, a system containing both EEP and the sodium aerosol species will be analyzed to observe and characterize their interaction. The effect of the carrier gas, specifically the concentration of oxygen, will be studied in regards to its quenching effect on the observed signal. Finally, time resolved measurements will allow for possible insight into the mechanism of photofragmentation for EEP and provide further information on the quenching of the observed signal by oxygen.
Methodology

Design of Experimental Configuration

The experimental set-up consists of a supply line of dry air, nitrogen supplied from a compressed gas cylinder, or a mix of pure nitrogen and pure oxygen supplied from compressed gas cylinders in a controlled ratio (Figure 3-1, c). The gas line is split with separate controlled flows running to a nebulizer (for aerosol generation), a bubbler (for introduction of EEP), and a diluting/drying coflow stream. The mass flow rate of each of the inlet gas streams was controlled with Alicat Scientific 16-Series Precision Gas Flow Controllers (MFCs) with an accuracy of +/- 1% of full scale and a repeatability of +/- 0.5% of full scale, with a full range of 0-1 SLPM on the bubbler line controller, 0-5 SLPM on the nebulizer line controller, and 0-10 SLPM on two MFC’s joined downstream controlling the coflow (yielding a total possible coflow rate of 20 SLPM).

Aerosol species were generated using a TSI Model 3076 Constant Output Atomizer (Figure 3-2). The atomizer consists of a glass reservoir of the particles of interest in a suspension of de-ionized water; as gas flows over the inlet tube from the reservoir, liquid is pulled upward and sprayed in a fine jet into the outlet tube. Large droplets impact the far wall of the tube and return back to the reservoir, leaving only fine droplets to continue on through the system. The concentration of analyte within suspension controls both the particle number density and size distribution of the resultant aerosol (Figure 3-3), with a more dilute suspension producing a greater number of smaller diameter particles. In order to minimize the laser energy required to evaporate liquid water, the output from the nebulizer was mixed with dry coflow gas in a mixing chamber (serving to both dry and dilute the stream) and further dried in a diffusion dryer consisting of a perforated gas flow tube embedded in silica desiccant to produce a very dry aerosol. It was discovered during the course of operation of the nebulizer that a shallow thread
depth on the cap sealing the glass reservoir resulted in leaks, therefore the glass reservoir was replaced with a similarly sized PVC tube and cap producing an airtight seal.

A bubbler was used to introduce vaporous EEP into the gas stream (Figure 3-4); this apparatus consisted of a PVC pipe sealed with caps at both ends. The top cap was drilled and tapped to accommodate two ¼” NPT to ¼” OD Swagelok fittings bored out to allow the insertion of a rigid ¼” tube running to the bottom of the liquid reservoir (on the inlet side) and a shorter ¼” tube terminating in the gas reservoir at the top of the bubbler (on the outlet side). Gas flowing through the inlet tube produces bubbles within the liquid reservoir that infuse with vaporous EEP as they float to the surface, filling the top of the bubbler with EEP doped gas which then flows through the outlet tube and into the sample chamber. Valves at the inlet and outlet of the bubbler allowed this apparatus to be closed off from the system for study of aerosol species without the presence of EEP.

The output from the nebulizer and the bubbler flowed into the sample chamber where photofragmentation takes place. Initially, this chamber consisted of a vacuum cross sealed at the top and bottom and containing windows at either end for optical access (Figure 3-1, a). During the course of operation, however, it was observed that this design produced eddies in the side chambers that effected the residence time of particles within the chamber. This design was replaced with a bored-out cube design that eliminated the regions of eddy formation (Figure 3-1, b). Both of these designs are described in further detail in the next sections. After passing through the sample chamber the gas/aerosol stream vented out through an exhaust tube.

The photofragmentation energy source was a GAM Laser, Inc., 193 nm EX5 ArF Excimer laser, operating at a wavelength of 193 nm and firing at a rate of 50 Hz. The laser passed through a pierced mirror and a lens that focused the beam to the center of the sample chamber,
with a beam dump past the chamber capturing the excess energy (Figure 3-1). Light emitted via
photofragmentation passed through the chamber window and was reflected by the pierced mirror
to a 4” diameter focusing lens that directed the light onto a fiber optic cable. The light
transmission through the focusing lens, composed of borosilicate crown optical glass, is 82%
transmission at 400 nm, 60% transmission at 350 nm, and falls below 10% for wavelengths less
than 300 nm. This cable carried the light to a Princeton Instruments CCD connected to an Acton
SpectroPro 300i spectrometer. Both the laser and the CCD were triggered using a delay
generator, with the CCD trigger set to a delay of 0.85 μs after the time of the laser trigger
determined experimentally to be the difference in time between the point at which the laser is
triggered and when it actually fires. The gate width of the CCD was set to 0.3 μs. Using
WinSpec software, the output spectra from the spectrometer in the wavelength range of interest
for the target species was captured and summed over 200 acquisitions to increase the signal-to-
noise ratio. The analysis of the spectra captured in this manner is described in a subsequent
section.

Vacuum Cross Sample Chamber

Initially the sample chamber consisted of a sealed vacuum cross (Figure 3-1, a and Figure
3-5; note that the apparatus itself is referred to as a vacuum cross because of its sealing
capabilities, in all cases this experimental set-up is run at atmospheric pressure and room
temperature). Both of the illustrations of the chamber are top views of the three-dimensional
cross; in reality, there are legs protruding from the top and bottom of the chamber as well that
were sealed off with end caps (e.g., the chamber has six symmetric spokes protruding from the
center section). The front and rear of the cross where the laser beam penetrates were sealed with
UV-grade quartz windows allowing optical access.
An interesting phenomena was observed while using this sampling apparatus. Following several runs of aerosol through the system it was noted that significant loss of particulate matter occurred to the walls of the sampling chamber. Given that the laden gas stream passes through a mixing chamber, diffusion dryer, and 2-3 meters of ½” OD tubing before entering the chamber, it can be presumed that the bulk of the aerosol that would be removed from the stream by the processes of impaction and diffusion would have done so before entering the chamber, therefore the relatively heavy deposition in the chamber was unexpected. It was determined that the optical table on which the experimental set-up rests is not electrically grounded, and a surface charge of ~0.5 mV builds up which is subsequently transferred to the chamber walls. Because particles generated from an atomizer such as the one used here have a charge distribution as they exit the nebulizer, this build-up of charge on the chamber wall led to a moderate electrostatic attraction between the aerosol and the surface and led to the heavy wall deposition. To correct this problem, the sample chamber was connected by wire to grounded metal infrastructure running over the optical table to remove the charge build-up; deposition on the chamber walls was observed to be virtually eliminated.

During the course of operation of this apparatus, it could be observed from the emission of light from the aerosol particles themselves during the filling and the clearing of the chamber that eddies were forming in the side chambers of the cross, thereby effecting the residence time of the aerosol and carrier gas within the sample chamber (Figure 3-5). Although this is not problematic for steady-state measurements (and thus several of the steady state results were obtained using this design), for any sort of time-resolved measurement it is necessary to eliminate these eddies so the residence time within the chamber is a controlled function of flow rate. To that end, the chamber was replaced with a more sophisticated bored-out cube design.
**Bored-Out Cube Sample Chamber**

In this design, a solid stainless steel cube was bored out with a tube of the same diameter as the gas inlet and outlet tubes so that flow could proceed through the chamber without the formation of large eddies (Figure 3-1, c; Figure 3-6). Optical access holes were drilled through the sides of the cube and into the gas flow chamber that could accommodate inset windows held in place by o-rings clamped down by stainless steel flanges; these windows were made of high transmission Suprasil UV grade quartz. The use of this design eliminates the large eddy formation that would cause an uncontrolled increase in residence time of the analyte within the chamber. As was done for the previous chamber design, the cube was grounded to minimize electrostatic losses to the walls. In addition, the cube was rotated so that inlet flow entered from the top of the chamber and exhausted out through the bottom so that any particulate matter deposited due to gravitational settling would collect downstream of the sample chamber (note that this was not observed to be significant).

**Issues of Energy Loss**

One of the principal issues discovered during the course of experimentation was the difficulty in maintaining high transmission of the laser energy through the access windows into the chamber. This effect was found to be considerably larger using the bored-out cube design despite the use of a higher grade of UV quartz. A noticeable drop in laser energy was observed with time both while analyte flowed through the chamber and to a reduced degree while the chamber was filled solely with filtered air (Figure 3-7). In this illustration, each point represents a different experimental measurement, with a time spacing between measurements of approximately 2 minutes (these data were recorded during a sequence of experiments with various aerosol analytes and/or EEP). Beam intensity was also recorded before the laser entered the sample chamber and was found to be nominally constant, therefore the decrease in energy is
a result of transmission losses within the chamber itself. This loss of energy is significant because the observed photofragmentation signal is a strong function of incident laser energy (Figure 3-8).

It was determined that this phenomena was the result of two contributing effects. The first loss is related to the presence of analyte within the chamber, specifically aerosol species. Over time, some aerosol is deposited on the quartz windows which attenuates the beam energy and reduces the overall transmission. By cleaning the windows with methanol, the transmission lost due to this effect can be restored. In order to minimize the loss of energy, the windows were cleaned in this manner between subsequent data runs.

In addition to the loss in transmission observed due to aerosol deposition on the quartz windows, it was determined that over time the energy loss in the bored-out cube chamber increased with time when the laser was fired through a chamber filled only with dry air; this effect cannot be explained by the deposition of particulate matter on the quartz, and transmission is not recovered by cleaning the windows. Although some loss in energy was observed for the quartz windows in the vacuum cross design, the effect was not observed to be as significant. The cause of the transmission loss was found to be degradation of the windows themselves under the intensity of the incident laser beam, despite them being constructed of UV grade quartz. In the bored-out cube design, the need to minimize eddy formation requires the optical access windows be close in distance to the focal point of the laser at the center of the chamber, thus causing the laser intensity to be significantly higher at the point of impact with the quartz than in the vacuum cross design. This high intensity UV energy is enough to degrade the quartz itself, an effect that can be observed both through transmission loss and through the visual observance of color deformities developing within the window. Unfortunately, given that the highest grade UV
quartz available was used in the construction of the chamber, there is no ready fix to this problem.

Because the effect is a relatively slow one, intensity of signal data taken within a reasonable period of time (2-3 hours) can be compared to observe trends in behavior, which forms the basis of some of the qualitative analysis performed during these studies (note also that the steady state measurements of individual species taken with the vacuum cross chamber are virtually unaffected by this phenomena). This problem, however, creates a change in condition that prohibits comparison of data sets taken on different days and reduces to some degree the quantitative analysis that can be performed on the magnitude of emitted intensity.

**Processing of Spectral Intensity Data**

As was previously mentioned, if the energy of the incident laser is sufficient photofragmentation of sodium containing aerosols such as those used in the present study produces excited sodium fragments which subsequently emit light at the characteristic wavelengths of 589.0 nm (16973.368 - 0 cm\(^{-1}\)) and 589.6 nm (16956.172 - 0 cm\(^{-1}\)) (Sansonetti et al, 2007). In the case of EEP, the photofragmentation of the complex hydrocarbon produces (as yet) undetermined fragments with excess energy contained and spontaneously emitted from the newly formed molecules (Table 3-1; Figure 3-9). Intensity peaks were observed at approximately 390 nm, multiple peaks in the region of 430-431 nm, 470 nm, 475 nm, 516 nm, and 559 nm. Note that the optics used in this experimental design would prohibit the observation of any peaks with a wavelength less than approximately 350 nm. Based on the chemical structure of EEP (C\(_7\)H\(_{14}\)O\(_3\); Figure 3-10) and known emitted wavelengths for various species the most likely candidates for all of these emissions are C\(_2\) and CH molecules. For the present study, the strongest two CH bands at 430.4 nm and 431.2 nm along with the strongest observed C\(_2\) band at 516.4 nm were considered for analysis (Figure 3-9; Table 3-1).
Processing of all spectral data was performed within Microsoft Excel and Mathworks Matlab software (Figure 3-11). In order to calculate the intensity of the peaks of interest, it is desirable to subtract off the background intensity observed independently of the presence of analyte species. This background profile can vary somewhat with wavelength due to “hot” pixels on the CCD array in addition to an underlying broadband intensity value that is a function of factors such as CCD temperature. In order to correct for this background, multiple “blanks” were taken with no analyte species present within the chamber; in order to most closely approximate the conditions of experimentation sans aerosol/EEP, the nebulizer was filled with de-ionized water only and all flow rates were maintained at the same values as for the experimental runs in order to obtain the blanks. All of the blanks were averaged together to obtain the wavelength dependent background intensity. In order to correct for the constant background intensity, an average value was taken in a range of wavelengths outside of the observed intensity peaks in both the spectral signal of interest ($I_{\text{signal,raw}}$) and in the blank signal ($I_{\text{blank}}$) and the ratio of backgrounds was taken (Equation 3-1; Figure 3-11).

$$R_f = \frac{I_{\text{signal,raw}}}{I_{\text{blank}}}$$  \hspace{1cm} (3-1)

The background corrected spectral signal ($I_{\text{signal, corrected}}$) was then calculated by subtracting the average blank signal corrected by the above ratio to account for changes in constant background signal (Equation 3-2).

$$I_{\text{signal,corrected}} = I_{\text{signal,raw}} - I_{\text{blank}} \cdot R_f$$  \hspace{1cm} (3-2)

The integrated intensity value for each wavelength of interest was then calculated by summing the intensity values within the peak to obtain a single value per observation per wavelength of interest.
Time Resolved Measurements

In addition to the integrated intensity measurements described above, time resolved photofragmentation data were collected. In this case, the spectral signal from the spectrometer was fed to a LeCroy Waverunner LT372 oscilloscope. The oscilloscope was triggered by a phototube (Hamamatsu R2949 No. QQ0181) collecting a portion of the 193-nm incident light passing through one of the mirrors which turned the beam and directed it into the sample chamber. Because the laser beam has a finite (if rapid) velocity in air and the signal itself takes time to transverse both the fiber optic cable and the electrical cable connecting the various instrumentation, there will be some lag in time between when the oscilloscope registers the trigger of the laser firing and when the beam reaches the sample chamber. In order to calibrate for this delay, the fiber optic cable used to collect emitted light was positioned at the exact distance from the sample chamber as it normally sits during experimentation, removing the focusing optics which filter out the 193-nm light from the collected signal. The delay time between the trigger and collected 193-nm scattered light was found to be 43.8 s; this time is therefore the delay between the laser signal observed on the oscilloscope and the observed photofragmentation signal due purely to the finite travel times of the laser in air and the light/electrons in the transmission cables. This value of time was subtracted off the observed photofragmentation signal so that any delay observed is due to the process of photofragmentation itself. In other words, the zero point in time for the observed signal has been corrected to correspond exactly to the moment of laser excitation.

Results

The data obtained allowed for qualitative investigation of the use of PF/FD for the detection and monitoring of sodium aerosols and the representative solvent, EEP, along with trends observed for systems containing both the aerosol and vapor species. In addition, oxygen
as a quenching species for the C₂ and CH signals observed following photofragmentation of EEP
was investigated using both time integrated and time resolved measurements. These results are
further elaborated in the present section. It is noted that oxygen quenching/photofragmentation
studies were carried out using the bored-out cube sample chamber design whereas the other
measurements were taken using the vacuum cross chamber.

**Sodium Signal Variation with Aerosol Species and Concentration**

Sodium containing aerosols were generated from several different concentrations of four
species in solutions, including NaCl, Na in HNO₃, Na₂SO₄, and NaOH (Figures 3-12, 3-13). The
resulting sodium concentration in the carrier gas (C_{Na,\text{gas}}, in \mu g/L) was calculated from the
volumetric flow rate of the nebulizer (determined experimentally as the change in liquid volume
with time to be 0.0667 ± 0.013 mL/min), the mass concentration of the analyte within the
solution (C_{sol}, in \mu g/mL), the total flow rate of air through the system (\dot{V}, in L/min), and the
mass fraction of sodium within each species (y_{Na}; Equation 3-3).

\[
C_{Na,\text{gas}} = \frac{0.0667 \cdot C_{sol}}{\dot{V}} \cdot y_{Na}
\]  

(3-3)

The mass fraction of sodium within each species was calculated as the percentage
contribution of sodium to the total molecular weight of the species calculated from the sum of
each component species (MW_i; Equation 3-4).

\[
y_{Na} = \frac{MW_{Na}}{\sum_i MW_i}
\]  

(3-4)

As an example, for a 120 \mu g/mL solution of Na₂SO₄ with a total flow rate of 14 L/min the
sodium mass fraction in the aerosol would be calculated from the molecular weights of sodium
(22.99 g/mol), sulfur (32.07 g/mol), and oxygen (16.00 g/mol; Equation 3-5).
The concentration of sodium in air of the resultant nebulized aerosol can then be calculated (Equation 3-6).

\[
Y_{Na} = \frac{2 \cdot 22.99 \, [g/mol]}{2 \cdot 22.99 \, [g/mol] + 32.07 \, [g/mol] + 4 \cdot 16.00 \, [g/mol]} = 0.3237
\]  

(Equation 3-5)

Note that data comprising Figures 3-12 and 3-13 come from different days, therefore as previously discussed only the behavioral trends within each plot may be considered and quantitative comparisons of the order of magnitude of the observed signal between the two plots cannot be made.

Within these two plots, it can be observed that a photofragmentation signal is seen at the sodium 589.6 nm line for NaCl, Na in HNO₃, and NaOH, with a larger concentration of aerosol producing a larger resultant signal, whereas no signal is seen from Na₂SO₄. Similar results were also observed for the sodium line at 589.0 nm. This result is unsurprising when one considers the energy threshold required to break down each of the considered aerosols, shown Table 3-2. A 193-nm laser is sufficient to break apart the Na, NaCl, and NaOH species and leave them in the excited state required for prompt emission photofragmentation, with higher concentrations of particles within the system resulting in an increased observed signal; however, insufficient energy is present to break down Na₂SO₄ therefore no signal is observed at any concentration of aerosol. These results are consistent with previous findings in the literature where photofragmentation of Na₂SO₄ aerosols with a 193-nm laser produced only a weak Na emission at very high incident laser energies, attributed to multiphoton interaction; NaCl and NaOH produced much stronger Na emission even at lower incident laser energies (Hidalgo Núñez et al., 2001). It is also interesting to note that the observed Na signal is linear as a function of incident
laser energy (Figure 3-8), indicating that photofragmentation in this case is the result of a single photon process. The observed signal for aerosol species is not, however, linear as a function of sodium concentration (Figures 3-12, 3-13), an unsurprising result given that PF/FD does not result in complete vaporization of the analyte particle (as opposed to LIBS). Because the aerosol is not completely vaporized, the technique only probes surface regions of the particle and not the bulk volume, resulting in non-linear surface area-to-volume ratio effects on the observed signal.

**Drop in Ethyl-3-Ethoxypropionate Signal In the Presence of Aerosol Species**

As previously discussed, vaporous EEP was introduced to the sample chamber by saturating gas flowing through a bubbler type apparatus at 1 L/min and diluting the stream by mixing with either 13 L/min of N₂ (if considering EEP alone) or 4 L/min of an aerosol stream generated as previously described and 9 L/min of coflow (e.g., in all cases the total flow rate of gas is 14 L/min). In this case N₂ was used as the carrier gas in place of air because a decrease in strength of the EEP signal (e.g., quenching) was observed as a result of oxygen present in the system; this effect was further quantified with additional experimentation with results described later in this section. Various particulate concentrations were considered for each of the three aerosol species which produce Na emission from 193-nm photofragmentation, with results for the observed EEP signal at the CH bands of 430.4 nm and 431.2 nm shown in Figures 3-14 and 3-15 and results for the C₂ band at 516.4 shown in Figure 3-16. As previously mentioned, one of the difficulties inherent with this experimental set-up is the loss of signal observed over time due to degradation of the optical windows by the incident UV radiation. To ensure that any drop in signal was a result of adding aerosol to the system, experiments consisted of taking an initial signal for isolated EEP followed by measurements for each concentration/species of aerosol species and lastly a final signal of isolated EEP; both initial and final standalone EEP measurements are shown in Figures 3-14, 3-15, and 3-16.
For all three EEP bands observed, the intensity signal drops significantly with the presence of even a small concentration of aerosol species. Increased aerosol loading magnifies this effect, with larger concentrations of aerosol resulting in a greater loss of the EEP signal. The sodium signal observed from each of the considered species (not shown) was unaffected by the presence of EEP and showed no drop in signal for any of the concentration levels. The signal observed from the EEP/aerosol mixtures is significantly reduced even over the final measurement of EEP taken after the course of experimentation, verifying that the signal loss is due to the presence of aerosol within the system and is not a result of a decrease in optical window transmission.

**Oxygen Quenching of Ethyl-3-Ethoxypropionate Signal**

During the course of experimentation, it was discovered that the EEP signal observed in both CH and C2 bands was significantly lower using a carrier gas of air as opposed to pure N2, whereas the sodium signal observed from the aerosol species was unaffected by the presence of oxygen. To quantify this observation, photofragmentation studies were carried out using mixtures consisting of N2 and O2 at various concentrations ranging from pure nitrogen to 80% nitrogen, 20% oxygen (approximately the composition of air; higher concentrations of oxygen were not considered given the potentially flammability of the EEP vapor in more oxygen rich environments). The spectra observed for EEP is shown in Figures 3-17 and 3-18, and the spectra observed for an example sodium species (NaCl) is shown in Figure 3-19. In the case of EEP, a dramatic decrease in signal for intensity bands in both the ~430 nm range and the ~516 nm range is observed with increased concentration of oxygen, whereas the sodium signal is unaffected by the change in percentage of O2. The observed C2 band at 516.4 nm almost completely disappears with only a modest concentration of oxygen present, 4%.
To further investigate oxygen as a quenching species, Stern-Volmer plots of the intensity of the EEP signal were created. The information obtained from this type of plot derives from the relationship of the quantum efficiency of a fluorescent species (\(\Phi_F^0\)) to the quantum efficiency of the same fluorescent species in the presence of a quencher (\(\Phi_F\)) of a given concentration \([Q]\) as a function of the Stern-Volmer quenching constant (\(K_q\); Equation 3-7; Ingle and Crouch, 1988).

\[
\frac{1}{\Phi_F} = \frac{1}{\Phi_F^0} + \frac{K_q}{\Phi_F^0} [Q]
\]  

(3-7)

A linear relationship between the inverse of observed fluorescence and the concentration of another species indicates that the second species is acting as a quencher. Stern-Volmer plots for the emission originating from the two CH bands (430.4 nm and 431.2 nm) are shown in Figures 3-20 and 3-21, with a plot for the EEP signal from the strongest C2 band (516.4 nm) shown in Figure 3-22 and a plot for the sodium band at 589.6 nm shown in Figure 3-23 (similar results were observed for the sodium band at 589.0 nm). Note that the EEP signal at 516.4 nm virtually disappears at oxygen concentrations higher than 4%, therefore this plot is truncated at this value and the large standard deviation observed in comparison to the other bands is a result of the poor signal-to-noise ratio. For all of the EEP bands, the inverse of the integrated peak shows a linear relationship with the concentration of oxygen, indicative that oxygen is a quenching species. As was observed in the spectral plots, the sodium signal itself is unaffected by the presence of EEP.

**Photofragmentation Timing**

In order to gain insight into the mechanism of the photofragmentation of EEP itself along with the quenching of the signal by oxygen, time resolved measurements of the observed intensity were taken as previously described. In the case of quenching, those species which act as static quenchers (e.g., a non-fluorescent complex is formed between quencher and fluorophore) do not effect the fluorescence lifetime, whereas those species which participate in
dynamic quenching (e.g., collisions between the quencher and the fluorophore result in non-radiative energy transfer and decay to the ground state) do result in a reduction in the fluorescence lifetime (Ingle and Crouch, 1988). An example of this type of measurement is shown in Figure 3-24 as a percentage of the maximum intensity observed at each wavelength along with the time-resolved laser signal (corrected for the delay between the time of measurement of the pulse and the time the pulse is incident on the sample chamber) for each of the three wavelengths of emitted intensity considered for EEP, 430.4 nm, 431.2 nm, and 516.4 nm. Examples for the sodium bands at 589.0 nm and 589.6 nm emitted from NaCl are also shown along with a sample EEP emission (at 431.23 nm) for comparison in Figure 3-25. The sodium signal can be observed to be much narrower, with a peak width of just over 20 ns that is only slightly longer than the laser pulse itself. By comparison, the signal observed in each of the observation bands of EEP are much wider, with a sharp increase in signal during the time of the incident laser pulse and a long decay time of over 100 ns.

To quantify this result, the decay time constant ($\alpha$) for each observed band was calculating by fitting an exponential to the decay in observed intensity signal as a function of the maximum signal ($y$, unit-less; Figures 3-24, 3-25) as a function of time ($t$, in ns; Equation 3-8).

$$y = \exp(-\alpha \cdot t)$$

(3-8)

Note that a larger decay time constant corresponds to a more rapid decay of the signal. The time constant calculated in this manner for the EEP bands of interest at varying concentrations of oxygen in nitrogen are shown in Figures 3-26, 3-27, and 3-28, and the time constants for the 589.0 nm and 589.6 nm sodium lines are shown in Figures 3-29 and 3-30. In each of these plots the $y$-scale is the same, allowing comparison between the values for the different species/wavelengths. It can be observed that in addition to the EEP signal showing a
drop in intensity with increased oxygen concentration as previously discussed the decay time constant also increases, indicating a faster drop in signal intensity and a shorter fluorescence lifetime. Even at the highest concentration of oxygen tested, however, the decay time is still longer for EEP than either the 589.0 nm or 589.6 nm band of sodium, found to be insensitive to the presence of oxygen in the carrier gas.

**Discussion and Conclusions**

**Detection of Sodium Species/EEP and Observation of Adsorption**

Several trends in behavior emerged from the UV photofragmentation studies on vaporous EEP and aerosol sodium species. The first is that, as would be expected, an increase in the aerosol loading of sodium species produces a larger signal for both the 589.0 nm and 589.6 nm sodium bands for those species with a breakdown energy threshold below that corresponding to the incident laser wavelength of 193-nm (6.4 eV); Na$_2$SO$_4$, with a breakdown threshold energy corresponding to a shorter wavelength of light, cannot be photofragmented with 193-nm light and produces no signal. PF/FD is also shown to be capable of detecting the representative solvent EEP through multiple observed emission lines for both CH and C$_2$ molecules, demonstrating the technique as a valid method of monitoring noxious hydrocarbons. Secondly, the signal observed from EEP at wavelengths of energy corresponding to both CH and C$_2$ molecules decreases in the presence of aerosol loading, with higher concentrations of aerosol producing weaker signals. This signal loss is attributed to adsorption of the vapor species onto the aerosol molecules, the precursor step for heterogeneous chemical reaction in which the particulate matter acts as a reactant, catalyst, or reaction site. Although further modification to the experimental configuration would be required to eliminate the energy loss problem and allow for quantitative investigation of adsorption, this study demonstrates PF/FD as a valid
methodology for the study of heterogeneous chemistry involving vaporous and aerosol species that spontaneously fluoresce following laser photofragmentation.

**Proposed Photofragmentation Pathways for EEP**

Based on the molecules observed from the photofragmentation of EEP, it is possible to suggest the possible mechanisms of fragmentation and excitation/emission. All of the emission bands seen following PF/FD correspond to either C$_2$ or CH molecules, indicating conclusively that these species are produced from the parent molecule. It should be noted that not all fragments produced will retain enough energy to be in an excited state, not all excited states will decay via fluorescence, and the optics of the experimental set-up prohibit the observation of bands of wavelength less than approximately 350 nm, therefore this list of photofragments is not comprehensive.

Based on the molecular structure of EEP (Figure 3-10), two photofragmentation pathways are proposed. In the first, an ethyl radical (C$_2$H$_5$) is produced by cleaving one of the two single C-O bonds in the linear EEP chain, e.g.:

\[
C_7H_{14}O_3 \rightarrow C_2H_5 + C_5H_9O_3 \tag{3-9}
\]

The ethyl radical may then undergo subsequent hydrogen abstraction via collision with other molecules (N$_2$, O$_2$, or other photofragments) until it results in a C$_2$H (ethynyl) radical. From this point, subsequent hydrogen abstraction could result in the production of an excited C$_2$ which could then fluoresce and produce the lines observed corresponding to this molecule (Equations 3-10, 3-11; example given for the strongest line observed at 516.4 nm).

\[
C_2H \rightarrow C_2^* + H \tag{3-10}
\]

\[
C_2^* \rightarrow C_2 + h\nu_{516.43\text{nm}} \tag{3-11}
\]
The ethynyl radical has also been shown to react with oxygen in combustion systems to produce an excited CH molecule via the reaction (Equation 3-12; Turns, 1996).

\[ C_2H + O_2 \rightarrow CH(A^2\Delta) + CO_2 \]  

(3-12)

The excited CH molecule produced via this pathway will subsequently fluoresce to produce an observed emission line (Equation 3-13; Turns, 1996).

\[ CH(A^2\Delta) \rightarrow CH(X^2\Pi) + h\nu \text{431.7 nm} \]  

(3-13)

In the second proposed pathway, in the initial photofragmentation of EEP a methyl radical (CH₃) is produced by the cleaving of one of the C-C bonds located at either end of the linear parent molecule (Equation 3-14).

\[ C_7H_{14}O_3 \rightarrow CH_3 + C_6H_{11}O_3 \]  

(3-14)

Hydrogen atom abstraction of the methyl radical may produce an excited CH molecule which will subsequently fluoresce and result in the observed emission lines (Equation 3-15).

\[ CH_3 \rightarrow CH_2 \rightarrow CH^* \rightarrow CH + h\nu \]  

(3-15)

Note that the specific state of CH produced prior to fluorescence in the first proposed photofragmentation pathway (Equation 3-13) is only one possible excited state; excess energy may result in CH molecules emitting other wavelengths of light corresponding to decay from other excited states. Although insufficient data exists to confirm or exclude the above proposed photofragmentation pathways, both are consistent with the production of the observed signal of C₂ and CH molecules.

**Quenching of EEP Signal in the Presence of Oxygen**

Although sodium aerosol species are shown to be insensitive to the presence of the carrier gases considered, both C₂ and CH bands observed during photofragmentation of EEP are reduced with increasing concentrations of oxygen. This result is further illustrated through the
use of Stern-Volmer plots, in which a linear relationship is shown between the magnitude of the observed emission for each of the bands observed from the photofragmentation of EEP and the concentration of oxygen. In addition, time-resolved measurements of the C₂ and CH fluorescence illustrate that oxygen reduces the lifetime of the excited states, indicating O₂ dynamically quenches each of the excited photofragments.

It is interesting to compare this result to observations made in regards to the behavior of CH(A²Δ) in combustion systems both alone and in the presence of other species, a summary of which is provided in Table 3-3. Both oxygen and other species can interact with the excited CH molecule to result in non-fluorescent decay to the ground state; however, the order of magnitude of the forward rate constant for the reaction with oxygen (4.1 \times 10^{13} \text{ m}^3\text{mol}^{-1}\text{s}^{-1} \text{ at } T = 293 \degree \text{K}) is two orders of magnitude greater at the same temperature than that observed for reaction with any other species, for example, nitrogen (6.8 \times 10^{11} \text{ m}^3\text{mol}^{-1}\text{s}^{-1} \text{ at } T = 293 \degree \text{K}). The oxygen quenching reaction is also seven orders of magnitude greater than the rate at which the excited species will fluoresce to return to the ground state (1.9 \times 10^6 \text{ s}^{-1} \text{ at } T = 293 \degree \text{K}). This increased reaction rate is consistent with the quenching behavior observed in the photofragmentation system; although N₂ or other photofragments may be colliding with the excited CH molecules and resulting in non-fluorescent decay, the rate of reaction between oxygen and the excited CH molecules is orders of magnitude faster, resulting in both the reduced magnitude of fluorescent signal (due to reduced quantum efficiency) and the shorter lifetime seen in time resolved measurements.

Overall, PF/FD has been demonstrated to have potential as a possible monitoring technique for both sodium containing aerosols and hydrocarbon solvents and as a study mechanism for heterogeneous chemistry involving these species. Although limitations of the current experiments prevent quantitative calibration for parent species concentration, a qualitative
relationship of increased PF/FD signal is observed for larger concentrations of aerosols and a
reduction of the EEP signal is seen in the presence of particles as a result of vapor loss via
adsorption. An investigation into the effect of oxygen on the observed signal has demonstrated
that this species acts as a quencher for the photofragments generated in PF/FD analysis of EEP,
indicating that any efforts to quantitatively determine solvent concentrations must take carrier
gas composition into account when performing calibration.
Table 3-1. Observed bands of interest from molecules generated from the photofragmentation of sodium containing aerosols and EEP.

<table>
<thead>
<tr>
<th>Species</th>
<th>Transition</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na I</td>
<td>16973.4 – 0 cm⁻¹</td>
<td>589.0 nm</td>
</tr>
<tr>
<td>Na I</td>
<td>16956.2 – 0 cm⁻¹</td>
<td>589.6 nm</td>
</tr>
<tr>
<td>CH</td>
<td>A²Δ→X²Π</td>
<td>430.5 nm</td>
</tr>
<tr>
<td>CH</td>
<td>A²Δ→X²Π (0,0)</td>
<td>431.4 nm</td>
</tr>
<tr>
<td>C₂</td>
<td>A¹Π₉→X³Πₙ (1,2)</td>
<td>516.5 nm</td>
</tr>
</tbody>
</table>

Data from Sansonetti et al. (2007) and Pearse and Gaydon (1963).

Table 3-2. Photofragmentation pathway of each of the considered aerosol species along with the threshold energy and corresponding wavelength to produce Na fragments in the lowest ²P state.

<table>
<thead>
<tr>
<th>Photofragmentation Pathway</th>
<th>ΔH [Kcal/mol]</th>
<th>λₘₜ [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl → Na* + Cl</td>
<td>149.9 ± 2</td>
<td>196 ± 6</td>
</tr>
<tr>
<td>NaOH → Na* + OH</td>
<td>130.0 ± 5</td>
<td>220 ± 15</td>
</tr>
<tr>
<td>Na₂SO₄ → Na* + NaSO₄</td>
<td>160.1 ± 5</td>
<td>179 ± 15</td>
</tr>
</tbody>
</table>

Data from Hidalgo Núñez et al. (2000).

Table 3-3. Possible decay mechanisms for the excited CH molecule both alone and in the presence of other species. The forward rate constant is given by the equation k=ATⁿexp(-Eₐ/RₜT), where T is the temperature [K], Eₐ is the activation energy [kJ/gmol], and Rₜ is the universal gas constant.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Forward Rate Coefficients</th>
<th>Forward Rate Constant (k) (T = 293 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(A²Δ) → CH(X²Π) + hν</td>
<td>A 1.9 x 10⁶  N 0  Eₐ 0</td>
<td>1.9 x 10⁶ [s⁻¹]</td>
</tr>
<tr>
<td>CH(A²Δ) + M → CH(X²Π) + M</td>
<td>A 4.00 x 10¹⁰  N 0.50  Eₐ 0</td>
<td>6.8 x 10¹¹ [m³ mol⁻¹ s⁻¹]</td>
</tr>
<tr>
<td>CH(A²Δ) + O₂ → CH(X²Π) + O₂</td>
<td>A 2.40 x 10¹²  N 0.50  Eₐ 0</td>
<td>4.1 x 10¹³ [m³ mol⁻¹ s⁻¹]</td>
</tr>
</tbody>
</table>

Data from Turns (1996).
Figure 3-1. Design of the photofragmentation experimental test configuration (c) along with diagrams showing the initial vacuum cross sample chamber (a) and the modified bored-out cube sample chamber (b).

(a) Vacuum chamber design

(b) Cube chamber design

(c) Experimental configuration

Figure 3-1. Design of the photofragmentation experimental test configuration (c) along with diagrams showing the initial vacuum cross sample chamber (a) and the modified bored-out cube sample chamber (b).

Figure 3-4. Schematic of the bubbler used to dope the inlet gas stream with EEP.
Figure 3-5. Schematic of the vacuum cross chamber design. Eddies can form in the side branches of the chamber effecting the residence time for the analyte in the laser beam.

Figure 3-6. Schematic of the bored-out cube chamber design. Note reducing eddy formation makes the residence time in the laser beam a direct function of flow rate.
Figure 3-7. Plot showing the increase in energy loss through the chamber as a function of time due to window degradation. Each individual point marks one data point; time between each data point is approximately 2 minutes.
Figure 3-8. Plot of the integrated sodium signal as a function of laser energy for a solution of 1 g/100 mL of NaCl.
Figure 3-9. Example of the measured spectral signal emanating from photofragmentation of EEP and a sodium aerosol, in this case NaCl.
Figure 3-10. Chemical structure of EEP. Note that a hydrogen molecule completes each of the unlabeled branches, yielding a chemical formula of $C_7H_{14}O_3$.

Figure 3-11. Method of removing the background from the spectral signal in order to integrate the peak of interest. Example shown for the 431.2 nm peak of EEP.
Figure 3-12. Integrated sodium signal at the 589.6 nm peak for aerosols generated from solutions of NaCl, Na in HNO₃, and Na₂SO₄ as a function of sodium concentration in air.
Figure 3-13. Integrated sodium signal at the 589.6 nm peak for aerosols generated from solutions of NaCl, Na in HNO₃, and NaOH as a function of sodium concentration in air.
Figure 3-14. Drop in EEP signal at 430.4 nm with the addition of different concentrations of sodium aerosol originating from varying source species.
Figure 3-15. Drop in EEP signal at 431.2 nm with the addition of different concentrations of sodium aerosol originating from varying source species.
Figure 3-16. Drop in EEP signal at 516.4 nm with the addition of different concentrations of sodium aerosol originating from varying source species.
Figure 3-17. EEP spectra around 430 nm for varying mixtures of O$_2$ and N$_2$. Note the strong reduction in signal with increased oxygen concentration.
Figure 3-18. EEP spectra around 516 nm for varying mixtures of O₂ and N₂. In this case, even a small amount of oxygen results in total suppression of the signal.
Figure 3-19. Sodium spectra generated from NaCl in varying mixtures of O$_2$ and N$_2$. Note that the sodium signal is unaffected by the presence of oxygen.
Figure 3-20. Inverse of the integrated EEP peak at 430.4 nm as a function of the percentage of oxygen.
Figure 3-21. Inverse of the integrated EEP peak at 431.2 nm as a function of the percentage of oxygen.
Figure 3-22. Inverse of the integrated EEP peak at 516.4 nm as a function of the percentage of oxygen.
Figure 3-23. Inverse of the integrated sodium peak at 589.6 nm as a function of the percentage of oxygen for NaCl. Note that the presence of oxygen has virtually no effect on the observed signal.
Figure 3-24. Example of the time dependent signal emitted from EEP at the various wavelengths of interest along with the scattering signal of the laser itself at 193 nm.
Figure 3-25. Example of the time dependent signal emitted from NaCl at the two wavelengths of interest along with the scattering signal of the laser itself at 193 nm. Also shown is an example of an EEP signal emitted at 431.2 nm; note the relatively wide time delay over which a signal is observed from the hydrocarbon as opposed to the sodium species.
Figure 3-26. Decay time constant for EEP at 430.4 nm. The asterisk and error bars shown the mean and standard deviation for the decay time constant calculated independently for each experimental run, the triangle shows the decay constant calculated from the intensity signal first averaged over all of the runs.
Figure 3-27. Decay time constant for EEP at 431.2 nm. The asterisk and error bars show the mean and standard deviation for the decay time constant calculated independently for each experimental run, the triangle shows the decay constant calculated from the intensity signal first averaged over all of the runs.
Figure 3-28. Decay time constant for EEP at 516.4 nm. The asterisk and error bars shown the mean and standard deviation for the decay time constant calculated independently for each experimental run, the triangle shows the decay constant calculated from the intensity signal first averaged over all of the runs.
Figure 3-29. Decay time constant for NaCl at 589.0 nm. The asterisk and error bars shown the mean and standard deviation for the decay time constant calculated independently for each experimental run, the triangle shows the decay constant calculated from the intensity signal first averaged over all of the runs.
Figure 3-30. Decay time constant for NaCl at 589.6 nm. The asterisk and error bars shown the mean and standard deviation for the decay time constant calculated independently for each experimental run, the triangle shows the decay constant calculated from the intensity signal first averaged over all of the runs.
The goal of the current study is to further explore flow cytometry as a detection and quantification scheme for single particles in suspension with particular attention paid to applying the technique for the identification of cancer-indicative circulating tumor cells. To that end, an experimental rig has been constructed that flows particles in suspension through a quartz sample tube where they can be illuminated with visible light to create an image, pass through a 594-nm laser to produce a scattering signal, and/or pass through a 355-nm excitation laser to induce particle fluorescence. Test particles consisting of silica beads and fluorescent-dye doped polystyrene particles demonstrated the ability of the experimental set-up to image particles and detect the scattered light and fluorescence signal. In order to quantify the effect of spatial position within the viewing area and the effect of suspending particles in fluid and to investigate fluorescence lifetime, static measurements were also taken of the test beads under dry and wet conditions for comparison with the dynamic measurements taken in a flowing stream. A statistical investigation into the theoretical percentage of observed particles and total volume sampled at a given concentration and laser rep rate was undertaken and compared to the actual number of particles seen using the dynamic set-up. The results of these tests are described below.

**Methodology**

**Test Particles**

In order to test the effectiveness of the flow cytometry rig, two types of test particles have been examined. The first are 2.4 μm silica beads, used to determine if particles within the sample tube could be visually imaged and recorded using the test bed camera. These beads were diluted in an ultra-purified deionized water suspension to a concentration of 4.2e4/mL. The
second are 5.26 μm diameter fluorescent-dye doped particles (Bangs Labs Glacial Blue polystyrene carboxyl fluorescent microspheres, FC06F/8184) diluted from a standard solution of 5.5e6/mL. These particles have an absorption band of light centered at 350 nm, and when excited emit light centered at 447 nm, allowing the fluorescence detection capabilities of the rig to be investigated.

**Dynamic Experimental Set-Up**

The dynamic flow cytometry experimental set-up consists of a pumping system, various illumination light sources, and collection optics (Figure 4-1; Table 4-1). The particles in suspension are pumped by a Bioanalytical Systems (BAS) Baby Bee syringe pump (Figure 4-2) through a short length of Teflon tubing (~10 cm) before transitioning through a stainless steel connector tube and passing into a 100 μm diameter UV-grade quartz capillary tube. The metal transition tube fits snugly into the Teflon tubing to produce a watertight seal, while the capillary tube is sealed to the metal connector with epoxy (Figure 4-3). The controller can depress the syringe plunger to produce a flow rate varying from 0.1-100 μm/min, which for the 1 mL syringe used in these experiments will produce a fluid velocity varying from approximately 0.1-85 cm/s within the capillary tube (noting that these values are an approximate average value, individual particle velocity will vary parabolically from the tube walls to the center line as dictated by Poiseuille flow). Particles within the pumping syringe are kept in suspension through the use of an automated agitation mechanism (Appendix A).

A number of light sources are used to illuminate particles in the system. A white lamp is directed onto a blank white card beneath the sample tube for backlighting, allowing the tube and particles to be visually seen and recorded when the image is magnified. A 594-nm HeNe laser is directed through the end of the capillary tube which acts as a light pipe, causing a scattering signal to be emitted from the particles as they move along the length of the tube. This
configuration was used to minimize the scattering off the quartz sample tube, found to be significant when the scattering laser was directed through the tube wall. Despite this effort to minimize the noise and background in the scattering signal, although visually it is possible to distinguish individual particles as they pass through the system, the spatially integrated (over the length of the tube) spectrometer signal does not allow for particle identification because of a small signal-to-noise ratio and the fact that at any point in time the scattered light from at least one particle is almost always present. Here the scattering was useful for visualization, but not for triggering. An image-based trigger would be viable, but was not implemented. Finally, a frequency tripled 355-nm Nd:YAG laser is focused onto the sample tube (through the quartz wall, which fluoresces negligibly), providing a fluorescence light source for the fluorescent-dye doped test bead particles.

Magnification of the tube/particle image is done by a 50x magnification microscope mounted above the capillary tube. The image and scattered/emitted light from the sample area passes from the microscope to a mirror which turns the beam from vertical to horizontal (done for convenience purposes). The light then enters a 50/50 beam splitting cube, with half of the light/image passing through to short connection tube mounted on the other side. A 60 nm width bandpass filter centered at 447 nm may be inserted or removed into this tube; with the filter in place, the scattered 355-nm light as well as other ambient sources are removed to allow fluorescence from the fluorescent-dye doped particles to be detected, whereas with the filter in place the tube and particles can be seen and imaged (visually). The light passes through the connection tube to a Hitachi Color CCD KP-D20B camera which transmits the captured image in real time to the connected PC computer. National Instruments Visual Acquisition software allows for either continuous viewing of the transmitted images or the capturing of an individual
snapshot when triggered manually. The other half of the light is turned 90° and lens-coupled into a fiber optic cable connected to an Acton SpectroPro 300i spectrometer. For time resolved measurements, the signal from the spectrometer is passed to a PMT (Hammatsu R2949 No. QQ0181) and recorded with a LeCroy Waverunner LT372 digital oscilloscope. The oscilloscope is triggered by a delay generator synced in time with the 355-nm Nd:YAG laser used to excite the fluorescent-dye doped particles, therefore any particles passing through the capillary tube at the time of the laser firing should appear as peaks of light at the emitted wavelength of approximately 447 nm.

Alternately, individual spectra could be recorded centered around the peak wavelength of 447 nm using an intensified CCD array detector and captured using WinSpec software, again triggered to be synchronized with the firing of the Nd:YAG. When a particle is present within the sample volume, the spectra will display a significant fluorescence signal above a negligible background intensity, whereas only the background will be present if no particles were sampled. Using Matlab, the spectra were processed to separate “hits” from “misses”, with a hit defined as any spectra where the average intensity of the 25 pixels centered at the peak fluorescence wavelength of 447 nm was 10% greater than the background intensity defined as an average of the 25 pixels on either edge of the viewing window. Using this methodology, it was possible to both isolate individual spectra containing the signal from dye-doped particles as well as to objectively quantify the number of particles observed in the flowing stream in a given time frame.

**Static Experimental Set-Up**

In order to quantify the fluorescence signal from the dye-doped test particles independently of any flow effects, static measurements were also taken. In this experimental configuration, a suspension of particles was introduced onto a quartz flat and dried, noting that the flat itself
produces negligible fluorescence from the 355-nm excitation source. The flat replaced the capillary tube under the microscope objective, and was mounted to a stage that allowed translation along the x, y, and z axes for quantification of the effects of position within the viewing region of the microscope on the observed signal. In order to isolate the region of laser excitation to the spatial area below the microscope, a 150 µm aperture was inserted in the beam path, thus reducing noise generated by light emitted from excited particles outside of the microscope viewing area. Three sample configurations were taken to examine the effects of carrier fluid on the observed signal (Figure 4-4). In the first condition, the solution on the quartz flat was allowed to dry completely before being examined with no cover in place between the sample and the microscope. In the second condition, a thin plastic separation ring was placed on the quartz flat around the dry sample and a second flat was placed on top to examine the effects of the laser and fluorescence signal passing through the quartz cover on the observed signal. In the final condition, the sample was not allowed to dry before the separation ring and cover were put into place, effectively trapping the wet sample on the flat and allowing for study of the particles under actual suspension conditions. Note that the stage was used to adjust the z-position of the particles to account for the change in height of the focal plane due to the addition of the quartz flat, and the zero height position in each data set corresponds to the plane at which the particles are in focus.

Results of Static Experimental Configuration

Effects of Particle Position in Viewing Region on Observed Signal

Under the dynamic condition of a suspension flowing through the capillary tube it was not possible to determine the specific location of an individual particle within the viewing region, with the current set-up, therefore potentially some variation in signal could exist depending on the position of the particle within the tube. Although a triggering system could be used to time
the excitation laser to ensure particles are at a specific location, static measurements of particle
signal variation with position were first considered to determine if this effect was significant. An
isolated individual particle was identified and centered within the viewing area, and the emitted
signal integrated from 50 shots (taken at a laser firing rate of 10 Hz) was measured as a baseline.
The aforementioned stage was used to systematically move the particle in the x-, y-, and z-
direction, taking the same 50 shot integrated signal at each location. Measurements were done
using dry particles to prevent Brownian motion from causing the particles to move independently
of the action of the stage. In between measurements the laser shutter was closed to prevent
excessive excitation from diminishing the fluorescence capability of the test particles (i.e.,
photobleaching).

The results of this investigation for three individual particles are shown in Figure 4-5, with
each symbol (x, +, and *) representing a separate particle; the results have been normalized as a
percentage of the maximum observed signal for each particle. The capillary tube diameter is also
shown as a reference in each panel, noting that the panels have different x-axis scales. Dashed
lines indicate the cutoff where the signal from each particle drops as it moves outside of the
range of the viewing area. It is interesting to note that during the course of investigation it was
determined that the region visually imaged by the camera system is smaller than the area
interrogated by the spectrometer, therefore particles may be observed by their fluorescence
signal even if they are no longer present in the camera’s field-of-view. Within Figure 4-5 it can
be observed that the signal observed from an individual particle is relatively constant while the
particle is within the viewing area and drops off sharply as it moves outside of this region. In the
x-, y-, and z-direction the width of this viewing region is wider than the diameter of the sample
tube, indicating that no variation in signal will occur due to spatial variation of excited particles within the capillary tube.

**Variation in Signal of Wet vs. Dry Particles**

Another question of interest is if the signal from an individual particle varies depending on if it is dry or in solution. In addition, it is desirable to confirm that the addition of multiple particles within the viewing window results in the expected linear or near linear increase in observed signal. As previously mentioned, the effect of dry vs. wet conditions was investigated by comparing the signal observed from a single particle on a dry quartz flat, within a wet cell containing suspension, and from within a dry cell (to isolate any effects of the carrier liquid from a change in signal due to the presence of the top flat). The results of this investigation are shown in Figure 4-6, with the error bars representing plus or minus one standard deviation for each data set. It can be observed that the presence of a quartz flat cover does not alter the signal. In addition, the signal from a single bead in suspension is not significantly different from a dry particle, indicating that no quenching is occurring as a result of the water. It should be noted that although measurement error may contribute somewhat to the standard deviation observed within each data set, there is also size variation in the fluorescent dye doped particles themselves (Figure 4-7), leading to a variation in the observed signal from particle to particle.

In addition to comparing wet vs. dry measurements, the effect of the number of particles within the viewing window on the observed signal was investigated for both wet and dry conditions (Figure 4-8). A general linear trend can be seen with an increase in the number of particles resulting in an increase in the observed signal. Although there is some variation in this trend, most notably for the 4-particle dry case, the variation between the signal from individual particles can explain the minor deviation observed. Note that the error bars for measurements of between one and five particles show plus and minus one standard deviation for multiple
measurements; for higher numbers of particles only one measurement was taken due to the lack of additional isolated clusters of the that number of beads within the samples.

In summary, the static experimental set-up has revealed the following:

- Dry particles produce the same intensity signal as particles in suspension
- The observed intensity is linear with the number of particles for both dry particles and particles in suspension
- There is no variation in signal strength with spatial translation within the imaged sample volume

**Time Resolved Measurements**

In order to investigate the fluorescence lifetime of the dye-doped particles time-resolved measurements were taken using the LeCroy oscilloscope as previously described for both dry samples and particles in suspension. For both dry and wet particles the fluorescence signal decays almost immediately (e.g., is coincident with the laser pulse), as shown in Figure 4-9. De-convolution of the observed signal resulted in a calculated fluorescence lifetime of ~1.8 ns, although it should be noted that the temporal resolution of the oscilloscope is insufficient for this quantitative result to be considered robust. This observation is, however, consistent with the actual fluorescence lifetime of these particles, stated by the manufacturer as about one nanosecond.

**Results of Dynamic Experimental Configuration**

**Imaging of Silica Particles**

Although the particles themselves may be visually observed on the display screen when passing through the capillary tube, even at the lowest pumping velocity they move in and out of screen too rapidly to allow an image of one to be manually captured. In order to illustrate what the particles look like, however, the flow stream through the tube was stopped, the inlet tubing disconnected, and the solution allowed to dry so that some particles would remain trapped within
the tube (Figure 4-10). This layout allowed for visual confirmation that particles in solution are indeed passing through the quartz capillary tube when the syringe mechanism is pumping.

Although this result is rather basic, an important observation was made during the initial testing of the system. When the pumping mechanism is initially turned on, particles are observed to pass through the tube at regular intervals; however, after a period of 20-30 seconds the particles begin to pass through with reduced frequency, ultimately decaying to no particles observed after several minutes. It has been determined that this problem is a result of the gravitational settling of particles out of suspension to the bottom of the pumping syringe, leaving only deionized water to pass through the sample tube. When the pumping mechanism and syringe were mounted on end (e.g., particles would settle toward the needle of the syringe and theoretically be pumped through the system), it was found that too high a concentration of particles entered the connecting tubing and clogged the flow. In order to resolve this problem during initial tests, the pumping mechanism was mounted sideways and the syringe and connecting tubing were occasionally manually agitated to maintain the flow of particles through the system. An automated system was later constructed that rotated the syringe from side to side, keeping the particles constantly in suspension (Appendix A).

**Comparison of Observed Signal with Static Measurements**

To synergize the results of the static and dynamic measurements, the spectra observed from a single particle in the flowing system was compared to the spectra obtained for wet and dry particles using the static system. Note that although typically in the dynamic set-up there is no aperture present in the laser beam in order to maximize the amount of energy incident on the tube, the aperture was inserted for comparison to the static measurements when reducing the size of the laser beam is necessary to prevent the excitation of particles outside of the sample viewing area (not required when the particles are confined in the sample tube). Although the spectra
from a flowing particle is understandably more noisy than that for the static case, the observed signal is of the same order of magnitude as the static measurement of a particle (Figure 4-11). This result confirms that results of the static measurements, most notably that translocation of the particle within the sample volume does not effect the observed signal, can be extended to the dynamic system.

**Theoretical and Experimental Investigation of Optimal Detection Conditions**

Using the dynamic experimental rig there are several parameters that may be optimized to maximize the number of particles that are detected within the system, including the concentration of the original sample (which may be adjusted with dilution), the repetition rate of the laser, and the flow rate through the tube. Ideally, (i) every particle within the stream would be detected once and only once, and (ii) 0% of the flow volume would pass through the tube without the laser firing, thus providing a complete count of the total number of particles that have passed through the system. From a statistical standpoint, these two conditions can be considered separately.

The first half of the problem, the probability that at the time of the laser firing there will be some specific number of particles within the sample volume (for example, a single particle, two particles, no particles, etc.), can be calculated using Poisson statistics as a function of the concentration of particles within the stream. The greater the original concentration, the less likely it is that the sample volume is empty, and at even greater concentrations the higher the likelihood that multiple particles will be in the sample volume at any given time. The probability \( P_n \) of any given number of particles \( n \) being located within the sample volume at a given time can be calculated using the Poisson distribution (Equation 4-1).

\[
P_n = \frac{\mu^n \exp(-\mu)}{n!}
\]  

\[(4-1)\]
In this equation, $\mu$ is a function of the sample volume ($V$) and the concentration ($C$; Equation 4-2).

$$\mu = C \cdot V$$ (4-2)

In our case, the sample volume is fixed based on the diameter of the tube ($d = 100 \, \mu m$) and the length of the tube in the field of view ($L = 250 \, \mu m$) and is therefore calculated as $1.96e-6 \, mL$. The probability of hitting any number of particles can be calculated by subtracting the probability of hitting no particles ($P_0$) from unity (Equation 4-3).

$$P_{\text{any}} = 1 - P_0 = 1 - \exp(-\mu)$$ (4-3)

A plot of the percentage of shots which would theoretically sample a single particle, two particles, or any number of particles (i.e., field of view is not empty) is presented in Figure 4-12 as a function of the particle concentration. From a single particle analysis perspective, it is desirable to trade a loss in efficiency (i.e., some shots strike no particles) for a loss in accuracy caused by striking multiple particles at once (e.g., $P_2$ or greater). For this reason, a maximum preferred concentration was calculated as the value for which the probability of hitting two particles fell to 0.5%. This concentration is $5.5e4$ particles/mL, and was used for all dynamic experiments using the test beads. It should be noted that although a lower concentration results in more empty shots (i.e., no particles are present), the number of multiple shot hits decreases even further, which is preferred. In the case of human blood, although the concentration of cells normally found in the bloodstream is higher than this threshold value (Table 4-2), the concentration of circulating tumor cells is considerably lower (averaging 8/mL) therefore the probably of counting cells multiple times is negligible (Allard, 2004).

The second half of the problem is calculating the percentage of the flow volume that is sampled, which is a function both of laser frequency and flow rate. Ideally, this value will be
100%, with a value less than 100% indicating that the flow rate is too fast relative to the laser firing rate and hence a portion of the sample is passing through without being illuminated. Conversely, if the laser fires too frequently relative to the flow rate, some particles may be illuminated by multiple laser shots and thus be erroneously counted twice.

In order to calculate the processed volume, the average flow rate of the particles ($\bar{v}$, in cm/s) must first be calculated from the volumetric flow rate ($\dot{V}$, in mL/s) of the fluid and the cross sectional area of the tube ($A_c$, in cm$^2$; Equation 4-4). It should be noted that this represents an average velocity of the particles, however, due to the parabolic flow profile within the tube, particles in the center will move faster than those at the tube edge.

$$\bar{v} = \frac{\dot{V}}{A_c} \quad (4-4)$$

Using the particle flow rate, the average amount of time ($\bar{t}$, in s) any given particle spends within the sample volume can be calculated from the length of the sample space ($L$, in cm; Equation 4-5).

$$\bar{t} = \frac{L}{\bar{v}} \quad (4-5)$$

The fraction of the total volume which passes through the tube which is processed by the laser ($V_p$) is then calculated from the laser firing frequency ($v$, in Hz; Equation 4-6).

$$V_p = \frac{\bar{t}}{1/v} \quad (4-6)$$

As the flow rate increases or the laser firing frequency decreases, the percentage of flow volume that is sampled by the laser will decrease (Figure 4-13). As previously mentioned, the ideal case would be if 100% of the volume that flows through the tube is sampled, which would occur at very low flow rates (less than 2 μL/min) for practical laser firing frequencies (~20 Hz).
of the current system. Physical constraints exist, however, on the flow rate; at very low flow rates, the particles settle out of the flow stream in the connecting tubing before reaching the sample chamber. Commercial systems may run in the 100s of Hz to kHz range, enabling 100% of the sample volume to be processed at much faster flow rates and thereby reducing processing time dramatically.

To determine the optimal flow conditions that may be physically achieved with the current system for the test particles, a parametric study of the percentage of particles found as a function of flow rate and laser firing frequency was conducted. Three different laser firing frequencies were used (5 Hz, 10 Hz, and 20 Hz) using three different flow rates (2 μL/min, 4 μL/min, and 10 μL/min), with the concentration of the beads fixed at 5.5e4 particles/mL to minimize the probability of multiple particles occupying the test volume at the time of the laser firing as previously discussed. For any given condition, the total flow volume passing through the sample tube ($V_{tot}$, in μL) was calculated from the flow rate ($V$, in μL/min) and the time of the experiment ($t_{exp}$, in s; Equation 4-7). The time of the experiment (from first firing of the laser to the last) was calculated from the laser frequency ($ν$, in Hz) and the total number of laser shots (2500 in most cases unless spectra were removed due to errant spikes caused by stray light).

$$V_{tot} = V \cdot \frac{t_{exp}}{60} = V \cdot \frac{\nu}{60} \cdot 2500 \cdot ν \quad (4-7)$$

The theoretical number of particles within that flow volume was calculated from the concentration of the original sample, and the fraction of particles counted was calculated as a percentage of this value (Table 4-3, Figure 4-14) and compared to the percentage of flow volume processed for each set of conditions (Figure 4-15). As the laser frequency increases, the percentage of particles counted increases as a result of the increased amount of volume illuminated at the higher rep rate (Figure 4-13). Note, however, that although theoretically at a
fixed laser frequency a slower flow rate should increase the percentage of particles counted, in fact the highest percentage of particles is captured at a flow rate of 4 μL/min. As previously mentioned, this deviation from the statistical calculations is due to the increased loss of particles at very slow flow rates due to settling out of the flow stream (Appendix C).

In summary, the optimal experimental conditions for single particle detection have been investigated both theoretically and experimentally. In order to minimize the amount of time multiple particles occupy the sample volume (probability of less than 0.5%) the particle concentration should be kept below a value of 5.5e4 particles/mL, as determined with Poisson statistics. The percentage of flow volume sampled with the laser, ideally 100%, was calculated as a theoretical function of laser firing frequency and flow rate, and verified by comparison with a parametric study of percentage of particles observed at various flow rate/laser firing frequency combinations. Experimentally, it was determined that at very low flow rates (< ~4 μL/min) a significant percentage of particles settle out of solution, setting a practical limit on this parameter. In the present experimental configuration the laser should fire at its fastest frequency (~20 Hz) to maximize the number of particles detected at this flow rate.

**Discussion and Conclusions**

The primary goal of the present flow cytometry study was the construction of a test bed capable of detecting fluorophores moving dynamically in suspension for the eventual purpose of constructing a system for rapidly identifying rogue circulating tumor cells *in situ*. To that end a set-up has been constructed that pumps a solution through a capillary tube where it is illuminated by visible light and with a scattering and fluorescence-inducing laser. A shaker mechanism has been designed and constructed to prevent the particles from settling out of solution within the pumping mechanism (Appendix A).
In order to verify that spatial variation within the sample tube would not influence the observed fluorescence signal, static measurements were taken of a single particle in multiple locations in the x-, y-, and z-planes. At the scale of the capillary tube no variation in signal was observed with translocation, validating that the data would be consistent irregardless of where a particle was located at the time of the laser firing. In addition, static tests were used to confirm that the carrier fluid (in this case, deionized water) had no quenching effect on the signal from a single particle. The signal collected from single particles in the static system was compared to those taken using the dynamic system to confirm that the change in experimental conditions had no effect on the signal. Static experiments were also done to investigate the fluorescence observed from 355-nm excitation of cancer cells in bulk solution (Appendix B).

In order to optimize the dynamic experimental set-up, a statistical investigation into the optimal operating conditions for particle detection was undertaken. Poisson statistics were done to determine the maximum particle concentration for which the probability of multiple particles occupying the sample tube would be negligible. This maximum value was calculated to be 5.5e4#/mL, with a corresponding probability of 0.5% for two particles occupying the sample volume at any given time. Any concentration less than this value increases the number of times the tube is empty at the time of the laser firing, but further reduces the chance of undercounting particles. In the case of circulating tumor cells, the concentration is considerably less than this maximum value, on the order of magnitude of less than 10 cells per mL on average ranging up to 1000’s of cells per mL. At these low concentrations there will be a large number of empty laser firings, but the odds of counting multiple particles as one are extremely low.

Statistical calculations were also done to maximize the volume processed by the system as a function of laser firing frequency and flow rate. If the flow rate is too fast or the laser fires too
infrequently, portions of the sample volume will pass through the tube without being illuminated. Conversely, if the flow rate is too slow for the laser firing rate, portions of the flow volume will be illuminated multiple times and potentially lead to a single particle being counted multiple times. It was determined that a relatively slow flow rate (< 5 μL/min) maximizes the processed volume at laser firing rates of less than 20 Hz. These results were consistent with the data obtained during a parametric experimental study on the effects of flow rate and laser firing frequency on the percentage of particles counted. During the experimental validation, however, it was determined that at very slow flow rates (~ 2 μL/min) the percentage of particles counted is relatively low compared to the percentage obtained at higher flow rates, attributed to particles settling out of suspension before reaching the sample chamber at the slower flow rate. A theoretical investigation into the settling of particles within the system was conducted (Appendix C). In terms of detecting circulating tumor cells, where ideally the amount of total sample volume required would be minimized to prevent excessive blood from being drawn from a patient, a laser firing frequency considerably higher than achievable with the current system would be required in order to maximize the percentage of volume processed and minimize the number of tumor cells passing through the system undetected.
Table 4-1. Specifications for each of the key components in the flow cytometry experimental rig.

<table>
<thead>
<tr>
<th>Component</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe Pump</td>
<td>Bioanalytical Systems (BAS) MD-1001 Baby Bee</td>
</tr>
<tr>
<td>Syringe Pump Controller</td>
<td>BAS MD-1000 Worker Bee Syringe Pump Controller</td>
</tr>
<tr>
<td>Syringe</td>
<td>BAS MDN-0100 1.0 mL Bee Stinger Gastight Syringe</td>
</tr>
<tr>
<td>Capillary Tube</td>
<td>VitroCom Inc. CV1017Q</td>
</tr>
<tr>
<td>Microscope Objective</td>
<td>Mitutoyo M Plan Apo 50X</td>
</tr>
<tr>
<td>Beam Splitter</td>
<td>Newport 10BC17MB.1 Beamsplitter</td>
</tr>
<tr>
<td>Camera</td>
<td>Hitachi Color CCD KP-D20B</td>
</tr>
<tr>
<td>Spectrometer</td>
<td>Acton SpectroPro 300i</td>
</tr>
<tr>
<td>Fluorescence Inducing Laser</td>
<td>Continuum Minilite Nd:YAG laser (355 nm)</td>
</tr>
<tr>
<td>Scattering Laser</td>
<td>JDS Uniphase 1677 (594 nm)</td>
</tr>
</tbody>
</table>

Table 4-2. Range of concentrations of various types of cells in the human bloodstream.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells(^1)</td>
<td>4.2e-9-5.9e9 #/mL</td>
</tr>
<tr>
<td>White Blood Cells(^1)</td>
<td>4.3e-6-18.8e6 #/mL</td>
</tr>
<tr>
<td>Platelets(^1)</td>
<td>150e6-400e6 #/mL</td>
</tr>
<tr>
<td>Circulating Tumor Cells(^2)</td>
<td>0.25-3.1e3 #/mL (Mean 8 #/mL)</td>
</tr>
</tbody>
</table>


\(^2\)Data from Allard et al, 2004.

Table 4-3. Results of parametric study of flow rate and laser frequency on the number of particles counted in the stream.

<table>
<thead>
<tr>
<th>Flow Rate [μL/min]</th>
<th>Laser Frequency [Hz]</th>
<th>Flow Volume [μL]</th>
<th>Calculated # of Particles</th>
<th>Counted # of Particles</th>
<th>% of Particles Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5</td>
<td>16.7</td>
<td>919</td>
<td>41</td>
<td>4.5%</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>33.1</td>
<td>1821</td>
<td>139</td>
<td>7.6%</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>75.5</td>
<td>4153</td>
<td>126</td>
<td>3.0%</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>8.3</td>
<td>457</td>
<td>29</td>
<td>6.3%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>16.7</td>
<td>919</td>
<td>135</td>
<td>14.7%</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>41.7</td>
<td>2294</td>
<td>147</td>
<td>6.4%</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>4.2</td>
<td>231</td>
<td>28</td>
<td>12.2%</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>8.3</td>
<td>457</td>
<td>111</td>
<td>24.2%</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>20.8</td>
<td>1144</td>
<td>105</td>
<td>9.2%</td>
</tr>
</tbody>
</table>
Figure 4-1. Design of the flow cytometry test bed. Note that part of the diagram is in side view and part is in top view, with a mirror turning the light from the particles from vertical to horizontal at the transition in view in the actual rig.
Figure 4-2. Diagram and picture of the syringe pump used to inject particles in suspension into the capillary tube. The pump mechanism is depressed to produce the desired flow rate by an automated controller (not shown). Diagram modified from an image on the Bioanalytical Systems website (http://www.bioanalytical.com/products/iv/bee.php, last accessed March, 2008).
Figure 4-3. Picture of experimental set-up.
Figure 4-4. Static experiment sample holder configurations. In (I), the dry particles are exposed to the air; in (II), the dry particles are confined in a sample cell consisting of a thin plastic ring sandwiched between two quartz flats; in (II), the same cell is used to confine a sample of wet particles.
Figure 4-5. Spatial variation in observed signal for three individual particles.
Figure 4-6. Signal observed from a single particle for the three static test conditions (dry without a quartz flat cover, dry containment cell, and wet containment cell).
Figure 4-7. Image taken of multiple fluorescence dye-doped particles illustrating the size variation between individual beads.
Figure 4-8. Variation in observed intensity with the number of particles in the sample volume for both wet and dry conditions.
Figure 4-9. Example of a time-resolved measurement of a dry particle. Note that the fluorescence decays virtually instantaneously.
Figure 4-10. Image of silica particles within the sample tube.
Figure 4-11. Comparison of the spectra taken of a single particle flowing through the dynamic system with the spectra (averaged over 50 shots) for a static particle.
Figure 4-12. Percentage of shots which would theoretical strike one particles, two particles, or any number of particles as a function of concentration.
Figure 4-13. Percentage of the flow volume sampled as a function of the flow rate for several laser frequencies.
Figure 4-14. The percentage of particles passing through the sample volume counted by the laser as a function of laser frequency and flow rate.
Figure 4-15. The percentage of flow volume processed as a function of flow rate (black line) and the percentage of particles counted (black circles) for the three laser frequencies considered.
Although traditional analysis methods predominantly characterize the average properties of a system such as temperature, pressure, and so forth, in some applications the data of interest comes from a relatively small portion of analyte with a signal that is lost when averaged with bulk parameters. In the extreme, single particles may need to be detected and analyzed; for example, circulating tumor cells provide an early indication of the presence of cancer, but due to a very low concentration in the bloodstream (typically on the order of $10^{10}$/mL vs. $10^6 - 10^9$/mL for normal blood cells) they are impossible to detect using bulk analysis. Other examples include the detection of toxic species in ambient air, which may represent parts per trillion on a mass basis, yet exist as discrete and detectable particles, and the quantification of specific species of phytoplankton, which may constitute a small fraction of similarly sized biota in a spatial region. Optical techniques are uniquely suited for these types of applications, in that such methods are both sensitive enough to detect single cells/molecules, and are rapid enough to allow for analysis of large systems in a reasonable time scale. In the present work a number of achievements have been made in the application of laser-based diagnostic methods for single particle detection and analysis for both environmental and medical applications.

Through the use of a numerical model of a Laser-Induced Breakdown Spectroscopy (LIBS) plasma interacting with a single aerosol particle, it has been demonstrated that the processes of particle evaporation and diffusion through the plasma occur on finite time scales relative to the delay times at which spectroscopic measurements typically occur. The implication of this result is that aerosol analyte species encounter local plasma conditions (temperature and free electron density) varying from the bulk plasma, and that in using LIBS for the analysis of single aerosol particles the timing and design of experimental measurements must be carefully
considered to insure robust results. These results may also extend to the use of LIBS for bulk analysis of solid, liquid, and gaseous systems, where local conditions again may dominate the observed signal. Key findings are summarized below:

- A finite rate of diffusion results in the aerosol analyte interacting with local conditions that may not be well represented by the properties of the bulk plasma.
- Heat and mass diffusion occur on similar time scales, as evidenced by a Lewis number calculated on the order of unity.
- Care must be taken in regards to the time delay at which measurements are taken in that the concentration of aerosol species will vary with time throughout the inhomogeneous plasma.

Although the present model has qualitatively captured the behavior of a LIBS plasma interacting with a single aerosol particle, the inherent assumptions of the model (relative to the present state-of-the-art in laser-induced plasma modeling) and the need for further validation presents several opportunities for expansion on the current studies, outlined below:

- Incorporate the current heat conduction and mass diffusion processes into more complex hydrodynamic plasma models that account for such phenomena as shock waves, particle explosion, etc.
- Develop a more realistic model of the detected plasma intensity accounting for an optically thick plasma and instrument collection optics.
- Validate the model results with experimental trials with the modeled analyte aerosol species.

As an alternate method to plasma-based aerosol analysis, experimental studies were undertaken to explore the application of prompt emission photofragmentation/fragment detection (PF/FD) to the detection and quantification of alkali aerosols, the vaporous hydrocarbon EEP, and the interaction between the two in a system containing both. The study has found that PF/FD is capable of detecting both alkali aerosols and the representative solvent, although the UV laser radiation required for the technique is such that constructing a durable test bed is challenging due to window contamination. Despite this challenge, however, it was demonstrated
that the presence of the aerosol species reduces the signal generated from the EEP, which is attributed to adsorption of the EEP vapor onto the aerosol surface. Such a finding speaks to the potential of this technique to monitor heterogeneous aerosol chemistry, an important problem for aerosol science. Oxygen was also determined to be a quenching species in the case of the emitted EEP signal, with no quenching effect found on the sodium signal generated by the aerosol particles. Fragmentation pathways are proposed to account for the observed photofragments and the observed quenching effect. Specific findings are summarized:

- PF/FD has been demonstrated as a detection technique for both aerosol species and vaporous hydrocarbons.
- Photofragmentation pathways have been proposed for the representative hydrocarbon considered in the current study, ethyl-3-ethoxypropionate (EEP).
- Adsorption of a noxious vapor onto aerosol particles has been observed, indicating the potential for PF/FD for the study of heterogeneous chemistry.
- Oxygen has been quantified as a quenching species of the EEP signal, resulting in both a decrease in signal and a shortening of the fluorescence lifetime.

As an expansion to the present work validating proof-of-concept for PF/FD as a detection method for aerosols and noxious hydrocarbons, future studies should include the following:

- Design and construction of a more robust test bed that eliminates both eddy formation and is not subject to optical window degradation.
- Quantified studies of the interaction of aerosols and vaporous hydrocarbons, considering the effects of particle volume/surface area on vapor adsorption and determining uptake rate coefficients for the interaction of specific species.
- Extension to other types of aerosol particles and hydrocarbons.
- Testing of PF/FD in more advanced systems including multiple particulate and vaporous components.
- Extend collection optics into the UV range, allowing detection and analysis of additional photofragment species including, for example, the hydroxyl radical (OH).
A final single particle analysis technique using laser-induced fluorescence for the detection of particles in suspension was considered with the specific application of identifying circulating tumor cells in the blood stream. An experimental set-up was designed and constructed allowing for the illumination of a suspension with visible light and with both a scattering and fluorescence-inducing laser. A complementary static system was also employed to verify that the signal detected in the dynamic system would be independent of the spatial location of the target particle within the test volume; in addition, the static system demonstrated that water as a carrier fluid had no quenching effect on the signal observed from the test beads used to validate the dynamic system. The aforementioned test beads were detected in the dynamic system, demonstrating the set-up’s effectiveness for use with flowing particles in suspension. A statistical investigation was undertaken to maximize the number of particles detected as a function of original concentration, laser firing frequency, and flow rate, and the results were confirmed experimentally. The highlights of the study are summarized here:

- Development of an experimental set-up capable of real-time detection of single fluorescent particles.
- Validation of the dynamic detection system through static experiments verifying that the observed signal is invariant with position within the sample volume.
- Statistical investigation into the maximum concentration of particles in suspension to prevent multiple particles from being illuminated simultaneously by the laser.
- Statistical and experimental investigation into the optimal volumetric flow rate and laser firing frequency to insure that 100% of the sample passing through the system is interrogated.

Based on the present state of this research several recommendations are made for continuing the current study:

- Investigation into using the present system for the detection and differentiation of different types of cancer.
• Experimental investigation into the number of particles within the sample volume as a function of flow rate and laser firing frequency using two different types of test particles fluorescing at different wavelengths (technique described in Wu et al., 2007).

• Use of both inherent fluorophores and dyes for circulating tumor cell detection.

• Experimental validation with human blood and in clinical trials using the technology to differentiate between healthy individuals, persons with cancer in remission, and those with an active form of the disease.

• Investigation into using the current technique for the quantification of circulating tumor cells in an individual with cancer and the possibility of using CTCs as a diagnostic and prognostic tool.

Overall, the present study represents advancement in both the fundamental understanding and the practical application of optical techniques for single particle detection and quantification in fluid (liquid and vapor) suspension for both aerosol and biomedical applications where traditional bulk analysis is incapable of effectively characterizing the system of interest.
APPENDIX A
SYRINGE AGITATOR DESIGN

It was determined during initial test runs with silica beads that particles in the pumping syringe settle out of solution over the course of a few minutes, preventing them from flowing through the experimental set-up with the pumped fluid. Although it is possible to manually rotate the syringe and pumping mechanism to alleviate this problem, for convenience purposes an automatic agitator was designed (Figure A-1).

The magnetic base of the pumping mechanism is supported on a steel plate that rocks back and forth through 180° to keep the particles in suspension (the plate rotates from a point where the syringe pump is vertical, through the horizontal position where it is upright, to the point where it is vertical on the other side and back). The plate is affixed by two small stainless steel support columns to a pivoting axle with a gear affixed to one end. This gear is connected to a second, larger gear, which is in turn connected by a rigid rod to a pin affixed to the rotating shaft of a DC motor. The length of the connection rod and gear sizes have been calculated (Figure A-2) so that the gear connected via rod to the motor shaft sweeps out an angle of ~56° (an angle small enough to prevent the mechanism from seizing) yet produces a full 180° of rotation of the upper gear and hence the syringe pump itself.
Figure A-1. Picture of agitator mechanism.
Figure A-2. Schematic of the agitator mechanism showing rotation angle of connector pin and diagrams of gear/pin mechanism at points of maximum displacement.
APPENDIX B

FLUORESCENCE OBSERVED FROM CANCER CELLS IN BULK SOLUTION

As a preliminary evaluation of the flow cytometry system’s capability to detect circulating tumor cells, the fluorescence emitted from cancer cells in bulk solution was investigated. 4TO7 mouse mammary adenocarcinoma cells at a concentration of $10^5$ particles/mL were prepared in a phosphate buffer solution. A 355-nm Nd:YAG laser was used as the excitation source, and the emitted fluorescence was collected with a fiber optic cable connected to the same spectrometer used in the flow cytometry system. 100 individual spectra were averaged for the cancer cell solution using several spectral windows to obtain intensity values over the range of 360-560 nm. The same windows were used to obtain the spectra emitted from the phosphate buffer solution with no cancer cells; these spectra were subtracted from the signal observed from the cancer cell solution to isolate the signal emitted purely from the cells themselves (Figure B-1). Although the high frequency variations in the intensity may be attributed to instrument function, a broad band of fluorescence can be clearly observed.

Using an estimated laser interrogation volume of 0.002 cm$^3$, calculated using an optical fiber bundle diameter of 1 mm and a laser beam thickness of 2.5 mm, the number of cells within the sample volume can be calculated from the concentration ($10^5$ particles/mL) as 200 cells. For the peak intensity observed of ~200,000 counts (a.u.) at 440 nm, the intensity collected from a single shot of a single cell is estimated as about 10 counts (a.u.); however, given that the collection optics in the dynamic experimental set-up are approximately 10 times more efficient than those used to collect the signal from the bulk solution, this value would be expected to be more on the order of magnitude of 100 counts (a.u.). This value is a detectable quantity using the set-up instrumentation, indicating that the experimental design should be capable of quantifying the fluorescence emitted from the inherent fluorophores of cancer cells.
Figure B-1. Signal emitted from cancer cells in bulk solution. Note that the background intensity emitted by the phosphate buffer solution has been subtracted and individual spectra have been cropped to remove errors at the edge of each spectral window.
APPENDIX C
THEORETICAL INVESTIGATION OF PARTICLE SETTLING IN FCM SYSTEM

During experimental runs, particles in the flow cytometry system were observed to cease to flow through the sample volume if the syringe pump was not agitated or the pumping flow rate was too slow (less than ~4 μL/min). This situation is a function of the settling velocity of the particles; if the settling velocity is sufficiently fast that the particles settle out of solution before they reach the sample volume, they cannot be interrogated by the laser and will not be detected.

The terminal settling velocity of a particle (v_t, in m/s; Equation C-1) is a function of the particle density and diameter (ρ_p, in kg/m³; d_p, in m), the acceleration due to gravity (g, 9.8 m/s²), the viscosity and density of the carrier fluid (μ, in N·s/m²; ρ, in kg/m³), and the dimensionless slip correction factor (C).

\[ v_t = \frac{ρ_p g d_p^2}{18\mu} \left[ 1 - \frac{ρ}{ρ_p} \right] \]  

(C-1)

The diameter of the fluorescent-dye doped test beads is 5.26e-6 m, with a density equal to approximately 1050 kg/m³; for these beads suspended in a water solution (ρ = 1000 kg/m³, μ = 855e-6 N·s/m²), the terminal settling velocity is calculated as approximately 1 μm/s assuming a slip correction factor of unity. Although this velocity may initially seem slow, this value is of the same order of magnitude as the average lateral velocity (̅v = 2 μm/s) of the particles moving through the syringe at the highest pumping rate used in the current study, 10 μL/min, calculated from the volumetric flow rate and the cross sectional area of the ~1 cm diameter syringe (Table C-1).

The inner diameter of the Teflon tubing connecting the syringe to the capillary tube is ~1 mm, resulting in a cross sectional area two orders of magnitude smaller (and hence an average flow velocity two orders of magnitude larger) than in the pumping syringe (Table C-1).
average time any given particle will spend traversing the 10 cm length tubing will be 39, 20, and 8 minutes for the volumetric flow rates considered of 2, 4, and 10 μL/min, respectively. By comparison, the time required for a particle to travel 1 mm (the diameter of the tubing) at the terminal settling velocity of 1 μm/s is 17 minutes. Although these values are approximate (noting that the terminal settling velocity is sensitive to the exact density of the Teflon beads, that the lateral velocity of the particles varies parabolically with the radial location in the tubing, and that particles will enter the tube at different radial locations, etc.), a comparison of the settling time to the time required for particles to pass through the length of tubing for each of the flow rates considered qualitatively confirms what was experimentally observed: at slow flow rates, particles settle out of solution before reaching the sample volume.

A final question for consideration would be whether the flow velocity is sufficient to resuspend particles that have settled out of solution, or to initiate bedload transport (i.e., particle motion along the surface of the tube). The force exerted by the fluid on the particles (F) is calculated from the particle diameter (dp, in m) and the fluid viscosity and velocity (μ and v, in N·s/m² and m/s, respectively) as shown in Equation C-2.

\[ F = 3\pi\mu d_p v \]  

(C-2)

The force exerted on the test particles using the average velocity for each volumetric flow rate considered is on the order of 10^{-12} N (Table C-1), noting that the velocity near the wall will be slower than the average velocity and thus the value calculated would be higher than the true force. The detachment force for polystyrene particles is on the order of 10^{-7}-10^{-9} N (Noordmans et al., 1997), indicating that the fluid is not likely to reinitiate motion in particles that have settled to the tubing wall, although the exact adhesion force is difficult to estimate due to uncertainty in the true particle surface functionality and the true surface functionality.
Table C-1. Velocity and force parameters for various volumetric flow rates.

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LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Patricia (Soupy) Dalyander was born in 1977, in Camden, South Carolina, and grew up in Charlotte, North Carolina, graduating from Providence High in 1995. She earned her Bachelor of Science degree with a dual major in physics and mathematics, in 1999, from Eckerd College in St. Petersburg, Florida, carrying out a theoretical study of the properties of $m$-accretive operators in Banach spaces. From there she went on to earn her master’s degree in oceanography, from Oregon State University, working in the Coastal Imaging Lab and developing a new method of extracting and analyzing data from time-lapse surf zone images. Soupy then worked for several years as an oceanographer with the U.S. Geological Survey in Woods Hole, Massachusetts, studying both Massachusetts Bay and the Hudson Shelf Valley. She then returned to school to obtain a master’s degree in mechanical engineering from the University of Florida, in 2006, before continuing her work in laser-diagnostics applied to single particle analysis for her Ph.D.